

UNIVERSIDADE FEDERAL DE VIÇOSA

ANDRÉ DE OLIVEIRA MENDONÇA

**ENHANCING BIOSAFETY MANAGEMENT AND GOVERNANCE: A
COMPREHENSIVE ASSESSMENT OF HIGH-CONTAINMENT BIOLOGICAL
LABORATORIES IN BRAZIL**

**VIÇOSA – MINAS GERAIS
2024**

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Thesis submitted to the Biochemistry Applied
Graduate Program of the *Universidade
Federal de Viçosa* in partial fulfillment of the
requirements for the degree of *Doctor
Scientiae*.

Adviser: Cláudio Lísias Mafra de Siqueira

Co-advisers: Donald Hugh Bouyer
Kurt Allen Zuelke

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*To the Creator of the Universe, King of Glory, Lord
of Lords, my Father, and beloved Redeemer. To
Him all honor, glory, and praise.*

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UFMS – *Hospital Universitário*
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'No job will be considered so important that it cannot be done safely'.

(Arnold G. Wedum)

'Biosafety culture is the set of values, beliefs, and patterns of behavior instilled and facilitated in an open and trusting environment by individuals throughout the organization who work together to support or enhance best practices for laboratory biosafety. This culture is crucial for the success of a biosafety program and is built from mutual trust and the active engagement of all personnel across the organization, with a clear commitment from the organization's management. Establishing and maintaining a biosafety culture provides a foundation for developing a successful biosafety program'.

(World Health Organization)

ABSTRACT

MENDONÇA, André de Oliveira, D.Sc., Viçosa Federal University, April, 2024. **Enhancing Biosafety Management and Governance: A Comprehensive Assessment of High-Containment Biological Laboratories in Brazil.** Adviser: Cláudio Lísias Mafra de Siqueira. Co-advisers: Donald Hugh Bouyer and Kurt Allen Zuelke.

High-containment biological laboratories are crucial in preventing and addressing high-risk infectious diseases, encompassing human and animal health. This comprehensive study presents an in-depth analysis of such laboratories in Brazil, covering their quantity, distribution, infrastructure, scope, regulatory compliance, and associated challenges. From a preliminary list of 92 presumed high-containment biological laboratories in Brazil, a total of 66 laboratories from 54 institutions currently operating under characteristics of a biosafety level 3 facility were identified, with 32 participating in the current research by responding to a comprehensive questionnaire. Key findings indicate a lack of official data, emphasizing the need for a National Biosafety and Biosecurity Policy and a strategic plan to assess and optimize the functionality and distribution of these facilities across the country. The study revealed that many high-containment biological laboratories may not comply with essential biosafety and biosecurity standards due to the absence of a national regulatory framework specifically addressing these issues. Furthermore, the sustainability of these laboratories is often jeopardized by financial constraints, particularly those associated with operational costs not covered by research grants. The establishment of a centralized system for laboratory oversight, akin to models in the US and Canada, emerges as a crucial recommendation. Such a framework would ensure standardized practices, facilitate sharing resources and information, and enhance Brazil's overall biosafety and biosecurity culture. In conclusion, the study advocates for urgent reforms in Brazil's approach to managing high-containment biological laboratories, including the development of a robust regulatory framework, enhanced government oversight, and the promotion of a strong culture of safety and security within the scientific community. These measures are deemed essential for safeguarding public and animal health and ensuring the country is equipped to address current and future biosecurity challenges.

Keywords: High-Containment Biological Laboratories; Biosafety and Biosecurity; Regulatory Framework. Risk Management in Laboratories; Laboratory Sustainability; National Biosafety and Biosecurity Policy; Capacity Building in Biosafety; Biocontainment Facilities; Laboratory Oversight and Governance; Biorisk Management System.

RESUMO

MENDONÇA, André de Oliveira, D.Sc., Universidade Federal de Viçosa, abril de 2024. **Aprimorando a Gestão e Governança em Biossegurança: Uma Avaliação Abrangente de Laboratórios de Alta Contenção Biológica no Brasil.** Orientador: Cláudio Lísias Mafra de Siqueira. Coorientadores: Donald Hugh Bouyer e Kurt Allen Zuelke.

Os laboratórios de alta contenção biológica desempenham um papel estratégico na prevenção e combate às doenças infecciosas de alto risco, tanto na saúde humana quanto na defesa agropecuária. O presente estudo apresenta uma análise detalhada a respeito da operação desses laboratórios no Brasil, abordando sua quantidade, distribuição, infraestrutura, escopo, conformidade regulatória e desafios associados. De uma lista preliminar de 92 laboratórios brasileiros presumivelmente equipados com ambientes de alta contenção biológica, identificamos um total de 66 laboratórios, distribuídos em 54 instituições, que atualmente operam sob características compatíveis com laboratórios de nível 3 de biossegurança. Desses, 32 participaram da pesquisa ao responderem um amplo questionário. Os principais achados indicaram a ausência de dados oficiais, ressaltando a necessidade urgente de uma Política Nacional de Biossegurança e Bioproteção, bem como de um plano estratégico para avaliar e otimizar a funcionalidade e distribuição dessas instalações no país. O estudo também revelou que muitos desses laboratórios não cumprem certos padrões essenciais de biossegurança e bioproteção devido à falta de um arcabouço regulatório nacional específico para essas questões. Além disso, a sustentabilidade desses laboratórios é frequentemente comprometida por restrições financeiras, especialmente os custos operacionais não cobertos por recursos provenientes de projetos de pesquisa. Recomendamos o estabelecimento de um sistema centralizado para supervisionar os laboratórios de alta contenção biológica, similar aos modelos existentes nos EUA e Canadá, a fim de garantir práticas padronizadas, facilitar o compartilhamento de recursos e informações, e fortalecer a cultura de biossegurança e bioproteção na comunidade científica. Essas medidas são cruciais para proteger a saúde pública, a defesa agropecuária e para assegurar uma estrutura adequada para enfrentar os desafios atuais e futuros relacionados à biossegurança e bioproteção.

Palavras-chave: Laboratórios de Alta Contenção Biológica; Biossegurança e Bioproteção; Arcabouço Regulatório; Gestão de Riscos em Laboratórios; Sustentabilidade Laboratorial; Política Nacional de Biossegurança e Bioproteção; Capacitação em Biossegurança; Instalações Biocontidas; Supervisão e Governança Laboratorial; Sistema de Gestão de Riscos Biológicos.

LIST OF ACRONYMS AND ABBREVIATIONS

ABIN	<i>Agência Brasileira de Inteligência</i> – Brazilian Agency of Intelligence
ABNT	<i>Associação Brasileira de Normas Técnicas</i> – Brazilian Association of Technical Standards
ABSA	American Biological Safety Association
AIDS	Acquired Immunodeficiency Syndrome
AMexBio	Mexican Association of Biosafety
ANAC	<i>Agência Nacional de Aviação Civil</i> – Brazilian National Civil Aviation Agency
ANVISA	<i>Agência Nacional de Vigilância Sanitária</i> – Brazilian National Health Surveillance Agency
A-PBA	Asia-Pacific Biosafety Organization
APELS	Accidental Pathogen Escape from Laboratory Settings
APHL	Association for Public Health Laboratories
ASF	African swine fever
BFG	Brazilian Federal Government
BioGib	Ibero-American Biosafety Group
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BO	Biosafety Officer
BSC	Biosafety Cabinet
BSL	Biosafety Level
BWC	Biological Weapons Convention
CAD	Canadian dollar
CAPES	<i>Coordenação de Aperfeiçoamento de Pessoal de Nível Superior</i> – Brazilian Coordination of Superior Level Staff Improvement
CBS	<i>Comissão de Biossegurança em Saúde do Ministério da Saúde</i> – Health Biosafety Commission of the Brazilian Ministry of Health
CDC	Centers for Disease Control and Prevention
CEPEM	<i>Centro de Pesquisa em Medicina Tropical</i> – Tropical Medicine Research Center
CFR	Code of Federal Regulations
CH	Hospital Complex
CIBio	Internal Biosafety Committee
CQB	Certificate of Quality in Biosafety
CNPISA	<i>Centro Nacional de Pesquisa de Suínos e Aves</i> – Brazilian National Swine and Poultry Research Center
COMBIOLAB	<i>Comissão Permanente de Gestão de Riscos Biológicos e Biossegurança em Laboratórios da Rede Nacional de Laboratórios Agropecuários que manipulem agentes biológicos e suas partes, vírus e suas partes e príons de interesse em saúde animal</i> – Permanent Commission for the Management of Biological Risks and Biosafety in Laboratories of the National Veterinary Laboratories that manipulate biological agents and their parts, viruses and their parts, and prions of interest in animal health
CONCEA	<i>Conselho Nacional de Controle de Experimentação Animal</i> – National Council for Animal Use Control
CREDEN	<i>Câmara de Relações Exteriores e Defesa Nacional</i> – Brazilian Chamber of Foreign Affairs and National Defense

CRPHF	<i>Centro de Referência Professor Hélio Fraga</i> – Reference Center Professor Hélio Fraga
CSF	Classical swine fever
CTNBio	<i>Comissão Técnica Nacional de Biossegurança</i> – Brazilian National Biosafety Technical Commission
CWA	CEN (European Committee for Standardization) Workshop Agreement
DNA	Deoxyribonucleic Acid
DURC	Dual-use Research of Concern
EBSERH	<i>Empresa Brasileira de Serviços Hospitalares</i> – Brazilian Hospital Services Company
EDS	Effluent Decontamination System
EDPLN	Emerging and Dangerous Pathogens Laboratory Network
EMBRAPA	<i>Empresa Brasileira de Pesquisa Agropecuária</i> – Brazilian Agricultural Research Corporation
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FAPESP	<i>Fundação de Amparo à Pesquisa do Estado de São Paulo</i> – São Paulo Research Foundation
FBI	Federal Bureau of Investigation
FINEP	<i>Financiadora de Estudos e Projetos</i> – Funding Authority for Studies and Projects
FIOCRUZ	<i>Fundação Oswaldo Cruz</i> – Oswaldo Cruz Foundation
FMD	Foot-and-mouth disease
FMT	<i>Fundação de Medicina Tropical Doutor Heitor Vieira Dourado</i> – Dr Heitor Vieira Dourado Tropical Medicine Foundation
FREQ	Frequency
FSAP	Federal Select Agent Program
FUNED	<i>Fundação Ezequiel Dias</i> – Ezequiel Dias Foundation
FURG	<i>Universidade Federal do Rio Grande</i> – Rio Grande Federal University
GHRC	Global Health Research Complex
GHS	Global Health Security
GMO	Genetic Modified Organism
GSI/PR	<i>Gabinete de Segurança Institucional da Presidência da República</i> – Brazilian Institutional Security Office of the Presidency of the Republic
HC	Clinical Hospital
HCBL	High-Containment Biological Laboratory
HEPA	High-Efficiency Particulate Arrestance
HHS	United States Department of Health and Human Services
HPAI	High Pathogenic Avian Influenza
HU	University Hospital
HVAC	Heating, Ventilation, and Air Conditioning
IACUC	Institutional Animal Care and Use Committee
IAL	<i>Instituto Adolfo Lutz</i> – Adolfo Lutz Institute
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
IBEx	<i>Instituto de Biologia do Exército</i> – Brazilian Army Biology Institute
IEC	<i>Instituto Evandro Chagas</i> – Evandro Chagas Institute
IEGBBR	International Expert Group for Biosafety and Biosecurity Regulation
IHR	International Health Regulations
IN	<i>Instrução Normativa</i> – Normative Instruction

INCA	<i>Instituto Nacional do Câncer</i> – National Cancer Institute
INPA	<i>Instituto Nacional de Pesquisas da Amazônia</i> – Amazon National Research Institute
ISO	International Organization for Standardization
IVBW	International Veterinary Biosafety Workgroup
JEE	Joint External Evaluation
LACEA/ICC	<i>Laboratório de Criação e Experimentação Animal/Instituto Carlos Chagas</i> – Animal Breeding and Vivarium/Carlos Chagas Institute
LACEN	<i>Laboratório Central de Saúde Pública</i> – Brazilian Public Health Central Laboratory
LAI	Laboratory-acquired infection
LAT	Laboratory Assessment Tool
LFDA	<i>Laboratório Federal de Defesa Agropecuária</i> – Brazilian National Veterinary Laboratory
LRN	Laboratory Response Network
MAPA	<i>Ministério da Agricultura e Pecuária</i> – Brazilian Ministry of Agriculture and Livestock
MCTI	<i>Ministério da Ciência, Tecnologia e Inovação</i> – Brazilian Ministry of Science, Technology, and Innovation
MD	<i>Ministério da Defesa</i> – Brazilian Ministry of Defense
MEC	<i>Ministério da Educação</i> – Brazilian Ministry of Education
MS	<i>Ministério da Saúde</i> – Brazilian Ministry of Health
NADC	National Animal Disease Center
NIH	National Institutes of Health
NMP	New Public Management
NR	<i>Norma Regulamentadora</i> – Regulatory Standard
NRC	National Research Council
NSABB	National Science Advisory Board for Biosecurity
NTI	Nuclear Threat Initiative
PAAR	Plant and Animal Agrosecurity Research
PAPR	Powered Air Purifying Respirator
PCMSO	<i>Programa de Controle Médico de Saúde Ocupacional</i> – Brazilian Medical Control Program for Occupational Health
PDCA	Plan–Do–Check–Act
PF	<i>Polícia Federal</i> – Brazilian Federal Police
PGR	<i>Programa de Gerenciamento de Riscos</i> – Risks Management Program
PI	Principal Investigator
Plansic	<i>Plano Nacional de Segurança de Infraestruturas Críticas</i> – Brazilian National Critical Infrastructure Security Plan
PNB	<i>Política Nacional de Biossegurança</i> – Brazilian National Biosafety Policy
PNBB	<i>Política Nacional de Biossegurança e Bioproteção</i> – Brazilian National Biosafety and Biosecurity Policy
PPE	Personal Protective Equipment
PROCAD	<i>Programa Nacional de Cooperação Acadêmica</i> – Brazilian National Academic Cooperation Program
PUCRS	<i>Pontifícia Universidade Católica do Rio Grande do Sul</i> – Pontifical Catholic University of Rio Grande do Sul
PVS	Performance of Veterinary Services

RAV3N	Research Alliance for Veterinary Science and Biodefense BSL-3 Network
RG	Risk Group
RO	Responsible Official
SARS	Severe Acute Respiratory Syndrome
SB3	<i>Sociedade Brasileira de Biossegurança e Bioproteção</i> – Brazilian Society for Biosafety and Biosecurity
SENAI	<i>Serviço Nacional de Aprendizagem Industrial</i> – National Service for Industrial Training
SISLAB	Sistema Nacional de Laboratórios de Saúde Pública – Brazilian National System of Public Health Laboratories
SOP	Standard Operating Procedure
Sq. ft.	Square feet
TAMU	Texas A&M University
TG	Technical Group
UEM	<i>Universidade Estadual de Maringá</i> – Maringá State University
UENF	<i>Universidade Estadual do Norte Fluminense ‘Darcy Ribeiro’</i> – ‘Darcy Ribeiro’ State University of Northern Rio de Janeiro
UFABC	<i>Universidade Federal do ABC</i> – Federal University of ABC
UFAM	<i>Universidade Federal do Amazonas</i> – Amazonas Federal University
UFC	<i>Universidade Federal do Ceará</i> – Ceará Federal University
UFCA	<i>Universidade Federal do Cariri</i> – Cariri Federal University
UFCG	<i>Universidade Federal de Campina Grande</i> – Campina Grande Federal University
UFES	<i>Universidade Federal do Espírito Santo</i> – Espírito Santo Federal University
UFF	<i>Universidade Federal Fluminense</i> – Fluminense Federal University
UFG	<i>Universidade Federal de Goiás</i> – Goiás Federal University
UFJF	<i>Universidade Federal de Juiz de Fora</i> – Juiz de Fora Federal University
UFLA	<i>Universidade Federal de Lavras</i> – Lavras Federal University
UFMA	<i>Universidade Federal do Maranhão</i> – Maranhão Federal University
UFMG	<i>Universidade Federal de Minas Gerais</i> – Minas Gerais Federal University
UFMS	<i>Universidade Federal do Mato Grosso do Sul</i> – Mato Grosso do Sul Federal University
UFPA	<i>Universidade Federal do Pará</i> – Pará Federal University
UFPE	<i>Universidade Federal do Pernambuco</i> – Pernambuco Federal University
UFPI	<i>Universidade Federal do Piauí</i> – Piauí Federal University
UFPR	<i>Universidade Federal do Paraná</i> – Paraná Federal University
UFRJ	<i>Universidade Federal do Rio de Janeiro</i> – Rio de Janeiro Federal University
UFS	<i>Universidade Federal de Sergipe</i> – Sergipe Federal University
UFSM	<i>Universidade Federal de Santa Maria</i> – Santa Maria Federal University
UFV	<i>Universidade Federal de Viçosa</i> – Viçosa Federal University
UFVJM	<i>Universidade Federal dos Vales do Jequitinhonha e Mucuri</i> – Jequitinhonha and Mucuri Valley Federal University
UK	United Kingdom
UN	United Nations

UNESP	<i>Universidade Estadual de São Paulo ‘Júlio de Mesquita Filho’</i> – ‘Júlio de Mesquita Filho’ São Paulo State University
UNICAMP	<i>Universidade Estadual de Campinas</i> – Campinas State University
UNIFESP	<i>Universidade Federal de São Paulo</i> – São Paulo Federal University
UNIFOR	<i>Universidade de Fortaleza</i> – Fortaleza University
UPS	Uninterruptible Power Supply
US	United States of America
USD	US dolar
USDA	United States Department of Agriculture
USG	United States Government
USP	<i>Universidade de São Paulo</i> – São Paulo University
UTMB	University of Texas Medical Branch
UV	Ultraviolet
VHP	Vaporized Hydrogen Peroxide
WHO	World Health Organization
WOAH	World Organisation for Animal Health

LIST OF SYMBOLS

%	Percentage
£	Pound
R\$	Real
®	Registered trademark

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1. INTRODUCTION

High-Containment Biological Laboratories (HCBLs) are pivotal for diagnosing, researching, or producing biological materials involving high-risk biological agents. These laboratories are instrumental in detecting and managing infectious diseases, yet they also present a health risk if biosafety is not strictly adhered to¹⁻³. The literature is replete with instances of Laboratory-acquired infections (LAI) and accidental releases of pathogens, evidencing significant health and economic ramifications and underscoring the critical nature of biosafety⁴⁻⁶.

Biosafety denotes the adoption of safety measures to mitigate or prevent harm from exposure to infectious agents, ensuring protection for those in the laboratory, the surrounding community, animals, and the environment. It encompasses a biosafety program that includes policies and plans for the secure handling and containment of infectious materials⁷. Distinguishing between biosafety and biosecurity, the former pertains to laboratory safety practices to prevent accidental exposure or release of agents. In contrast, biosecurity concerns the measures against the loss, theft, misuse, or intentional dissemination of these materials².

This discourse is interwoven with the global apprehension surrounding the potential use of biological agents as weapons and the controversial 'gain-of-function' research that could increase pathogen virulence⁸.

While Biosafety Level (BSL) 3 and BSL-4 are designed for high and maximum-containment, respectively, the World Health Organization (WHO) and the World Organization for Animal Health (WOAH) have questioned the effectiveness of the BSL classification, leading to a move away from these categories towards a more comprehensive risk analysis approach. Such risk analysis is foundational to biosafety, influencing the design and functioning of laboratories^{2, 9}, a stance also reflected in Brazilian legislation that requires risk assessment-based containment in laboratories¹⁰.

The intricacies of laboratory biosafety management are outlined in international standards such as the ISO (International Organization for Standardization) 35001:2019 Standard¹¹, the WHO Laboratory Biosafety Manual², and the Biosafety in Microbiological and Biomedical Laboratories (BMBL), which is the primary reference for biosafety practices in the United States of America (US)¹.

The strategic role of HCBLs in public and animal health and the significant investment they require underscores the need for active government involvement. Thus, the WHO has published guidelines to aid in adopting laboratory biosafety and biosecurity standards¹².

The US has established the Federal Select Agent Program (FSAP), supervising the management of high-risk pathogens for over two decades, marking a significant step in enhancing national biosafety and biosecurity¹³⁻¹⁴. In Brazil, the enhancement of biosafety and biosecurity measures has been highlighted by their inclusion in Brazilian's Chamber of Foreign Affairs and National Defense (CREDEN) list of critical structures¹⁵ and the adoption of the National Critical Infrastructure Security Plan (Plansic), which appoints the Ministry of Health (MS) with developing sectoral biosafety plans¹⁶.

Despite these advances, the Brazilian Federal Government (BFG) needs more comprehensive legal frameworks and standardization to construct and oversee HCBLs¹⁷⁻²⁰. The lack of official data on the operation of these laboratories and insufficient legal mechanisms for risk monitoring exacerbates this challenge²⁰. This

deficit is particularly alarming in global health security and national sovereignty, where well-structured laboratory networks are essential²¹⁻²³.

Given Brazil's geographical and demographic scale and agricultural prominence, the COVID-19 pandemic has intensified discussions on the necessity of a robust laboratory network to manage emerging diseases and bioterrorist threats. The country must bolster its public laboratory network for research, diagnosis, and biological production²⁴. Amidst these endeavors, the responsible management of risks associated with high-risk pathogens is critical and a legal imperative²⁵. Hence, the situation calls for a multidisciplinary and inter-institutional response, integrating actions within the 'One Health' concept and fostering cooperation among governmental agencies².

This thesis addresses these issues, aiming to:

- a. Identify operational HCBLs in Brazil according to their self-assessed biosafety levels;
- b. Survey the Brazilian HCBLs infrastructure, primary roles, management, and operational aspects, evaluating their structures and procedures in light of international guidelines;
- c. Conduct a comparative study of the legislative, standard, and manual frameworks governing biological risk management in Brazil and other countries, identifying areas for enhancement;
- d. Develop an interactive platform to disseminate information on operational HCBLs in Brazil.

2. LITERATURE REVIEW

2.1 The importance, role, and risks of High-Containment Biological Laboratories

2.1.1 Global trends for public health

Humanity has witnessed increased outbreaks of emerging and re-emerging infectious diseases in recent decades. These include influenza, dengue, chikungunya, Zika virus, poliomyelitis, meningitis, measles, yellow fever, hantavirus, Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS), Nipah virus, Hendra virus, viral hemorrhagic fevers such as Ebola, Marburg, Lassa fever, Crimean-Congo hemorrhagic fever, and those caused by viruses identified in South America, such as Junin, Machupo, Sabia, and Guanarito, and diseases associated with antimicrobial resistance such as tuberculosis and campylobacteriosis²⁵⁻²⁹. The world has recently been battling the severe COVID-19 pandemic³⁰⁻³¹.

It is estimated that 25% of all deaths worldwide are caused by infectious diseases³², and in 2019, six of the top ten causes of death in low-income countries were due to infectious diseases³³.

The situation in the veterinary context is also alarming, given the recent outbreaks of High Pathogenic Avian Influenza (HPAI) and African Swine Fever (ASF), which have led to the loss of more than 300 million birds between 2005 and 2021³⁴ and more than two million pigs in 2020 alone³⁵. It is noteworthy that, like HPAI, approximately 75% of emerging and re-emerging diseases are zoonoses²⁷.

There is a consensus that the global scenario, both in terms of public and animal health, is expected to deteriorate in the next few years due to factors such as:

- a. An increase in global population and demographic density^{27-28, 30, 36-37};
- b. Greater demand for food production due to population growth and economic development, leading to more intensive animal husbandry^{27, 30, 36-37};
- c. An increase urbanization and encroachment into forested and wildlife-rich areas^{28, 36};
- d. Climate change caused by deforestation and the burning of fossil fuels, promoting the proliferation and spread of vectors^{27, 30, 37};
- e. Environmental degradation and habitat destruction, resulting in an uptick in natural disasters and alterations to ecosystems and biodiversity^{27, 37};
- f. The emergence of multidrug-resistant biological agents due to the indiscriminate use of antibiotics in both humans and animals²⁸;
- g. Changing patterns of human mobility due to the intensification of global trade and globalization^{27-28, 36-37};
- h. An increase in the legal and illegal trade of live animals and animal products³⁷;
- i. The intensification of social inequality leads to a growing number of individuals living in poverty and without adequate access to basic sanitation and healthcare³⁰;
- j. Civil conflicts and an uptick in the number of refugees and the move of their animals^{27, 30}.

In the case of Brazil, there is significant pressure on ecosystems that are still preserved, which may result in the discovery of new highly lethal viruses. It was demonstrated by the Arbovirology Session of the Evandro Chagas Institute (IEC), which isolated 1,090 arbovirus samples with the potential to cause encephalopathies from samples collected primarily in the Amazon region between 1954 and 2022³⁸.

2.1.2 Strategic importance of laboratories

Given the ongoing sanitary threats, many countries have invested in improving their laboratory infrastructure. Laboratories are considered strategic for providing rapid and accurate disease diagnosis, researching pathogens and their interactions with hosts, working with Genetically Modified Organisms (GMOs), and producing medicines and vaccines³. The COVID-19 pandemic has underscored the importance of robust laboratory infrastructure in combating the most significant health crisis of the last decades. Countries with more advanced laboratory systems achieved faster and more effective results in their efforts to contain the spread of the virus and develop treatments and vaccines³⁹.

The benefits of adequate laboratory infrastructure can be measured by estimating disease outbreaks' financial impacts and consequences⁴⁰. A recent study, which considered different epidemiological scenarios for an HPAI outbreak in Brazil, estimated a cost-benefit ratio of maintaining laboratory activities and agricultural surveillance of over three. Despite the high operational costs associated with laboratories, the benefits of investing in these facilities have been demonstrated⁴¹.

However, more than having a laboratory infrastructure that is adequate for the country's demand is required. This infrastructure needs to be used as efficiently as possible. Considering that public resources are always limited, particularly in developing countries, the need for adequate policy and strategic planning to use the available laboratory network means that scarce resources are often applied in an uncoordinated and ineffective manner. It is particularly evident in the case of laboratories dedicated to the diagnosis of infectious diseases and research with high-risk biological agents²⁵.

2.1.3 Risks related to laboratory activities in High-Containment Biological Laboratories

It is crucial to consider the risks involved and follow appropriate biosafety procedures to ensure the safety of laboratory workers and prevent the intentional or accidental release of biological materials into the environment. It requires adequate biological containment structures, commonly called 'High-Containment Biological Laboratories'¹⁻².

Although serological and molecular biology methods using inactivated materials like antigens and synthetic nucleic acids may suffice in some cases, working with active pathogens is essential in the context of emerging and re-emerging infectious diseases. Therefore, HCBLs are crucial as they provide emerging and re-emerging disease surveillance and control conditions⁴².

2.1.4 High-Containment Biological Laboratories in the world

Countries with a high level of development usually have structured networks of HCBLs, as is the case with the US, which already had at least one public HCBL in each state in 2009⁴³. A study conducted in the same year on 48 isolation units for treating infectious diseases in 16 European Union (EU) countries showed that all had at least one HCBL available in their own country to diagnose viral diseases. In contrast, this percentage reached 98% of the units for bacterial diseases⁴⁴.

The number of HCBLs installed worldwide is still being determined, but it is estimated that there are at least 3,204³. Another literature review-based study concluded that at least eight countries have more than 10 HCBLs. They are the US, China, Spain, France, Germany, India, Japan, and South Korea. Although the study

did not intend to map the number of laboratories in different countries, it indicates some countries that invest the most in these structures⁴⁵.

It is estimated that there are more than 1,300 HCBLs in the US⁴⁵ and between 16 and 44 in India⁴⁶. China aims to install at least one HCBL in its 22 provinces by 2025⁴⁷⁻⁴⁸.

In addition to the countries mentioned above, it is known that the United Kingdom (UK)⁴⁹, Canada⁵⁰, Russia, South Africa, Australia, and Sweden³ also have a significant number of HCBLs.

The states with former weapons programs (US, UK, Germany, Russia, Canada, China, South Africa, and France) have together at least 2,595 HCBLs and maximum-biocontainment laboratories³.

Data collection on the number and type of HCBLs in Latin America represents a significant challenge due to the need for standardized information. Various sources have been identified for each country, and the data provided by these sources often conflicts with the data from the only centralized document that seeks to gather information on the various biocontainment laboratories in the world, which is the King's College London Global Biolabs report⁵¹. This lack of uniformity and coherence in the data makes it difficult to obtain an accurate picture of the infrastructure dedicated to the investigation of dangerous pathogens in Latin America, which suggests the possibility that there are unknown laboratories under construction or others whose information is not available or is challenging to access⁵².

Regardless of the uncertainty on the number of HCBLs worldwide, there is no doubt that since September 2011, this number has increased exponentially³.

Although HCBLs play a crucial role in diagnosing and controlling infectious diseases, paradoxically, their existence represents a health risk that cannot be ignored^{3, 53-54}.

2.1.5 Importance of planning in the construction of new High-Containment Biological Laboratories

Another challenge associated with these structures is their high construction and operation costs. HCBLs are estimated to have operating costs three times higher than conventional laboratories⁵⁵. This cost is estimated to represent 5 to 15% of the construction value annually⁵⁶⁻⁵⁸ and can reach up to USD 2,000/m²/year in the US⁵⁹. These costs are primarily related to the high consumption of electrical energy, mainly for the operation of the HVAC (Heating, Ventilation, and Air Conditioning) system, periodic equipment and system certifications, replacement of HEPA (High-Efficiency Particulate Arrestance) filters, and labor costs for maintenance of high-complexity equipment and systems⁵⁵.

Considering the earlier points, the construction of HCBLs should be preceded by institutional planning for the medium and long term, considering sustainability, effectiveness, and safety requirements. It means that the institution must be capable of raising resources to maintain the high operational costs, and the laboratory should contribute in a relevant and timely manner to meet the country's health demands, with a high level of reliability of results and appropriate methodologies, complementing the existing networks and national and regional capacities. The inability to maintain or improve laboratory performance to leverage investments made by federal governments and donors undermines laboratory activities' safety, quality, and security^{46, 53, 60}.

The WOA's Ad Hoc Group on Sustainable Laboratories defines a sustainable laboratory network as one that can continuously deliver specialized services in a manner that is efficient, timely, accurate, consistent, secure, and safe; is in line with international standards and best practices; is provided at an acceptable cost; responds to clients' needs across sectors (public or private); and benefit One Health goals and the overall One Health system⁶⁰.

In the context of public laboratories, authorities should prioritize consolidating existing laboratory networks rather than expanding them, including providing training resources and stimulating international partnerships. Depending on the demands, improving conventional laboratories or allocating resources to other aspects of public health is often more beneficial than building HCBLs. They tend to generate more expenses than revenues; therefore, oversized or unnecessary laboratories should be avoided. Considering that in some contexts, there is more availability of resources for construction works than for maintenance expenses, it is not uncommon to have excellent structures that become unusable shortly due to a lack of maintenance¹⁷.

The proliferation of HCBLs in locations where specialized equipment and infrastructure services are difficult or impossible to access has meant that donation recipients neither have the financial nor human capital to maintain the laboratory and to keep the equipment and systems operational, leading to inevitable engineering failures and increasing the potential for accidental laboratory releases of dangerous pathogens⁶⁰.

Even in developed countries, there are difficulties in maintaining HCBLs, mainly in the academic context, despite the demonstrated scientific advances they bring. Traditional financial support tools, such as grants and allowances for project execution, cannot cover the high operational costs for universities. In the specific case of the US, no program includes government replacement of resources for operating and maintaining laboratories that handle high-risk pathogens⁵⁸.

On the other hand, HCBLs can also generate substantial income, especially considering the biotechnology industry³.

Faced with these complex challenges, Chatham House, an independent British non-profit organization, developed a tool to assist public managers in deciding about building a new HCBL, considering the following factors:

- a. Strategic planning: Which national strategies will be covered by the construction of the laboratory? Are there any other similar laboratories being planned in the country?
- b. Financial sustainability: Which government agencies are involved in the project, and what financial commitments can each make? What will be the construction and operation costs, and how will these costs be covered in the short, medium, and long term? Are there partnership opportunities, such as public-private partnerships? Will activities be carried out that allow for revenue generation? Will these revenues be sufficient to cover the expenses?
- c. Infrastructure and utilities: What are the best locations for the laboratory, considering environmental issues, accessibility, safety, and licenses to be obtained? What will be the infrastructure requirements? How will waste disposal be handled? How will the supply of electricity and drinking water be provided? Are there sources of clean energy (e.g., photovoltaic systems or wind energy)? How will the supply of electricity be maintained in case of power outages? What are the plans for telecommunications?
- d. Work plan: What will be the laboratory's primary function (research, teaching, diagnosis, biobank, epidemiological surveillance)? What activities will be carried out, and what contributions will be made compared to the current scenario? What

methods will be employed in routine work? Will the laboratory have any role in responding to outbreaks? Which pathogens will be worked on? What type and volume of samples will be received? What alternative plan will be if government priorities change or resources become scarce?

e. Human resources: What skills and expertise will be necessary, including during the construction phase? What is the size of the current workforce? Will it be sufficient for the laboratory's operational phase? How will any gaps be filled?

f. Biosafety and quality management: What biosafety and biosecurity measures will be adopted based on risk analysis? What approach will be adopted regarding the adoption of a Quality Management System?⁶¹

The US Government (USG) had a similar concern after observing a significant increase in HCBLs in the early 2000s when around USD 57 billion were invested. The National Congress was tasked with evaluating the following issues:

- a. How many laboratories are needed?
- b. What is the required federal investment for these laboratories?
- c. Should the laboratories be centralized or distributed throughout the national territory?
- d. What plans currently exist to coordinate the efforts of different agencies in expanding the capacity of HCBLs?
- e. Does expanding laboratory capacity increase the risk of accidents and malicious use?
- f. What is the balance between the benefits of these laboratories and the risks they pose?^{43, 62}

Later, the USG established a list of criteria to be considered by public entities at different levels when planning the construction of high and maximum-containment laboratories. The tool is divided into four parts:

- a. Demonstrate the need for the project after a careful evaluation of other alternatives;
- b. Demonstrate commitment and ability to operate, maintain, and sustain the laboratory in a safe and contained manner;
- c. Demonstrate commitment to meeting the requirements established in biosafety manuals;
- d. Ensure engagement of the local community before the start of construction⁶³.

2.1.6 The Brazilian scenario

Although in much more modest proportions than the US, the BFG also prioritized the construction of HCBLs in the early 2000s, with financial support from the World Bank. At the time, faced with the precarious biosafety conditions of public health laboratories, the MS established a National Biosafety Program with three main goals:

- a. Biosafety training for all laboratories in the public health network;
- b. Adaptation of 12 HCBLs to strengthen the Public Health Central Laboratory (LACEN) network in the states of Amazonas, Bahia, Ceará, Distrito Federal, Minas Gerais, Pará, Pernambuco, Rio de Janeiro, Rondônia, Rio Grande do Sul, and São Paulo;
- c. Development of biosafety standards for public health institutions⁶⁴⁻⁶⁵.

During the same period, six laboratories belonging to the Virus Genetic Diversity Network were constructed in the State of São Paulo, supported by the São Paulo Research Foundation (FAPESP), to prepare the country for potential SARS outbreaks and influenza pandemics⁶⁶.

The Ministry of Agriculture and Livestock (MAPA) took a leading position in agricultural defense by constructing HCBLs belonging to the National Veterinary Laboratory Network (LFDA) and the Brazilian Agricultural Research Corporation (EMBRAPA) for the diagnosis and study of animal diseases of mandatory notification²⁰. The LFDA-SP invested approximately R\$ 30 million^a in constructing an HCBL that was launched in 2018. This facility is considered one of the most modern in the country and has been a world reference laboratory for diagnosing Newcastle disease and HPAI since 2016⁶⁷.

Other structures that work with high-risk pathogens are linked to the Ministry of Defense (MD), universities, and private entities, mainly dedicated to producing vaccines against Foot-and-mouth disease (FMD)²⁰.

According to the MD, the Brazilian Army has fixed and mobile laboratories that can identify biological agents or collaborate with national and international agencies. Additionally, units are equipped to recognize and decontaminate materials, ensuring protection against a potential threat⁶⁸.

One of these facilities is known as 'LabMove' (The Mobile Laboratory for Identification of Chemical and Biological Agents). It is a versatile device designed to swiftly and securely detect and identify chemical and biological agents on-site in samples suspected of contamination. It is housed within a standard container and has been purposefully adapted for easy transport, allowing for seamless movement via land, sea, or air transportation⁶⁹. It is worth noting that this device is not considered an HCBL.

Despite the challenges faced by Brazilian scientific and technological development programs, health research exhibited signs of vigor by integrating new structures and technologies. Some institutions took advantage of the opportunity to implement modern biotechnological methods for public health studies, including the Oswaldo Cruz Foundation (FIOCRUZ), the IEC, the Adolfo Lutz Institute (IAL), the Butantan Institute, and the National Cancer Institute (INCA). However, during the initial years of operation of these laboratories, there was a lack of investment in public health, no prioritization of laboratory biosafety, and a lack of centralized management, among other factors, resulting in a heterogeneous scenario where some institutions maintained excellent biosafety profiles while others observed a significant decline in the quality of structures and procedures⁶⁴.

Although official publications do not provide information on how many HCBLs exist in the country, their operational status, or how well they comply with international biosafety and biosecurity standards, a preliminary study estimated the presence of 58 self-declared HCBLs in Brazil. This study concluded that they are present in some of the most advanced research centers, including federal and state universities such as USP, UNIFESP, UFRJ, UFMG, UFPE, and UNESP (São José do Rio Preto campus), which conduct activities to support human and animal health²⁰.

In the context of the Ministry of Education (MEC), it is worth mentioning that the Brazilian Hospital Services Company (EBSERH) as a critical component of the BFG's initiatives aimed at restructuring hospitals associated with federal higher education institutions. Since its establishment, the EBSERH, a public company affiliated with the MEC, has assumed responsibility for managing federal university hospitals. The company's responsibilities include:

^a Equivalent to USD 7,742,335.09, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2018.

- a. Coordinating and evaluating the execution of hospital activities;
- b. Providing technical support for the development of management improvement tools;
- c. Formulating the resource distribution matrix for these hospitals.

The network of federal university hospitals comprises 51 hospitals affiliated with 36 federal universities. Out of these, 41 hospitals are part of the EBSEH Network^b. Part of them is equipped with HCBLs.

More recent initiatives have been undertaken to strengthen the structure of Brazilian HCBLs, focusing on restructuring the National Public Health Laboratory Network⁷⁰. The Public Call of Funding Authority for Studies and Projects (FINEP), an agency linked to the Ministry of Science, Technology, and Innovation (MCTI), published in July 2020, aimed to select proposals for financial support for the execution of institutional projects for the adaptation or implementation of the physical infrastructure of HCBLs and vivarium for research, development, and innovation for the development of vaccines, treatments, and studies of the pathogenesis of the SARS-CoV-2 virus and other emerging and re-emerging viruses⁷¹. The same agency released another Public Call in 2022 to evaluate proposals to provide financial support for establishing, adapting, and enhancing controlled environments and cleanrooms dedicated to research development within the existing spaces⁷². Both opportunities supported HCBLs to respond to the COVID-19 pandemic.

On the other hand, there is no news regarding a comprehensive government assessment of demands, installed capacity, and aspects related to planning and management of HCBLs operating in Brazil¹⁷⁻¹⁸.

In practice, it is observed that each ministry defines its strategies and criteria for constructing new laboratories based on its priorities, the prevalence of a particular biological agent, regional demands, and institutional capacity to develop the project without any coordination with other agencies. Moreover, universities operate independently, and evaluate the criteria adopted to identify the need for constructing HCBLs is challenging¹⁷⁻¹⁸.

As a result of this fragmented approach to planning the network of HCBLs in Brazil, existing laboratories in public universities lack a well-defined purpose and remain underutilized. These facilities could otherwise contribute to expanding the capacity to respond to health emergencies. There is also a shortage of laboratories capable of conducting animal experimentation in high containment, essential for pre-clinical trials in developing vaccines and antivirals²⁴.

The situation becomes even more challenging as evidence suggests that Brazilian HCBLs face significant operational problems resulting from design and construction deficiencies, certification procedures, equipment maintenance, lack of specialized personnel, and other aspects related to laboratory biosafety and biosecurity management. Budgetary constraints often exacerbate these issues^{20, 73-74}.

Before exploring the aspects and challenges related to laboratory biosafety management and regulation, it is essential to present some fundamental concepts.

^b Available at: <https://www.gov.br/ebserh/pt-br/hospitais-universitarios/sobre-os-hospitais-universitarios-federais>. Accessed on: December 27, 2023.

2.2 Fundamental concepts on laboratory biosafety and biosecurity

Biosafety, as a science, emerged during the US biological weapons program. After World War II, three US Army laboratories were established as permanent facilities for researching and developing biological weapons: the Biological Research Laboratories at Camp Detrick; the Biological Production and Development Laboratories at Pine Bluff Arsenal; and the Biological Assessment Laboratories at Dugway Proving Grounds⁷⁵. At that point, it became necessary to develop measures to protect the employees at the laboratory and the neighboring communities from the infectious agents being manipulated there. This program continued during the Cold War and ended in 1969⁷⁶.

Good biosafety practices were inherent in developing biological weapons, and the knowledge produced there began to be shared with other laboratories⁷⁶.

It is worth noting that the first technical meetings on laboratory biosafety were held from 1955 onwards, initially with exclusive participation from US military laboratories. The event's fourth edition was the first to include representatives from the United States Department of Agriculture (USDA) in 'non-confidential' sessions. From 1964 onwards, these conferences began to have increasing number of participants, including representatives from the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). The conferences became more open for sharing experiences and information on biosafety, addressing essential topics for biological containment laboratory operations. These conferences formed the basis for creating the American Biological Safety Association (ABSA) in 1984⁷⁵.

The fundamental concepts of laboratory biosafety were widely disseminated for the first time in the NIH manual, published in 1976. This manual served as the foundation for the first version of the WHO Biosafety Manual, published in 1983, and the first edition of the BMBL, issued the following year by the CDC in partnership with NIH⁷⁶.

Conceptually, 'Laboratory Biosafety' refers to the consistent implementation of measures to minimize or prevent harm to people working in the laboratory, other occupants of the facility, the general population, animals, and the environment from exposure to infectious material, infected animals, or toxins handled in the contaminated area. A biosafety program comprises institutional policies and planning that facilitate the safe handling and storage of infectious materials and toxins, preventing their release or escape from the contaminated area^{2, 7}.

While laboratory biosafety refers to the principles and practices for preventing the unintentional escape of biological agents and toxins or accidental exposure to them, 'Laboratory Biosecurity' is an equally important concept that refers to preventing loss, theft, misuse, unauthorized access, or intentional release of biological agents and toxins².

The current edition of the BMBL¹, considered the primary laboratory biosafety manual in the US and one of the most widely referenced worldwide, identifies four BSLs, as described in Chart 01.

Chart 01 – Overview of applicability and critical features for each Laboratory Biosafety Level (BSL).

BSL	Applicability and key features
1	Generally appropriate for undergraduate and secondary educational training and teaching laboratories and for other laboratories that work consistently with defined and characterized strains of viable biological agents not known to cause disease in healthy adult humans. BSL-1 represents a basic level of containment that relies on standard, microbiological best practices and procedures with no particular primary or secondary barriers other than a door, a sink for handwashing, and non-porous work surfaces that are cleanable and easy to decontaminate.
2	Applicable to laboratories in which work is performed using a broad spectrum of biological agents and toxins associated with causing disease in humans of varying severity. Work done with human, animal, or plant-derived specimens where a biological agent or toxin may be unknown can often be safely conducted under conditions typically associated with BSL-2. Even though the biological agents and toxins routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential are conducted within primary containment equipment, such as a Biosafety Cabinet (BSC) or safety centrifuge cups. Waste decontamination capabilities to reduce the possibility of environmental contamination and separating laboratory spaces from office and public spaces to reduce the risk of exposure to other personnel should be considered.
3	Applicable to laboratories in which work is performed using indigenous or exotic biological agents with a potential for respiratory transmission and those that may cause severe and potentially lethal infection. Infectious materials are manipulated within a BSC or other primary containment device. No work with open vessels is conducted on the bench. Secondary barriers for BSL-3 laboratories include those previously mentioned for BSL-1 and BSL-2 laboratories. They also have enhanced ventilation strategies to ensure inward directional airflow and controlled access zones to limit access to only laboratory-approved personnel. They may contain anterooms, airlocks, exit showers, and exhaust HEPA filtration.
4	Applicable primarily for laboratories working with dangerous and exotic biological agents that pose a high individual risk of life-threatening disease that may be transmitted via the aerosol route and for which there is no available vaccine or therapy. The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III or a Class II BSC with a full-body, air-supplied positive-pressure personnel suit. Secondary barriers for BSL-4 laboratories should include those previously mentioned for previous BSLs. Additionally, the BSL-4 facility is often a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems for solid and liquid waste to prevent the release of hazardous biological agents into the surrounding community and the environment.

Source: Centers for Disease Control and Prevention and National Institutes of Health¹

Four BSLs for activities involving biological agents and toxins conducted on experimental animals are also described. This classification is known as ABSL-1, ABSL-2, ABSL-3, and ABSL-4¹.

Another classification, BSL-Ag, refers to activities involving biological agents, with or without zoonotic potential, and toxins that have significant implications for agriculture. The economic impact of Accidental Pathogen Escape from Laboratory Settings (APELS) must be considered in risk assessment as it can cause morbidity or mortality in production animals or trade restrictions. Additionally, due to the animals' size, disposition in the laboratory, and behavioral characteristics, workers may suffer injuries beyond those expected from handling conventional laboratory animals^{1, 77}.

In addition to the characteristics described in the BMBL for each BSL, other international references provide more detailed information on the recommended construction characteristics for HCBLs. These include recommendations from institutions, government agencies, organizations, and associations in the US⁷⁸⁻⁷⁹, Canada⁸⁰, the EU⁸¹, and Spain⁸². The Australian and New Zealand manuals contain more detailed information on engineering aspects and equipment calibration⁸³.

It is worth noting that a similar BSL classification exists for greenhouses dedicated to research involving high-risk plant pathogens⁸⁴⁻⁸⁵. However, this topic will not be addressed in the present study.

Specific guidelines for constructing and operating mobile and modular HCBLs must still be provided^c. Due to the unique nature of these types of laboratories, some concepts related to conventional laboratories may only sometimes be applicable⁸⁶.

The applicability of these models should be considered in specific contexts. For instance, mobile laboratories proved helpful in combating the Ebola epidemic in Sierra Leone in 2014⁸⁶⁻⁸⁷, an outbreak of dengue in Senegal between 2017 and 2018⁸⁸, and the COVID-19 pandemic in East African countries, such as Uganda, Kenya, Burundi, Rwanda, South Sudan, and Tanzania, from 2020 onwards³¹.

The primary advantages of these models are their shorter manufacturing time and the possibility of using them in remote areas, allowing for the analysis of samples in situations where logistical aspects would make it challenging to send them to laboratories located in large urban centers. Moreover, mobile laboratories eliminate the biological risks associated with transporting samples. However, there are difficulties in replenishing reagent stocks, maintaining and calibrating equipment (especially for BSCs), acquiring diesel for the electrical generator, defining the inactivation methods, providing support for solving various operational problems, waste disposal, and personnel training. In addition, due to personnel, equipment, and space restrictions within the laboratory, work is typically limited to small volumes^{86, 88-89}.

Personnel working in mobile laboratories must pay close attention to safety procedures, particularly regarding the use of Personal Protective Equipment (PPE), since the structure of these laboratories does not offer the same level of safety as conventional ones. Additionally, biosecurity aspects must be emphasized. These labs are often located in remote and high-risk areas and may become targets for individuals or groups with criminal, political, and ideological motives⁸.

The BSL classification should not be seen as a ranking to be achieved but rather as a minimum requirement. It should also be used more than just for defining project funding. Thus, classifying laboratories into four BSLs may not always be suitable for different scenarios and demands, which has led to the development of intermediate classifications such as BSL-2 'enhanced' or BSL-3 'plus'¹⁷.

An illustrative example is the laboratories that handle HPAI, especially when conducting animal inoculations. In these cases, ABSL-3 laboratories are recommended, along with additional biosafety measures such as the use of isolators or Class 3 BSCs, redundancy of exhaust air filtration with HEPA filters in series, additional training for staff, more rigorous emergency response plans, enhanced respiratory protection for personnel against aerosols, adherence to shower-out protocols, pass-through autoclaves, Effluent Decontamination System (EDS), and strengthened access controls and monitoring^{51, 83}.

Therefore, we have chosen to avoid using the BSL classification in this study and instead adopt the term 'High-containment Biological Laboratories' (HCBL) for structures that have biosafety features superior to BSL-2 laboratories but inferior to BSL-4 laboratories, which we will refer to as 'maximum-biocontainment laboratories.'

^c The use of these structures in Brazil is restricted to the military sphere (biodefense and related chemical, biological, radiological, and nuclear operations) and sporadic research conducted in remote regions, which explains their exclusion from the present study.

In this context, conducting risk analysis to define construction requirements and adopting procedures to ensure biosafety and biosecurity is crucial, as discussed in the next session.

2.3 Risk assessment as an essential component of a laboratory biosafety program

2.3.1 Risk assessment x Biosafety Levels

The classification into BSLs is a commonly used didactic tool that helps identify the minimum requirements for laboratories handling biological agents. However, there may be better strategies when planning the construction of new laboratories. A laboratory can meet only part of the requirements of a specific BSL depending on the type of microorganism and the activities to be performed. Therefore, a thorough risk assessment should be conducted to determine the appropriate containment level and to document the necessary conditions^{76, 90-91}.

The risk assessment approach promotes the construction of economically sustainable laboratories that are designed to meet local needs. Recent research has shown that human error rather than structural malfunctions causes most LAI. Nevertheless, countries need to develop effective oversight mechanisms to evaluate the technical solutions employed in each case⁹².

The effectiveness of the requirements established in globally disseminated manuals and standards for each BSL is questioned due to the lack of technical-scientific evidence that supports their ability to promote biosafety. Some authors conclude that some constructions are unnecessarily expensive, which presents a significant issue, particularly in developing countries. Conversely, constructing low-cost facilities can pose substantial risks to users and the environment when risks are not appropriately evaluated and treated^{25, 76, 93}.

The construction of a more flexible laboratory that can handle a wide range of pathogens and for various purposes over time requires careful consideration of the most stringent requirements and additional biosafety measures, resulting in higher construction and operating costs⁷⁸.

Therefore, recent versions of biosafety manuals from WHO and WOAHA have moved away from the classification of laboratories based on BSLs and are now advocating for risk assessments tailored to the specific installation^{2, 9}. WHO views risk analysis as the foundation of biosafety and specifies the minimum aspects that should be considered in this analysis:

- a. Activities carried out in the laboratory;
- b. Competence and qualification of the technical staff;
- c. Concentration and volume of the biological agent and potentially infected materials to be manipulated;
- d. Characteristics of the biological agents, such as transmission routes, range of hosts, stability in the laboratory and external environment, and infective dose;
- e. Characteristics of the diseases caused by the biological agents, such as transmissibility, severity, local availability of treatment and prophylactic measures, susceptibility of laboratory personnel, and endemicity in the local population;
- f. Minimum requirements for the building infrastructure, including equipment and systems, to provide adequate biosafety and biosecurity conditions⁹⁰.

Other factors to consider are the generation of aerosols, the use of GMOs and oncogenic organisms, the presence of engineering monitoring and control systems, the availability of supplies, the HCBL's capacity to meet occupational health requirements, and compliance with other relevant legislation and regulations^{6, 94}.

The diagnosis of tuberculosis serves as a prime example of how the type of activity conducted in the laboratory affects biosafety measures. In 2009, a group of WHO experts discussed specific laboratory biosafety measures for diagnosing this disease. According to the manual published as a result of this meeting, laboratories can be categorized into three levels of risk based on the diagnostic method employed:

- a. Low-risk level (direct sputum smear microscopy and sample preparation for automated nucleic acid amplification test);
- b. Moderate-risk level (direct antimicrobial susceptibility testing and sample processing for inoculation into primary culture media);
- c. High-risk level (manipulation of cultures for identification, antimicrobial susceptibility testing, or probe assay from isolated cultures and manipulation of multi and extensively drug-resistant strains)⁹⁵.

In response to this conference, appropriate design features were described for each level. A separate area from public space and a unidirectional airflow is recommended for the low-risk level. For the moderate-risk level, installing Class II BSC, a backup power generator, and centrifuges equipped with sealed rotors and buckets is essential. For the high-risk level, an anteroom for laboratory access and an autoclave installed inside the laboratory are required⁹⁵⁻⁹⁷.

2.3.2 Conducting a risk assessment

Risk assessment is a unique exercise for each institution and should guide management decisions and the application of mitigation measures. In many countries, conducting a risk assessment is a legal obligation in the laboratory's certification process. A risk assessment should be documented appropriately even when no such obligation exists. Given the subjective nature of specific evaluations, professionals often rely on their experience and technical knowledge to decide on the acceptability of particular risks. It makes conducting the analysis and the auditing process complex tasks that require a great deal of responsibility. Questions such as 'How tolerable is a certain risk?' or 'How sufficient is a certain risk mitigation procedure?' should be answered based on technical biosafety requirements^{76, 98}.

It is crucial to note that the quality of the analysis depends on the accuracy of the collected information. When there is not enough information to determine the risks, it is prudent to consider more conservative alternatives until new information becomes available⁹⁹.

Several tools are available for conducting a risk assessment for HCBLs. The manual published by the Canadian government is one of the most comprehensive¹⁰⁰. The Canadian government has even made an online course available for conducting this risk assessment. However, the absence of a standardized methodology at a global level represents an additional challenge for method harmonization⁷⁶.

Given the criticality and complexity of this stage in the process, a risk assessment must be carried out by trained personnel and periodically reviewed, or as required, such as when there are changes in the fundamental nature of the activities or the physical structure¹⁰¹⁻¹⁰⁴. A multidisciplinary team should be involved in the process, comprising the Biosafety Officer (BO), the technical team involved in the laboratory routine activities, the engineering and maintenance team, the occupational safety personnel, administrative staff, and managers, among others. Other stakeholders who can be involved are the Institutional Biosafety Committee (IBC), the Environmental Committee, and the Institutional Animal Care and Use Committee (IACUC)^{76, 101}.

Risk assessment may employ mathematical models to evaluate the pathogen transmission rates in case of an APELS^{98, 102, 105}. Alternatively, it may assess the economic impact of a potential outbreak arising from such an escape¹⁰⁶. It is worth mentioning that specific pathogens that do not cause human diseases can inflict significant financial losses on agriculture and the food industry, with severe impacts on product exports and, subsequently, the trade balance, as observed in outbreaks of FMD, ASF, Classical Swine Fever (CSF), and other illnesses¹⁰⁷⁻¹⁰⁸.

Some of the controls that can be implemented based on a risk assessment include:

- a. Elimination or substitution of the risk, which may involve the decision not to work with a particular pathogen;
- b. Implementation of engineering solutions, such as the installation of redundant HEPA filters to exhaust air from the laboratory;
- c. Installation of equipment and systems, such as fumigation cameras, EDS, electrical power generators, and biodigesters;
- d. Adoption of administrative measures, such as developing SOPs (Standard Operating Procedures);
- e. Utilization of additional PPE;
- f. Provision of supplementary training;
- g. Mandatory showering upon leaving the laboratory^{77, 104}.

When conducting a risk assessment for HCBLs, it is crucial to consider how communication and trust-building with the local community will be managed. For instance, the BSL-4 laboratories in Musashi-Murayama, Japan¹⁰⁹, and Boston, US¹¹⁰, had to operate as BSL-3 for 35 and eight years, respectively, due to restrictions imposed by the local community. In both cases, community resistance was attributed to inadequate communication, which should have considered the laboratory's importance and inclusive strategies with a continuous dialogue with the neighborhood¹¹¹. Examples of other laboratories facing similar challenges are Central Public Health in Canada and the University of California in the US¹¹².

A thorough risk assessment for HCBLs should include two main aspects. The first is biosafety, which covers the prevention of LAI, accidents, and incidents. The second is biosecurity, which considers the potential use of microorganisms present in the laboratory as a biological weapon of mass destruction^{76, 113-114}. These different perspectives will be further explored.

2.4 Laboratory-acquired infection and Accidental Pathogen Escape from Laboratory Settings

Since the beginning of the 20th century, LAI has been systematically recognized and recorded as an occupational hazard in various countries.

Studies have identified 168 deaths and 4,079 LAI in the US between 1930 and 1978, representing a range of one to five infections per million hours worked¹. Data from other countries between 1979 and 2004 showed the notification of 1,448 symptomatic infections and 36 deaths⁴. Another study estimates an incidence of 16.2 LAIs per 100,000 people per year⁹⁸. However, these figures are likely underestimated since most countries need more effective mechanisms for reporting LAIs^{93, 115}.

A recent study reported LAIs in 309 individuals worldwide between 2000 and 2021, with eight fatalities caused by infection with *Neisseria meningitidis* (n = 3), *Yersinia pestis* (n = 2), *Salmonella enterica* serotype Typhimurium, Ebola virus, and due to bovine spongiform encephalopathy⁶.

Currently, the incidence of tuberculosis in laboratory personnel is three to 100 times higher than that in the general population⁹⁸. Other pathogens frequently associated with LAI include *Brucella* spp., *Francisella tularensis*, *Salmonella enteritidis*, and Vaccinia virus^{6, 116}. In China, 37 cases of laboratory-acquired brucellosis were identified between 2006 and 2017, with 27 involving students¹¹⁷.

Numerous other pathogens have been associated with LAIs, including *Shigella* spp., *Escherichia coli*, *Klebsiella*, *Coxiella burnetii*, hantavirus, hepatitis B and C viruses, Venezuelan equine encephalitis virus, dengue virus, *Rickettsia* spp., *Chlamydia psittaci*, Newcastle disease virus, *Burkholderia pseudomallei*, *Cryptosporidium* spp., *Blastomyces (Ajellomyces) dermatidis*, *Sporothrix schenckii*, West Nile virus, *Staphylococcus aureus*, Crimean-Congo hemorrhagic fever virus, the Human Immunodeficiency Virus (HIV), *Toxoplasma gondii*, Mimivirus^{4, 6, 93, 116, 118-120}, and more recently, SARS-CoV-2^{6, 121}.

In Brazil, the first recorded LAI dates back to 1935, involving two researchers from the Butantan Institute who succumbed to Rocky Mountain spotted fever. They contracted the disease while handling ticks during research to develop a vaccine for this illness¹²². Additional incidents include eight technicians contaminated with the Venezuelan equine encephalitis virus in 1943⁶⁴ and a researcher who contracted the Sabia virus in 1992 while working with biological samples¹²³. In 2002, an LAI occurred due to the accidental inoculation of the Vaccinia virus¹²⁴.

The exposure of three laboratory workers to *Brucella abortus* was reported in 2020, but they did not develop the infection. According to the authors, implementing a working group due to a new local regulation making the notification of human brucellosis mandatory allowed for improvement in the detection of laboratory accidents in Parana State¹²⁵.

Other recent LAIs reported in Brazil were associated with the Zika virus, and *Leishmania (Viannia) naiffi*⁶.

High and maximum biocontainment laboratories present a particular risk for LAIs. For instance, cases of SARS were reported in Singapore¹²⁶ and Taiwan¹²⁷, Ebola in Russia¹²⁸, and bubonic plague in the US¹²⁹, with some resulting in fatalities. In the UK, over five years, more than 100 accidents and incidents occurred in high and maximum-containment laboratories¹³⁰. In Canada, between 2015 and 2020, 289 incidents involving 596 people were reported in laboratories handling risk groups (RGs) 2, 3, and 4 pathogens, with 13 resulting in LAI. In 2020, most incidents occurred in hospital (45.2%) and academic (39.1%) laboratories^{120, 131}.

Human error is the primary cause of accidents involving workers' exposure to pathogens in HCBLs, responsible for over 65% of accidents^{4, 6, 93, 132-133}. The most common failures are the non-use of PPE and non-compliance with good laboratory practices and biosafety procedures^{76, 93, 117, 120, 130}.

Inhalation of aerosols is the most common LAI transmission route, responsible for 84-99% of cases^{93, 120, 134}.

In 2014, the USG reported three serious incidents associated with high and maximum-containment laboratories that could have had catastrophic consequences. Two of them were related to the incorrect transfer of high-risk pathogens to other laboratories (HPAI and *Bacillus anthracis*)¹³⁵, and the other one was the detection of vials containing live smallpox virus stored in a location without proper biosafety¹³⁶.

There are suspicions that the COVID-19 pandemic originated from an APELS from a maximum-biocontainment laboratory in Wuhan, China. However, there is currently no definitive evidence about this¹³⁷. Other events involving influenza viruses

may have also originated from laboratory escape, such as the outbreak of the Asian flu in 1977 and the emergence of the H1N1 strain in Mexico in 2009¹³⁸.

The APELS of the FMD virus from a maximum-biocontainment laboratory located in England resulted in an outbreak with losses exceeding £ 3 billion^d to the public sector and a further £ 5 billion^e to the private sector⁵⁴. Studies concluded that this incident resulted from a series of procedural and structural failures that culminated in the escape of the virus from the EDS piping^{5, 107, 139}.

At least 13 other incidents involving the possible APELS of FMD viruses have been reported worldwide in the past 50 years¹⁰⁶. There was also a report of APELS of the CSF virus from a US laboratory, resulting in the infection and subsequent euthanasia of two animals¹¹⁶.

A probability of 20% occurrence of an HPAI virus escape in 10 years was estimated to originate from one of the ten laboratories that handle it in the US. It is also estimated that a 20% probability exists for one of these events to cause a flu pandemic¹³².

Between 2020 and 2021, 56 events related to unintentional exposure to pathogens were recorded in laboratories in England, Scotland, and Wales¹⁴⁰.

A study in the US with BSL-3 laboratories concluded that 148 incidents were reported in three years, corresponding to 1.55 incidents per 100 hours of work. The proportion of incidents involving trainees was significantly higher. The most common types of incidents were spills (72%, with 98% occurring within a BSC), non-compliance with SOPs (10%), failure to wear PPE (9%), equipment failure (5%), and growth of microorganisms on environmental exposure plates (4%)¹⁴¹.

Another study reported 16 APELS worldwide between 2000 and 2021, mostly involving RG 3 pathogens in research or university laboratories. The majority was caused by human errors⁶.

Considering that human error is the leading cause of accidents and incidents in HCBLs, investing in training and promoting a biosafety culture represents the best strategy for reducing these occurrences^{4, 93, 98, 134, 141}.

Furthermore, the existence of tools that facilitate the notification of accidents and incidents in HCBLs and transparent communication of these incidents is essential. It is not only to generate reliable information regarding the frequency of such occurrences but also to generate learning that prevents recurrences. When the root cause is identified and mitigating actions are put into practice, there is a consequent reduction in the occurrence of similar incidents^{115, 134, 141-142}.

Recording even seemingly minor incidents is essential, as they can lead to implementing mitigating measures that prevent more severe events and enhance biosafety culture. Additionally, the need for recording offers an opportunity to discuss biosafety and biosecurity aspects with the team^{53, 134, 141}.

A standardized and centralized system for registering accidents and incidents across different agencies and institutions is highly desirable, as it enables information sharing and learning from mistakes and negative experiences and mitigating measures^{43, 119, 143}. The *Laboratory Incident Notification Canada* (LINC), an electronic registration system developed by the Canadian government, is an excellent example of such an initiative¹²⁰, as is the ABSA International online LAI database¹¹⁵. Unfortunately, only a few countries invest in such tools^{142, 144}.

^d Equivalent to USD 6,029,400,000.00, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2007.

^e Equivalent to USD 10,049,000,000.00, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2007.

It is essential to create an environment where professionals feel motivated to report incidents and accidents, which involves ensuring that reporting does not lead to punishment or judgments^{141, 145}. Due to the frequency of these behaviors, many workers opt not to report accidents out of embarrassment or the fear of facing penalties, such as dismissal. Additionally, some may refrain from reporting to prevent financial repercussions for the institution, such as the loss of research funding. Other factors that may discourage reporting are social pressure and the complexity, lack of standardization, and automation of the registration process, which can be time-consuming and labor-intensive^{50, 142}. It is estimated that 30% of zoonotic LAIs are not reported¹³⁴.

Notably, according to Brazilian legislation, any accident involving releasing biological materials shall be notified¹⁴⁶.

2.5 Biological weapons and bioterrorism^f

The use of biological agents as weapons of war is nearly as old as humanity itself. The most significant event in terms of loss of human life occurred in the 1940s when Japan used biological weapons in its war against China¹⁴⁷⁻¹⁴⁸. Researchers and professionals working in high-risk biological agent handling laboratories must be vigilant of the possibility of malicious use of biological assets and the technologies and information generated, particularly those derived from microbiology and biotechnology advancements¹⁴⁹.

In addition to high-risk human pathogens, pathogens of interest to agriculture can also be used as biological weapons due to their strong economic and social impact. Pathogens involved, such as the viruses causing CSF, AFS, FMD, bovine plague, and plant pests like Asian soybean rust¹⁵⁰ and witches' broom, as in the agro-bioterrorism crime involving cocoa plantations in southern Bahia in 1989¹⁵¹, can be highlighted as examples.

Biological agents not associated with zoonoses are safer to handle, and the spread of diseases can be facilitated in intensive farming with poor biosafety. Attacks on agribusiness may have different motivations from conventional terrorism, such as ideological issues against using GMOs and economic problems associated with competition between companies and price manipulation¹⁵².

In an all-hazards approach to biological threats, the veterinary and law enforcement sectors have important roles to play alongside public health, and they must jointly plan to prevent and respond to criminal activities affecting primarily animal health in the form of agro-crime acts, including agroterrorism. Agriculture is critical to national security, and preparedness for agro-crime must also be integrated into contingency planning at both national and international levels. Preventing and tackling agro-crime and agroterrorism will contribute to mitigating the risk of spillover of diseases from animals into human populations¹⁵³.

The international community has made several attempts to prevent the use of chemical and biological weapons, dating back to the Brussels Declaration of 1874. This declaration was the foundation for the Hague Conventions of 1899 and 1907. The Geneva Protocol was signed in 1925 following the extensive use of chemical weapons during World War I. While it condemned the use of biological weapons, it did not prohibit their development, which allowed for their use in retaliation. In 1972, with the signing of the Biological Weapons Convention (BWC) under the auspices of

^f Bioterrorism is understood as the 'intentional use of a biological agent with a political, economic, religious, or social objective, or to coerce, intimidating, or sending a message to an audience beyond the immediate victims.'¹⁸.

the United Nations (UN), the explicit prohibition of the production and use of chemical and biological weapons was formalized¹⁵⁴⁻¹⁵⁵. Only nine countries in the world have not signed this Convention¹⁵⁶.

One initiative aimed at identifying potential biological weapon pathogens and preventing their spread was the creation of the 'Australia Group.' This informal group was founded in 1985 during the war between Iran and Iraq, a time when chemical weapons were being produced using precursors and technologies that were imported through legal trade mechanisms. The group's primary objective was to ensure that member countries' industries did not intentionally or unintentionally support countries investing in chemical weapons production. The group's scope was later expanded to include biological weapons. The 42 member countries are required to have a robust system for export control and demonstrate adherence to multilateral treaties related to the use of chemical and biological weapons. This group maintains a list of chemicals and biological agents that must be controlled in export processes, as well as equipment, technologies, and software that could be used in the production and proliferation of biological weapons¹⁵⁷. The Australia Group's actions represent a global advancement in compliance with the BWC. It should be noted that Brazil is not a member of this group.

Following the intensification of terrorist attacks in the early 2000s, the UN emphasized the need for member countries to establish regulatory mechanisms to prevent the development, acquisition, manufacture, possession, transportation, and transfer or use of nuclear, chemical, or biological weapons and their delivery systems through Resolution 15.40 in 2004. This resolution differs from the BWC in two crucial aspects: the first is its scope (it covers all UN member states), and the second is its verification mechanism, through which countries must submit reports informing about the status of their implementation¹⁵⁴. Brazil internalized Resolution 15.40 through Decree n. 7,722/2012¹⁵⁸.

Countries can demonstrate their commitment to Resolution 15.40 by implementing biosafety and biosecurity measures in laboratories, especially regarding monitoring biological assets production, use, storage, and transportation. Additionally, it is crucial to adopt measures to ensure the physical protection of facilities, including access control. However, in 2008, 159 countries submitted reports on the legal framework related to weapons of mass destruction, with very few demonstrating compliance with Resolution 15.40. The most common reasons for this were a need for more understanding of their obligations and a lack of capacity to meet the requirements of the Resolution¹⁵⁹.

The WHO has emphasized the importance of countries implementing biosafety and biosecurity measures through Resolution 58.29, which was approved at the General Assembly in 2005. However, there is currently no independent international mechanism to monitor adherence to this resolution, and countries are not obligated to report on their implementation efforts¹⁵⁹⁻¹⁶⁰.

Some argue that the use of biological weapons has historically been infrequent, with a trend towards even less military use, especially with the decline of terrorist group activity. In Brazil, the country's democratic nature, non-involvement in international conflicts, and commitment to defending human rights and condemning political terror contribute to further lowering the risk of biological weapon use. Despite their low production cost, biological weapons become unattractive when compared to chemical weapons because even those responsible for producing them can lose complete control over their use after the pathogen spreads. It makes their application

dangerous, even for the producers themselves, and they are considered morally unacceptable and politically indefensible^{18, 147, 154, 161}.

Furthermore, transforming biological agents into weapons of mass destruction requires technological refinement, scientific knowledge, and financial resources to research, produce, and develop such artifacts³. In addition to isolating and perfecting the lethal capacity of the agent itself, making it a weapon of mass destruction requires the development of a delivery mechanism coupled with an explosive device. It enables the biological agent to disperse without being deactivated by the heat, pressure, or shockwave generated by the explosion¹⁶².

Despite these considerations, the threat of biological weapons cannot be dismissed, which is why the US CDC maintains a list of biological agents with potential use in bioterrorism, categorized by priority according to their potential impact on society¹⁶³. This list is smaller than the one maintained by the Australia Group and should not be confused with the one referred to by the FSAP.

HCBLs play a dual role in bioterrorism: they can function as its source and the primary line of defense against it³. Countries such as the US and France require laboratories to maintain an updated inventory of biological assets relevant to biosecurity^{55, 164}. In contrast, a few, such as Uganda, have developed tools to ensure this information is periodically communicated to the government. One of the main advantages of centralizing this type of information is the ability to identify researchers who can support the country in an outbreak or threat involving a particular pathogen¹⁶⁵.

At least 32 countries possess some biodefence programs. Finland, Japan, South Korea, Switzerland, the Philippines, the UK, and the US explicitly mentioned bioterrorism as the reason for their biodefence programs³.

The UK Biological Security Strategy, quoting the 2023 Integrated Review of Security, Defence, Development and Foreign Policy, stated that it is likely that a terrorist group will launch a successful Chemical, Biological, Radiological, or Nuclear attack by 2030⁵⁴.

While bioterrorism incidents are improbable in Brazil¹⁸, the country is actively taking proactive measures to mitigate potential risks. To this end, the MCTI's General Coordination of Sensitive Goods has compiled a comprehensive list of pathogens that could be used to develop biological weapons or have dual-use capabilities. This list includes equipment such as BSCs, and BSL-3 and BSL-4 'Complete Containment Units.' However, the current approach emphasizes aspects related to export control, with no specific legislation on bioterrorism and no robust legal mechanisms for monitoring the handling of high-risk pathogens in laboratories¹⁵⁴.

2.6 Laboratory biosafety management: primary reference documents

Implementing a laboratory biosafety management system is crucial in promoting an organization's culture of biosafety and biosecurity. This culture comprises an assembly of beliefs, attitudes, and patterns of behavior of individuals and organizations that can support, complement, or enhance operating procedures, rules, and practices, as well as professional standards and ethics designed to prevent the loss, theft, misuse, and diversion of biological agents, related materials, technology, or equipment, and the unintentional or intentional exposure to (or release of) biological agents. This culture should be promoted through management systems that include policies, processes, procedures, and programs to prioritize biosafety and biosecurity for management. A strong biosafety culture involves viewing safety as a natural way of working rather than just complying with rules. It is more easily

achieved with a robust biosafety program within the institution and leadership committed to the topic¹⁶⁶.

Research on laboratory accidents commonly finds that the causes are more related to the absence of a strong biosafety culture than physical barriers or regulations and the lack of a reliable incident reporting system with monitoring, evaluation, and sharing of lessons learned. Once the culture is established, people will follow procedures regardless of regulations and act responsibly in scenarios representing risks and threats¹⁶⁶.

Despite countries' concerns regarding threats related to biological agents, no minimally standardized regulation deals with laboratory biosafety management, as the WHO desires. Although most countries agree with the proposal for a global regulation on biosafety and biosecurity, priorities and approaches vary among countries to the extent that the subject still requires further discussion and maturity¹⁴⁰.

The primary references currently available for guidance on laboratory biosafety management are the WHO Biosafety Manual² and the BMBL, used as a reference by the USG¹.

The WHO emphasizes that countries must establish their own regulations and monitoring mechanisms, utilizing the manual as an additional source of information for biosafety management. The current edition of the WHO Biosafety Manual, on its core material, covers the following topics:

- a. Risk assessment;
- b. Core requirements;
- c. Heightened control measures;
- d. Maximum-containment measures;
- e. Transfer and transportation;
- f. Biosafety program management;
- g. Laboratory biosecurity;
- h. National and international biosafety oversight².

This manual also includes supplementary monographs with more detailed information on the following topics:

- a. Biosafety program management;
- b. Risk assessment;
- c. BSCs and other primary containment devices;
- d. PPE;
- e. Laboratory design and maintenance;
- f. Decontamination and waste management;
- g. Outbreak preparedness and resilience².

In a distinct position, the BMBL maintained, in its latest edition, the classification of laboratories into BSLs, describing the recommended construction requirements, equipment, and operational procedures for each level. The manual also presents the main characteristics, associated risks, and recommendations for handling numerous pathogens¹.

Like the WHO manual, the BMBL has annexes that cover complementary topics such as:

- a. Primary containment for biohazards: selection, installation, and use of BSCs;
- b. Decontamination and disinfection of laboratory surfaces and items;
- c. Transportation of infectious substances;
- d. Biosafety and biocontainment for pathogens affecting agricultural animals and animals that are loose-housed or in open penning;

- e. Arthropod containment guidelines;
- f. Select agents and toxins;
- g. Integrated pest management;
- h. Working with humans, non-human primates, and other mammalian cells and tissue;
- i. Guidelines for work with toxins of biological origin;
- j. NIH oversight of research involving recombinant biosafety issues;
- k. Inactivation and verification;
- l. Sustainability;
- m. Large-scale biosafety;
- n. Clinical laboratories¹.

Another document widely disseminated and used as a reference for managing HCBLs is the ISO 35001:2019 (*Biorisk management for laboratories and other related Organisations*), which originated from the CWA (CEN – *European Committee for Standardization* – Workshop Agreement) 15793. This standards' main prerogatives are leadership commitment and continual improvement through a cycle of planning, implementing, reviewing, and improving the processes and actions an organization undertakes to meet its goals (PDCA cycle: Plan-Do-Check-Act)¹¹.

As for the operational aspects of laboratory biosafety management, this standard addresses the following topics:

- a. Operational planning and control;
- b. Commissioning and decommissioning;
- c. Maintenance, control, calibration, certification, and validation;
- d. Physical security;
- e. Biological materials inventory;
- f. Good microbiological technique;
- g. Clothing and PPE;
- h. Decontamination and waste management;
- i. Emergency response and contingency planning;
- j. Transport of biological materials¹¹.

Also, it brings recommendations regarding the evaluation and continuous improvement of management, which includes conducting internal audits, inspections, and other monitoring mechanisms, such as measuring incidents and recording and addressing non-conformities¹¹.

It also recommends designating a professional to act as a BO. Although the 35001:2019 states some duties of a BO (also known as biosafety advisor, biosafety manager, biosafety coordinator, or biorisk management advisor), a more comprehensive document that details these competencies is the CWA 16335:2011. According to the CWA 16335:2011, the key competencies and expertise of a BO are:

- a. General principles of microbiology, biochemistry, and cell biology;
- b. General principles of molecular biology and genetic engineering;
- c. Biological and other hazards in the work area;
- d. Occupational health and biosafety;
- e. Human factors (e.g., behavior, reliability, ergonomics);
- f. Containment principles;
- g. Biorisk assessment and management;
- h. Environmental safety;
- i. Facility design, construction, commissioning, decommissioning, validation, operation, and maintenance;
- j. Selection, validation, certification, and maintenance of equipment;

- k. Good microbiological techniques;
- l. PPE;
- m. Infection control, disinfection, decontamination, and sterilization;
- n. Biological waste management;
- o. Emergency preparedness and response;
- p. Incident and accident investigation;
- q. Biorisk management programme;
- r. Inventory monitoring and control;
- s. Physical security;
- t. Training (to understand training needs and to develop, deliver, and validate an internal biosafety and biosecurity (re)training program tailored to different audiences);
- u. Communication skills and information/knowledge systems;
- v. Audits and inspections;
- w. Packaging, shipping, transport, import, and export of biological material;
- x. International and national regulatory framework, standards, guidelines, and conventions;
- y. Bioethics¹⁶⁷.

Although private institutions in countries such as the US and the UK grant accreditation certificates to specialists in biological risk management¹⁶⁸⁻¹⁶⁹, formal courses aimed at BOs are generally lacking. As a result, the designation is often based on professional experience rather than academic qualifications. However, depending on the size and complexity of the laboratory, the BOs must dedicate exclusively to this activity and have the independence and authority to implement the biological risk management program^{25, 167, 170-171}.

Due to its comprehensive and flexible nature, some authors have advocated for adopting ISO 35001:2019 as a normative reference by countries that lack specific regulations for laboratory biosafety management, including the possibility of laboratory accreditation under this standard. For labs operating in countries without comprehensive biosafety and biosecurity laws and regulations, it provides a roadmap to best practices in biorisk management^{17, 51, 76, 140, 172-174}. The WHO, for instance, uses ISO 35001:2019 as a reference in periodic inspections conducted in the two authorized laboratories for storing the human smallpox virus¹⁷⁴.

The adoption of ISO 35001:2019 received encouragement during the 2020 BWC Convention¹⁷⁵. Ten countries proposed to enhance biological risk management by implementing this standard. According to this document, the implementation of the standard can contribute to the achievement of BWC goals, as it provides:

- a. Increased awareness within the scientific community about the risks associated with the dual-use of biological materials and, consequently, greater responsibility in research involving hazardous biological materials;
- b. Reduction of the risk of unauthorized access to biological agents and materials;
- c. Greater assurance that institutions will comply with the prohibitions and obligations of the BWC;
- d. Responsible management of biorisk, even in cases where national legislation and regulations do not meet the requirements;
- e. Facilitation of international transactions involving biological materials¹⁷⁵.

2.7 Laboratory biosecurity management and dual-use research

The management of laboratory biosecurity is an aspect that is commonly overlooked in HCBLs as well as in regulatory frameworks^{144, 176}.

The WHO and the Organisation for Economic Cooperation and Development (OECD) have published manuals on good practices related to laboratory biosecurity, emphasizing the importance of the physical security of facilities and the possession of biological materials of interest. These materials require special monitoring and accountability procedures due to their economic and public health importance and the potential risk of malicious use. Such precautions should primarily focus on controlling the use, biological asset use, stockpiling, and transfer¹⁷⁷.

The Public Health Agency of Canada published the 'Canadian Biosafety Guideline – Conducting a Biosecurity Risk Assessment', which can be used as a roadmap to carry out a biosecurity risk analysis¹⁰⁰.

A complete biosecurity management program includes standardized procedures for:

- a. Risk analysis considering the consequences of potential intentional exposure to pathogens present in the laboratory;
- b. Armed or unarmed guard;
- c. Access controls to the laboratory (individual password, proximity cards, or biometrics) with different levels of access privileges for different environments;
- d. Prior evaluation of visitors' profiles and continuous monitoring during their visit;
- e. Definition of physical perimeter barriers (e.g., walls and fences) and electronic monitoring through presence sensors and security cameras;
- f. Evaluation of personnel reliability, including criminal record checks, social media activity profiles, and previous work experiences;
- g. Inventory and transportation of biological assets;
- h. Development of a personnel training plan for biosecurity and emergency response;
- i. Communication and response to biosecurity-related accidents and incidents;
- j. Information security, namely 'cyberbiosecurity'^{14, 178-180}.

The following data are examples of sensitive information:

- a. Biological asset inventory and storage location;
- b. Sensitive equipment inventory;
- c. Surveillance procedure routines;
- d. Laboratory access reports;
- e. Received sample data;
- f. Employee personal information;
- g. Access passwords¹⁷⁸.

Another critical aspect to consider in the context of laboratory biosecurity is Dual-use Research of Concern (DURC), which refers to research that produces knowledge, information, methods, products, or technologies that can be used for peaceful and legitimate purposes but may also be exploited maliciously to cause harm to humans, animals, plants, agriculture, or the environment⁸.

One of the most common examples of DURC is 'gain-of-function' research, where a microorganism is genetically modified to enhance its biological functions, aiming to advance the understanding and treatment of infectious diseases. However, this type of research can lead to unexpected findings, such as the unforeseen increase in virulence of a particular biological agent during an experiment unrelated to this objective. Furthermore, the misuse of research results by third parties or deliberate misconduct by the researcher can also lead to biosafety risks. The National Research Council (NRC), the operational branch of the US National

Academies of Sciences, Engineering, and Medicine, has identified the types of DURC, the gain-of-function, and biosafety risks:

- a. Render a vaccine ineffective;
- b. Confer resistance to therapeutically helpful antibiotics or antiviral agents;
- c. Enhance the virulence of a pathogen or render a nonpathogen virulent;
- d. Increase the transmissibility of a pathogen;
- e. Alter the host range of a pathogen;
- f. Enable the evasion of diagnostic or detection modalities;
- g. Enable the weaponization of a biological agent or toxin⁸.

Most gain-of-function research is conducted in BSL-3 laboratories⁸³. Evaluation of these facilities has revealed studies indicating questionable biosafety situations, poor occupational health monitoring, and little experience in high-biocontainment operations, highlighting the need for greater regulation on the subject at the national or international level^{168, 181}.

Effective management of biosecurity depends on the following factors:

- a. The values, principles, and training of the scientists directly involved in research (the research culture);
- b. Active management of biosafety and laboratory biosecurity risks by institutions;
- c. The government initiatives that set out the responsibilities and obligations of individuals, institutions, and other relevant stakeholders⁸.

In this sense, regulatory acts are crucial in ensuring research institutions understand their responsibility in effectively managing biological risks, recognizing that this aspect is as important as academic or commercial ones. Funding and financing agencies for research are also relevant in this process, as they could mandate compliance with specific requirements for funding approval of research projects involving high-risk pathogens. The increasing involvement of the private sector in research projects indicates that supervision mechanisms should cover both the public and private sectors⁸.

WOAH also expressed concern regarding this issue and stressed that risk assessment on DURC should be included in routine procedures for risk analysis in veterinary laboratories. According to WOA, this assessment should be performed by the same group responsible for evaluating laboratory biosafety and biosecurity, usually the IBC. Among the recommendations, regulation was highlighted as necessary to safeguard society from risks related to particular research and to provide legal security for researchers and institutions. Regulation promotes standardization of supervision criteria and sanctions for non-compliance with these criteria¹⁸².

Different government policies and strategies for monitoring and regulating activities in high and maximum-containment laboratories can be identified in this sense. For instance, in the Netherlands, a DURC Quick Scan toolkit was developed to help researchers identify potential DURC issues and increase awareness of the related issues. The survey is based on 15 questions related to the following topics:

- a. High-risk biological agents;
- b. Host range and tropism;
- c. Virulence;
- d. Stability;
- e. Transmissibility;
- f. Absorption and toxicokinetics;
- g. Drug resistance;
- h. Population immunity;

- i. Detection methodology and diagnostics;
- j. Reconstruction;
- k. Harmful effects;
- l. Knowledge and technology;
- m. Economic consequences;
- n. Consequences for society¹⁶⁹.

2.8 Government policies and strategies for monitoring and regulating activities in high and maximum-containment laboratories

2.8.1 The role of the State

Over the past decades, significant and ongoing changes in the society have resulted in complex social, economic, political, and technological transformations. In response, the study of administration and public policy has sought to understand the impact of these changes on the functioning and results of government actions¹⁸³.

The state's role has evolved from being a service provider to ensuring the production of public goods, from a directing or managing state to a cooperative state that works with other actors to produce public goods. This transition has generated political debates about the scope of state activities¹⁸⁴.

Laboratory biosafety is a critical issue that falls within the state's responsibilities. The new state action model recognizes the state's role in providing this service, considering the high costs and specialized demands involved in designing, constructing, operating, and maintaining HCBL. It requires a well-structured state governance process⁵⁵.

Given the complexity and potential economic impact of laboratory biosafety, it requires the integrated and collaborative participation of various actors, such as representatives from the areas of public health, national defense and security, agriculture, trade, intelligence, justice, industry, and finance, as well as professionals from academia, non-governmental organizations, local institutions, the media, and civil society⁴⁰.

Several issues need to be addressed at the governmental level to define the governance model to be adopted. WHO states that controlling biological risks begins nationally by establishing legislation and regulations for authorized laboratories' control measures. Countries that have developed such legal frameworks have also established mechanisms to monitor and supervise compliance with rules by these laboratories².

Unfortunately, in many cases, laboratory biosafety and biosecurity design and implementation are outdated and inconsistent, with insufficient or nonexistent legal frameworks for the construction, certification, and operation of HCBLs. Additionally, government agencies often fail to be involved in their maintenance, resulting in degraded structures that are unsafe or out of operation when built in places without sufficient resources for their maintenance in the medium and long term^{46, 185-186}.

2.8.2 Developing governance mechanisms

In 2009, the US Congressional Research Service published a report that focused on the regulation of HCBLs by the USG. According to this document, HCBLs play a crucial role in the biodefense effort, offering the potential for more effective responses to bioterrorism threats and a deeper understanding of the risks posed by bioterrorism. However, they also present the possibility of increasing the risk of a biological attack by serving as a potential source of materials or training for malicious

actors. Therefore, rather than relying solely on self-regulation, policymakers might consider enhancing oversight through additional regulations for HCBLs.

Implementing a rigorous oversight program that includes facility and personnel licensure, mandatory training, and restrictions on constructing new facilities could enhance security. In contrast, it might also result in regulatory burdens, increased federal expenditures, and potential impediments to scientific progress in countermeasure research, forensics, and public health¹⁸⁷.

Perkins et al. (2019) argued that enhancing responsibility in the life sciences is possible. In an ideal scenario, responsible science should integrate scientists as part of the solution rather than part of the problem. However, implementation of social responsibility largely hinges on education and self-regulation by scientists (e.g., through IBCs, ethics, and DURC committees). The authors also caution that a top-down approach by the government may become inevitable if no consensus within the research community effectively matches genuine self-regulation with public views¹⁶⁶.

BSL-4 facilities are subject to more government and international oversight than their BSL-3 counterparts. However, the large number of BSL-3 facilities makes monitoring all of them adequately challenging³.

The topic is so complex and significant that the WHO has recently published a guide to assist countries in developing regulatory mechanisms to ensure the adoption of biosafety and biosecurity standards in laboratories¹².

In this document, the WHO cautions that the scarcity of legislation, regulations, and standards on managing biological risks and their implementation is the central issue for governance, which impedes the establishment of a more robust biosafety culture. Governance, as defined by the WHO, encompasses a set of norms, values, and rules governing public management to guarantee transparency, participation, inclusion, and appropriate response to a specific subject. Governance includes formal mechanisms (such as laws and regulations) and informal mechanisms (such as codes of conduct, manuals, guides, best practices, and training) and should have tools to address violations. The goals of laboratory biosafety governance are:

- a. Reduce incidents and accidents and enable a rapid response when they cannot be prevented;
- b. Reduce violations and facilitate their rapid detection and treatment;
- c. Reduce future opportunities for research, development of tools, and generation of knowledge with criminal intentions;
- d. Encourage information exchange and learning¹².

In this regard, the action of governments is essential to strengthen the regulatory framework, allocate resources, and require various actors to fulfill their roles and responsibilities. As such, the WHO guideline outlines seven steps for conducting this process:

- a. Mobilize national commitment and resources;
- b. Conduct a national evaluation;
- c. Establish national institutions and operational mechanisms and develop best-fitting regulations;
- d. Strengthen regulatory expertise;
- e. Implement and enforce regulations;
- f. Establish national information exchange networks and international partnerships;
- g. Review performance and adaptability¹².

During this process, some crucial questions will have to be answered, such as:

- a. Is there a pre-established government infrastructure to support the program, or does this structure need to be developed?
- b. What will be the costs of the program? Is there budget availability? What will be the source of the resources?
- c. How comprehensive will the program be? Should it include monitoring specific biological agents or the registration and certification of HCBLs in general? Will it address only biosafety aspects, or will it also include biosecurity?
- d. Who will be monitored, and who will conduct monitoring activities? Will participation in the program be voluntary or compulsory?
- e. What will be the scope of the monitoring process? Will only documentation be evaluated, or will on-site audits and inspections be carried out? If audits and inspections are to be conducted, is there availability of qualified personnel?
- f. Will only federal agencies be evaluated, or will the scope be universal, including private entities?
- g. Will evaluations occur only at the time of authorizing the operation of a laboratory? Will periodic assessments be conducted? What criteria will be adopted to define priorities?
- h. What will be the legal basis for monitoring? Will national and international manuals and guides be adopted? Will a checklist need to be developed?
- i. What sanctions will be applied in case of non-compliance?
- j. How will the program be evaluated, and what indicators will be developed?¹²

2.8.3 The United States legislation

Learning from the successes and failures of initiatives implemented by other countries with established regulations, such as the US, is essential when developing a regulatory framework. The FSAP defined the governance mechanism for laboratory biosafety and biosecurity in the US, which was established more than 20 years ago. The program aimed to develop a list of biological agents that could be used as weapons (known as 'select agents') and regulate their transfer. After the terrorist attacks in 2001, the program became more rigorous and restrictive^{14, 43}.

Defining the pathogens included in the FSAP list is a complex task, as exemplified by the difficulty in classifying SARS-CoV-2, which meets some criteria but not all. Despite being theoretically usable for bioterrorism purposes, HIV is not included in the list. Therefore, the requirements must be periodically revisited, with the list of agents reviewed every two years. The current criteria established for selecting pathogens include:

- a. Virulence, pathogenicity, or toxicity of the organism;
- b. Availability of treatments such as vaccines or drugs to control the consequences of a release or epidemic;
- c. Transmissibility of the organism;
- d. Ease of preparing the organism in sufficient quantity and stability for use as a bioterrorism agent;
- e. Ease of disseminating the organism in a bioterrorism event to cause mass casualties;
- f. Public perception of the organism;
- g. Known research and development efforts on the organism by national bioweapons programs¹⁸⁸.

The current version of the FSAP list encompasses pathogens and toxins that affect plants, animals, and humans, with some being common to the latter two groups. Any person or institution intending to possess, use, or transfer select agents must hold a CDC and/or USDA registration certificate. For these individuals or institutions, the Federal Bureau of Investigation (FBI) conducts a risk analysis to evaluate the profile of professionals with access to select agents. Every institution must appoint a Responsible Official (RO), whose duties include ensuring compliance with all regulatory requirements regarding the possession, use, and transfer of select agents¹⁸⁹⁻¹⁹¹.

Institutions registered in the FSAP must implement a biosafety plan under the NIH guideline (if they manipulate GMOs) and the BMBL manual. Other requirements comprise:

- a. Biosafety plan according to risk assessment;
- b. Incident response plan;
- c. Training program;
- d. Prior authorization from CDC and/or USDA for transfer of biological assets or toxins;
- e. Maintenance of a complete inventory of biological assets and toxins;
- f. Immediate notification of events such as theft, loss, or exposure of select agents;
- g. Pathogens considered most dangerous are classified as 'Tier 1' and require additional biosafety procedures, including an occupational health plan for the involved staff^{143, 189, 191}.

Audits conducted under the FSAP encompass a comprehensive evaluation of safety and security concerning biological asset possession, use, and transfer based on an audit checklist derived from recognized regulations and manuals (primarily from the CDC and NIH). Inspectors evaluate documentation related to biosafety management, physical structure, biosecurity components, incident responses, and training records. Reports are generated at the end, and laboratories are given a period to present their considerations and corrective actions. Additional audits may be scheduled depending on non-conformities severity¹⁹².

FSAP employs an electronic system to maintain the program's database, including the names and locations of registered entities, the biological agents each has in stock, and the names of researchers authorized to access such agents. Implementing this system has led to a notable enhancement in the efficiency and effectiveness of the program. Information exchanges regarding action plans derived from inspections are also facilitated through the electronic system¹⁹³.

Registration of laboratories and professionals who handle select agents must be renewed every three years, with initial accreditation and renewals granted only after an audit. In 2021, there were 233 registered laboratories, with 198 registered with the CDC and 35 with APHIS. Of these, 82 were academic, 67 were non-federal government, 40 were commercial, 30 were part of USG, and 14 were private (a privately owned non-profit company). Most registered laboratories (n=187) were BSL-3 or ABSL-3, of which 69 belonged to academic institutions. At the same time, 8,672 professionals were registered in the program^{50, 193}.

In the fiscal year of 2016, the CDC's budget for the FSAP was USD 21 million, and the APHIS's was USD 5.5 million. In 2021, 206 audits were conducted, mostly without prior notice, with a duration ranging from one day to two weeks, depending on the complexity of the process. The average time required to complete reports is 36 days, but it may take over 100 days in some cases. Between one and ten auditors participate in these audits, and multidisciplinary teams with experts in

human health, veterinary health, maintenance/engineering, biological risk management, and automation are desirable. In 2017, there were 51 trained auditors in the CDC and 11 in the APHIS^{50, 193}.

Depending on the results, three levels of sanctions can be applied:

- a. Administrative actions, including suspension or denial of registration to work with select agents;
- b. Referral to the CDC or APHIS for investigation, which may result in fines or warnings;
- c. Referral to the FBI for investigation may result in up to five years in prison or fines⁵⁰.

Between 2003 and 2016, the program suspended or denied ten registrations⁵⁰. Fines for violations involving select agents in the US can reach up to USD 250,000 for individuals and USD 500,000 for companies. Until 2009, the CDC had applied about USD 2 million in fines, and the APHIS had applied almost USD 550,000⁴³.

The FSAP has been periodically evaluated by US authorities, mainly by the Federal Experts Security Advisory Panel, a committee created in 2010 under the US Department of Health and Human Services (HHS), to identify gaps and needs and propose improvements in biosafety and biosecurity in the country. In 2014, this committee presented numerous recommendations, like:

- a. Create and strengthen a culture that emphasizes biosafety, laboratory biosecurity, and responsible conduct in the life sciences;
- b. Require that all research institutions in which human, plant, and/or animal infectious agents and toxins research is conducted have an appropriate organizational and governance structure to ensure compliance with biosafety, biocontainment, and laboratory biosecurity regulations and guidelines;
- c. Require that an appropriately constituted and qualified review entity validates local policies, laboratory protocols, and mitigation plans involving the inactivation, sterilization, or decontamination of biohazardous materials;
- d. Create an expert panel or Federal Advisory Committee to serve as an external group that could share best practices or make recommendations to the FSAP;
- e. Support the development and implementation of security awareness education programs/curriculum;
- f. Release aggregate information on laboratory incidents on an annual basis;
- g. Identify a mechanism for sharing best practices;
- h. Enhance the international engagement to explore the harmonization of pathogen security standards, including convening and expanding membership of the International Expert Group for Biosafety and Biosecurity Regulation (IEGBBR);
- i. Develop and maintain a robust federally-supported program of applied biosafety research to create additional evidence-based practices and technologies and to update existing practices and operations;
- j. Recommends the development of an approach to improve the consistency of the inspection process;
- k. Improve guidance for biosafety plans;
- l. Amend guidance documents to suggest that entities consider establishing policies on maximum work hours for high containment workers;
- m. Determine the appropriate number of HCBLs installed in the US¹⁹⁴.

Although the annual reports of the FSAP published to date demonstrate that the program has significantly contributed to strengthening laboratory biosafety and biosecurity in the US, due to the low number of reported incidents and serious

accidents, it is essential to note that the program does not cover HCBLs that do not manipulate GMOs either select agents^{3, 13}. Another criticism of the program is the high bureaucratic burden it places on researchers and institutions, particularly regarding strict inventory control and transfer of biological assets, the requirement for continuous training of human resources, and the long time required to obtain a license, as well as the double regulation received by laboratories that handle pathogens that belong to both the CDC and APHIS lists⁵⁸. Additionally, some consider the individual licensing of researchers unnecessary and undesirable¹⁴. These challenges have decreased the number of laboratories registered in the program since 2015, reducing research involving select agents¹⁹³. A similar trend was observed in France, where the number of laboratories working with select agents decreased by 54% between 2011 and 2012, along with a 71% decrease in the number of licenses granted to researchers during the same period³².

It is worth noting that the US *National Biodefense Strategy* was published in 2018, with a second version released in 2022 as the *National Biodefense Strategy and Implementation Plan for Countering Biological Threats, Enhancing Pandemic Preparedness, and Achieving Global Health Security*. The document states that the USG must address the range of biological threats: emerging and re-emerging infectious diseases affecting humans, animals, plants, and the environment; risk of intentional misuse of advances in biotechnology; accidental release of biological agents; and threats posed by terrorist groups or adversaries seeking to use biological weapons¹⁹⁵.

Additionally, the US National Biodefense Strategy highlights that while research with high-risk pathogens is vital for developing countermeasures and understanding and predicting future outbreaks, laboratories with insufficient biocontainment or biosafety protocols and practices exacerbate the risk of an outbreak through LAIs or accidental release of a pathogen into the environment. In this sense, the document contemplates the following initiatives to prevent nationally or internationally significant biological incidents:

- a. Minimizing the chances of laboratory accidents;
- b. Reducing the likelihood of deliberate use or accidental misuse;
- c. Ensuring effective biosafety and biosecurity practices and oversight;
- d. Promoting responsible research and innovation;
- e. Reducing the likelihood of animal-to-human spillover of zoonotic pathogens;
- f. Ensuring all facilities in the US or funded by the USG that conduct life sciences research, development, manufacturing, or diagnostic activities with hazardous biological materials are implementing and maintaining practical, transparent, rigorous, and comprehensive oversight, training, and monitoring programs for biosafety, biosecurity, and responsible and ethical conduct in science;
- g. Ensuring the workforce at these facilities is trained to recognize, respond to, and report unsafe practices and threats;
- h. Accelerating laboratory biosafety and biosecurity innovation by supporting efforts to identify and address the research gaps needed to improve evidence-based laboratory biological risk management in the US and globally¹⁹⁵.

2.8.4 The Canadian legislation

The 'Human Pathogens and Toxins Act'¹⁹⁶, the 'Human Pathogens and Toxins Regulations'¹⁹⁷, the 'Health of Animals Act'¹⁹⁸, and the 'Health of Animals Regulations'¹⁹⁹ are the primary legislation related to the handling of pathogens and toxins in Canada and the following guidelines and manuals complement them:

'Canadian Biosafety Handbook'⁷, 'Canadian Biosafety Standard'⁸⁰, 'Canadian Biosafety Guideline – Conducting a Biosecurity Risk Assessment'¹⁰⁰, 'Canadian Biosafety Guideline – Pathogen Risk Assessment'²⁰⁰, 'Containment Standards for Facilities Handling Plant Pests'⁸⁵, and 'Containment Standards for Facilities Handling Aquatic Animal Pathogens'²⁰¹.

According to this legal framework, possessing, handling, using, producing, storing, permitting access, transferring, importing, exporting, releasing, and disposing of a human pathogen (RG 2 or higher) or toxin is considered a controlled activity, that requires a license issued by the federal government. The permit is issued to organizations; otherwise, accessing high-risk pathogens (RGs 3 and 4) demands a security clearance issued to every person^{196, 202}.

The license also permits the importation and subsequent transfer of indigenous terrestrial animal pathogens under the Health of Animals Act²⁰².

The license holder shall notify immediately the federal government when they have reason to believe that:

- a. A human pathogen or toxin has been released inadvertently;
- b. An incident has or may have caused disease in an individual;
- c. A human pathogen or toxin has been stolen or is otherwise missing¹⁹⁶.

The license must set out:

- a. The license holder's identification;
- b. The period during which the license is in effect;
- c. A description of the facility in which controlled activities are authorized under it;
- d. A description of each part of the facility that is subject to the regulation;
- e. The toxins, human pathogens, or the RGs of the human pathogens, in respect of which controlled activities are authorized under it¹⁹⁶.

The obligations of the license holder include maintaining a list of persons authorized to access the facility to which the license applies and supervising and escorting persons who do not have a security clearance to access part of the facility in which controlled activities are authorized to human pathogens that fall into RG 3 or 4. The facility where the controlled activities are carried out shall be licensed, and a BO, who also may hold a license, shall be designated¹⁹⁶.

The maximum period during which a license may be in effect is the following:

- a. Five years for activities with RG 2 pathogens;
- b. Three years for activities with RG 3 pathogens;
- c. One year for activities with RG 4 pathogens¹⁹⁷.

The government conducts the following record checks to issue a security clearance:

- a. A criminal record check;
- b. A check of the relevant files of law enforcement agencies, including intelligence gathered for law enforcement purposes;
- c. A Canadian Security Intelligence Service security assessment;
- d. A credit check¹⁹⁶.

The government designates inspectors who may:

- a. Examine the laboratory and any material or equipment;
- b. Require any person to produce, in the manner and form requested by the inspector, any material or equipment;
- c. Seize and detain any material, equipment, or document;
- d. Open and examine any receptacle or package;
- e. Take or require any person in the place or conveyance to produce, free of charge, a sample of any material found there;

- f. Direct the owner or the person having possession, care, or control of any material, equipment, or document found in the place or conveyance to move it or, for any time that may be necessary, not to move it or to restrict its movement;
- g. Conduct, or require any person to conduct, any test or analysis or take any measurement of any material or equipment;
- h. Take photographs or make recordings or sketches;
- i. Examine and make copies, in whole or in part, of any book, document, or another record;
- j. Require any person to produce any book, document, or another record for examination or copying;
- k. Use or cause to be used any computer system or other device found there to examine information;
- l. Reproduce any information;
- m. Use or cause to be used any copying equipment¹⁹⁶.

The primary objective of the legal framework is to manage risk and guide the regulated party toward compliance through a graduated continuum of enforcement activities. However, some situations may be more appropriately addressed with more robust and immediate actions²⁰². Every person who contravenes the regulation concerning a human pathogen that falls into RG 2 must pay a fine of not more than CAD 50,000^g. The fine would be not more than CAD 250,000^h for a subsequent offense. Additionally, the person may be in prison for up to three months. When the situation evolves higher RG pathogens, the fine can be CAD 10,000ⁱ and imprisonment for two years. Every person who intentionally releases a human pathogen or toxin and, as a result, creates a risk to the health or safety of the public is guilty of an indictable offense and is sentenced to imprisonment for a term of not more than 10 years¹⁹⁶.

The guidelines and manuals regulate the following issues:

- a. Containment levels for human pathogens or toxins;
- b. The decontamination of material, equipment, places, conveyances, and persons contaminated by human pathogens or toxins;
- c. The safety and security of controlled activities;
- d. The conditions that must be met for a license to be issued;
- e. The conditions that must be complied with under a license;
- f. The renewal, suspension, and revocation of a license and the variation of the conditions of an existing license;
- g. The location, design, construction, layout, and upgrading of facilities, as well as the material and equipment at those facilities, including HVAC systems and BSCs;
- h. Access control procedures;
- i. Specification of the human pathogens and toxins RGs;
- j. The conditions to be met by an applicant for a security clearance;
- k. The qualifications, powers, and functions of the BOs;
- l. The establishment, content, and maintenance of inventories¹⁹⁶.

^g Equivalent to USD 36.938,53, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 30, 2022.

^h Equivalent to USD 184.692,67, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 30, 2022.

ⁱ Equivalent to USD 7.387,71, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 30, 2022.

The Canadian Biosafety Standard is used by the human and animal health authorities to verify the ongoing compliance of regulated facilities with the applicable legislation, supporting license applications, renewals, animal pathogen import permits, and, where appropriate, the facility certification (and re-certification) of containment zones. The Canadian Biosafety Handbook is a companion document to the Canadian Biosafety Standard that provides core information and guidance on how the biosafety and biosecurity requirements can be achieved⁸⁰.

All license applicants looking to conduct scientific research with pathogens and toxins must submit a Plan for Administrative Oversight describing how biosafety and biosecurity risks will be managed²⁰². The Plan should demonstrate how institutions or organizations identify, assess, and manage DURC, including the gain-of-function research, addressing the following elements:

- a. Commitment from Senior Management to manage and control biosafety and biosecurity risks;
- b. Delineation of the roles and responsibilities of committees, individuals, departments, etc., that have a role in the control and management of biosafety and biosecurity risks;
- c. Establishment of a single point of contact to provide guidance on the Plan and a senior level who can represent biosafety issues on their behalf;
- d. Overview of how biosafety and biosecurity risks, including those from DURC, are identified, assessed, managed, and controlled at the institution/organization;
- e. Description of all work areas and individuals covered by the Plan;
- f. Summary of how the Plan is communicated;
- g. Overview of the procedures to review and monitor the Plan²⁰².

As of March 2019, 986 organizations were licensed (916 for RG 2 pathogens, 65 for RG 3, two for RG 4, and 3 for toxins) and 439 people with a security clearance in Canada. Most licenses were issued for the industry (464), followed by hospitals (192), academic institutes (201), and governmental laboratories (34 for public health, 30 for environmental health, 27 for animal health, and 38 for others)²⁰².

Between 2018 and 2019, 78 licensed organizations were inspected. The inspection frequency is determined by the risks associated with pathogens or toxins. The target was to inspect at least 5% of the organizations licensed to RG 2 pathogens^j while RG 3 and toxins licenses are inspected once every three years, and RG 4 licenses are inspected annually²⁰².

The top deficiencies found during the inspections were:

- a. Records and documentation (e.g., inadequate inventory in long-term storage);
- b. Training (e.g., no annual refresher training on emergency response procedures);
- c. Work practices (e.g., no verification of the integrity of primary containment devices);
- d. Surface finishes and casework (e.g., inadequate replacement, removal, or repair of worn-down surfaces and materials);
- e. Performance and verification tests (e.g., the integrity of primary containment devices not tested by procedures)²⁰².

The Public Health Agency of Canada provides regulated parties with a range of tools, resources, and guidance to support them in identifying and mitigating biosafety and biosecurity risks, like training courses, newsletters, pathogen safety sheets, webinars, guidelines, checklists for internal audits, pre-licensing inspections,

^j A risk-based approach selects and prioritizes the RG 2 sites to be inspected. The algorithm considers various risk factors, including an organization's compliance history, the robustness of its biosafety and biosecurity program, and the role of the BO²⁰².

and conferences. Besides capacity building, these initiatives aim to provide transparency, better communication with the laboratories, and enhance their engagement with the program²⁰².

A negative aspect of the Canadian regulation is that Canadian agri-food researchers have noted misalignment and duplication between the regulatory regimes for human and animal pathogen oversight²⁰².

2.8.5 The legislation in the European Union

In the EU, Directive 2000/54/EC²⁰³ is widely used as a reference document for defining policies and procedures related to laboratory biosafety and biosecurity. Initially drafted in 1998, the directive presents a concise list of containment measures corresponding to each laboratory BSL. These measures are categorized as either specified, recommended, or unnecessary²⁰⁴.

However, several countries have independently established supplementary detailed instructions that offer additional insights into meeting the specified requirements, often outlining specific methods for achieving compliance. Consequently, there needs to be more harmonization in implementing these guidelines²⁰⁴. Some countries have incorporated additional references such as the BMBL and Canadian manuals⁸³.

Germany and Switzerland have the most comprehensive legislation on laboratory biosafety and biosecurity among EU countries. German directives provide detailed construction requirements and establish minimum criteria to be met by the Principal Investigator (PI) and BO. Laboratories are required to submit an annual report to the government, which conducts yearly inspections and certifications⁸³.

In Switzerland, laboratories that handle pathogens of RG 2 must notify the government of their activities, while those managing agents of RGs 3 and 4 must apply for an operating license⁸³.

The UK systematically monitors HCBLs through *Great Britain's Health and Safety Executive*, the only regulatory body in the country. It brings benefits in terms of standardization of procedures and legislation and avoids duplication of control, resulting in less bureaucracy for laboratories handling pathogens of human and veterinary interest. Inspections are prioritized according to a risk analysis that considers the degree of risk of the pathogen, how it is dealt within the laboratory, and the impact of a possible incident. In this sense, diagnostic laboratories are audited less frequently than research laboratories⁵⁰.

The development of biosafety legislation published by the government of Denmark in 2008 is another case that deserves to be studied in greater depth. The construction process was so remarkable that it motivated the publication of a book to serve as a model for other countries. The authors suggest that a list of controlled agents, both human and animal interest, should be defined, as well as a licensing and auditing system for facilities that intend to handle such pathogens. Penalties should be defined in case of violation, and periodic audits are an essential part of the monitoring work for compliance with this law. Given the workload and the governmental staffing limitations, it is highly probable that priority levels should be established. In this sense, more vulnerable structures or those requiring a higher level of security for handling higher-risk pathogens or in larger volumes and concentrations should be prioritized. Audits should have, especially at the beginning of the establishment of the program, a more educational than punitive character, and it is recommended that they be conducted every three or four years, with reasons for

additional audits when there is suspicion of any violation or at the request of the laboratory itself²⁰⁵.

The authors also proposed the mandatory assignment of BOs in each laboratory, responsible for implementing and maintaining regulations. These professionals must participate in training events offered by the government. Institutions must have security plans and vulnerability assessments for sensitive areas, systems for reporting accidents and incidents, and a contingency plan to deal with crises. However, the authors emphasized that the most critical element that should permeate the regulation application is the culture of biosafety, and the scientific-academic community represents a key piece in this process by setting an example in complying with the law²⁰⁵.

Before the enactment of this legislation, the Danish government conducted a questionnaire to identify the number of institutions working with biological agents, the types of facilities involved, the effectiveness of biosafety systems, and the main challenges faced. The survey identified 94 institutions, of which 22 were visited. A preliminary review of existing legislation was conducted to prevent conflicts, contradictions, and overlaps. The Danish government opted to create a comprehensive Biosafety Law to minimize bureaucracy and conflicts and highlight the significance of biosafety²⁰⁵.

2.8.6 The legislation in Asian countries

In the Asian continent, the situation varies significantly among countries. However, there needs to be more regulation on laboratory biosafety and biosecurity. A 2008 study conducted by the WHO concluded that only two out of the 11 evaluated countries had any regulation on the subject. As a result, BOs are rarely designated, and documentation on incidents and LAIs is minimal. In many cases, laboratories recognized as BSL-3 barely meet the requirements to be classified as BSL-2. For instance, in India, there are many manuals, but there is a lack of legislation to encourage their use by laboratories⁸³.

However, some countries are much more advanced in this regard. For example, in South Korea, BSL-3 laboratories are inspected annually during shutdowns for decontamination and maintenance, and licenses are renewed every three years by the *Korean Center for Disease Control and Prevention*¹⁷.

After a laboratory accident involving the SARS virus in Singapore, the government implemented new legislation requiring the Ministry of Health to authorize the operation of HCBLs from 2006 onwards. These facilities must be recertified annually^{17, 83}.

In Hong Kong, BSL-3 laboratories are periodically certified by independent certification bodies based on criteria established by the BMBL or the manuals of Australia and New Zealand⁸³.

The Chinese Biosecurity Law, which came into force in 2021, is a comprehensive, systematic, and over-arching law involving biosecurity risks, national biosecurity governance, and systems for minimizing biosecurity threats. China's implementation of the Biosecurity Law marked the establishment of a three-in-one biosafety risk and governance system incorporating national biosafety strategies, national biosafety laws, and national biosafety policies in the field of biosafety²⁰⁶.

In 2019, China had 49 standards for biosafety laboratory management, including 24 international standards and 25 Chinese national standards²⁰⁷.

According to Chinese legislation, all BSL-3 and BSL-4 laboratories must be accredited. Before applying for accreditation, the laboratory must pass a construction

evaluation process conducted by the Ministry of Science and Technology. After accreditation, monitoring audits are conducted 12, 30, and 48 months later. Qualified experts conduct these audits with a multidisciplinary team that includes representatives from public health and agriculture-related agencies. The experts must have experience in different areas such as management, research, diagnostic routine, and pharmaceuticals. They also hire advisors in microbiology, public health, veterinary, management, construction, HVAC, and automation²⁰⁸.

After an LAI of SARS occurred in Taiwan in 2003, the government took the following measures:

- a. Promotion of international exchange;
- b. Development of manuals for the construction, management, inspection, training, and operation of BSL-3 laboratories;
- c. Enhancement of biosafety culture through training programs and minimum grade requirements for professionals authorized to work in BSL-3 environments;
- d. Formation of a private network through the establishment of the Taiwanese Biosafety Society;
- e. Requirement of government pre-approval for new BSL-3 laboratories to commence operations;
- f. Implementing new regulations, including forming a permanent interministerial committee responsible for harmonizing biosafety policies under the Ministry of Health¹²⁷.

Since 2004, the Taiwan CDC has been actively developing laboratory biosafety regulations and guidelines. They have also strengthened the management responsibilities of entities' IBCs and regularly inspected high and maximum-biocontainment laboratories. Additionally, they have developed verification regulations for RG 3 and 4 pathogen storage and transfer. Approximately 100 laboratories are operating with this system in Taiwan²⁰⁹.

Taiwan also implemented a select agent program, with 36 pathogens and four toxins on its list. Among them, 13 pathogens and one toxin are deemed highly dangerous ('Tier 1'). The program is more stringent for the select agents than general RG 3 pathogens and biotoxins. If entities will possess, store, or use select agents, they must apply to the Taiwan CDC for review in advance. An entity would submit draft documents that detail training and assessment of the relevant personnel, planning of the laboratories or storage facilities, expected items and purposes, and development of biosafety, biosecurity, and incident response plans. After documentary review and on-site inspection, the Taiwan CDC will approve the entity if no defects are identified or the deficiency is fixed. There are currently seven entities registered in the select agent program²⁰⁹.

To make the program management more effective and complete, the Taiwan CDC has developed some guidelines. These include the 'Guidance for Suitability Assessment of Select Agents and Toxins Staff', the 'Guidance of Select Agents and Toxins Security Plan', the 'Select Agents and Toxins Incident Response Plan Guidance', the 'Guidance for Drills and Exercise of Select Agents and Toxins', the 'Select Agents and Toxins Biosafety/Biocontainment Plan Guidance', the 'Guidance on the Inventory of Select Agents and Toxins', and the 'Guideline for Review Research Programs of Highly Dangerous Pathogens and Biotoxins'²⁰⁹. The regulations include the following requirements:

- a. Assign a RO;
- b. The Taiwan CDC conducts the on-site inspection every year;

- c. The new addition and transfer of the select agents and toxins must be approved by the IBC and RO, and finally by the Taiwan CDC;
- d. Laboratories that possess, store, or use hazardous agents must conduct annual insider threat awareness training and suitability assessment every three years;
- e. Conduct drills at least annually and exercises every three years to test and evaluate the effectiveness of their security, biosafety, and incident response plans²⁰⁹.

In 2018, a management interface was added to the information system, including the entity application review, transfer review and report, the select agent or toxin-positive specimen (from the infectious disease patient or proficiency testing), incident notification and disposal report, inventory management, and the DURC of the hazardous agents²⁰⁹.

In 2019, the Taiwan CDC completed the annual laboratory biosafety and biosecurity inspection for 14 BSL-3 laboratories and RG 3 pathogens storage facilities. In addition, under the supervision of Taiwan CDC, the local government health bureaus have completed the laboratory inspection for 83 BSL-2 microbiology laboratories of other institutions²¹⁰.

2.8.7 The legislation in African countries

There are at least 14 BSL-3 laboratories across Africa²¹¹. According to the regional office of WHO for Africa, there is no information on legislation, regulations, or guidance on HCBLs design in the African region, except in South Africa, whose Health Act contemplates the registration of microbiological laboratories and the acquisition, importation, handling, maintenance, and supply of human pathogens. Other countries in the region are also developing legislation in this area. A document on clinical laboratories from Nigeria lists BSL-3 laboratory requirements, which was modified following US guidance. Some information was obtained about activities with GMOs. One of the goals of the African Biological Safety Association is to support emerging legislation and standards in this area²⁰⁴.

Therefore, for many African countries wishing to build a BSL-3 facility, the ordinary course of action is to follow the WHO Biosafety Manual² and guidance provided by high-income countries, who often act as donors. The primary guidance documents and regulations are from the US, Canada, EU, and Japan²⁰⁴.

In 2022, the African Union and the Africa CDC released a document titled 'Regulatory and Certification Framework for Institutions Handling High-Risk Pathogens'. This document highlights the consistent concern and significant challenge of the need for regulation in laboratory biosafety and biosecurity efforts across African countries. The specifications for high and maximum-containment laboratories developed in higher-income countries, have often been unattainable for resource-constrained regions where infrastructure and suitably trained personnel are lacking²¹².

Consequently, a framework was developed to establish minimum standards for high and maximum-containment laboratories, create a checklist for assessing these facilities, and select a Regional Certification and Recognition Framework. The framework covers planning, design principles, biorisk management systems, good laboratory practices, maintenance, performance testing, and verification. It does not aim to replace national regulations but serves as a tool for self-assessment against internationally accepted rules and procedures for handling high-risk biological assets²¹².

The standard tool for evaluating compliance is a scored checklist that specifies biosafety and biosecurity requirements. The level of compliance is

determined by the score and recognized on a scale of 0 to 5. Facilities with zero to two stars are not authorized to perform any functions associated with high-risk pathogens. Facilities with three or four stars receive provisional authorization and are given three to six months to submit corrective actions. Facilities with five stars receive a certificate of approval for five years, with surveillance assessments in the third and fifth years. It is important to note that Africa CDC is not an accreditation body; therefore, an Independent Advisory Committee will be established to govern the implementation of this program²¹².

2.8.8 The legislation in Latin American countries

A significant challenge in identifying national regulations in Latin America has been the lack of clear information on the institutions responsible for applying and supervising these regulations in each country, making it difficult to search for and obtain the corresponding regulations at the institutional level. In many cases, the information is dispersed among various government entities, institutions in charge of the laboratories, and regulatory bodies, which complicates identifying the sources to obtain updated and reliable information⁵².

For instance, in Argentina, the norms and regulations of laboratories are dispersed and not easily accessible. No official source lists the type and number of existing HCBLs⁵².

Some countries have developed national standards, as observed in Mexico, which has a total of 32 official standards published by the Ministry of Health, and in Colombia, where there is a solid set of regulations compiled in the document *General Guidelines for Biosafety and Biocontainment for the laboratories of the National Laboratory Network*⁵².

In Chile, laboratory regulations are based on a specific biosafety guide for clinical laboratories, which seeks to guide the implementation of the corresponding measures according to the biosafety level. This document contains guidelines for managing biological risk according to containment levels, using personal protection elements, waste management, and transporting samples and infectious substances, among others. In addition to the guide, laws related to the operation of clinical laboratories have been established. However, as in other countries in the region, the lack of clarity in supervisory responsibilities and the absence of a consolidated registry of laboratories represent a significant obstacle to guaranteeing effective management and a comprehensive understanding of Biosafety⁵².

The following countries have self-declared HCBLs: Colombia (n = 1), Paraguay (n = 1), Mexico (n = 18), Argentina (eight BSL-3 and one BSL-4, the last one being commissioned), Venezuela (n = 2, one in container), and Cuba (n = 1, in container) (Cláudio Mafra, personal communication).

2.9 Governance over Dual-use Research of Concern

The COVID-19 pandemic has highlighted how biosafety must be a strategic issue for governments, given the damages and consequences involved. Just as the September 11, 2001, attack on the Twin Towers prompted governments to rethink their approach to terrorist threats, the emergence of COVID-19 should motivate governments to reevaluate their strategies and structures for dealing with intentional or unintentional biological threats²⁸.

In the US, for example, in addition to the FSAP, there are legal instruments to supervise DURC, according to recommendations from an advisory body called the *National Science Advisory Board for Biosecurity* (NSABB). HHS established NSABB

after the 2001 anthrax attacks in the US to consider so-called dual-use research that could be used for both good and ill²¹³.

Research may be suspended, scope reduced, or financial support canceled when non-compliance is detected. However, this regulation does not affect studies carried out with private resources²¹⁴⁻²¹⁵.

As new pandemics are likely to emerge more frequently, the national structures of HCBLs and their regulatory frameworks should be part of governments' strategic planning for future pandemics²⁸.

This issue requires a cohesive perspective on biosafety in industry and research organizations, with a regulatory framework that focuses on minimizing physical risks associated with biological containment limitations and governance of research conducted. The measures taken should not impede research and technological innovation, particularly a country's ability to respond to new biological threats⁵⁴.

In general, risks will always be associated with under and over-regulation, making it difficult to balance. Advocates for greater regulation argue that priority should be given to methods that use attenuated strains, computational models, or 'pseudo-viruses'. This debate culminated in the publication of the American policy on the subject, '*Potential Pandemic Pathogens Care and Oversight (P3CO)*', in 2017. This policy requires a panel designated by the HHS to review any NIH-funded study that could generate a gain-of-function²¹⁶. This policy is more flexible than the one related to DURC, as it has a broader scope and is not limited to a specific list of pathogens. However, this greater flexibility brings uncertainty and subjectivity regarding which research should be included in the monitoring process²¹⁷. Both policies are under review by the NSABB, which published numerous recommendations in January 2023, like:

- a. Enhance the definition of 'potential pandemic pathogens';
- b. Remove current blanket exclusions for research activities associated with surveillance and vaccine development or production;
- c. Include and articulate specific roles, responsibilities, and expectations for investigators and institutions;
- d. Develop principles and guidelines that can be applied and implemented to ensure there are no feasible alternative methods of obtaining the relevant benefits from proposed research that pose less risk, unnecessary risks have been eliminated, and the potential benefits justify the remaining risks;
- e. Increase transparency in the review process;
- f. Develop analogous policies and processes for identification, review, evaluation, and ongoing oversight of relevant research involving enhanced pathogens likely to pose severe threats to human health, food security, economic security, or national security by its impacts on animals or plants or to animal or plant products;
- g. Include international institutions receiving USG support for life sciences research;
- h. Continue to facilitate sharing experiences and best practices regarding DURC policy implementation;
- i. Engage relevant stakeholder and publishing groups to encourage the development and adoption of more uniform editorial policies, review processes, and best practices for identifying material that may raise significant biosecurity and biosafety concerns;
- j. Promote and ensure that all research meeting the scope of the policy frameworks conducted within the US or supported by the USG be subject to equivalent oversight regardless of funding source;

k. Minimize the potential for duplicative or parallel institutional or federal review processes²¹⁴.

Similarly, the US Congress has identified some critical gaps when reviewing policy regarding gain-of-function studies, such as:

- a. There is a lack of transparency in determining which types of research should be reviewed by the panel designated by the HHS, which could result in specific research submissions being omitted;
- b. Criteria are absent, and therefore, there is a lack of transparency in determining the composition of the panel of experts;
- c. The panel of experts in research processes developed by the agency may lack independence, as NIH specialists themselves could be part of this panel;
- d. The conducting agency needs more authority, as the agency that funds the project rather than the government authority makes the final decision on measures to be adopted;
- e. It is difficult to include new pathogens on the list of select agents, as this could require compliance with regulatory requirements of the FSAP, causing delays in the execution of fundamental research to address a public health emergency. This dilemma was observed during the COVID-19 pandemic, balancing the need for quick solutions to an emergency with laboratory biosafety and biosecurity precautions;
- f. There is limited capacity to regulate research conducted with private resources, as only research involving select agents undergoes some form of government supervision. The volume of activities related to DURC that is not subject to any regulation in the US is unknown²¹⁶.

It is worth mentioning a statement from the US 'Committee on laboratory security and personnel reliability assurance systems for laboratories researching biological select agents and toxins' which addresses the potential conflict between biosafety and biosecurity regulations and research:

*'It is common to talk about the need for a 'balance' between science and security. However, this committee rejects the notion that science and security are inversely related and that there is an inherent tension between these two elements. While there may be specific circumstances when a particular action conducted to enhance security may be seen at odds with the conduct of scientific research, the two aims are not fundamentally opposed. Rather, the policies that support science and those that promote security operate in different but overlapping spheres'*¹⁹².

Many people advocate for a broad global discussion on supervising DURC without impeding technical and scientific development, such as the one initiated by a task force led by the *Bulletin of the Atomic Scientists*²¹³.

Like in most countries, Brazilian legislation must address this issue extensively. However, the BFG has made significant progress in recent years concerning other laboratory biosafety and biosecurity issues, as discussed below.

2.10 Laboratory biosafety and biosecurity in Brazil: regulatory aspects

Law n. 11,105/2005²¹⁸ established the National Biosafety Policy (PNB). It was regulated by Decree n. 5,591/2005²¹⁹, which sets safety standards and monitoring mechanisms for activities involving GMOs and their derivatives. This legislation addresses safety standards and monitoring mechanisms related to the construction, cultivation, production, manipulation, transport, transfer, import, export,

storage, research, commercialization, consumption, environmental release, and disposal of GMOs and their derivatives. The guidelines of this law aim to promote scientific advancement in biosafety and biotechnology, safeguard human, animal, and plant life and health, and adhere to the precautionary principle for environmental protection. The law establishes safety standards and monitoring mechanisms through the National Biosafety Technical Commission (CTNBio), a multidisciplinary collegiate body affiliated with the MCTI. CTNBio is entrusted with the analysis and decision-making on issues involving GMOs in Brazil²¹⁸.

In practice, any laboratory activity involving GMOs and their derivatives requires authorization from CTNBio, which is granted by issuing the Certificate of Quality in Biosafety (CQB). Installing an Internal Biosafety Committee (CIBio) is mandatory, and an on-site inspection by CTNBio may be necessary²¹⁸.

Despite being titled the 'Biosafety Law,' the Law n. 11,105/2005 focuses explicitly on GMOs-related aspects. This law was established to implement an international policy through the Cartagena Protocol on Biosafety to the UN Convention on Biological Diversity. To guide its actions, CTNBio has defined four BSLs for work involving GMOs and has described the requirements for them²²⁰.

Although the current legislation satisfactorily addresses aspects related to the regulation of GMOs and the use of human embryonic stem cells for research and therapy purposes, it does not sufficiently cover other aspects related to laboratory biosafety and biosecurity^{17, 18, 20, 64, 221-222}.

To address this gap, the MS has issued more specific regulations, such as the Technical Biosafety Standard for Public Health Laboratories, published in 2010. This standard stipulates that those IBCs and laboratories linked to the National System of Public Health Laboratories (SISLAB) must comply with several obligations, like:

- a. Develop a biosafety management system that includes a Biosafety Manual and a set of associated documents;
- b. Ensure the implementation and monitoring of institutional biosafety policies through financial, human, and technological resources;
- c. Establish an IBC linked to the highest managerial level;
- d. Monitor and evaluate the effectiveness of the implementation of institutional biosafety policies through performance indicators;
- e. Establish containment levels for activities based on risk assessments and provide suitable facilities in compliance with the 'General Directives for the Containment Work with Biological Agents'²²³;
- f. Conduct periodic (preferably annually) internal biosafety audits;
- g. Implement a program and procedures for occupational health surveillance, including reporting accidents and incidents;
- h. Implement a continuous education program on biosafety¹⁰.

The 'General Directives for the Containment Work with Biological Agents', was prepared by the MS Health Biosafety Commission (CBS), to guide the physical structure, human, and material resources necessary to safely handle biological agents in laboratories and health units. It also describes the general characteristics of the different containment levels (BSL-1 to BSL-4) and provides recommendations for the national and international transport of biological assets²²³. However, this regulation does not include monitoring or overseeing pathway⁶⁴.

The MS also published a Portuguese translation of the fourth edition of the BMBL²²⁴ but did not publish this manual's fifth (2009) or sixth (2020) editions.

Another essential publication of the MS refers to the classification of pathogen RGs^k based on criteria described in Chart 02. The list of pathogens, categorized according to their RGs, is published through an Ordinance⁹¹ that undergoes periodic review.

Chart 02 – Overview of the criteria for each pathogen Risk Group, according to the Brazilian Ministry of Health.

RG	Key criteria	Examples
1	Low individual and community risk. It includes biological agents known not to cause disease in healthy humans or adult animals.	<i>Lactobacillus</i> spp. <i>Bacillus subtilis</i>
2	Moderate individual risk and limited risk to the community. It includes biological agents that cause infections in humans or animals, whose potential for community spread and environmental dissemination is limited, and for which there are known effective prophylactic and therapeutic measures.	<i>Schistosoma mansoni</i> Rubella virus
3	High individual risk and a moderate risk to the community. This category includes biological agents that can spread, mainly through respiratory transmission, and cause potentially lethal diseases in humans or animals. There are usually prophylactic and therapeutic measures available for these agents. They pose a risk if disseminated in the community and environment and can spread from person to person.	<i>Bacillus anthracis</i> AIDS virus
4	High individual and community risk. It includes biological agents with high transmissibility, primarily through the respiratory route. No effective prophylactic or therapeutic measures are available for infections caused by these agents. They cause severe human and animal diseases, with a high capacity for dissemination in the community and the environment. This category primarily comprises viruses.	Ebola virus Smallpox virus

Source: MS⁹¹

It is important to note that the direct correlation between risk classification and BSL is not mandatory, as specific procedures may require a higher or lower level of containment than the pathogen's RG. For example, even though SARS-CoV-2 is an RG 3 pathogen, non-propagative laboratory activities can be performed in BSL-2 laboratories^{91, 225-226}. In contrast, methods that involve handling large volumes or the inoculation of *Trypanosoma cruzi*, an RG 2 pathogen, require BSL-3 facilities⁹¹.

The MAPA has defined a list of mandatory notifiable diseases²³⁰ for animal health pathogens but still needs to establish pathogen risk classifications. Laboratory biosafety requirements for these diseases can be found in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*⁹.

Still, in the context of MAPA, the technical biosafety regulation for handling the FMD virus details the construction, operational, and procedural requirements for HCBLs, mainly serving as a basis for inspections in FMD vaccine factories²³¹.

Regarding biodefense aspects, the BFG has internalized the BWC²³² and the UN Resolution n. 15.40¹⁵⁸, demonstrating its commitment to combating the production and storage of chemical and biological weapons. In addition, as mentioned in the session on biological weapons and bioterrorism, regulations have been established on the monitoring of the export of biological, chemical, and

^k Specific biosafety requirements for each pathogen can be obtained from different sources, like the Public Health Agency of Canada²²⁷⁻²²⁸ and ABSA International²²⁹.

radiological materials that may be used as raw materials to produce weapons of mass destruction²³³⁻²³⁴.

Other relevant national legislation related directly or indirectly to laboratory biosafety includes:

- a. Transport of biological assets²³⁵⁻²³⁶;
- b. Waste management²³⁷⁻²³⁹;
- c. Occupational health²⁴⁰;
- d. Ethics in the use of experimental animals²⁴¹⁻²⁴².

The national legislation provides little support for inspecting and monitoring activities in HCBLs. ANVISA (the National Health Surveillance Agency) is responsible for inspecting clinical laboratories, both public and private, as they offer health services to the population, as well as public and private laboratories that produce medicines or other biological materials. Therefore, inspections are limited to these services, even if the laboratory performs different activities, such as research. For example, laboratories located in universities are not subject to regulation, nor are public laboratories that do not produce medicines or carry out clinical exams. The *Technical Regulation for the Operation of Clinical Laboratories* primarily emphasizes implementing biosafety and quality control measures. However, it does not mandate incorporating biosecurity measures in laboratories or emphasize conducting inspections focused on biosafety and biosecurity¹⁸.

The lack of a monitoring system for HCBLs in Brazil may impact its credibility with international organizations and countries with which Brazil maintains important commercial relations, given the absence of information on the laboratories and reports of incidents and accidents, as well as the lack of assurance that research in Brazil with high-risk pathogens is being carried out within a safe manner¹⁶⁰.

Despite the gaps and limitations mentioned, the theme of biosafety and biosecurity has been increasingly important in the context of BFG's strategic decisions. Since 2018, it has been considered, along with Energy, Transportation, Water, Communications, Finance, and Defense, as one of the priority areas of Critical Infrastructure within CREDEN²⁴³.

It is important to note that the BFG defines such facilities, services, and assets as those that, if interrupted or destroyed, would cause serious social, economic, political, international, or national security impacts²⁴⁴.

With the inclusion of biosafety and biosecurity as a priority area and seeking to standardize this area, CREDEN created Technical Groups (TGs) to:

- a. Address the security issues related to critical infrastructures, including the research, identification, survey, and assessment of threats and vulnerabilities in the priority area of biosafety and biosecurity;
- b. Define the National Biosafety and Biosecurity Policy (PNBB);
- c. Develop a proposal for the construction of a National BSL-4 Laboratory²⁴⁵.

The TG's activities should adhere to the guidelines outlined in the National Strategy for Critical Infrastructure Security (Ensic) established in 2020. This document identifies the main actions required to ensure the integrity of services essential to Brazilian society and sets principles for critical infrastructure security activities. Additionally, it outlines the challenges, structural axes for effective implementation, strategic objectives, and initiatives that guided the Plansic development. This Plan, approved in September 2022, designated the MS as responsible for developing a sectoral plan for biosafety and biosecurity¹⁶.

These sectoral plans will complement Plansic and address critical infrastructure security measures related to each sector. It should, at a minimum:

- a. Establish objectives and goals to ensure adequate levels of protection for the sector;
- b. Define sector partners, relevant authorities, legislation that supports the sectoral plan, and the specific roles and responsibilities of all actors involved;
- c. Establish or list procedures for sectoral interaction, information sharing, coordination of efforts, and partnerships;
- d. Identify the specific sectoral approach or methodology to be used;
- e. Establish the creation of specific plans for coordination and cooperation, including incident response;
- f. Provide training for employees in areas such as risk management, crisis management, business continuity management, and service management;
- g. Ensure alignment with the National Cybersecurity Strategy¹⁶.

The actors involved in the implementation of the sectoral plan should:

- a. Continuously monitor and assess the status of critical infrastructure;
- b. Implement protection measures when necessary;
- c. Exchange information to aid in crisis management;
- d. Maintain constant communication channels with partners;
- e. Issue timely alerts¹⁶.

The continued implementation of the risk management structure, partnerships, and information-sharing networks of Plansic offers mechanisms for a rapid assessment of the impact of incidents on critical infrastructure, assistance in establishing priorities for restoring their services, and sharing information about incidents¹⁶.

It is essential to highlight the significant contributions made by the Brazilian Association of Technical Standards – ABNT – regarding regulating biosafety and biosecurity. Since 2013, the Biological Containment Study Commission (CE 046:000.02) has been actively involved in developing standards for construction requirements as part of the CB-46 Committee – Clean and Controlled Areas. Furthermore, the Special Study Commission on Biosafety and Biosecurity (CEE-244) was responsible for the release of the ABNT NBR 17069-1 – Biosafety and Biosecurity – Laboratory Infrastructure – Part 1: Specific Requirements for Biosafety Level 1 (BSL-1)²⁴⁶. Currently, the same Commission is engaged in two additional projects:

- a. The biorisk management system for laboratories and other related organizations;
- b. Biosafety and biosecurity – Intra-institutional transport of biological material – General requirements²⁴⁷.

The advancement of regulatory mechanisms must be complemented by efforts to strengthen the culture of biosafety and biosecurity in the country, as there is a risk of having well-crafted legislation that fails in practice. In this regard, educational institutions, national biosafety commissions, and laboratory networks play a fundamental role²⁴⁸⁻²⁴⁹.

2.11 National Biosafety Commissions

In addition to CTNBio, which is linked to MCTI, other ministries have biosafety commissions. Unlike CTNBio, these commissions go beyond issues related to GMOs. The following bodies have such commissions: MS, MAPA, and MD.

The MS created the CBS in 2002²⁵⁰, and its internal regulations were defined in 2005²⁵¹. It comprises representatives from various organizations, including the Secretariat of Science, Technology, Innovation, and Health Complex, the Secretariat of Specialized Health Care, the Secretariat of Health and Environment Surveillance,

the Secretariat of Work and Health Education Management, the International Health Affairs Special Advisory Office, FIOCRUZ, and ANVISA²⁵².

The CBS has several responsibilities, including:

- a. Participating in the development and reformulation of biosafety and biosecurity standards at the national and international levels and submitting proposals to the authorities responsible for issuing them;
- b. Providing advice on activities related to the formulation, updating, and implementation of the PNB and other policies regarding biosafety and biosecurity;
- c. Organizing public debates on biosafety and biosecurity;
- d. Publishing the classification of RGs of pathogens of interest to public health²⁵².

At MAPA, the COMBIOLAB (Permanent Commission for the Management of Biological Risks and Biosafety in Laboratories of the National Veterinary Laboratories that manipulate biological agents and their parts, viruses and their parts, and prions of interest in animal health) was created in 2017 with the following responsibilities:

- a. Analyzing and monitoring projects for the construction, renovation, and adaptation of physical structures in laboratories that perform animal diagnosis in the National Veterinary Laboratories;
- b. Classifying the levels of biosafety and biosecurity required for manipulating biological agents and their parts, viruses and their parts, and prions that are considered a risk to animal health²⁵³.

The General Coordination of Veterinary Laboratories oversees the Commission. In addition to the executive committee, which is comprised of MAPA's employees, there is an ad hoc group that includes representatives from various organizations, such as the Institutional Security Office of the Presidency of the Republic (GSI/PR), the Brazilian Agency of Intelligence (ABIN), the Pan American FMD Disease Center (PANAFTOSA), the Food and Agriculture Organization of the UN (FAO), the Federal Police (PF), MS, and MD²⁵³.

It is worth noting that within the scope of MAPA, the standardized organizational structure for all LFDAs defines the existence of a Biological Risk and Chemical Risk Management Nucleus. This Nucleus is responsible for promoting, executing, and monitoring biosafety activities. The Biological Risk and Chemical Risk Management Sector was created at the central level to coordinate biosafety activities in MAPA's laboratory network²⁵⁴.

The Biosafety Commission of the MD (CBio-MD) was created in 2012 and has several responsibilities, including:

- a. Conducting a survey and analysis of technical issues related to biosafety to identify their impacts and correlations with biological defense and national security;
- b. Identifying biosafety topics whose developments may require MD's preventive action²⁵⁵.

This Commission is coordinated by the Biological Security Division of the Department of Health and Social Assistance of the Personnel, Education, Health, and Sport Secretariat of the MD²⁵⁵.

2.12 Laboratory networks and their role in the context of laboratory biosafety and biosecurity

Delivering diagnostic services within integrated and functional national laboratory networks is a recommended strategy to meet demands more effectively and efficiently, especially during a shortage of financial resources²¹⁻²³. This need becomes even more critical for HCBLs, where a centralized management system,

preferably under a single agency, is desirable to register and monitor ongoing research, among other essential information^{143, 256}.

Some of the benefits of working in a network include:

- a. Encouraging complementarity and reducing duplication of efforts by adapting the operational capacity of each laboratory to meet national demands for the diagnosis of infectious diseases, thereby optimizing human and financial resources;
- b. Providing quick and effective responses to health threats;
- c. Facilitating international cooperation;
- d. Sharing experiences, best practices, protocols, and research results;
- e. Exchanging samples, reference controls, and other materials;
- f. Supporting less prepared laboratories by providing training;
- g. Transferring methods;
- h. Sharing equipment^{26, 110, 257}.

When a government agency coordinates such a network with authority and competence for decision-making in public health, additional benefits can be achieved, such as:

- a. Determining the ideal number of laboratories in the country, considering the risks and benefits of new constructions;
- b. Controlling and monitoring these structures;
- c. Evaluating how laboratories can collaborate in an integrated way in addressing biological threats present in the country;
- d. Knowing the installed capacity in the country for research and diagnosis;
- e. Better geographical distribution of laboratories in the national territory and better coordination of their activities according to the demands present in each region;
- f. Developing technical and constructive standards and guides;
- g. Standardizing training and defining concepts and terminologies;
- h. Defining inventory control mechanisms;
- i. Defining and standardizing mechanisms for addressing the reliability of professionals⁴³.

Demonstrating the existence of laboratory networks tends to strengthen trust relationships in international relations, particularly in a world where biotechnology is increasingly advanced and biological threats are persistent and global, demanding multilateral and multidisciplinary cooperation. In this sense, information produced by laboratory networks is more reliable than that generated by laboratories working independently, given that working in a network presupposes the adoption of control and supervision measures²¹⁻²².

The organization of a network presupposes the existence of reference laboratories, which play an essential role in harmonizing methods and procedures crucial in combating emerging and re-emerging diseases. Reference laboratories should implement appropriate quality, biosafety, and biosecurity protocols (including constructive requirements), serving as a model for other laboratories in the network²⁵⁸.

2.12.1 The Brazilian scenario

It should be noted that Brazil currently needs an interinstitutional network that brings together HCBLs. However, there are some initiatives for networking within different ministries, like the MS SISLAB and MAPA's LFDA networks.

The SISLAB comprises four national laboratory networks focusing on Epidemiological Surveillance, Environmental Health Surveillance, Sanitary Surveillance, and High Complexity Medical Care to support the MS initiatives. This

system is organized hierarchically, with actions executed at the federal, state, and municipal levels in the principles of the Unified Health System. The sub-networks are structured by disease or program, identifying the reference laboratory, geographic area of coverage, and definition of competencies. The reference laboratories can be classified at the National, Regional, State (LACENs), Municipal, Local, or Border levels²⁵⁹.

The MS, through the General Coordination of Public Health Laboratories (CGLAB), is responsible for providing technical advice and cooperation to the states and the Federal District in implementing the National Laboratory Network. It coordinates with other competent units and helps promote permanent education and training of health surveillance professionals²⁶⁰.

Supporting MAPA's actions, the LFDA Network is coordinated by the General Coordination of Veterinary Laboratories (CGAL), which is linked to the Technical Services Department of the Secretariat of Veterinary Defense. This network is complemented by recognized laboratories, which can be public, private, or university ones²⁶¹. There are six LFDAs, all with national coverage, governed by the same organizational chart and located in Belém, Pará State (LFDA-PA), Recife, Pernambuco State (LFDA-PE), Goiânia, Goiás State (LFDA-GO), Pedro Leopoldo, Minas Gerais State (LFDA-MG), Campinas, São Paulo State (LFDA-SP), and Porto Alegre, Rio Grande do Sul State (LFDA-RS). The recognized laboratories are authorized to perform official assays provided they meet the requirements established by MAPA²⁶². There are approximately 400 accredited laboratories, with around 150 focused on diagnosing animal diseases. MAPA also has the laboratory structure of EMBRAPA, mainly for research and innovation activities, with a network consisting of 43 units²⁶³.

The recent creation by MCTI of the National System of Research Infrastructures with Biosafety (SISNIPE-BIO), which is still in the implementation phase, is also worth mentioning. Its objectives include:

- a. Foster scientific and technological progress and innovation within biosafety research infrastructures;
- b. Promote coordination and integration of scientific research and technological development efforts across biosafety research infrastructures;
- c. Maximize the efficiency of infrastructures, basic and applied research development, and high-level biosafety activities;
- d. Ensure universal access of the country's scientific, technological, and innovation community to research infrastructures with biosafety measures in place;
- e. Encourage the training and qualification of human resources to work in biosafety environments;
- f. Support the regulation of biosafety practices in the country;
- g. Stimulate the implementation of laboratory quality systems for biosafety research infrastructures;
- h. Disseminate knowledge on biosafety environments²⁶⁴.

2.12.2 The worldwide scenario

The IEGBBR comprises biosafety and biosecurity regulatory authorities from eleven member countries with well-established biosafety and biosecurity regulatory oversight systems. The WHO and WOAHA are non-member observers. Since 2007, the group has been meeting every 18-24 months to facilitate the exchange of knowledge and experience regarding biosafety and biosecurity oversight issues related to human and zoonotic animal pathogens. This forum also aims to strengthen

international biosafety and biosecurity by sharing expertise and lessons from established regulatory systems with the global community²⁶⁵.

Additionally, the IEGBBR develops reference tools and materials, including a training model of Standardized Regulatory Practices for Biosafety and Biosecurity Incidents, which was launched in September of 2023 and is hosted on the training portal of the Public Health Agency of Canada (PHAC)²⁶⁵⁻²⁶⁶.

There are initiatives aimed explicitly at high-biocontainment veterinary laboratories, such as the International Veterinary Biosafety Workgroup (IVBW), which brings together over 130 laboratories installed worldwide²⁶⁷.

Major international organizations have collaborating centers that serve as a global reference for biological risk management, as listed in Chart 03.

Chart 03 – Global reference centers for biological risk management.

International body	Reference centers	Location
FAO ⁱ	Centro de Investigación en Sanidad Animal/Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria	Valdeolmos, Spain
	Australian Centre for Disease Preparedness/Commonwealth Scientific and Industrial Research Organisation	Geelong, Australia
WHO ^m	Instituto de Diagnóstico y Referencia Epidemiológicos 'Dr. Manuel Martínez Báez'	Mexico City, Mexico
	UK Health Security Agency	Salisbury, UK
	CDC	Atlanta, US
	Public Health Agency of Canada	Ottawa, Canada
WOAH ⁿ	Sandia National Laboratories	Albuquerque, US

It is worth mentioning initiatives other countries and regions adopted to implement networks of high and maximum-biocontainment laboratories.

The US began structuring a laboratory network to respond to possible terrorist attacks in 1999 by creating the Laboratory Response Network (LRN), a joint effort of the CDC, FBI, and APHL (Association for Public Health Laboratories). The LRN aims to maintain and integrate a network of laboratories equipped to respond quickly to terrorist acts, emerging infectious disease outbreaks, and other public health threats¹¹⁴. The network comprises federal laboratories (CDC, USDA, Department of Defense, FBI, FDA – Food and Drug Administration – and other federal agencies), state, military, food analysis, environmental analysis, veterinary laboratories, and international partners²⁶⁸.

To integrate the network, the laboratories at the base of the LRN pyramid need to meet the following requirements:

- Follow the standards of the American Society for Microbiology;
- Demonstrate competence by participating in proficiency testing;
- Have class II or III BSCs duly certified;
- Comply with correct practices for the use of BSCs and other OSHA (Occupational Safety and Health Administration) regulations;

ⁱ Available at: <https://www.fao.org/animal-health/fao-reference-centres/en>. Accessed on: February 20, 2024.

^m Available at: <https://apps.who.int/whocc/List.aspx?f=dP8rigGIDPNZEZbpEfjUssyK5wgxKLLp2P6lsYnp0>. Accessed on: December 27, 2023.

ⁿ Available at: <https://www.woah.org/en/what-we-offer/expertise-network/collaborating-centres/#ui-id-3>. Accessed on: December 27, 2023.

e. Comply with FSAP regulations¹¹⁴.

The second level of the pyramid is formed by approximately 160 American reference laboratories that confirm the results obtained in the sentinel laboratories. At the top of the pyramid are national laboratories, some of which have BSL-4 facilities. National laboratories organize periodic practical training applied in LRN laboratories. About 1,000 to 1,200 laboratories participate in this exercise every two years. The exercises demonstrate that training and infrastructure are fundamental to a good result¹¹⁴. In 2005, the LRN had 120 BSL-3 laboratories. In 2011, this number rose to 150²⁶⁹.

It is worth mentioning the Research Alliance for Veterinary Science and Biodefense BSL-3 Network (RAV3N), which brings together 18 federal and academic veterinary laboratories equipped with high and/or maximum-biocontainment laboratories, including ABSL-3Ag facilities²⁷⁰.

The Chinese government planned to establish a network of high and maximum-biocontainment laboratories to face epidemics, motivated by the SARS outbreak in 2002 and 2003. In 2015 the first BSL-4 laboratory was completed in Wuhan, and its certification was finished in 2018. Simultaneously, the Chinese government promulgated a series of regulations, norms, and manuals related to biosafety laboratories to standardize the procedures related to design, construction, certification, and management. By 2018, 57 high and maximum-biocontainment laboratories were in this network^{57, 208}.

Non-governmental networks are being created in different regions of the world to facilitate the exchange of information between high and maximum-biocontainment laboratories. The European Research Infrastructure on Highly Pathogenic Agents (ERINHA) is a network that studies emerging and re-emerging pathogens with severe consequences. Created in 2017 and based in Brussels, Belgium, its primary function is to provide the capacity to conduct projects that require a broad and ambitious approach that no structure or country could provide alone, as well as to recommend the use of resources and optimize the functioning of equipment and facilities. It currently has ten members, with six BSL-3 and four BSL-4 laboratories²⁷¹.

The Emerging and Dangerous Pathogens Laboratory Network (EDPLN) is a WHO-supported network that connects 14 BSL-3 reference laboratories across Africa. Its primary objectives are to promote collaboration, facilitate the exchange of information and biological materials, conduct external quality management evaluations, and establish a regional biobank²¹¹.

The Canadian Consortium of Academics Biosafety Level 3 Laboratories brings together Canadian researchers working with RG 3 pathogens, with the main objectives of promoting good biosafety practices and providing training for its members²⁷².

In the Latin American context, it is noteworthy to mention the Ibero-American Biosafety Group (BioGib), established in 2010, which unites 19 laboratories from seven Latin American countries and Spain. Brazil is a founding member and has always had representatives on the executive board. One of the group's objectives is to discuss engineering solutions for HCBLs that are financially feasible for low and middle-income countries²⁷³.

2.13 Occupational Health and High-Containment Biological Laboratories

No specific regulations or standards address occupational health for workers in HCBLs worldwide²⁷⁴. However, prioritizing the occupational health of personnel working in this environment, including their mental health, can significantly improve productivity and compliance with procedures²⁷⁵.

The critical elements of an effective occupational health program include:

- a. Risk assessment;
- b. Medical assessment and surveillance;
- c. Access to clinical health services and management;
- d. Hazard communication²⁷⁶.

It is crucial not only for managers to understand the significance of implementing an occupational health program but also for employees themselves to recognize the importance of taking care of their health and that of their colleagues. An effective occupational health program for HCBLs should consider the specific risks of the activity, such as conducting regular medical examinations (or whenever an incident occurs) by specialist professionals that include clinical exams and laboratory tests to detect diseases and conditions directly associated with the laboratory's activities. Any specific risks to handling experimental animals should also be addressed where necessary. The program should include vaccination against diseases that may be acquired in that particular laboratory (e.g., rabies, tetanus, flu, anthrax, and hepatitis B), serological monitoring and antibody titration, as well as evaluations related to the use of PPE, especially masks and respirators, such as lung capacity testing and mask fit testing. Aspects related to mental health should also be considered, which may include periodic interviews with specialists in an environment of transparency and trust^{94, 277-279}.

Blood tests to monitor the use of illicit drugs and psychological tests to address behavioral deviations may be considered depending on the risks involved^{93, 143, 192, 257}. However, this is controversial since some costly approaches have unproven benefits²⁸⁰.

Countries such as the US, the UK, Germany, and Australia have developed personnel reliability programs as a necessary step for the clearance of professionals to work in high-biocontainment environments¹⁹². The American program involves evaluating and monitoring of the physical, mental, and emotional health and the behavior and involvement with suspicious or criminal activities of professionals working in HCBLs. There is a mechanism for reporting situations involving the employees themselves and concerning worrying attitudes and behaviors of colleagues. The program is not only about punishment but also about establishing a culture of trust. Any investigation must be conducted with respect, consistency, objectivity, and confidentiality. The occupational health team is responsible for providing toxicological tests and assessing the employee's health status, collecting information about depression, drug use, and chronic diseases. The human resources staff is also involved in the process, responsible for evaluating the employee's professional history, academic qualifications, financial status, and criminal background and registering any cases of sexual harassment. Another professional involved is an FBI representative, who presents a report on the employee's profile²⁸¹.

Once the employee is approved for the program, they begin a continuous monitoring process, which includes regular evaluations and a renewal process, which includes:

- a. Self-declaration regarding changes in initial status about health conditions, abusive use of alcohol and drugs, legal proceedings, divorce, financial problems, or any other event that may impact professional performance;
- b. Reports from supervisors and coworkers regarding changes in behavior or events that may affect reliability;
- c. Periodic medical evaluation;
- d. Random toxicological tests;
- e. Annual meeting with the program manager²⁸¹.

2.13.1 The Brazilian scenario

In Brazil, the occupational health of workers in laboratories associated with healthcare services is regulated by Regulatory Standard NR n. 32 – Safety and Health at Work in Health Establishments. Although there are no specific requirements for HCBLs, the standard establishes that the Risks Management Program (PGR) must identify biological risks, considering the following characteristics related to microorganisms and their relationship with humans:

- a. Sources of exposure and reservoirs;
- b. Routes of transmission;
- c. Transmissibility, pathogenicity, and virulence;
- d. Persistence of the biological agent in the environment;
- e. Epidemiological studies or statistical data²⁸².

The PGR must be reviewed annually or whenever a change in working conditions occurs that could alter exposure to biological agents or when the analysis of accidents and incidents determines it. NR n. 32 also mandates that the Medical Control Program for Occupational Health (PCMSO) should include:

- a. The recognition and evaluation of biological risks;
- b. Medical monitoring of workers potentially exposed to such risks;
- c. A vaccination program²⁸².

Regarding the possibility of accidental exposure to biological agents, it should be included in the PCMSO:

- a. Procedures to be adopted for the diagnosis, monitoring, and prevention of diseases;
- b. Measures for decontaminating the workplace;
- c. Emergency medical treatment for workers;
- d. Identification of those responsible for implementing relevant measures;
- e. List of healthcare establishments that can assist workers;
- f. Methods of transportation for workers to receive medical care;
- g. List of healthcare establishments that store immunoglobulins, vaccines, necessary medications, and other special materials²⁸².

The PCMSO also recommends that PPE be available in sufficient numbers at workstations, ensuring immediate supply or replacement. Employers must ensure that workers are trained by healthcare professionals familiar with the risks inherent to biological agents. This training must be adapted to the evolution of knowledge and the identification of new biological risks and should include:

- a. Available data on potential health risks;
- b. Control measures to minimize exposure to biological agents;
- c. Standards and procedures for hygiene;
- d. Use of PPE, work clothing, and collective protection equipment;
- e. Measures for preventing accidents and incidents;
- f. Actions to be taken by workers in case of incidents and accidents²⁸².

The NR n. 32 requires workers who perform cleaning services in healthcare establishments to be initially and continuously trained about biological risks. Similarly, maintenance workers must be familiar with the principles of biological risks, types of collective protection equipment, and PPE. The air conditioning systems in the units must undergo preventive and corrective maintenance procedures to preserve the integrity and efficiency of all components and provide thermal comfort²⁸².

Furthermore, NR n. 32 stipulates that any worker must immediately report accidents or incidents with possible exposure to biological agents²⁸². The mandatory notification of work accidents involving exposure to biological material is also included in the Ordinance that deals with the national list of compulsory notification of diseases, injuries, and public health events¹⁴⁶.

Institutions with well-structured occupational health programs tend to have a more effective accident and incident reporting process, including those associated with LAIs⁹⁸.

As reported below, particular attention should be given to occupational health care when the laboratory manipulates experimental animals.

2.14 Laboratory biosafety and experimental animals

Conducting experimental infections of animals in high-biocontainment environments is essential for assessing the effectiveness of drugs and vaccines and studying various aspects of viral replication, antibody development, viral excretion, transmission, and pathogenesis, among other studies²⁸³.

However, using animals in infectious agent research poses a greater risk than in vitro experiments, mainly when working with animals that cannot be confined in controlled environments, such as isolators and ventilated racks, where the room acts as the primary barrier²⁸³. In such cases, additional safety measures must be implemented, especially since the concentration of aerosol-transmitted pathogens can reach up to 10,000 colony-forming units/m³¹⁰³.

The laboratory design should consider in advance the animal species to be used, how they will be housed, and the appropriate environmental characteristics. Whenever possible, calmer and easier-to-handle animal breeds should be used. Before experiments begin, all animals should properly acclimate to the laboratory environment's characteristics, such as high airflow, temperature, lighting, and acoustics. Certain species, such as non-human primates, require specific housing environments. Bedding decontamination is a critical issue in these facilities due to the large volume of organic matter, requiring a prior definition of the decontamination process and validation. Microisolators equipped with HEPA filters are recommended for rodents' use as they are dismountable, sterilizable, and easily stored. Ventilating racks offer additional animal protection, eliminate cross-contamination, and increase caretaker safety²⁸⁴.

Risk assessment should consider the specific risks related to handling animals, depending on the species-specific characteristics. Specialized professionals, such as representatives of the IACUC, researchers, veterinarians, and animal caretakers, should contribute to this evaluation²⁷⁷. This analysis should consider potential conflicts between animal welfare concepts and biosafety and biosecurity requirements²⁸⁵.

Working with animals in HCBLs requires highly trained personnel in biosafety aspects and animal handling and welfare^{29, 285}. Few HCBLs have the infrastructure to work with insects and ticks due to additional protection barrier requirements based on the risk level of the involved pathogens²⁸⁶⁻²⁸⁷. Some diseases requiring insect testing

in high-biocontainment environments include dengue, chikungunya, Zika virus, West Nile fever, yellow fever, and malaria⁸⁷.

2.15 Commissioning and certification of High-Containment Biological Laboratories

It is expected to need clarification regarding the terms ‘commissioning’ and ‘certification’ of laboratories and the tests that should be conducted at each stage²⁸⁸. Commissioning is the process through which a new laboratory undergoes a series of tests and evaluations, from the conceptual design and, including the performance of systems and equipment, to ensure that it can operate according to the specifications and definitions provided in the design. Commissioning also applies after the renovation or expansion of HCBLs^{2, 80, 94}. The duration of commissioning can range from a few weeks to several months, depending on the size and complexity of the facility²⁸⁹. The commissioning is part of the certification process⁹⁴.

Certification involves attestation by government agencies or third parties based on a structural and documentary evaluation. The laboratory meets biosafety requirements as applied according to the standards and manuals used as a reference. While commissioning is carried out from the initial stages of the project, certification must be performed before the operation or reactivation of a laboratory and repeated annually^{2, 80}.

The commissioning process of an HCBL must ensure that structural and system components are installed, inspected, tested for performance, and verified to meet pre-established performance requirements based on reference documents. Individual components must be shown to work correctly and that different systems are functioning correctly in complex situations. Commissioning planning involves defining the conditions under which tests should be performed, specifications, mode of implementation, expected values, and required resources. The tests should include laboratory equipment (such as autoclaves, BSCs, and EDS), various piping, HVAC systems (airflow, dampers, pressure, and HEPA filters), tightness, airlocks, door operation, general supplies, drainage, emergency power supply (Uninterruptible Power Supply – UPS – and generators), fire fighting systems, alarms, operation of redundant systems, among others. Visual inspections and verification of the specifications of materials used on floors, wall painting, furniture, and other materials used to finish the work should be carried out. Tests should also be conducted on system failures, such as air supply and exhaust, and power supply, to verify atmospheric pressure behavior under these conditions. The tests should be performed under the supervision of the laboratory’s technical and maintenance team and in the presence of manufacturers if adjustments need to be made^{80, 94, 289-291}.

The commissioning process can take several weeks to months, depending on the size and complexity of the laboratory and the experience and competence of the design and construction team²⁸⁹.

Certification involves an evaluation conducted by a competent authority, which may be carried out through an audit. Certification entails evaluating the technical aspects of the construction and operation of the laboratory, as well as verifying the biosafety management system documents and the commissioning process²⁹⁰.

Commonly recommended procedures and tests include:

- a. Assessment of the design and commissioning report;
- b. Verification of the biorisk management policy and documentation;
- c. Verification of human resources capabilities and training;

- d. Verification of biosecurity issues, including access controls;
- e. Verification of the PPE;
- f. Visual inspection to identify damages to floors and walls;
- g. Verification of pressure differentials and calibration of equipment used for monitoring;
- h. Verification of airflow, including the number of air changes per hour and the functioning of exhaust motors;
- i. Verification of HEPA filter integrity;
- j. Verification of damper functioning;
- k. Verification of environmental parameters (noise level, temperature, humidity, and lighting);
- l. Certification of autoclaves and other pressure vessels as EDS;
- m. Testing of electrical systems, including UPS and generators;
- n. Verification of door interlocking system functioning;
- o. Structural and centrifuge seal tightness tests;
- p. Verification of automation system and its alarms;
- q. Verification of communication systems;
- r. Validation of decontamination systems for rooms and airlocks;
- s. Verification of the functioning of emergency lighting and fire-fighting systems;
- t. Verification of the emergency plan;
- u. Certification of BSCs and other primary containment equipment, such as ventilated cages, racks, and isolators;
- v. Confirmation of emergency equipment functioning, such as showers and eyewash stations, where applicable;
- w. Confirmation of anti-reflux system functioning^{55, 80, 94, 289, 292-293}.

After issuing the certification report, the laboratory should document the corrective actions required to address the identified issues^{55, 292-293}. An independent entity not involved in the laboratory's design or construction must conduct the certification process. National certifiers are available in some countries, such as Canada and Singapore, providing a more cost-effective alternative to international certification entities¹⁷.

Many laboratories have an annual shutdown to facilitate the certification testing, including decontaminating the laboratory (a procedure known as 'shutdown'). This procedure is typical in research laboratories that can plan their activities to allow for a shutdown. The process may sometimes be impractical in diagnostic laboratories, and certification testing is performed while the laboratory is in operation, bringing additional risks to maintenance technicians⁷⁶.

Only laboratories that have been certified should house dangerous pathogens, and each state should have a single government agency responsible for monitoring and certifying all their HCBLs, including private laboratories. It could be done through a mandatory certification and inspection process, where the government has access to the relevant information on the type of research being conducted. However, this could pose issues for companies working on proprietary research. Laboratories should be checked frequently for equipment failures and be required to recertify periodically³.

Nevertheless, national legislation often needs more requirements and parameters for commissioning and certification tests. Thus, it is necessary to consult international references such as the NSF/ANSI (National Sanitation Foundation/American National Standards Institute) standard n. 49, used for BSCs

certification, and manuals from the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) for HVAC system testing^{66, 294-295}.

2.16 Assessment tools for evaluating the conformity of laboratories to international biosafety and biosecurity standards

There is currently no international organization specifically responsible for addressing the aspects of laboratory biosafety and biosecurity, as there is, for example, the International Atomic Energy Agency for the nuclear energy field¹⁶⁹. As a result, there needs to be a mechanism to manage the disparate efforts currently underway to address these risks in a comprehensive, sustained, and impactful manner. While creating a single, powerful entity with the authority and capability to strengthen biorisk management globally is intellectually appealing, it is a long-term project requiring a great deal of political capital with an uncertain outcome⁵¹. Kazakhstan proposed the creation of an 'International Agency for Biological Safety' during the UN General Assembly in 2020; however, negotiating the realization of this type of proposal is quite complex¹⁷⁴.

The presence of such an organization could not only enable the registration of HCBLs but also foster the enhancement of global governance, ensuring that research involving high-risk pathogens, including potential pandemic pathogens, is conducted safely, securely, and responsibly manner⁵¹. The COVID-19 pandemic is a prime example of this. Due to a lack of transparency and trust among nations, the world may never know the truth about the origin of SARS-CoV-2. This governance implies that the work developed in high and maximum-biocontainment laboratories should be made public, as transparency nurtures and sustains trust. Unfortunately, international organizations do not have the authority to perform this oversight or to require governments to do so, so the decision to monitor falls to each country²².

Regardless of whether this new agency will be created or not, it is essential to strengthen the UN and its agencies (such as FAO, WHO, and WOA) to deal with the challenge of new epidemics and pandemics, which requires cooperation and integrated international actions, such as joint research with high-risk pathogens and political actions³⁰. In this sense, in 2022, WHO created a Technical Advisory Group on Biosafety to propose strategic actions. The group is expected to meet at least once a year⁹.

International organizations have tools for a voluntary assessment of countries' capacities to deal with challenges to public and animal health, such as the Joint External Evaluation (JEE), the Laboratory Assessment Tool (LAT), the Performance of Veterinary Services (PVS), and the Global Health Security Index (GHS).

The JEE is a voluntary and comprehensive process of collaboratively evaluating a country's public health capacity across 19 technical areas between the country's experts and the external evaluation team. The JEE creates a baseline assessment, enabling governments to better understand their gaps and weaknesses in health security and to prioritize their efforts to close those gaps. Although JEE has limitations in accuracy and consistency across the process, it provides an informative and practical assessment of IHR (International Health Regulations) obligations²⁹⁶⁻²⁹⁷.

The WHO developed the LAT to aid in assessing national laboratory systems and individual laboratories. It is founded on internationally recognized standards and

⁹ Available at: <https://www.who.int/news-room/articles-detail/technical-advisory-group---biosafety>. Accessed on: December 27, 2023.

best practices governing laboratory services. The assessment consists of two phases: the first phase evaluates the structure, organization, and regulations, while the second phase assesses the facilities. Both phases include a section dedicated to biorisk management⁹⁵.

The WOA's PVS pathway empowers national Veterinary Services by comprehensively understanding their strengths and weaknesses using a globally consistent methodology based on international standards. It is a valuable external perspective that can reveal gaps, inefficiencies, and opportunities for innovation through a voluntary, broad-based, long-term, and collaborative process. Based on the results of the PVS Evaluation, the WOA has identified priority gaps at a global level. To address these gaps, the organization has developed a suite of targeted support tools as further options for PVS Pathway engagement by its Member Countries, including PVS Sustainable Laboratory Support. The PVS Sustainable Laboratories Mission was developed to provide an in-depth analysis of the pertinence, efficiency, and sustainability of the national laboratory network, including an appropriate balance of personnel, infrastructure, equipment, and operational resources, based on national diagnostic needs^{37, 298}.

The GHS Index was developed by the Nuclear Threat Initiative (NTI) in partnership with the Johns Hopkins Center for Health Security. It was launched in 2019 and is considered by the WHO to be a valuable tool for decision-makers in guiding investment prioritization in biosafety. It conducts a comparative assessment of the ability of 195 countries to prevent and respond to biological threats. The second edition of the survey was published in 2021, and the third is expected to be published in 2024. The latest assessment concluded that although many countries rapidly developed capabilities to deal with the COVID-19 pandemic, all countries remain unprepared for future epidemics or pandemics^{40, 299}.

While these tools are comprehensive, they only cover certain aspects related to biosafety and biosecurity. Thus, countries that desire a more detailed assessment of these areas should establish specific mechanisms. For instance, biosafety and biosecurity are merely one of the 19 categories evaluated by the JEE. Furthermore, the JEE concentrates on the national level rather than individual laboratory practices.

Nonetheless, JEE assessments have demonstrated a correlation between a country's economic development level and laboratory biosafety enhancement. The evaluations have pinpointed several primary gaps, which include:

- a. Absence of a legal framework for biosafety and biosecurity, as well as manuals addressing the handling and containment of pathogens;
- b. Lack of mechanisms for coordinating and supervising laboratories at the federal level;
- c. Problems with the transport, handling, management, and disposal of biological assets and toxins;
- d. Absence of mapping of capacities for handling high-risk pathogens;
- e. Insufficient resources for training;
- f. Lack of clarity in defining the roles and responsibilities of the different stakeholders involved;
- g. Lack of modern and effective methods for diagnosing diseases that do not require the isolation of agents;
- h. Lack of mechanisms for interaction between public health, animal health, and defense to investigate biological attacks³⁰⁰.

FAO has developed a Laboratory Mapping Tool for assessing veterinary laboratories with a specific biosafety and biosecurity requirements module. The tool

was initially tested in 34 laboratories from 19 countries belonging to two distinct geopolitical regions from 2015 to 2017. The results indicated that the laboratories generally only minimally met the requirements advocated by internationally recognized biosafety manuals. In one region, the compliance percentage was 41.3%, and in the other, it was 28.1%. The worst results in both areas were related to engineering and administrative controls, while the best were related to using PPE. National reference laboratories presented superior results in both regions¹⁸⁵.

A group of CDC researchers developed another tool for assessing the management of biological risks in laboratories. This comprehensive questionnaire has 646 questions, 108 specifics to BSL-3 laboratories. The questions are divided into ten modules, covering a range of themes, including premises and workflow, people management and training, good laboratory practices, cleaning, disinfection, sterilization and waste management, emergencies, risk management, documentation and regulations, biosecurity, and other risks. The leading international manuals were used as a reference for developing the questionnaire, available online in several languages^P. The questionnaire was applied in laboratories from eight countries, and Kazakhstan used it as a national checklist³⁰¹.

The WHO regional office for Africa developed a laboratory assessment tool for BSL-3 laboratories. This tool was tested on a series of laboratory visits in Ghana, Uganda, and Kenya, and the final version was released in 2016²⁰⁴.

2.16.1 Results of assessing laboratory services in Brazil

Regarding the evaluation results of the tools mentioned above, the following results are highlighted for the Brazilian scenario:

- a. An evaluation based on the IHR conducted in 2005 revealed that only a minority of laboratories meet all the necessary biosafety procedures: 40.3% in state institutions, 49% in municipal institutions, and 50% in federal institutions³⁰²;
- b. A PVS evaluation conducted in 2007 highlighted the opening of the BSL-3 'enhanced' laboratory at LFDA-MG the previous year, although it was not yet fully operational. The report also pointed out the need to establish a broader biosafety structure in the country to address threats related to emerging diseases³⁰³. The PVS follow-up mission carried out in 2014 stated that the Brazilian Veterinary Services have a strong and competent laboratory network throughout the country and that all national laboratories are accredited under ISO 17025. This report did not add information on laboratory biosafety³⁰⁴. Brazil never received a PVS Sustainable Laboratory Support Mission;
- c. Results from the GHS index for 2021 showed that Brazil had an overall compliance rate of 51.2%, ranking 43rd out of 195 countries evaluated. Within the 'Prevention' evaluation item, there is a biosafety indicator that covers the existence of biosafety laws and regulations, the presence of an agency to enforce these regulations, and a standardized approach to biosafety training. Brazil scored a compliance rate of 25% for this indicator, ranking 62nd worldwide. The only requirement met in this item was the existence of regulation (probably related to the legal framework for GMOs). For the biosecurity indicator, Brazil scored a compliance rate of 48% (ranking 18th). For the indicator related to DURC, Brazil met 33% of the requirements (ranking 7th). Once again, the positive response was likely associated with GMO control. In the 'Detection' category, there is a laboratory systems indicator

^PAvailable at: https://www.iqls.net/docs/IQLS_Biosafety_LAT_v9_Oct2019_EN_FR_RU.zip. Accessed on: December 27, 2023.

for which Brazil had a compliance rate of 75% (ranking 38th). The indicator considers the laboratory capacity installed in the country to detect priority diseases and aspects related to laboratory quality management systems³⁰⁵;

d. In 2023, the Global BioLabs initiative published National Biorisk Management Scorecards, which assigned Brazil 18 points out of a maximum of 20 for biosafety, placing it behind only six countries (Australia, Canada, France, Germany, Japan, and the US). For biosecurity and DURC governance, Brazil received scores of 9 (out of 18) and 1 (out of 10), respectively. Brazil's overall ranking in the biorisk management score was 16th out of the 27 countries assessed. As for the GHS Index, the results probably considered the legal framework for GMOs⁵¹.

2.17 The importance of training and generating scientific knowledge in the management of laboratory biosafety and biosecurity

The construction of high and maximum-biocontainment laboratories must be accompanied by high-level training for all personnel involved, including managers, maintenance personnel, and laboratory users, to enable safe operation^{29, 57, 211}. For example, China trained approximately 100,000 people in biosafety over 15 years, but this number still appeared insufficient⁴⁷.

The CDC and APHL published a document outlining the minimum competencies for professionals working in high-biocontainment environments³⁰⁶. However, the need for standardized training from regulations and manuals posted by the USG is a commonly reported problem by laboratories in different countries^{171, 176, 307}.

Scientific civil associations often play a vital role in promoting biosafety and biosecurity training, such as ABSA (established in 1984), IVBW (established in 1991), EBSA (European BioSafety Association, established in 1996), IFBA (International Federation of Biosafety Associations, established in 2001), A-PBA (Asian-Pacific Biosafety Association, established in 2005), AMexBio (Mexican Association of Biosafety, established in 2009), and AEBioS (Spanish Association of Biosafety, established in 2011)²⁸.

In Brazil, the National Biosafety Association (ANBio), established in 1999, played a crucial role. In the first six years of its existence, the association organized three international congresses and three Latin American symposiums. It trained over three thousand professionals in biosafety procedures, focusing on GMOs. It also developed a postgraduate course in biosafety and biosecurity, with a workload of 180 hours, in partnership with CAPES (Coordination of Superior Level Staff Improvement), FIOCRUZ, and the USG^{17, 221}. The Brazilian Society for Biosafety and Biosecurity (SB3), founded in 2020, assumes this role and has organized several training events, including virtual seminars and numerous courses and workshops in partnership with the US non-profit organization Health Security Partners³⁰⁸.

Training programs for laboratory biosafety and biosecurity should cover the following concepts:

- a. Fundamental principles of biosafety and biosecurity;
- b. Specific information about biological agents and toxins of interest, including modes of transmission;
- c. Types and procedures for the proper use of PPE, including respirator training, if applicable;
- d. Biosafety equipment used in the laboratory, with emphasis on BSCs and centrifuges;
- e. Occupational health;

- f. Biosafety applied to experimental animals, including animal handling, carcass disposal, ethics, and animal welfare aspects;
- g. Legislation and national and international standards applied to biosafety and biosecurity;
- h. Overview of biological risk assessment, including potential laboratory hazards;
- i. Institutional policies and procedures, including access control, handwashing procedures, waste disposal, classification and transport of hazardous materials, emergency response (including handling spills), accident and incident management, and specific laboratory SOPs;
- j. Proper use of disinfectants and decontamination procedures;
- k. Specific work-related training;
- l. Biosecurity, including protection of sensitive information, asset control, identification of suspicious behaviors by colleagues and visitors, inventory control procedures, and DURC^{269, 278, 309-310}.

All users of high and maximum-biocontainment laboratories must receive theoretical and practical training before beginning their activities. This training should be repeated annually to refresh their knowledge. Ideally, the training should include visits and internships at other laboratories with similar biological containment levels^{13, 269, 278}.

In addition to the need for formal training opportunities, there has been a general shortage of scientific research related to laboratory biosafety and biosecurity³¹¹. A study conducted with ten countries, including Brazil, concluded that there is very little research being conducted on the effectiveness of decontamination methods for specific pathogens, the effectiveness of PPE use, behavioral aspects of employees working in high-biocontainment environments, inspections and audits, engineering-related elements such as laboratory equipment, systems, and operation, as well as incidents and accident rates¹⁶⁸.

Chart 04, which lists some of the prominent national scientific publications related to the topic, confirms the above statement, given the scarcity of publications by Brazilian researchers in international journals.

Chart 04 – Brazilian researchers' primary scientific publications on laboratory biosafety and biosecurity.

Title	Kind of publication	Year of publication
Memories of biosafety in Brazil: lessons to be learned ²²¹	Manuscript	2005
Biosafety applied to laboratories and health services* ³¹²	Book	2005
Architecture's contribution to the quality of spaces destined for biological containment laboratories ³¹³	Thesis	2008
Indicators of uncertainty for the decisions of architecture to laboratories NB3 – study of cases ⁷³	Manuscript	2008
Design and construction of low-cost BSL-3 laboratories* ⁶⁶	Dissertation	2008
Analysis of the building competence of Brazil toward the maximum-containment laboratory: reality and perspectives* ⁶⁴	Thesis	2008
Biosecurity in health: priorities and strategies action ³¹⁴	Book	2010
Biosafety: a multidisciplinary approach* ³¹⁵	Book	2010
General Directives for the Containment Work with Biological Agents ²²³	Manual	2010
Health surveillance, biosafety and emergence and re-emergence of infectious diseases in Brazil ³¹⁶	Manuscript	2010
Invisibility of biohazard situations in the field of Public Health: Challenges for biosafety and biosecurity ⁶⁵	Thesis	2011
Assessment of workers' knowledge and biosafety procedures in BSL-3 laboratories ⁷⁴	Thesis	2014
International standards for protection against bioterrorism and biocrimes: gaps and vulnerabilities in Brazil ¹⁵⁴	Dissertation	2014
Technical Fundamentals and the National Biosafety System in Biotechnology* ³¹⁷	Book	2015
Biocontainment: the risk management in environments of high biological containment NB3 and NBA3 ⁹⁴	Book	2015
Public health emergencies due to chemical, biological, radiological, and nuclear (CBRN) events from the perspective of strategic intelligence: recommendations in favor of inter-sectoral action in health security and biodefense* ¹⁸	Dissertation	2017
Laboratory Biosafety: consolidation and harmonization of national regulations and standards ³¹⁸	Book	2019
Biosafety in the development of vaccines, biopharmaceuticals and diagnostic kits ³¹⁹	Manuscript	2020
International workshop on the National Maximum-biocontainment Laboratory: final report* (LNMCB) ³²⁰	Report	2021
Accidental release of anthrax in a research laboratory: case study ¹⁰⁵	Thesis	2022
Thinking about a National Strategic Infrastructure. The Brazilian BSL-4 Laboratory ²⁰	Book	2023
Comparison of Brazilian High- and Maximum-Containment Laboratories Biosafety and Biosecurity Regulations to Legal Frameworks in the United States and Other Countries: Gaps and Opportunities ³²¹	Manuscript	2023
Policy and regulatory requirements for laboratory biosafety and biosecurity in Brazil ³²²	Manuscript	2024

* Free translation of the title

The low volume of publications is directly linked to the insufficient provision of postgraduate courses focused on health security in Brazil, which includes biodefense, biosafety, and biosecurity. Research funding agencies and schools of health and public safety must encourage discussions, academic research, and the

establishment of research centers, awards, and scholarships in health security, including biosecurity aspects, to address Brazilian-specific problems and solutions¹⁷.

To this end, the MD's partnership with CAPES deserves recognition. Some of these programs aim to advance technical-scientific research by creating postgraduate courses focusing on biosafety and biosecurity within the defense context. For example, the publication of Edict n. 15/2019 by CAPES³²³ made possible the funding of the proposal for a doctoral course focused on 'Management and Governance in Biosafety and Biosecurity' offered by the Postgraduate Program in Applied Biochemistry and Biotechnology at Viçosa Federal University (UFV) in collaboration with the University of the Air Force (UNIFA), IBEx, and UTMB. The present thesis is part of that program.

2.18 Open dissemination of information on operating High-Containment Biological Laboratories

The 'Global BioLabs' is an initiative of a group of researchers from King's College London and George Mason University, launched in 2021. Its goal is to identify the world's maximum-biocontainment laboratory. In 2023, their survey expanded to include BSL-3 enhanced facilities. The initiative maintains a website displaying a map of these facilities' locations. They also release reports evaluating countries' governance and biorisk management practices about these types of laboratories⁵¹.

However, some inconsistencies were detected in the data about Brazilian BSL-3 enhanced facilities. Consequently, there is an opportunity to develop a local tool to rectify these inconsistencies and encompass all operating HCBLs in the country.

3. HYPOTHESIS

The significant gaps in the Brazilian legal framework for managing HCBLs contribute to deficiencies in preventing and controlling infectious diseases and shortcomings in biosafety and biosecurity management. Investigating the current operational status of HCBLs in Brazil and assessing the effectiveness of their risk management practices will provide crucial insights for developing more robust and effective regulatory mechanisms.

4. OBJECTIVES

4.1 General objectives

Offer an overview of the current operational status of HCBLs in Brazil, evaluating their primary strengths, weaknesses, gaps, and challenges, and delve into potential solutions to enhance national laboratory biosafety and biosecurity regulations.

4.2 Specific objectives

- a. Identify operational HCBLs in Brazil according to their self-assessed biosafety levels;
- b. Survey the Brazilian HCBLs infrastructure, primary roles, management, and operational aspects, evaluating their structures and procedures in light of international guidelines;
- c. Conduct a comparative study of the legislative, standard, and manual frameworks governing biological risk management in Brazil and other countries, identifying areas for enhancement;
- d. Develop an interactive platform to disseminate information on operational HCBLs in Brazil.

5. MATERIALS AND METHODS

5.1 Identification of operational High-Containment Biological Laboratories in Brazil

No official sources provide a list of HCBLs currently in operation in Brazil. As a result, to identify as many laboratories as possible, the following sources of information were utilized:

- a. Literature review, especially the book 'Thinking about a National Strategic Infrastructure. The Brazilian BSL-4 Laboratory'²⁰;
- b. The FINEP's Public Call aimed to select proposals for financial support for the execution of institutional projects for the adaptation and implementation of the physical infrastructure of HCBLs⁷¹;
- c. Information collected through participation in the TG created by the GSI/PR responsible for addressing security issues related to national critical infrastructures;
- d. Internet search, especially institutional websites and news related to the opening ceremony of new HCBLs;
- e. Personal communication.

Since there is no official certification process to ensure a laboratory's biosafety and biosecurity status in Brazil, this study included all organizations that self-declared as having an HCBL and all facilities identified through the above sources.

5.2 Administration of a comprehensive questionnaire

A comprehensive questionnaire was developed to gather information about the current status of HCBLs, focusing on data organized according to the following sections:

- a. Laboratory identification;
- b. Laboratory scope and operation characteristics;
- c. Design process and construction details;
- d. Facility features;
- e. Water and gas supply;
- f. Access control system;
- g. HVAC system;
- h. Electrical systems;
- i. Airlocks;
- j. Decontamination methods;
- k. EDS;
- l. Autoclaves;
- m. BSCs;
- n. Freezers and refrigerators;
- o. Centrifuges and other equipment;
- p. Laboratory animal models;
- q. Uniforms and PPE;
- r. Human resources and training;
- s. Occupational health;
- t. Biosecurity aspects;
- u. GMOs activities;
- v. Risk assessment;
- w. Automation;
- x. Other management and administrative/financial issues.

The questions were prepared according to the main international manuals and standards, especially the BMBL¹, WHO Biosafety Manual², and ISO 35001:2019¹¹.

The questionnaire consisted of 382 questions (Appendix B), most of which were multiple-choice. After receiving feedback that the questionnaire was too lengthy, a second version was prepared with 186 questions.

The survey method and the Free Consent Agreement Form (Appendix C) were submitted to the Ethics Committee for Research with Human Beings (CEP) of UFV for evaluation. The approval was granted on November 05, 2021, after complying with some recommendations (Technical Advice n. 5.081.703).

After a preliminary list of 92 presumed HCBLs in operation in 81 institutes across Brazil was compiled, their respective directors, Pls, or BOs were contacted to explain the project's goals and invite them to participate in the survey. Those who agreed to participate were provided with a Free Consent Agreement Form to demonstrate the author's commitment to maintaining the confidentiality of the information provided. Additionally, they received PDF (Portable Document Format – Adobe Acrobat Reader) and Microsoft Word versions of the questionnaire and a link to a Google form template. Participants were allowed to choose the most convenient method for completing and submitting their answers.

Responses were collected from November 2021 to March 2023. Following data collection, follow-up sessions were conducted to resolve inconsistencies in the data as necessary. Respondents were not provided with any explanations to clarify concepts since it was assumed that they were familiar with the basic concepts of laboratory biosafety and biosecurity, as outlined in the primary international standards and guidelines. The answers provided were based on self-reporting and reflected each respondent's understanding and interpretation. The resulting data was compiled into a Microsoft Excel 2016³²⁴ file and unidentified for statistical analysis.

Since this study was a quali-quantitative assay, descriptive statistics, such as percentages and frequencies, were used to analyze categorical data. The Mann-Whitney U test is a non-parametric test used to compare two independent samples to assess whether they are likely to come from the same population or if one tends to have higher values than the other³²⁵. Due to the low frequency in some categories ($n \leq 5$), Fisher's Exact Test³²⁶ was utilized to examine the relationships and correlations among the variables in the statistical analysis. The Kruskal-Wallis H test³²⁷ was used to determine if there were statistically significant differences between two or more groups of a quantitative variable.

It was considered a significance level of 5% for all statistical analyses.

5.3 Scheduling of technical visits

Between June 2021 and April 2022, eight laboratories were visited, including one mobile laboratory in the states of São Paulo, Rio de Janeiro, and Minas Gerais.

These visits aimed to understand the infrastructure and particular features of laboratories affiliated with entities such as the state, universities, and hospitals. Additionally, these visits provided the opportunity to engage with various stakeholders and discuss the challenges and gaps related to the managing HCBLs in Brazil, yielding valuable insights that contributed to the discussion presented in this study.

5.4 Assessment of current legislation and guidelines in effect in Brazil and other countries

A thorough gap analysis was conducted to compare Brazilian biosafety and biosecurity regulations with those of other countries, focusing on the oversight and governance of HCBLs. In addition to examining legislation and complementary documents obtained from governmental websites and official reports, valuable insights were gathered through a comprehensive literature review. Additional sources of information included conversations with key stakeholders, participation in working groups, scientific events, and seminars, as well as searches on websites such as Biosecurity Central³²⁸, ABSA International³²⁹, IFBA³³⁰, CDC³³¹, FSAP³³², BioGib³³³, Government of Canada³³⁴, and SB3³³⁵, among others.

5.5 Comparative analysis between High-Containment Biological Laboratories in Brazil and those affiliated with the Research Alliance for Veterinary Science and Biodefense BSL-3 Network

The internship carried out between August 2022 and July 2023 at the Global Health Research Complex (GHRC), belonging to Texas A&M University (TAMU), allowed access to several HCBLs belonging to the RAV3N network, which is coordinated at GHRC.

The same questionnaire and Free Consent Agreement Form mentioned in Chapter 5.2 were applied to RAV3N laboratories.

Responses were collected between November 2022 and March 2023. The answers were based on self-reporting and reflected each respondent's understanding and interpretation. Subsequently, the collected data was compiled into a Microsoft Excel³²⁴ file and unidentified to facilitate statistical analysis.

The Fisher's exact test³²⁶ and the Mann-Whitney U test³²⁵ were employed for statistical analysis when assessing categorical and quantitative variables, respectively.

The statistical analysis involved calculating errors associated with responses like 'I don't know' and 'No response' was conducted to illustrate the impact of these responses in the comparative analysis, with a particularly notable influence in Brazil, where the highest percentages of non-response were observed. The following expression was employed, taking into account the matrix outlined in Chart 05: $a/(a+b)-((a+c))/(a+b+c+d)$.

Chart 05. Error coefficients matrix for 'I don't know' and 'No response' in comparative analysis between Brazilian vs. RAV3N HCBLs.

	Brazilian HCBL	RAV3N laboratories
Respondents	a	b
Non-respondents	c	d

5.6 Development of an interactive platform to disseminate information on the High-Containment Biological Laboratories in operation in Brazil

An interactive application has been developed using the *Shiny* framework³³⁶ for spatial visualization of HCBLs operating in Brazil, as identified in the current study. The development of the *Shiny* application followed the steps outlined below:

a. Data collection: The HCBLs were compiled according to the data outlined in Chapter 6.1.1 of the current study. The geographic coordinates (latitude and

longitude) were taken from the Google Maps application³³⁷ after inputting the address of each facility;

b. Development of the application: The interactive interface was developed using the R programming language³³⁸ and the *Shiny* framework³³⁶. The Leaflet library³³⁹ was utilized for the geographical visualization of the laboratories on a map;

c. Filter implementation: Filters have been implemented to enable users to select laboratories based on BSL and state, offering a customized experience;

d. Integration with *Shinyapps.io*: The application was hosted on *Shinyapps.io*, making it accessible online for analysis and public use.

The tool was named 'BrazilBioLabs'.

6. RESULTS AND DISCUSSION

6.1. Identification of operational High-Containment Biological Laboratories in Brazil

6.1.1 Results

Ninety-two laboratories belonging to 81 institutes were identified (Table 01).

Table 01 – List of presumed HCBLs in Brazil, according to the literature review and other sources, and those that filled in the questionnaire, part of the current study.

Body linked	Lab name	City	State	Number of High-containment Labs	Questionnaire filled in
MS	FIOCRUZ (CPqAM)	Recife	PE	1	1
	FIOCRUZ (CPqGM)	Salvador	BA	1	0
	FIOCRUZ (CRPHF)	Rio de Janeiro	RJ	1	1
	FIOCRUZ (ICC)	Curitiba	PR	2	1
	FIOCRUZ (IOC)	Rio de Janeiro	RJ	2	1
	FIOCRUZ (Biomanguinhos)	Rio de Janeiro	RJ	2	0
	IEC	Ananindeua	PA	2	1
	INCA	Rio de Janeiro	RJ	1	1
State Health Secretariat	Adolfo Lutz Institute	São Paulo	SP	1	1
	Butantan Institute	São Paulo	SP	2	0
	CEPEM	Porto Velho	RO	1	0
	Charles Merieux Infectology Center	Rio Branco	AC	1	1
	FMT	Manaus	AM	1	0
	FUNED	Belo Horizonte	MG	1	1
	LACEN-BA	Salvador	BA	1	0
	LACEN-CE	Fortaleza	CE	1	1
	LACEN-DF	Brasília	DF	1	1
	LACEN-RS	Porto Alegre	RS	1	1
MAPA	Pasteur Institute	São Paulo	SP	1	1
	EMBRAPA (CNPqG)	Campo Grande	MS	1	1
	EMBRAPA (CNPqSA)	Concórdia	SC	1	1
	LFDA-MG	Pedro Leopoldo	MG	2	2
	LFDA-PE	Recife	PE	1	0
MD	LFDA-SP	Campinas	SP	1	1
	IBEx	Rio de Janeiro	RJ	1	0
MCTI	INPA	Manaus	AM	1	1
State Agriculture Secretariat	Biological Institute	São Paulo	SP	1	1
	PUCRS (CPBMF/INCT-TB)	Porto Alegre	RS	1	0
Universities	UEM	Maringá	PR	1	0
	UENF	Campos dos Goytacazes	RJ	1	0
	UFABC	Santo André	SP	1	0
	UFC	Porangabucu	CE	1	0
	UFES	Vitória	ES	1	0
	UFF	Rio de Janeiro	RJ	1	0
	UFLA	Lavras	MG	1	1
	UFMG (CELAM/IB)	Belo Horizonte	MG	1	0
	UFMG (HC)	Belo Horizonte	MG	1	1
	UFPA	Belém	PA	1	0
	UFPE	Recife	PE	1	1
	UFPR	Curitiba	PR	1	1
	UFRJ (LBMV)	Rio de Janeiro	RJ	1	1
	UFRJ (PEMOP3/IBCCF)	Rio de Janeiro	RJ	1	0
	UFSC	Santa Maria	RS	1	1
	UFVJM	Mucuri	BA	1	0
	UNESP (Biology Department)	São José do Rio Preto	SP	1	0
	UNESP (Hemocenter)	Botucatu	SP	1	0
	UNESP (Biology Institute)	Botucatu	SP	1	0
	UNESP (LICBM-FCF)	Araraquara	SP	1	0
	UNESP (UNIPEX)	Botucatu	SP	1	1
	UNICAMP (Applied Virology and Biotechnology)	Campinas	SP	1	1
	UNICAMP (Emergent Virus)	Campinas	SP	1	1
	UNIFESP (Lab2ICB + Lab3FF)	São Paulo	SP	1	0
	UNIFESP (Mycobacterium)	São Paulo	SP	1	0
	UNIFESP (Retrovirology)	São Paulo	SP	1	1
	UNIFOR	Fortaleza	CE	1	0
	USP (FCF)	São Paulo	SP	1	1
	USP (FMRP)	Ribeirão Preto	SP	2	0
	USP (ICB/Parasitology)	São Paulo	SP	1	0
	USP (ICB/Virology)	São Paulo	SP	1	0
	USP (IMT)	São Paulo	SP	1	0
	USP (Pasteur Scientific Platform)	São Paulo	SP	2	1
Private companies	BIOVET	Vargem Grande Paulista	SP	1	0
	BOEHRINGER	Paulínia	SP	1	0
	CEVA	Juatuba	MG	1	1
	CPABR	Amparo	SP	3	0
	MERCK	Montes Claros	MG	2	1
	OURO FINO	Cravinhos	SP	1	1
	Paraná Molecular Biology Institute	Curitiba	PR	1	0
	SENAI (ISI/FIRJAN)	Rio de Janeiro	RJ	1	0
EBSERH	FURG (HU Miguel Riet Correa Junior)	Rio Grande	RS	1	0
	UFAM (HU Getúlio Vargas)	Manaus	AM	1	0
	UFCA (CH)	Juazeiro do Norte	CE	1	0
	UFCG (HU Alcides Carneiro)	Campina Grande	PB	1	0
	UFF (HU Antônio Pedro)	Niterói	RJ	1	0
	UFG (HC)	Goiânia	GO	1	0
	UFJF (HU)	Juiz de Fora	MG	1	0
	UFMA (HU)	São Luis	MA	1	0
	UFMS (HU Maria Aparecida Pedrossian)	Campo Grande	MS	1	1
	UFPI (HU)	Teresina	PI	1	0
	UFPR (CHC)	Curitiba	PR	1	0
	UFS (HU)	Aracaju	SE	1	0
TOTAL	81			92	36

After reviewing the available information and directly contacting as many laboratories as possible. It has been determined that 17 facilities from the initial list were not functioning as HBCLs during the research period. Furthermore, the status of nine laboratories managed by the EBSEH could not be confirmed due to difficulties in reaching them. Therefore, the total number of HBCLs considered in the present study was 66, belonging to 54 institutes.

Of the 66 HBCLs, 32 (48.5%) laboratories belonging to 31 institutes (59.2%) participated in the survey. Four additional laboratories responded to the questionnaire, but their data were excluded because, according to personal communication from their representatives, they were not functioning as high-biocontainment facilities at the time of the survey. It is important to note that the classification of these 66 laboratories was primarily based on self-reporting. Furthermore, it is essential to clarify that the purpose of this study was not to confirm their biocontainment status.

Data regarding the geographical distribution of HBCLs across Brazil reveals a notable concentration of these facilities in the southeast region (68.2%), particularly in the states of São Paulo, Rio de Janeiro, and Minas Gerais. These states, respectively, account for 34.9%, 18.2%, and 13.6% of the laboratories in operation in Brazil. Among the 27 Brazilian states and the Federal District, 15 (55.6%) have at least one HBCL, while the remaining 12 states (Alagoas, Amapá, Goiás, Maranhão, Mato Grosso, Paraíba, Piauí, Rio Grande do Norte, Rondônia, Roraima, Sergipe, and Tocantins) do not have any such facilities. Of these 12 states, half are in the Northeast region, followed by the North (three states) and Central-West (one state) regions. São Paulo and Rio de Janeiro are the cities with the highest number of HBCLs in the country, with 10 and 11 facilities, respectively (Figure 01 and Table 02).

Figure 01 – Geographical distribution of self-reported HCBLs in Brazil. Each symbol denotes an HCBL.



Table 02 – Distribution of self-declared HCBLs in Brazil, according to location.

Region	State	Acronym	Number of HCBLs	%
Midwest	Federal District	DF	1	1.5
	Goiás	GO	0	0
	Mato Grosso	MT	0	0
	Mato Grosso do Sul	MS	1	1.5
Sub-total			2	3.0
North	Acre	AC	1	1.5
	Amapá	AP	0	0
	Amazonas	AM	2	3.0
	Pará	PA	2	3.0
	Rondônia	RO	0	0
	Roraima	RR	0	0
	Tocantins	TO	0	0
Sub-total			5	7.5
Northeast	Alagoas	AL	0	0
	Bahia	BA	2	3.0
	Ceará	CE	3	4.6
	Maranhão	MA	0	0
	Paraíba	PB	0	0
	Pernambuco	PE	2	3.0
	Piauí	PI	0	0
	Rio Grande do Norte	RN	0	0
	Sergipe	SE	0	0
Sub-total			7	10.6
South	Paraná	PR	4	6.1
	Rio Grande do Sul	RS	2	3.0
	Santa Catarina	SC	1	1.5
Sub-total			7	10.6
Southeast	Espírito Santo	ES	1	1.5
	Minas Gerais	MG	9	13.6
	Rio de Janeiro	RJ	12	18.2
	São Paulo	SP	23	34.9
Sub-total			45	68.2
TOTAL			66	99.9

Most of the laboratories (31, 47.0%) are affiliated with universities, with 16 (51.6%) belonging to state universities, 13 (41.9%) to federal universities, and two (6.5%) to private institutions. A significant proportion of the laboratories (57, 86.8%) are publicly funded, with 32 (56.1%) being federal and 25 (43.9%) state-funded. The federal ones comprise 13 (40.6%) university-affiliated labs, 12 (37.5%) affiliated with the MS, five (15.6%) with the MAPA, one (3.1%) with the MD, and one (3.1%) with the MCTI. Among the state institutes, 16 (64.0%) belong to universities, eight (32.0%) to a State Health Secretariat, and one (4.0%) to a State Agriculture Secretariat. Among the private laboratories, four (40.0%) are FMD vaccine manufacturers, two (20.0%) are universities, and four (40.0%) are research centers. The national reference laboratory for the MS, FIOCRUZ, is the institute with the highest number of HCBL (9, 13.6%).

6.1.2 Discussion

Even with the data collected, it cannot be conclusively stated that the exact number of HCBLs operational in Brazil was 66 at the time of this study for the following reasons:

- a. Lack of official data;

b. The biosafety level status was self-declared, and the definition of an HCBL is not standardized in the country. Therefore, the manager or PI may consider the laboratory a BSL-3 lab without applying adequate criteria for that classification. It is important to note that it was not the purpose of this study to confirm the biocontainment status;

c. Quantifying the number of HCBLs within the same institute becomes more complex when multiple such laboratories are located within the same building with shared access.

Regarding the last topic, a report from the US Government Accountability Office concerning HCBLs recognized that counting the number of laboratories can be problematic due to variations in the term 'laboratory', mainly when the containment facility comprises independent rooms. Some HCBLs may classify each room as a separate laboratory, while others consider the entire complex a single laboratory⁴³.

The absence of official data on the operation of HCBLs in Brazil is a critical issue. The data relating to the facilities belonging to the MS, MAPA, MD, and MCTI are reliable, although not publicly accessible. Some additional information has been gathered from attendance at GSI/PR TGs, which include representatives from all these bodies.

Conversely, obtaining information on the status of the laboratories belonging to EBSEH, private companies, and universities is challenging. The BWC collects annual declarations under the treaty's confidence-building measures, including a form that requires information about maximum-biocontainment laboratories in the country or HCBLs if it does not have a BSL-4 lab. This information is considered confidential by the BFG³⁴⁰. According to personal communication from an MD representative, this list does not include university laboratories, only those linked to MS, MAPA, MD, and MCTI.

The EBSEH laboratories are under the management of the MEC and are in hospitals affiliated with Federal Universities throughout the country. They aim to support these universities' teaching, research, and services. Of the 41 hospitals linked to the EBSEH, 12 have laboratories initially classified as HCBLs. However, after being invited to participate in this study, three reported that they could not be categorized as a BSL-3 laboratory. Despite attempts to contact them, there was no response from the other nine laboratories. After reviewing information from MEC regarding their general features and purpose, none could likely be classified as an HCBL. It will be necessary to carry out a more detailed evaluation regarding the status of these laboratories.

The number of laboratories responsible for producing the FMD vaccine in the country has decreased due to MAPA's decision to grant the entire country a 'free of FMD without vaccination' sanitary status. MAFRA (2020)²⁰ mentioned that five of these companies were in operation in 2020, but according to personal communication from a MAPA representative, two have closed in recent years. MAPA has inspected these laboratories under IN n. 5/2012²³¹, the country's most comprehensive and strict legislation regarding biosafety and biosecurity requirements. Consequently, these laboratories were classified as 'BSL-4 WOA'. However, this classification is no longer valid, as the latest version of the WOA's Manual of Diagnostic Tests and Vaccines for Terrestrial Animals⁹ has excluded it. As a result, a more appropriate classification based on the BMBL¹ is 'BSL-3 enhanced'. Another possibility is to classify them as 'HCBL able to handle FMD virus according to the national regulations'.

Although the exact number of HCBLs installed in Brazil is unknown, the present study estimates it to be around 66. This number is 2.1, 4.4, and 3.3 times greater than those found in previous studies^{3, 74, 313}. Notably, Simonetti (2014)⁷⁴ excluded private and university laboratories from his survey. According to Vieira (2008)³¹³ and Simonetti (2014)⁷⁴, eleven states had at least one HCBL, whereas the present study identified four additional states. One of the 15 laboratories specified by Simonetti (2014)⁷⁴ and ten laboratories placed by Vieira (2008)³¹³ were not operating as HCBLs during the present study.

In a more recent survey, 47 HCBLs were identified as operational in Brazil, along with three additional mobile units²⁰. Of those 47 laboratories, 12 were not operating as HCBLs during the present study.

The data above demonstrate the variability of the number of HCBLs operating in the country due to the construction of new facilities and the decommissioning of others. According to personal communication from numerous employees from these laboratories, the main reason laboratories cease to operate as HCBLs is inadequate maintenance, primarily due to insufficient financial resources.

Compared to countries such as the US and the UK, which have approximately 1,300⁴⁵ and 600⁴⁹ HCBLs, respectively, Brazil has a relatively low number of HCBLs. However, this number is similar to Canada, which has 66 licensed high and maximum-containment laboratories across the country²⁰², and much higher than Argentina, which has less than ten BSL-3 laboratories³.

Abad (2018) highlighted the discrepancy between the numbers of HCBL in countries with different income levels and noted that BSL-3 facilities in countries such as Bangladesh, India, Indonesia, China, Brazil, and Mexico provide services to more than two-thirds of the world's population³⁴¹.

Similarly, Dickman et al. (2015) pointed out that countries with a high prevalence of disease often have limited resources and capacities to diagnose and monitor the diseases caused by these agents. In contrast, countries with low endemicity of high pathogenic agents tend to have costly infrastructure and maintenance for HCBLs. However, the authors stressed that the proliferation of laboratories is not necessarily the solution. Instead, an alternative framework is needed to establish a safer, more secure, and sustainable laboratory network⁵³.

HCBLs can be counterproductive if they are underutilized or used for research that does not align with public and animal health government policies. Furthermore, such labs can pose an unnecessary risk and increase the workload for government bodies monitoring them. Murray (1998) recommended that consolidating existing national laboratories and establishing a network among them would be a more effective strategy than constructing new HCBLs³⁴². This concept is especially relevant for diagnostic laboratories, given the recent development of new methods that do not require a high-biocontainment environment, particularly those utilizing molecular biology and histopathology⁴⁶.

On the other hand, Souza & Morel (2021) have drawn attention to the disruptive challenge posed by the requirement for HCBLs to conduct fundamental research on SARS-CoV-2 in Brazil. According to the authors, laboratories that excelled at generating essential knowledge during epidemics of Zika virus, dengue, and chikungunya have faced long waiting lines to access BSL-3 facilities in their own or collaborating institutions. They also highlighted the concentration of BSL-3 laboratories in the Southeast region and the need to strengthen the current network by establishing other federally funded BSL-3 and, significantly, ABSL-3 laboratories

within the system of Federal Universities to expand the capacity to respond to sanitary emergencies²⁴.

Although the concern raised by these authors is justifiable, it is essential to note that a better understanding of the current scenario is necessary before considering expanding the number of HCBLs in Brazil.

As concluded by the experts who attended the workshop 'Developing Norms for the Provision of Biological Laboratories in Low-Resource' promoted by the US Academies of Sciences, Engineering, and Medicine in 2019, knowing the current number, status, and location of HCBLs is crucial for any country intending to organize its national laboratory infrastructure and plan the construction of new facilities under a strategic plan. While most countries lack a comprehensive list of HCBLs, it would benefit decision-makers to have such a list to help distribute resources, identify gaps, avoid duplication of efforts, and foster a collaborative environment between the laboratories^{3, 46}.

Yeh et al. (2016) advised that when constructing a new facility, it is vital to consider the seamless integration of new capabilities into the country's existing public health architecture. It is crucial to avoid duplicating existing laboratory capabilities and national program efforts to ensure sustainability. These authors also emphasized that although challenging, documenting the progress of laboratory capability and operational development in a country is a necessary task³⁴³.

The main objectives of the overall national laboratory biosafety infrastructure must be delineated through a PNBB. Therefore, after publishing the PNBB, the BFG should consider establishing a TG to coordinate this discussion nationwide. According to the WHO, the national laboratory strategic plan translates the prerequisites for an ideal laboratory system formulated in the national laboratory policy into strategic objectives that must be undertaken to turn the policy statements into reality. The national laboratory strategic plan is thus the logical and essential next step after developing a national laboratory policy³⁴⁴. While this statement does not directly pertain to laboratory biosafety and biosecurity, it is highly relevant and applicable in this context.

The national strategic plan for laboratory biosafety should address the ideal number, type (BSL-3, ABSL-3, ABSL-3Ag, BSL-4, ABSL-4, etc.), distribution, linkage, and purpose of these facilities across the country, as well as the budget need to its implementation.

This TG in charge of the national strategic plan would address several essential questions, including the main gaps and challenges facing public and animal health regarding laboratory support. They would investigate whether these gaps and challenges are related to the overall laboratory operational capacity. The group would also discuss the need for HCBLs and whether the number of laboratories identified in the present study can address the identified gaps and challenges. Additionally, the group would explore management improvements that can encourage collaboration and communication between laboratories from different networks to achieve a high level of effectiveness and avoid overlapping while taking advantage of complementary expertise and equipment.

The concentration of laboratories in the country's Southeast region can be attributed to the region's demographics and numerous industries, universities, and reference laboratories. The same situation was observed in China, with a concentration of BSL-3 laboratories in the eastern and middle regions⁴⁷. When discussing the national strategic plan, it is essential to consider this issue, and the health authorities should assess the necessity of establishing additional laboratories

in other regions. This evaluation must consider the logistical challenges associated with sample transportation, particularly from remote areas. On the other hand, it should be regarded as the availability of qualified personnel and maintenance services. According to Cardoso (2008), establishing Brazil's BSL-3 health public laboratories network was based on epidemiological, technical expertise, demand, and geographic criteria⁶⁴. However, an update is necessary since this evaluation was conducted nearly 20 years ago.

Five HCBLs have been identified in the Amazon region, spanning the states of Pará, Amazonas, and Acre. This area holds strategic significance due to its potential for detecting novel pathogens, given the rich biological diversity of its ecosystem, as demonstrated by Wanzeller et al. (2023)³⁸.

The role of university laboratories is another critical topic that needs to be addressed. According to the present study, almost half of the HCBLs in the country are located in public universities. A survey involving 245 BSL-3 laboratories in the US yielded similar results. Among the 245 respondents, it was observed that academic HCBLs accounted for 52.3% of the total³⁴⁵. However, considering only the HCBLs registered in the FSAP program, the proportion of academic laboratories is lower, at 35.2% (82/233)¹⁹³. Out of 600 BSL-3 facilities in the UK in 2016, an estimated 150 are in universities (25.0%)⁴⁹. In Canada, the percentage of academic laboratories licensed to work with RG 2, 3, or 4 pathogens and toxins is even lower, at 20.4% (201/916)²⁰².

The BFG should evaluate the level of collaboration between university-based laboratories and assess their effectiveness in addressing national priorities for animal and public health. It includes evaluating whether these laboratories can fill the gaps that the current governmental laboratories cannot meet. It is essential to assess whether the higher prevalence of HCBLs in Brazilian universities, in contrast to countries such as Canada and the UK, is appropriate, particularly when considering the oversight gaps.

Another important topic for discussion is the need for BSL-4 and ABSL-3Ag facilities and how to approach this within a 'One Health' and collaborative framework since disconnected and fragmented actions lead to the wastage of scarce resources.

In this sense, Dhama et al. (2013) argued that multidisciplinary and collaborative efforts at global and local levels are required since increased antimicrobial resistance, extreme variability of RNA viruses, and involvement of domestic and migratory birds and wildlife in disease transmission have made the scenario of the spread of zoonotic diseases more complicated than ever before. The authors added that regional and local networking systems should use highly sensitive and specific methods to detect pathogens as early as possible²⁷.

The national strategy plan should include the identification of key stakeholders within the national context and define Brazil's role in the international arena, particularly in the international organizations that address biosafety and biosecurity.

Within the domain of MAPA, it may be beneficial to request a PVS Sustainable Laboratory Mission from WOA. It could help to identify the primary gaps and opportunities within the veterinary laboratory network and provide relevant recommendations to strengthen it, including the need to increase the number of HCBLs.

The recent initiatives of the BFG to highlight laboratory biosafety and biosecurity, primarily through the creation of the three CREDEN TGs²⁴⁵, are relevant.

Still, it is necessary to move forward from the immediate publication of the PNBB, followed by the elaboration of a national strategic plan for laboratory biosafety.

6.2 Administration of a comprehensive questionnaire

6.2.1 Participating laboratories

6.2.1.1 Results

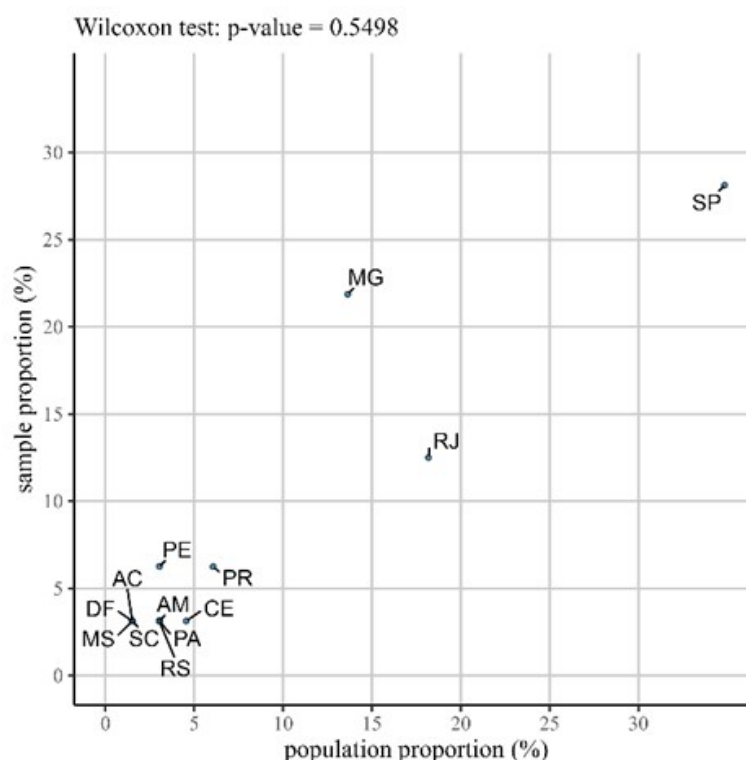
Considering the estimated number of Brazilian HCBLs, the overall participation rate reached 48.5% (32/66)^q. Although four additional laboratories submitted their responses, their data was not included as they reported not operating as an HCBL during the survey period. Five HCBLs (5/32, 15.6%) opted to use a shortened questionnaire version. Nine HCBLs (9/32, 28.1%) were either in the final stages of construction or commissioning, which rendered some of the questions asked not applicable to them (Table 03 and Graph 01).

Table 03 – Proportion of HCBLs participating in the current survey by State, compared to the total number of Brazilian self-declared HCLBs.

State	Total number of HCBL	%	Sampling	%
DF	1	1.5	1	3.1
GO	0	0	0	0
MT	0	0	0	0
MS	1	1.5	1	3.1
AC	1	1.5	1	3.1
AP	0	0	0	0
AM	2	3.0	1	3.1
PA	2	3.0	1	3.1
RO	0	0	0	0
RR	0	0	0	0
TO	0	0	0	0
AL	0	0	0	0
BA	2	3.0	0	0
CE	3	4.6	1	3.1
MA	0	0	0	0
PB	0	0	0	0
PE	2	3.0	2	6.3
PI	0	0	0	0
RN	0	0	0	0
SE	0	0	0	0
PR	4	6.1	2	6.3
RS	2	3.0	1	3.1
SC	1	1.5	1	3.1
ES	1	1.5	0	0
MG	9	13.6	7	21.9
RJ	12	18.2	4	12.5
SP	23	34.9	9	28.1
TOTAL	66	99.9	32	99.9

^q All tables generated from the questionnaire are listed in Appendix A

Graph 01 – Comparative analysis between the sample and population proportions of HCBLs concerning their location.



The statistical analysis of the distribution of participating HCBLs according to their location indicated strong evidence that such proportions were similar to the total number of HCBLs despite an apparent higher proportion of laboratories in Minas Gerais State.

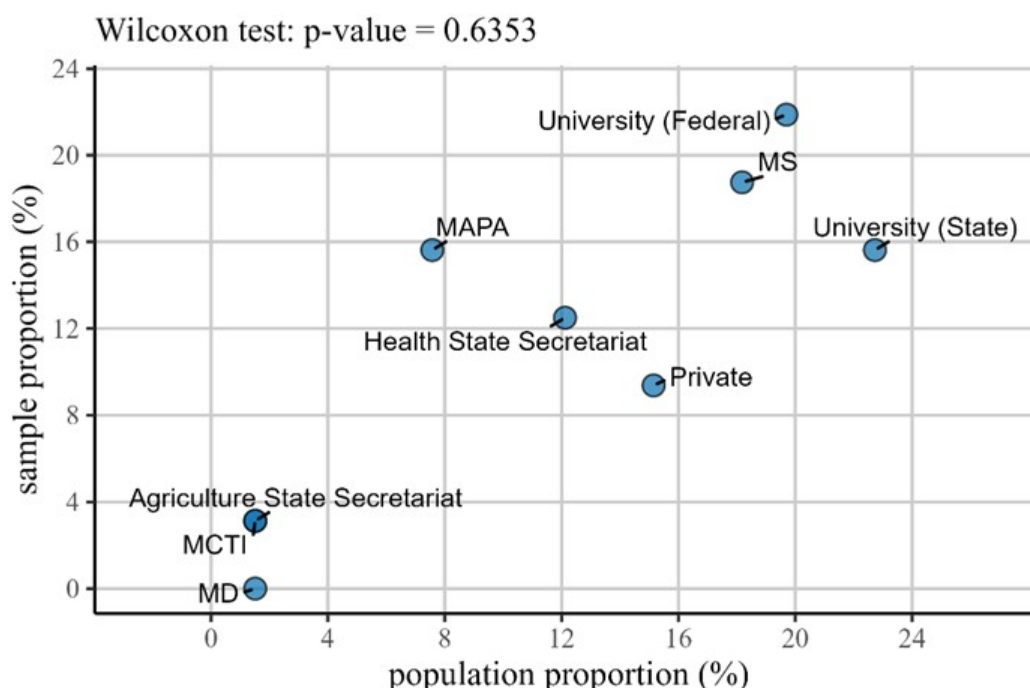
All HCBLs affiliated with MAPA (5/5, 100.0%), MCTI (1/1, 100.0%), and the State Secretariat of Agriculture (1/1, 100.0%) participated in the current survey. In contrast, half of the HCBLs affiliated with MS (6/12, 50.0%) and to the State Secretariat of Health (4/8, 50.0%) completed the questionnaire (Table A01). The participation rate for university-affiliated HCBLs was 41.4% (12/29).

However, the statistical analysis of the distribution of participating HCBLs according to their affiliation indicated strong evidence that such proportions were similar (Table 04 and Graph 02).

Table 04 – Proportion of HCBLs participating in the current survey by affiliation, compared to the total number of Brazilian self-declared HCLBs.

Body linked	Total number of HCBLs	%	Sampling	%
Agriculture State Secretariat	1	1.5	1	3.1
Health State Secretariat	8	12.1	4	12.5
MAPA	5	7.6	5	15.6
MCTI	1	1.5	1	3.1
MS	12	18.2	6	18.9
MD	1	1.5	0	0
University (Federal)	13	19.7	7	21.9
University (State)	15	22.7	5	15.6
Private	10	15.2	3	9.4
TOTAL	66	100.0	32	100.1

Graph 02 – Comparative analysis between the sample and population proportions of HCBL concerning their affiliation.



6.2.1.2 Discussion

The high level of engagement from MAPA's HCBLs was anticipated, given the author's professional affiliation. It is worth noting that only one HCBL was affiliated with MCTI and another with the Agriculture State Secretariat, making a comprehensive analysis infeasible. A similar situation applies to the MD, who has just one laboratory that didn't participate. The overall participation rate approached 50.0%, which is satisfactory when considering the questionnaire's length and the time required to complete it.

Barclay et al. (2002), studying maximizing response rates in postal questionnaire surveys, suggested that handwritten letters, telephone calls, or personal visits might be considered to boost participation. They also recommended sending a minimum of three reminders. Furthermore, they emphasized the importance of discerning differences between respondents and non-respondents to identify potential data bias³⁴⁶.

In line with the recommendations mentioned above, the following strategies were implemented to enhance the response rate:

- The invitation letter was sent via email and included a brief explanation of the study's objectives, along with a consent agreement term;
- Video calls were recommended for those participants seeking a deeper understanding of the survey;
- Visits, as outlined in Chapter 5.3 of this study, were planned but were ultimately limited due to financial and time limitations, as well as the restrictions imposed by the COVID-19 pandemic;
- Reminders were sent via email and WhatsApp on at least three occasions, especially when the contacted person initially expressed interest in participating in the study;

e. Although no specific analysis was conducted to examine potential differences between respondents and non-respondents and their impact on data bias, the absence of a biosafety officer and a biorisk management program may have contributed to non-responses. If this assumption holds, it suggests that the actual state of biorisk management may be more challenging than what was concluded in this study.

It is worth noting that quantifying the number of HCBLs within the same institute becomes more complex when multiple such laboratories are located within the same building with shared access.

It is also relevant to clarify that 'hybrid' refers to two laboratories resulting from partnerships between private and public entities.

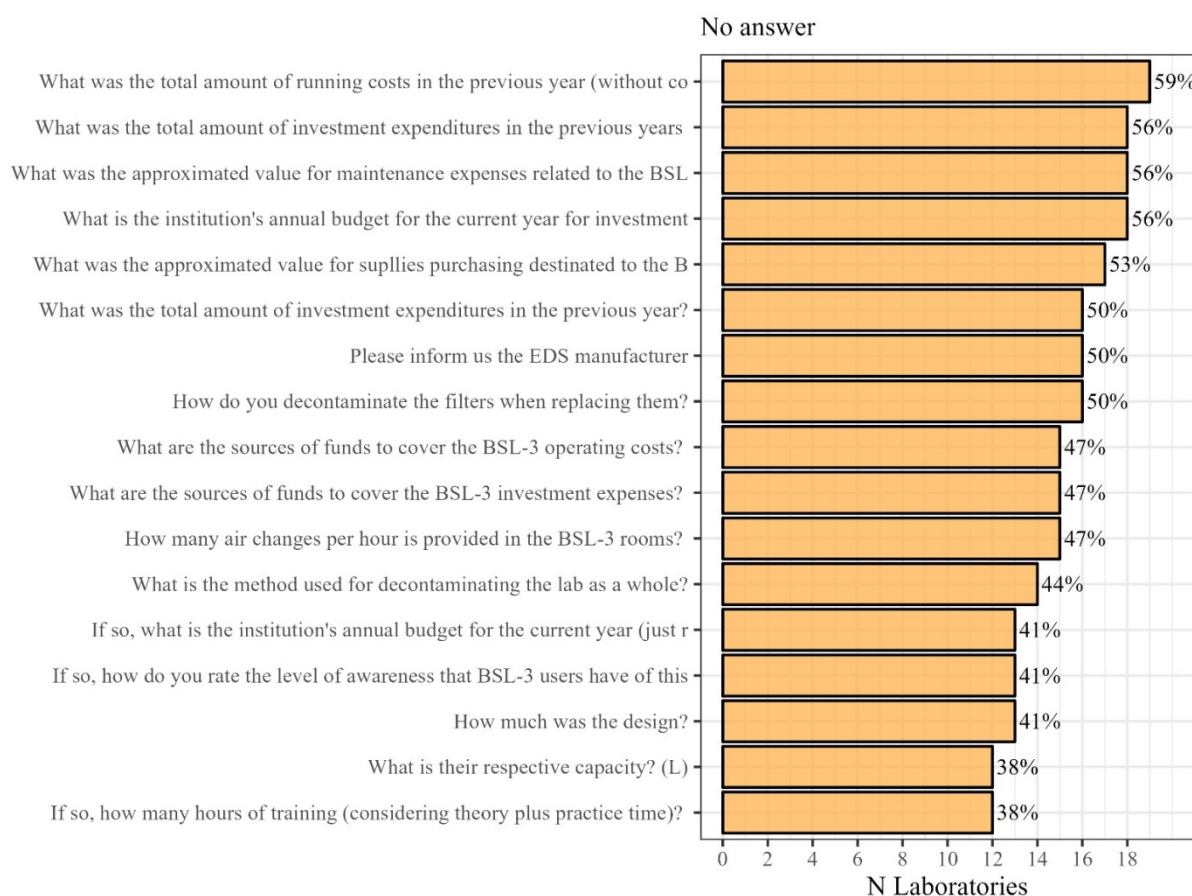
6.2.2 Valid and missed questions

6.2.2.1 Results

In the tables displaying the results from the questionnaire application, the term 'missing' was employed to signify answers that were not applicable. Questions left unanswered or answered with the option 'I don't know' were categorized as 'valid' questions. Therefore, the term 'valid' was utilized for the overall number of participants ($n = 32$), excluding the 'missing' responses for each question. The frequency values exclusively represent the responses categorized as 'valid'.

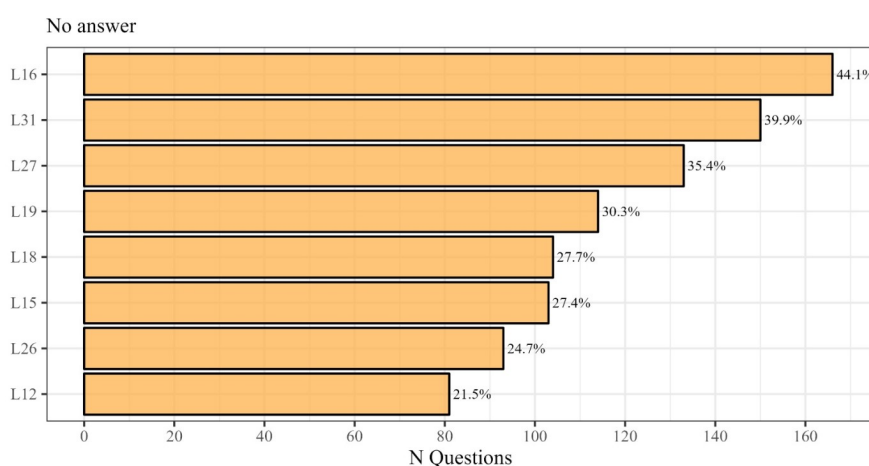
Out of the 382 questions, eight (8/382, 2.1%) were not completed by half or more than the participants (Graph 03).

Graph 03 – Questions with the highest non-response rate among those applied in the survey.



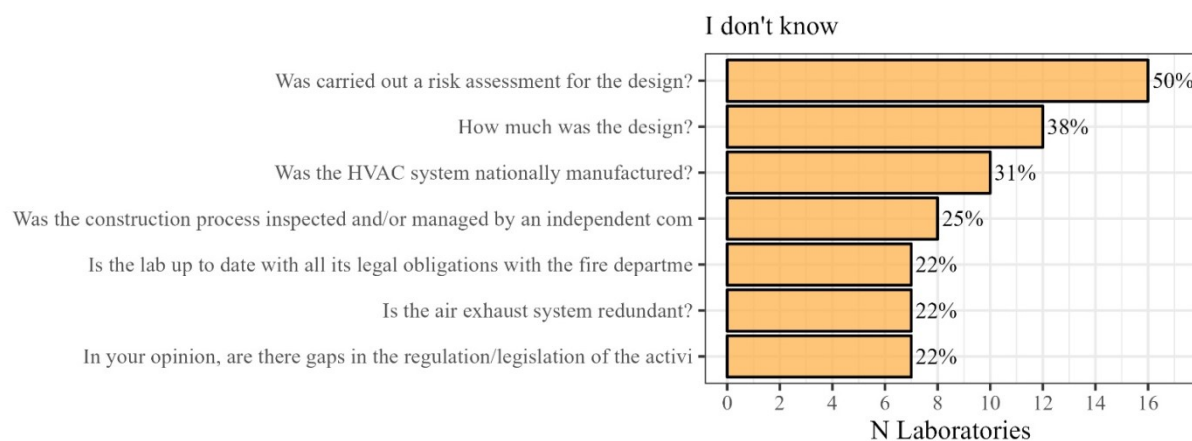
All HCBLs participating in the survey answered at least half of the questions. Among them, only three (3/32, 9.4%) did not provide any answer for more than one-third of the questions (Graph 04).

Graph 04 – Respondents with the highest number of unanswered questions among those who participated in the survey.



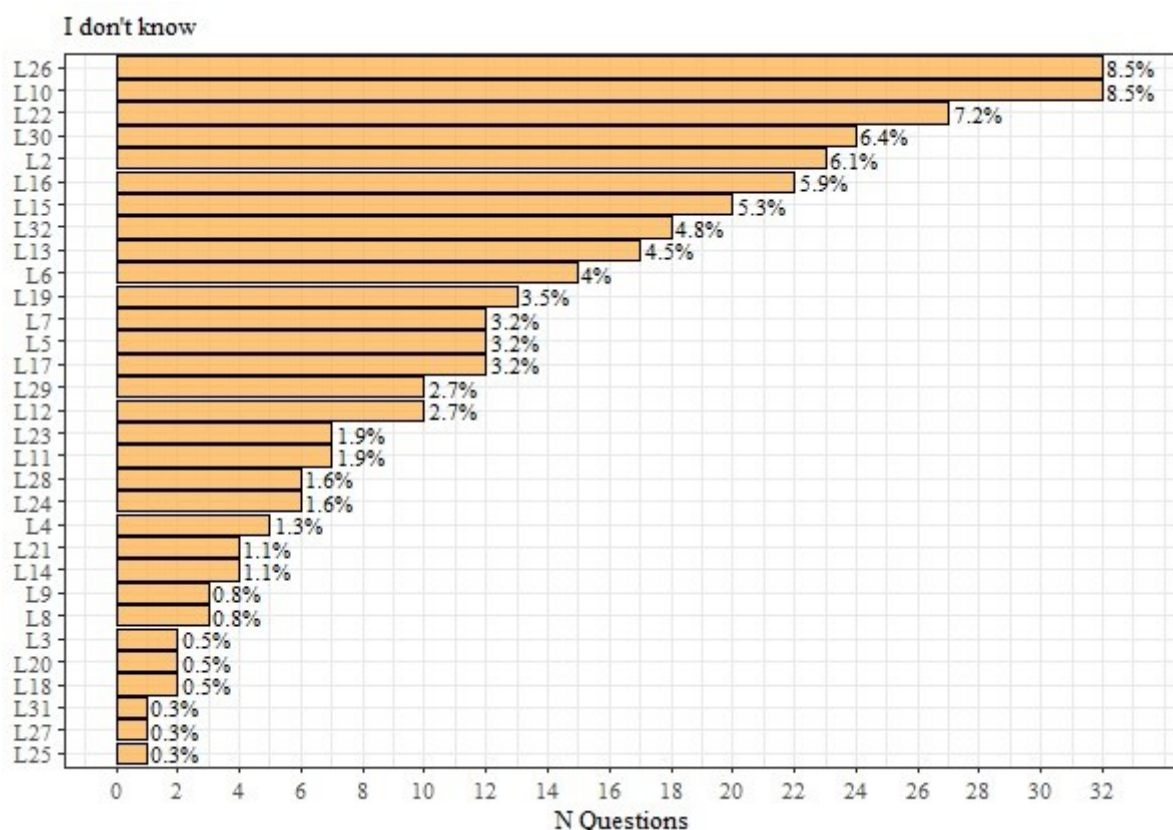
Among the 382 questions, only one (1/382, 0.3%) received answers in the form of 'I don't know' from more than half of the participants (Graph 05).

Graph 05 – Questions with the highest proportion of participants choosing the option 'I don't know' among those applied in the survey.



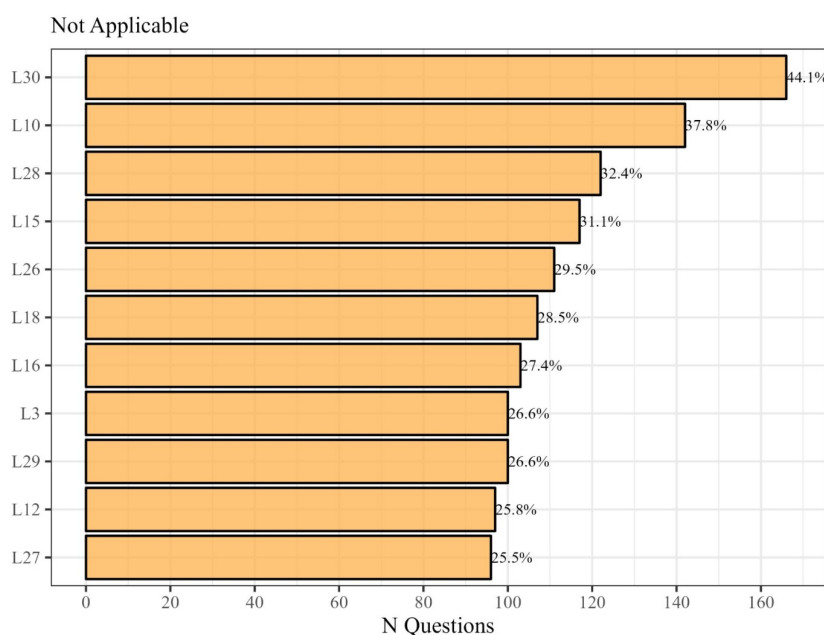
Additionally, two respondents reported not knowing how to answer 8.5% of the questions, representing the highest incidence of this response (Graph 06).

Graph 06 – Respondents with the highest proportion of questions not knowing how to answer them among those that participated in the survey.



The highest proportion of not-applicable questions observed during the present study was 44.1%. Eleven HCBLs (11/32, 34.4%) considered more than 25% of the questions unapplicable (Graph 07). The answers to 23 questions were deemed not applicable for 75% or more respondents.

Graph 07 – Respondents with the highest proportion of questions considered ‘not applicable’ among those who participated in the survey.



6.2.2.2 Discussion

Only questions marked as ‘Not applicable’ were treated as missing and excluded from frequency calculations. The accounting for unanswered questions and those for which respondents expressed uncertainty was essential as they provided valuable insights for this study.

The unanswered questions primarily pertained to budgetary and other financial matters. Three main factors might explain this:

- Data may have been lacking or unavailable;
- Respondents might not have been directly involved in the subject or lacked the initiative or interest to seek such information;
- Some data may have been classified as confidential.

Hence, additional research is imperative to gain a deeper understanding of the financial management of HCBLs, as the information gathered in the present study yielded limited conclusions.

It was assumed that when a respondent selected the option ‘I don’t know,’ it indicated a lack of knowledge or an inability to answer the question. Other explanations may account for blank responses, such as unintentional oversight, a reluctance to share the information, unavailability of data, or insufficient time to retrieve it. It was evident that the extensive number of questions posed a significant obstacle to completing the questionnaire.

Given these considerations and taking into account the respondents’ position in their respective institutes, it is likely that for the three HCBLs who left more than one-third of the questions unanswered, their reasons were not necessarily a lack of knowledge but rather the other factors mentioned earlier.

The motivation to participate in the survey and complete the questionnaire to the best of one’s ability was primarily driven by an awareness of the study’s significance. No form of reward or financial compensation was offered. Sometimes, a video call was scheduled with the respondent to elucidate the study’s objectives and

address any queries. The rate of unanswered questions indicates that this awareness-building effort was partially successful.

Some respondents offered positive feedback regarding their participation in the present study. They found that filling out the questionnaire allowed them to evaluate their facility and procedures, drawing attention to critical points they had not previously recognized.

It is essential to highlight that five of the six HCBLs with over 25% unanswered questions utilized a shorter version of the questionnaire.

Unsurprisingly, the two questions with the highest rate of 'I don't know' responses were related to the design because, in many cases, respondents did not actively participate in this process, mainly when the design had been established several years ago. Additionally, two other questions about the HVAC system also had a notable proportion of uncertain answers, which indicated that many respondents needed to be better versed in these topics, often leaving them to the maintenance staff.

However, two other questions raised some concerns. It was unexpected that over 20% of the respondents could not answer questions concerning legal obligations with the fire department and gaps in the regulations for activities conducted in HCBLs. These are issues that all HCBL workers, regardless of their position, should be aware of as they pertain to critical safety matters. The lack of opinions on regulatory gaps underscores the need to enhance the critical thinking of HCBL users, given that specific gaps are evident and can significantly impact biosafety and biosecurity. Therefore, there is an urgent need to provide training opportunities related to national regulations on this subject.

No respondent selected the 'I don't know' option for more than 8.5% of the questions, which appears entirely satisfactory. This proportion was significantly lower than the number of unanswered questions.

The most common reasons for questions being marked as 'not applicable' pertained to topics related to the usage of experimental animals. It was mainly because most HCBLs participating in this study exclusively conducted in vitro assays.

Another critical reason for 'Not applicable' responses was related to HCBLs in the final phase of construction or commissioning. These facilities were not operational when the questionnaire was filled in, making it impossible to address questions related to aspects such as assessing service providers' quality or implementing specific procedures.

However, other topics also had high rates of 'Not applicable' responses, revealing notable gaps and weaknesses identified in the HCBL participating in this study, as discussed in the following Chapters. These topics included:

- a. Implementing energy and water-saving mechanisms;
- b. Evaluating worker well-being;
- c. Establishing international networking;
- d. Providing training to IT staff.

6.2.3 Respondents' profile

6.2.3.1 Results

Of the professionals who responded to the questionnaire, around half (15/32, 46.9%) were professors or researchers. Other job titles mentioned were federal inspector, BO, pharmacist, technologist, executive assistant, finance manager, general manager, industrial director, lab manager, analyst, supervisor, and technical director. In terms of their role related to HCBL, half of them are the head of the laboratory (16/32, 50.0%), followed by BOs or equivalent (7/32, 21.9%) and managers (6/32, 18.8%). Other roles mentioned were engineer, researcher, and veterinarian (Table A02).

6.2.3.2 Discussion

Although multiple individuals answered the questionnaire in many cases, given the extensive range of questions, there was a designated focal point responsible for compiling and sending the data. As many participants were affiliated with universities and research institutes, it is unsurprising that most respondents were professors, researchers, and often heads of their respective laboratories.

The high-level positions held by the respondents instill confidence in the obtained results. However, it is noteworthy that the number of responses indicating 'I don't know' or providing no answer was higher than expected, considering the respondents' positions and roles. Even laboratory heads apparently lacked knowledge of specific technical aspects or budgetary data.

It became evident that only a few individuals possessed a comprehensive knowledge of biorisk management and other areas such as construction, maintenance, and finances. The apparent lack of knowledge regarding certain subjects among BOs and managers can be attributed to the burden of their tasks, which is a common situation in public institutes where only some individuals are exclusively dedicated to HCBL-related activities. In other cases, it may reflect the high level of specialization and lack of interest in topics beyond one's educational background. For example, researchers or managers with a background in biological sciences may need more interest in maintenance issues, which are more closely associated with engineering sciences. The opposite can also be true.

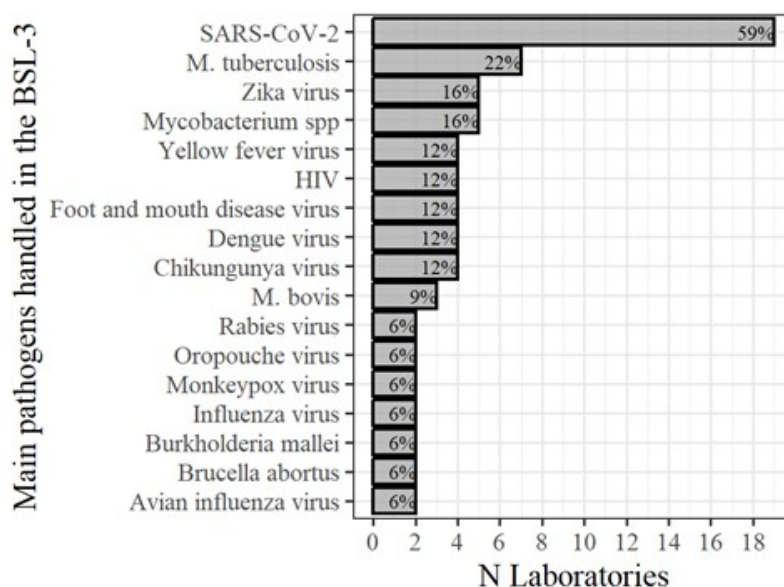
However, a well-functioning HCBL necessitates versatile professionals. Unfortunately, no career path in Brazil encompasses all the requirements of a BO or an HCBL manager.

6.2.4 Laboratories scope and networking

6.2.4.1 Results

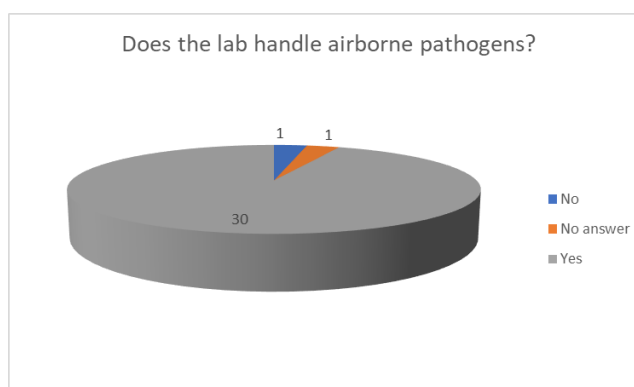
Graph 08 lists the pathogens handled in the participating laboratories. SARS-CoV-2 and *Mycobacterium tuberculosis* were the most often mentioned. The following pathogens appear only once: Adenovirus, ASF virus, *Bacillus anthracis*, Bluetongue virus, *Brucella* spp., *Burkholderia pseudomallei*, CSF virus, *Clostridium botulinum*, *Coccidioides immitis*, *Cryptococcus* sp, Hantavirus, Hepatitis virus, Infectious laryngotracheitis virus, *Leishmania*, Lentivirus, *Mycobacterium avium*, *Mycobacterium leprae*, Mayaro virus, Newcastle disease virus, *Plasmodium* sp, Retrovirus, Rickettsia spp., *Trypanosoma cruzi*, Vesicular stomatitis virus, and *Yersinia pestis*.

Graph 08 – List of most frequent pathogens handled in the laboratories participating in the survey.



All laboratories, except one, handle or intend to handle airborne pathogens. One laboratory did not answer to this question (Graph 09).

Graph 09 – Frequency of HCBLs participating in the survey handling airborne pathogens.



Most laboratories have national coverage (13/32, 40.6%). Less than half of the laboratories (13/32, 40.6%) belong to any national network ('Public Health National Laboratories Network' was the most common), and just five (5/32, 15.6%) to an international one (Table A03). The international networks mentioned were:

- WOAH;
- PAHO/WHO: Pan American Health Organization, cited by two HCBLs;
- Pasteur Institute International Network;
- The Arbovirus Diagnosis Laboratory Network of the Americas (RELDA/PAHO);
- ReLeVA/PAHO: Entomological Virology Laboratories Network of the Americas;
- RESUDIA: South American Laboratories Network for Avian Influenza and Newcastle Disease;
- GABRIEL Network from the Mérieux Foundation.

Two HCBLs stated participating in two networks.

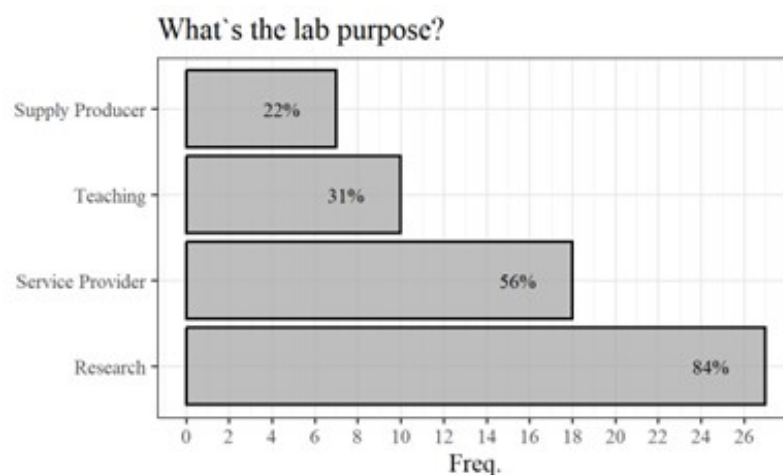
It was possible to detect a statistical difference between the HCBL affiliations regarding participation in national networks. While all of MAPA's HCBLs reported participating in at least one nationwide network, none of the university HCBLs did (Table 05).

Table 05 – Comparative analysis between the affiliation of the HCBLs participating in the survey with their participation in national networks.

Does the lab belong to any national network?	Governmental body to which is linked						Fisher's Exact Test
	Agriculture State Secretariat	Health State Secretariat	MAPA	MCTI	MS	University	
No	1	1	0	1	1	12	< 0.001
Yes	0	3	5	0	5	0	

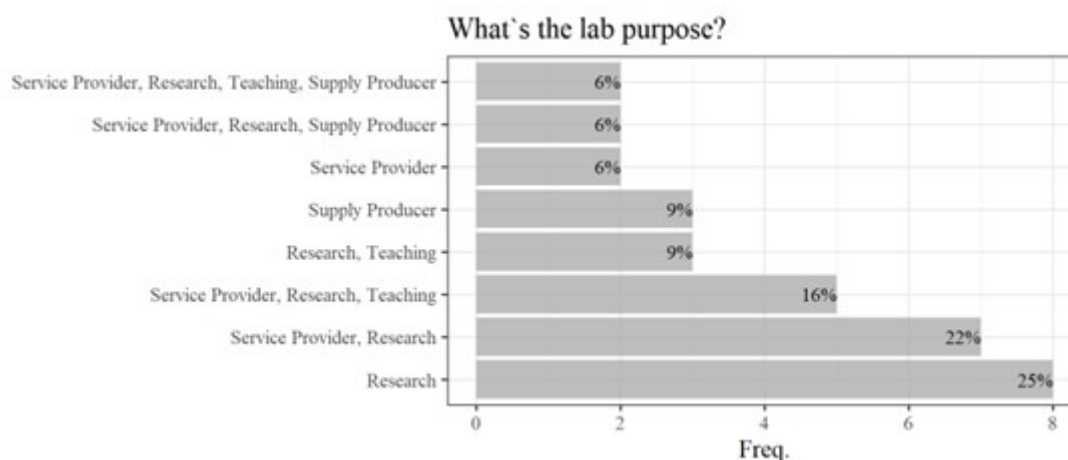
Most laboratories (19/32, 56.3%) have more than one purpose, and 27/32 (84.4%) of the HCBLs do research as part of their mandate. More than half (18/32, 56.3%) of the laboratories provide services like diagnosis, 10/32 (31.3%) are involved in teaching activities, and just seven (7/32, 21.9%) produce supplies like vaccines and reference materials. One-quarter of the HCBLs participating in the survey are primarily research laboratories (8/32, 25.0%) and 19/32 (59.4%) carrying out at least two kinds of activities (Graphs 10 and 11).

Graph 10 – Purpose of the HCBLs participating in the survey^r.



^r HCBLs could provide more than one answer

Graph 11 – Purpose of the HCBLs participating in the survey (detailed answers).



Fifteen of the 32 HCBLs provided information about their primary outputs (15/32, 46.9%). Among them, eleven reported the yearly average number of assays conducted, four provided details on the volume of supplies produced, and seven shared information regarding scientific production outputs.

The data concerning the number of assays conducted displayed notable variability. The range of assays performed annually spanned from 240 to 22,000, with an average of 8,858 and a median of 6,941 (Table A04). Interpreting these findings should be exercised with caution due to the absence of standardization in data collection and variations in the methodologies used to quantify activities across different laboratories. It is important to note that laboratory assays can encompass a wide range of complexity, making direct comparisons challenging.

A similar challenge emerges when assessing the volume of supplies manufactured, as these supplies may encompass various types of products, such as vaccines, antigens, antisera, and other items. Additionally, the number of HCBLs providing such information was limited, rendering the data scientifically inconclusive.

Regarding scientific output, the number of studies conducted within the HCBLs varied from one to ten per year, with an average of 3.2 and a median of 3.

6.2.4.2 Discussion

The only laboratory that stated not handling airborne pathogens is an FMD vaccine manufacturer. The answer is presumed to be incorrect since the FMD virus can be transmitted by aerosols³⁴⁷. Therefore, we can conclude that all laboratories handle airborne pathogens, which is unsurprising since most high-risk pathogens are transmitted via aerosols.

The high number of laboratories handling the SARS-CoV-2 virus resulted from the COVID-19 pandemic and the WHO's declaration of a global health emergency, which was in effect from January 2020 to May 2023³⁴⁸. However, it cannot be guaranteed that all activities related to the virus demand the use of an HCBL. The MS permits diagnostic assays in a BSL-2 laboratory for hematological and serological tests, tissue fixing, molecular biology, clinical pathology, processing of fixed or inactivated samples, electron microscopy, sample inactivation, and other low-risk activities⁹¹. Notably, according to the WHO, only proliferative assays developed with suspected samples of SARS-CoV-2 should be conducted in an HCBL²²⁵⁻²²⁶.

Nevertheless, at the beginning of the COVID-19 pandemic, many institutes that had HCBLs decided to use them for diagnostic purposes, especially for PCR assays, as a precautionary measure since the risks related to the sample handling were not fully understood. Meanwhile, other institutes took advantage of FINEP's Public Call, which provided grants to enhance biosafety in laboratories to renovate their existing HCBLs. The resources were allocated to institutes that already possess biocontainment facilities, at a minimum of BSL-2 level⁷¹.

These findings underscore the importance of HCBLs in emergency contexts and the significance of having flexible facilities. However, conducting low-risk activities in an HCBL could be considered a misallocation of resources.

Similar considerations can be made for work involving *Mycobacterium* spp., an RG 3 microorganism⁹¹ that is the second most frequently handled. According to the 'Guidelines for tuberculosis control in Brazil', conducting bacilloscopy with good microbiological practices presents a low risk of aerosol generation. It can, therefore, be performed on an open bench with adequate room ventilation. However, for other procedures that carry a higher risk of aerosol generation, such as sample preparation for culture, the guidelines recommend the use of an adequate BSC or a 'TB containment laboratory' (which need not necessarily be a BSL-3 facility) when handling cultures for sensitivity or genetic probe tests³⁴⁹.

Arboviruses, such as the Zika virus, and viruses associated with dengue, chikungunya, and yellow fever, form a significant group of viruses commonly found in Brazilian HCBLs. It was expected, given the importance of these diseases in the country. Although all of them are classified as RG 2 pathogens, the Brazilian regulation determines that when working with the wild yellow fever virus using techniques that produce a significant volume of infectious material and aerosols, it is advisable to incorporate additional safety measures into the procedures. In activities with high viral loads or substantial volumes, the wild yellow fever virus requires a BSL-3 facility. Additionally, manipulating the chikungunya virus using techniques that generate a significant volume of infectious material and aerosols should be done in BSL-3 laboratories⁹¹.

Further studies are necessary to evaluate whether activities conducted in Brazilian HCBLs involve manipulating arbovirus, SARS-CoV-2, and *Mycobacterium* spp. require BSL-3 laboratories or whether they could be performed in lower biosafety containment levels. The same concern applies to other RG 2 pathogens handled in HCBLs, as identified in the current survey, including Adenovirus, *Cryptococcus* sp., Hepatitis virus, *Leishmania* sp., Mayaro virus, *Plasmodium* sp., Retrovirus, *Trypanosoma cruzi*, and Oropouche virus.

Clostridium botulinum, *Bacillus anthracis*, *Rickettsia rickettsia*, *Salmonella enterica* subsp. *enterica* ser. *Typhi*, Hantavirus, and *Francisella tularensis* are classified as RG 3 pathogens⁹¹, and their associated diseases require notification¹⁴⁶. Surprisingly, these pathogens were omitted or mentioned just once in the current survey. Given their significance, one would have expected to find more HCBLs actively engaged in research involving them.

The most frequently handled animal pathogens include FMDv, *Mycobacterium bovis*, *Brucella abortus*, avian influenza virus, and *Burkholderia mallei*. These are associated with notifiable diseases²³⁰ and are considered RG 3 pathogens according to the e-pathogen website²²⁸. Some laboratories that handle FMDv are involved in vaccine production, and they will likely be closed when vaccination is no longer mandatory. The present study did not identify any laboratories handling other significant RG 3 animal pathogens, such as equine

encephalitis virus and Aujeszky disease virus. It may indicate the country's need for more dedicated HCBLs for animal diseases.

Notably, the absence of certain pathogens of interest for public and animal health in HCBLs does not necessarily mean they are not being handled in laboratories across the country. Due to governance gaps in the storage, transfer, and handling of these pathogens, it is possible that institutes not adhering to the minimum biosafety requirements are working with high-risk pathogens.

It is important to note that Brazil has no ABSL-3Ag laboratories^s. It is a significant vulnerability for the country because these facilities are necessary to conduct in vivo research (such as the development of new vaccines and drugs) and to produce supplies and reference materials such as antiserum for high-risk animal diseases (e.g., CSF, ASF, and FMD), including zoonoses (e.g., HPAI, anthrax, and brucellosis). The same can be stated regarding the absence of BSL-4 laboratories necessary to handle RG-4 viruses, like Ebolavirus, Marburgvirus, Hendra henipavirus, Nipah henipavirus, Crimean-Congo hemorrhagic fever orthonairovirus, and the Brazilian mammarenavirus (known as Sabia virus).

Other studies conducted worldwide have found a higher percentage of BSL-3 laboratories handling Hantavirus, *Bacillus anthracis*, *Yersinia pestis*, *Clostridium botulinum*, and CSF virus in Asia^{57, 350}; *Brucella melitensis*, *B. suis*, *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, and *Burkholderia pseudomallei* in the US¹⁹³, *Bacillus anthracis*, Viral Haemorrhagic Fever viruses (like Rift Valley phlebovirus), and West Nile virus in Africa^{211, 351}.

In studies carried out in Brazil before the COVID-19 pandemic, the authors found that *Mycobacterium tuberculosis* was the most frequently handled pathogen in HCBLs^{74, 313}. Still, one of the authors also found a high frequency of rabies virus, virus related to respiratory diseases, and *Yersinia pestis*³¹³.

The pathogens most handled in HCBLs usually represent the most significant public and animal health threats during a given time. Although the COVID-19 pandemic heavily influenced the data obtained, the BFG, particularly MS, and MAPA, should evaluate whether the list of pathogens and their proportions found in the present study align with national priorities and if the HCBLs are being utilized appropriately. Furthermore, it is worth noting that *Bacillus anthracis* appears on the list of most frequently handled pathogens in HCBLs worldwide but was only mentioned once in the present study.

The significant proportion of laboratories with international coverage (21.9%) reflects Brazil's leadership in the region and underscores these facilities' high level of responsibility. Due to the high expenses associated with the construction and operation of HCBLs, many are established within national and international reference institutes. Therefore, most laboratories (62.5%) have national or international coverage.

^s In the present study, the classification of laboratory biosafety levels from the 6th Edition of the BMBL was adopted¹. According to this manual, ABSL-3Ag is required for animals housed in open cages or pens infected with specific transboundary livestock or wildlife pathogens. There is sometimes confusion related to this classification, called 'BSL-4 OIE' in Brazil. This terminology is outdated and stems from former editions of the WOA's Manual of Diagnostic Tests and Vaccines for Terrestrial Animals⁹, considered the maximum-containment level for veterinary laboratories. It had distinct requirements compared to the BMBL definition for BSL-4 laboratories. 'BSL-4 OIE' laboratories necessitate additional procedures and structural features compared to a BSL-3 facility. The Brazilian regulation has retained the term 'BSL-4 OIE' to describe the requirements for handling the FMD virus²³¹. Although the current edition of the WOA's Manual no longer uses the BSL classification, the Brazilian regulation has not been updated to align with the WOA's current concept, which emphasizes risk assessment rather than using a BSL classification.

Unfortunately, most laboratories (17/32, 53.1%) do not belong to any national or international network. The scenario is more severe in universities.

Laboratory networks are essential in enhancing the laboratories' capabilities, especially in laboratory biosafety and biosecurity, as shown in Chapter 2.12 of the current study. According to the US National Biodefense Strategy and Implementation Plan, networking is crucial to getting a rapid response and limiting the impacts of incidents¹⁹⁵. There are some initiatives of MS and MAPA to establish their laboratory biosafety networks, but there is no network integrating laboratories from different bodies. Hopefully, the National System of Research Infrastructures with Biosafety recently created by MCTI²⁶⁴ can do that.

The RAV3N is an example of an HCBL network with the participation of laboratories from universities, USDA, HHS, and the US Department of Defense. Some of their activities include periodical conferences and webinars. The network has four working groups: Workforce Development and Capacity Building; Animal Husbandry and Health Management in Containment; Science Excellence and Capacity; Facilities Operations, Risk Management, and Compliance. These groups meet at least monthly to discuss common issues²⁷⁰.

In addition to these activities, a national HCBL network could provide discussions on the standardization of biosafety and biosecurity protocols and procedures, presentations and discussions on scientific publications, interchange of initiatives, creative solutions for typical problems, paperwork, etc.

Research is the primary purpose of Brazilian HCBLs, followed by providing services. The findings align with a survey conducted on 528 US BSL-3 laboratories, where 71.8% were engaged in research, 44.9% in diagnostics, and 4.5% in supplies production³⁴⁵.

A survey among African BSL-3 laboratories belonging to the EDPLN revealed that their primary function was diagnostics, with a majority (11 out of 13) also serving a research role. Only one facility reported involvement in antigen production²¹¹.

The primary goals of the EDPLN are to contribute to outbreak response, preparedness, and the rapid development of diagnostic assays for emerging and infectious pathogens worldwide. Thus, it is unsurprising that, except for two laboratories, all others provide diagnostic services and engage in research activities.

Diagnostics and research are globally recognized as the most critical purposes of HCBLs, and the results obtained in this study indicate that Brazil is no exception. However, considering the associated risks, it is concerning that over 30% of Brazilian HCBLs participating in the present survey are engaged in teaching activities, including those for undergraduate students (Cláudio Mafra, personal communication). Notably, according to international recommendations, only individuals with adequate training should be permitted to access an HCBL, making BSL-2 laboratories more suitable for capacity-building activities.

Another aspect that should be considered in this context is the lack of regulations for research in HCBLs in Brazil. The results of this study draw attention to the high percentage of HCBLs being utilized for research purposes, highlighting the need for the BFG to establish oversight mechanisms to address DURC.

Further studies are essential to evaluate the extent of activities undertaken by Brazilian HCBLs. The data collected in the present study do not permit conclusive insights, given the variations in the types and complexities of methodologies employed by the laboratories. A comprehensive study would prove invaluable in determining whether the current facilities are operating at maximum capacity or are

being underutilized. Examining the volume and significance of these activities, which includes scientific production and outcomes, is crucial for deliberating on the sustainability and strategic aspects of HCBLs.

6.2.4 Laboratories design and construction

6.2.4.1 Results

Only three laboratories (3/32, 9.4%) provided information about the design cost. One laboratory (1/32, 3.1%) considered this information confidential, and the design cost was included in the construction cost for three laboratories (3/32, 9.4%). Nearly all laboratories (28/30, 93.3%) continued to carry out most or all the activities planned initially during the design phase. In most cases, new laboratories were built (19/32, 59.4%), but a significant percentage involved expansion or remodeling (12/32, 37.5%), and over one-third of the constructions (12/33, 37.5%) included additional facilities alongside the BSL-3 laboratory. There were no negative responses to the question, 'Does the lab conduct periodic certifications/qualifications of the equipment/systems?'. However, three respondents (3/30, 10.0%) did not answer, and two (2/32, 6.2%) considered this question not applicable since the laboratory had not been launched yet. The percentage of respondents who identified relevant shortcomings in the design (11/32, 34.4%) was similar to those who did not (12/32, 37.5%) (Table A05).

The design shortcomings mentioned by the respondents included:

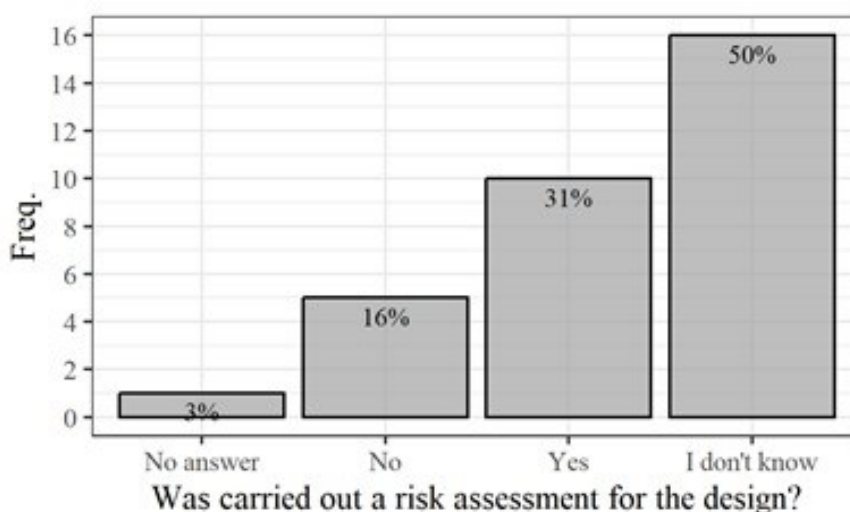
- a. The laboratory is located in a region that is difficult for service providers to access;
- b. The design phase did not account for financial resources for operation, training, and maintenance;
- c. The absence of showers in the changing room and sealed doors limit the usage of the laboratory for specific aerosol-transmitted pathogens;
- d. During cold days, the water in the first shower of the day is freezing;
- e. Structural issues, mainly related to electricity supply, have arisen due to a lack of communication between the construction companies involved;
- f. EDS and filter boxes are present in the same room;
- g. The laboratory lacks automation for EDS, access control, autoclave, and showers;
- h. There is no redundancy for the EDS;
- i. The EDS could be made more efficient;
- j. The laboratory lacks an EDS;
- k. There is no backup power system for BSCs or EDS;
- l. Some doors lack emergency opening devices;
- m. Some sealed doors were installed too close to the wall, which compromises the durability of the hoses;
- n. The autoclave lacks a safety valve for pressure relief;
- o. The mechanical part of the autoclave is located outside the biocontainment area;
- p. The pass-through does not have an automatic decontamination system;
- q. There is no monitoring of HEPA filter saturation linked to the automation system;
- r. The placement of air supply spots in the rooms could be improved;
- s. The dampers are not entirely sealed or pneumatic;
- t. The vivarium is not located in the room with the most negative air pressure;
- u. There is only one HVAC system for the entire laboratory, including the animal room;
- v. There are issues with the air pressure cascade;
- w. There are problems with air refrigeration;
- x. The vivarium airlock does not have sealed doors;

- y. The airlocks do not have sufficient space for donning and doffing PPE;
- z. The airlock is undersized;
- aa. There is no airlock for decontamination and equipment removal;
- bb. There are multiple workstations in the same room;
- cc. The ABSL-3 facility lacks a necropsy room;
- dd. There is dead space between the ceiling and the roof;
- ee. The type of flooring used needs to be improved.

The cost of construction of the HCBLs participating in the present study varied from R\$ 4,019.71/m²^t to R\$ 104,774.42/m²^u for new laboratories and from R\$ 10,000.00/m²^v to R\$ 46,666.67/m²^w for expansion/modeling. The average cost of the construction of the laboratories was R\$ 46,467.19/m²^x and R\$ 20,697.60/m²^y for new laboratories and expansion/modeling, respectively (Table A06).

Only 31.2% of the laboratories (10/32) conducted a risk assessment for the design (Graph 12).

Graph 12 – Proportion of the HCBLs participating in the survey that carried out a risk assessment during the design period.



The number of laboratories that were not inspected by an independent company during construction (12/32, 37.5%) was higher than those that underwent inspections either throughout or partially during the construction period (10/32, 31.2%) (Graph 13).

^t Equivalent to USD 791.22/m², as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^u Equivalent to USD 20,623.26/m², as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

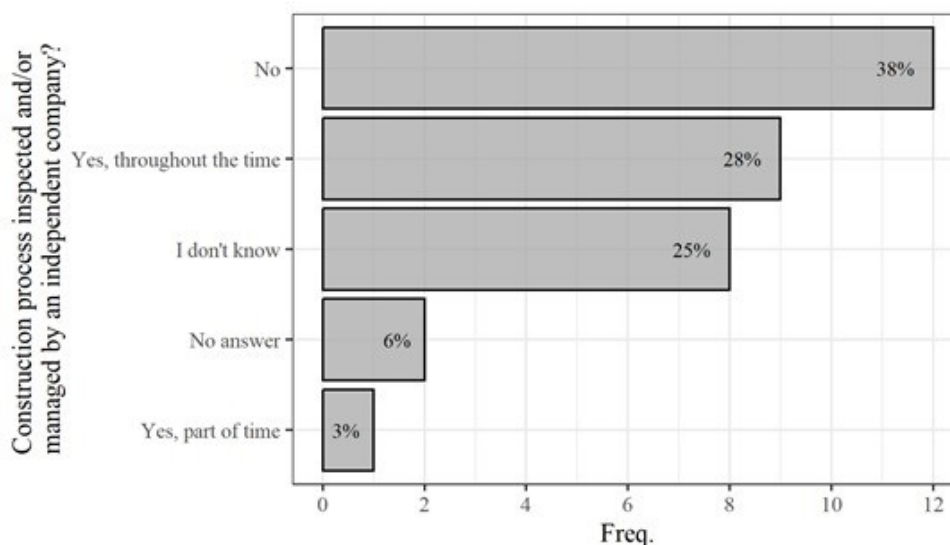
^v Equivalent to USD 1,968.35/m², as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^w Equivalent to USD 9,185.63/m², as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^x Equivalent to USD 9,146.36/m², as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^y Equivalent to USD 4,074.01/m², as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

Graph 13 – Proportion of the HCBLs participating in the survey that had their construction inspected or managed by a third-party company.



Some HCBLs that did not hire an independent company to conduct the inspection described how they monitored the construction. The following patterns were identified regarding the staff responsible for the monitoring process:

- The internal multidisciplinary committee designated for this purpose;
- Institute's engineering department;
- The organization's engineering central department;
- Engineering committee with representatives from different organizations;
- Informal inspection by the laboratory's employees;
- The company responsible for the design;
- Engineer designated by the constructor;
- Experts appointed by the company in charge of the financial resources management;
- Representatives from the organization's headquarters periodically visited the construction site.

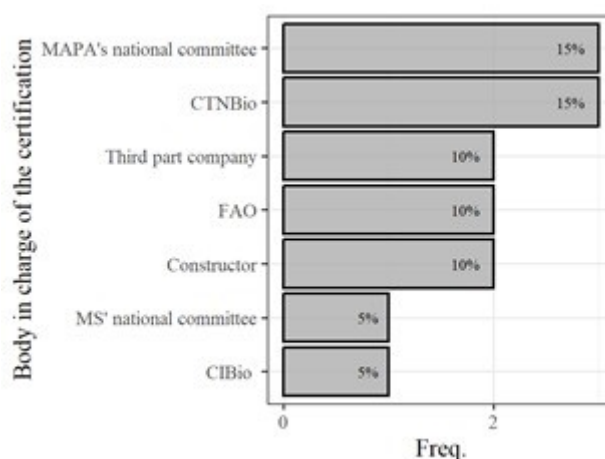
The percentage of HCBLs commissioned (18/30, 60.0%) slightly exceeded those certified before launch (15/29, 51.7%). None of the HCBLs has been certified without previous commissioning. There was no correlation between commissioning and the kind of construction, as shown in Table 06.

Table 06 – Correlation analysis between the frequency of HCBLs participating in the survey that reported being commissioned and their kind of construction.

Mark the most appropriate response to the kind of construction	Was the lab commissioned?		Fisher's Exact Test
	No	Yes	
Expansion or remodeling	1	7	1.000
New Lab	2	11	

Of the 15 laboratories that claimed to be certified (15/29, 51.7%), three did not disclose the responsible entity for the certification. Among the remaining, seven HCBLs were certified by official committees or bodies, one by the IBC, two by the constructor, and the other by a third-party company. The MAPA's National Committee was the most commonly responsible for the HCBLs certification (Graph 14).

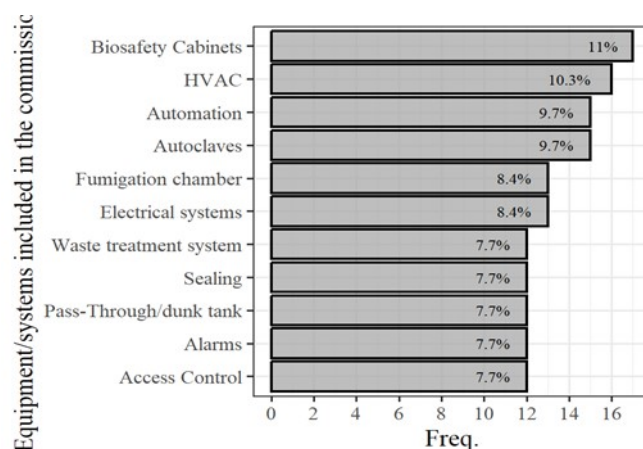
Graph 14 – Proportion of bodies reported as responsible for certifying the HCBLs participating in the survey.



Seven out of the 15 HCBLs (7/15, 46.7%) reported renewing their certification, with the majority doing so annually (4/7, 57.1%). One (1/7, 14.3%) indicated that the re-certification interval extends beyond two years, while the remaining two (2/7, 28.6%) did not provide this information. Some HCBLs that were not certified indicated that the constructor assigned the BSL designation (2/12, 16.7%). In contrast, others reported self-assignment (1/12, 8.3%), and for two HCBLs (2/12, 16.7%), the BSL was assigned by an MS committee. Six HCBLs (6/12, 50.0%) did not provide an answer to this question, and two (2/12, 16.7%) were unsure of the answer.

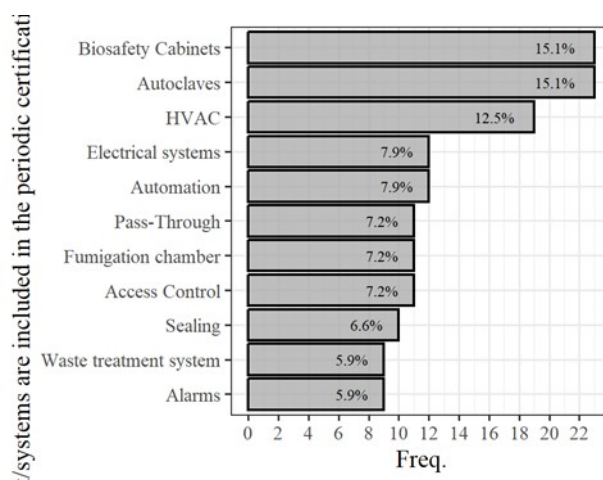
The BSCs and HVAC systems were the most mentioned systems/equipment included in the commissioning process (Graph 15).

Graph 15 – Proportion of equipment/systems contemplated in the commissioning process of the HCBLs participating in the survey.



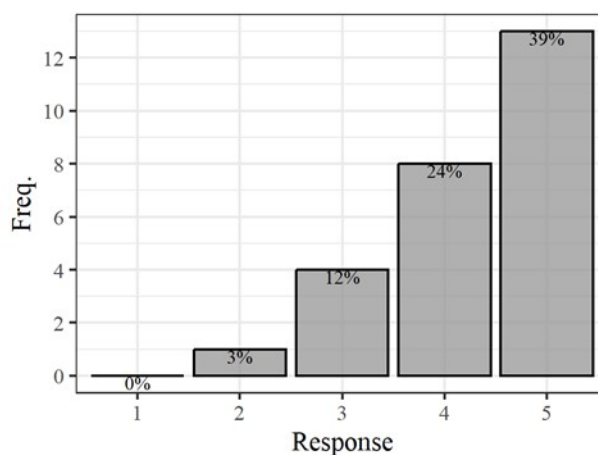
In contrast, BSCs, autoclaves, and HVAC were the most said systems/equipment in the periodic certification process (Graph 16).

Graph 16 – Proportion of equipment/systems contemplated in the periodic certification process of the HCBLs participating in the survey.



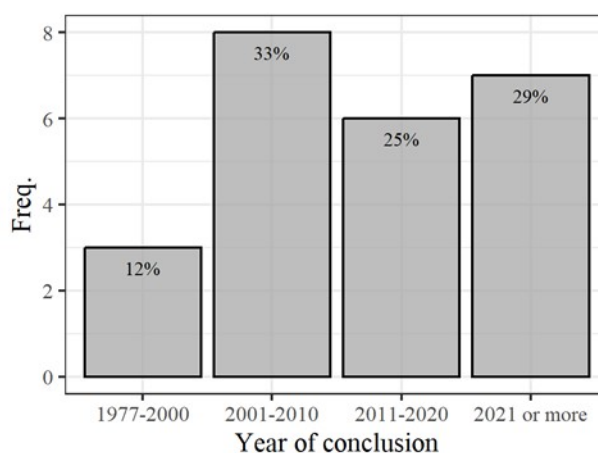
Of the HCBLs surveyed, the majority (21/32, 65.6%) rated the quality of roads providing access to the laboratory as high, indicating a score of 4 or 5 on a scale ranging from 1 (very bad) to 5 (excellent) (Graph 17).

Graph 17 – Results from evaluating the quality of roads providing access to the HCBLs participating in the survey^z.



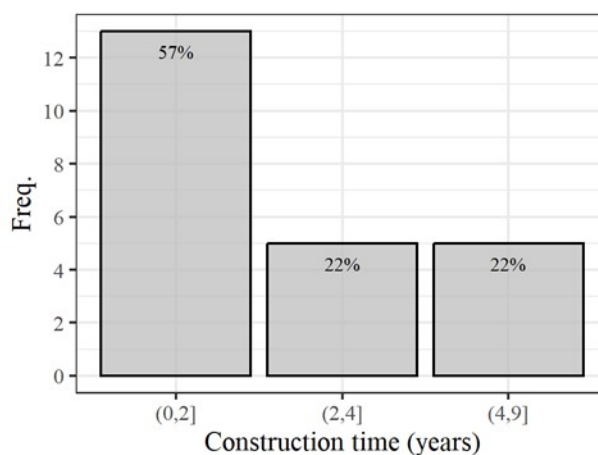
Most laboratories (21/24, 87.5%) were built after 2001 (Graph 18), and in most cases (13/23, 56.5%) the construction period was less than two years (Graph 19).

Graph 18 – Results regarding the completion year for the construction of the HCBLs participating in the survey.



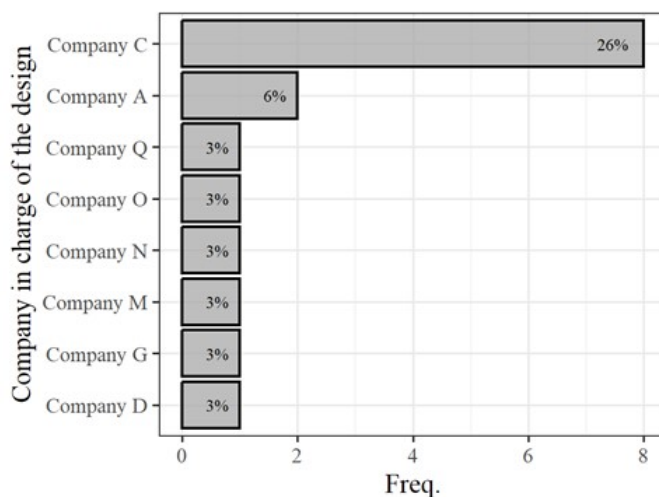
^z Scale ranging from 1 (very bad) to 5 (excellent).

Graph 19 – Results regarding the time to construct the HCBLs participating in the survey.

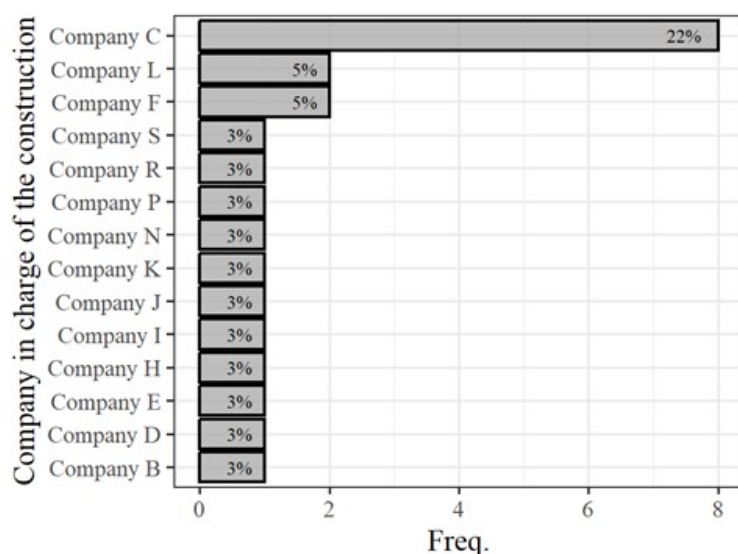


Among the laboratories that provided the name of the company responsible for the design, one company was mentioned eight times (8/16, 50.0%) (Graph 20). This same company was also responsible for constructing all these eight HCBLs (Graph 21).

Graph 20 – Results regarding the company in charge of the design of the HCBLs participating in the survey.

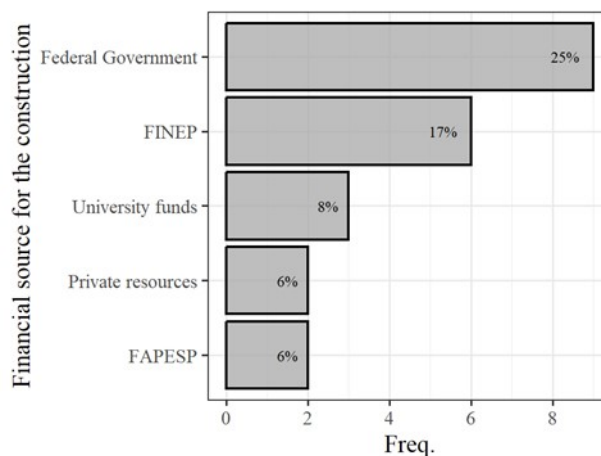


Graph 21 – Results regarding the company in charge of the construction of the HCBLs participating in the survey.



The BFG was the most common source of financial support for construction. Among the laboratories that answered this question, 9/27 (33.3%) mentioned the BFG, followed by FINEP, university funds, private resources, and FAPESP (Graph 22).

Graph 22 – Proportion of primary sources of financial support for the construction of the HCBLs participating in the survey.



Other sources mentioned once included BNDES (*Banco Nacional de Desenvolvimento Econômico e Social* – National Bank for Economic and Social Development), EMBRAPA, FAPEMIG (*Fundação de Amparo à Pesquisa do Estado de Minas Gerais* – Minas Gerais Research Foundation), research grant, and State Government.

6.2.4.2 Discussion

6.2.4.2.1 Risk assessment during the design phase

A comprehensive risk assessment should be incorporated in the HCBL design paperwork, as highlighted by Salerno & Gaudioso (2015). According to these authors, understanding the risk of working with hazardous biological materials prior to constructing or renovating a laboratory is vital to ensure the success of an effective biorisk management program. The design of the laboratory and the containment measures implemented can negatively impact a biorisk management program. Therefore, design decisions and selection of specific containment measures should be based on a comprehensive biorisk assessment as opposed to defaulting to predefined solutions that may not be suitable in all cases⁷⁶.

The data obtained in the present study indicate that the design documentation of most Brazilian HCBLs lacks a comprehensive risk assessment. This finding highlights the necessity of transforming the culture within responsible design companies, ensuring this document is incorporated into all projects. Additionally, there is a need to foster a culture shift within the institutes that should demand the inclusion of this document from the designing companies.

The risk assessment during the design phase should involve a multidisciplinary team, including researchers and other users. However, it is unfortunate that HCBL staff often do not actively participate in discussions with designers and construction companies during the preliminary stages of the project. As a result, they may lack awareness of the guidelines and regulatory requirements being employed^{211, 313}.

Furthermore, it is essential to note that, as stated by Patterson et al. (2014), when assessing risk, the typical assumption is that the operational lifespan of a facility is approximately 50 years. It is expected that beyond these 50 years, advancements in construction technology, building operations, safety systems, and ongoing improvements will render the results of the current analysis no longer applicable¹⁰².

6.2.4.2.2 Design and construction cost

It was impossible to analyze the data related to the design cost since 90.6% of the laboratories did not provide this information. The high percentage of non-responses (40.6%) and lack of knowledge (37.5%) demonstrates that such information is not readily available in Brazilian HCBLs, making establishing a national data bank challenging. It should be noted that public resources funded most laboratories through public bids and grants, which means that this information is typically available in public documentation. However, it is essential to note that older processes were not conducted electronically, making obtaining the data more challenging. Furthermore, three laboratories (9.4%) did not provide this information because the design cost was already included in the construction cost. This justification is illogical since even when the same company is responsible for the project and construction, the design cost should be explicitly identified in the budget.

The available literature provides limited information on the design and construction costs of BSL-3 laboratories in Brazil and worldwide. Due to the diverse characteristics of such constructions and the lack of regulatory requirements, the costs are expected to vary significantly. For instance, Ssengooba et al. (2015) reported that a 29.25m² BSL-3 laboratory constructed in Uganda cost USD 372,324.00. The construction expenses accounted for USD 130,000.00, while the

equipment costs reached USD 242,324.00. This laboratory focused on TB diagnosis and was completed within six months. It did not include an EDS⁹⁷.

In another study, Bridges et al. (2014) estimated the construction cost of a BSL-3 laboratory in Zambia to be around USD 200,000.00, with a projected construction timeline of 6 to 9 months³⁵².

The cost of constructing a BSL-3 laboratory in Mexico varies from USD 9,500 to USD 13,500/m², while the operational cost is estimated at USD 643/m² (Luis Liñares, personal communication).

Heckert et al. (2011) highlighted that while the requirement to identify and control disease outbreaks has increased diagnostic capacity, unfortunately, no corresponding increase in biosafety and biosecurity capacity has occurred, especially in countries with limited resources. According to these authors, while all countries need to operate HCBLs, not all countries have the ability or resources to apply appropriate biosafety and biosecurity principles and practices to laboratory operations. They concluded that engineering solutions should be local, practical, and sustainable and that collaboration is required to develop solutions appropriate for the specific needs and available resources within any given country⁵⁶.

Six HCBLs in Brazil were built according to a 'low-cost' concept, as detailed by Hernandez (2008)⁶⁶. Maehira & Spencer (2019) argued that there is a dilemma concerning whether there is a regulatory conflict in accepting laboratory buildings and facility infrastructure utilized in low and middle-income countries by personnel from those advanced countries where such buildings or facilities would not be accepted. This paper refers to maximum-biocontainment laboratories, but the concern may be the same for HCBLs. The authors concluded that harmonizing biosafety and biosecurity is challenging. However, occupational safety and health management standards should be pursued on a single, commonly adopted policy, for which a detailed factual and normative analysis of institutional guidelines and regulations can provide pragmatic insights²⁷⁴.

The same term 'low cost' for HCBLs was found on the website of a Brazilian constructor company, which stated that:

*'After years of researching and developing materials and products, the company has successfully integrated quality and technology while maintaining affordable prices for the construction of accessible BSL-3 biosafety laboratories, thereby transforming biosafety projects into reality. Our cost-effective BSL-3 biosafety laboratories encompass all the essential features required to comply with national and international biosafety regulations, ensuring a biosecure environment for executing your activities and procedures. By employing a modular construction concept, the company provides low-cost biosafety laboratories that can be customized to fit any available physical space and building structure'*³⁵³.

Hernandes (2008) stated that the cost of three 'classical' HCBLs constructed in Brazil between 2003 and 2007 ranged from R\$ 521,663.00^{aa} to R\$ 1,250,860.00^{bb}. Additionally, the cost of three 'low-cost' HCBLs built between 2004 and 2007 varied

^{aa} Equivalent to USD 294,508.55, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2007.

^{bb} Equivalent to USD 706,181.90, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2007.

from R\$ 100,435.00^{cc} to R\$ 150,000.00^{dd}. According to the author, it is possible to reduce the construction cost of HCBLs when the risk assessment allows the relinquishing of certain concepts and omitting of specific equipment such as sealed doors, redundant HEPA filters and exhaust fans, 100% air recirculation, EDS, and shower facilities in dry laboratories, and pass-through and double-door border autoclaves⁶⁶.

It is worth noting that the budget for the projects related to the 2020 FINEP's Public Call to adequate BSL-3 and ABSL-3 facilities, including upgrading BSL-2 and ABSL-2 ones, varies between R\$ 289,923.00^{ee} and R\$ 3,558,566.00^{ff 354}.

Since Brazil has no regulation or standard defining the constructive requirements for HCBLs, it is impossible to evaluate if the concept of 'low-cost' HCBL applied by Hernandez (2008)⁶⁶ and the company aforementioned³⁵³ is adequate.

While Brazil does not establish a regulatory framework for HCBL design and construction, there will be a risk of giving up safety requirements to reduce costs, especially in institutes where the bidding process is based on costs. According to the data obtained in the present study, more than one-third of the HCBLs identified relevant shortcomings in the design (34.4%), indicating that more attention should be given to this phase when designing new HCBLs in the country.

In the absence of national regulation on constructive requirements, institutes should develop technical solutions in compliance with a consensus of the most relevant international biosafety regulations²⁹⁰.

Emery et al. (1997) documented a situation in the US involving specific laboratories handling RG 3 pathogens in BSL-2 facilities in 1996-1997, a period predating the establishment of the FSAP program. Most of these laboratories were working with *Mycobacterium tuberculosis* and HIV. At that juncture, recognizing that some existing laboratories could not meet the facility-based criteria for BSL-3, the CDC and NIH introduced an option for achieving BSL-3 status by employing BSL-2 facilities and rigorously adhering to BSL-3 protocols³⁵⁵. The BFG may consider such an alternative approach, contingent upon a risk assessment of the activities conducted in specific laboratories, particularly those affiliated with the EBSEH network. This consideration can occur either in the absence of the publication of the PNBB or as an alternative during a transitional period for aligning with a new regulatory framework.

Another potential solution worth considering is the acquisition of modular HCBLs as an alternative to traditional construction. Their advantages and limitations were enumerated in Chapter 2.2 of this study.

6.2.4.2.3 Design shortcomings

The fact that only two HCBLs have made significant changes to the activities outlined in the design may be explained by the following possibilities:

a. The work plan developed during the design phase was well-executed and aligned with both current and future demands;

^{cc} Equivalent to USD 56,701.29, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2007.

^{dd} Equivalent to USD 84,683.57, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2007.

^{ee} Equivalent to USD 55,789.83, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2020.

^{ff} Equivalent to USD 684,774.18, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: December 31, 2020.

- b. The HCBL is new;
- c. The work plan was overly generalized, lacking specific details like the pathogens to be handled and methods to be applied in the laboratory;
- d. The respondents did not consult the original work plan when answering this question;
- e. The facility needs more flexibility to accommodate new demands.

The last option appears to be the least likely since most laboratories were involved in dealing with SARS-CoV-2, which was not anticipated in the original work plan. Additionally, considering that only 31.2% of HCBLs conducted a risk assessment during the design phase, option 'a' seems improbable. Options 'b', 'c', and 'd' appear to be the most plausible explanations.

The most common design shortcomings mentioned by the respondents were related to the EDS, HVAC system, and airlocks/pass-throughs. These findings serve as a valuable alert to design and construction companies, urging them to pay closer attention to these systems and equipment in future projects. Given that a significant portion of the respondents were able to identify relevant design shortcomings, it is crucial to emphasize the critical nature of the design phase. Managers should allocate additional effort and time to this step to prevent unnecessary expenses during construction and mitigate potential biosafety and biosecurity concerns during operation. It is a reminder that investing in a thorough and meticulous design phase can yield substantial long-term benefits and ensure the project's overall success. As stated by Heckert et al., 2011, unfortunately, there are too many examples of non-operational facilities and those that cannot be operated safely due to design or construction failure⁵⁶.

Appelt et al. (2020) identified similar concerns in European BSL-3 laboratories, with only 12 out of 25 (48.0%) of the surveyed laboratories found to be in full compliance with design and infrastructure requirements according to the Integrated European Checklist for Laboratory Biorisk Management³⁵⁶.

One significant factor impacting a project's quality is whether it entails constructing a new laboratory or renovating an existing BSL-2 facility to meet BSL-3 requirements. In our current study, most HCBLs were newly built. This scenario contrasts with the findings of Vieira (2008), who noted that most Brazilian HCBLs examined in 2008 were repurposed from spaces initially designed for other uses. In such instances, Vieira (2008) argued that these facilities faced limitations in terms of structure and layout, often constrained by the available space. This limitation, in turn, hindered the ability to establish a more suitable configuration for HCBL operations, impacting both the containment perimeter and the provision of utilities and services. Furthermore, this author also identified instances of underutilized spaces⁷³.

Sometimes, space constraints within an institution or cost considerations prompt managers to retrofit a BSL-2 facility rather than construct a new one. However, conducting a comprehensive and evidence-based cost-effectiveness analysis is crucial when making such decisions. This analysis should involve a multidisciplinary team of experts, including engineers and BOs, to mitigate the risk of structural issues that could compromise biosafety and biosecurity. The findings of this analysis should be meticulously documented as part of the preliminary studies, clearly outlining the advantages and disadvantages of each alternative.

The report concerning the Ethiopian government's plans to construct a BSL-3 National Reference Laboratory and associated facilities at the Ethiopian Public Health Institute is an excellent example of the preliminary studies that should be conducted before designing an HCBL. This report considers crucial aspects such as

a desk review of pertinent documents, interviews with specialists, site assessments and observations, public and stakeholder consultations, and secondary data collection to establish the environmental and socio-economic baseline conditions³⁵⁷. These comprehensive preliminary studies are essential for informed decision-making and ensure that the subsequent design phase aligns with the specific requirements and considerations of the project.

6.2.4.2.4 Facility location

The location of the laboratory is a crucial aspect that needs to be addressed during the preliminary studies. Several criteria should be considered, including the impact of highway and road quality on the accessibility of HCBLs, particularly in remote areas of Brazilian territory. The present study's findings revealed that only a few HCBLs face serious road access issues, likely because most laboratories were constructed in major urban centers such as São Paulo and Rio de Janeiro cities and prominent universities. These results are comparable to those obtained by Vieira & Salgado (2008), who concluded that most of the surveyed HCBLs at that time were satisfied with the security aspects concerning the location and access to the laboratories⁷³.

In the early days of constructing maximum-biocontainment facilities, there was a prevailing notion that safety could be ensured by situating them in remote island locations. However, while this approach instills a strong perception of safety, it comes with significant disadvantages and additional operational costs. As early as 1998, Murray (1998) acknowledged that a combination of primary and secondary barriers provides the best security as the understanding of biocontainment principles grew³⁴².

While some authors argue that HCBLs should be established in rural and low-density areas to minimize the risk of virus spread in the event of a breach³⁵⁸, others contend that locating them in urban centers offers advantages in terms of recruitment and retention of highly skilled technical and maintenance staff, as well as access to specialized service providers³⁵⁹.

During an International Conference on Risk Communication Strategies for BSL-4 Laboratories, it was observed that:

*'ambivalent perceptions can result if a facility is embedded within an urban setting surrounded by schools, traffic, and private dwellings; it implies the facility is so safe that further distancing measures are unnecessary. Conversely, if a BSL-4 facility is very isolated, it could mean that distance is the only way to keep the public safe, resulting in the somewhat ambivalent message that while it is safe, it is safer still if kept away from the public'*¹¹².

Although some of these comments were explicitly related to BSL-4 laboratories, similar reasoning can be applied to HCBLs. Even if a certain level of isolation is desired (acknowledging that accidents can occur even in well-equipped facilities with good biosafety and biosecurity measures), the quality of road access remains a critical consideration during the preliminary studies.

6.2.4.2.5 Construction monitoring and laboratory commissioning procedure

Effective construction monitoring plays a vital role in complex projects like an HCBL. It helps ensure alignment between the design and construction and guarantees the use of high-quality materials. However, due to limited engineering

staff in most institutes, full-time supervision becomes challenging. Therefore, it is highly recommended to engage a third-party company to oversee the construction process. Unfortunately, this practice is not widely adopted in Brazil, as only one-third of the HCBLs underwent inspection by an independent company during construction.

The respondents mentioned several alternative solutions for construction inspection. While hiring a third-party company is the optimal choice, other feasible options for less complex projects include establishing internal multidisciplinary committees or arranging periodic visits from representatives of the central engineering department. However, specific solutions mentioned are deemed unacceptable, such as inspections conducted by engineers designated by the constructor (due to conflicts of interest) or by non-engineering staff lacking expertise. The absence or inadequacies in the monitoring process are likely to result in significant deficiencies in the construction, consequently posing a high-risk of biosafety issues upon completion.

It is important to highlight that many HCBL users, upon receiving permission to work in these laboratories, often lack adequate or comprehensive training to operate within such environments. As a result, they may not fully comprehend the risks associated with these spaces, leading to a dangerously false sense of safety and security.

The commissioning procedures for HCBLs should be integrated into the preliminary studies and thoroughly documented in the design phase. At least two recent international standards outline the necessary tests during the commissioning phase: Chapter 5 of the 'Canadian Biosafety Standard'⁸⁰ and the Swiss 'Recommendation on Structural and Technical Safety Measures in BSL-3 Laboratories'²⁸⁹. However, due to the need for more regulations about HCBL commissioning in Brazil and worldwide and the scarcity of expertise in Latin America, it is advisable to visit laboratories worldwide to gather insights into their commissioning procedures³²⁰.

The present study highlights a concerning finding regarding the relatively low proportion of HCBLs commissioned before launch. However, this result is comparatively better than the observed compliance rate in the EU, where only 40.0% (10/25) of the BSL-3 laboratories fully adhered to biorisk management practices for commissioning/decommissioning³⁵⁶. It is important to note that in the Brazilian context, the commissioning process appears incomplete for some HCBLs, wherein critical equipment/systems such as access control and autoclaves were not included in the commissioning procedures. Therefore, if we considered only the laboratories that conducted the complete commissioning tests, the results would be worse than those found in the EU.

It is worth noting that the criteria employed by the respondents to answer the question about commissioning and certification were not addressed in the present study. Therefore, it is vital to highlight the possibility of incorrect answers stemming from a misunderstanding of concepts or a lack of knowledge on the part of the respondents. This concern applies to the entire questionnaire.

6.2.4.2.6 High-Containment Biological Laboratories certification

Despite the absence of national regulations regarding the certification of HCBLs in Brazil, over 50% of the laboratories participating in this study claimed to be certified. However, in the absence of such regulations, critical questions arise regarding the validity and authority of these certifications. It is essential to determine

the entities responsible for carrying out the certifications and assessing whether they hold significance.

MS and MAPA have committees that possess the authority, per their internal regulations, to evaluate HCBLs and approve their operations, which can be considered a form of the certification process. Similarly, two LFDAs obtained certification from the FAO. However, it should be noted that certifications provided by CTNBio and private companies cannot be regarded as official certifications for biosafety and biosecurity levels. CTNBio focuses primarily on GMOs, and certifications provided by private companies lack legal support.

The significant number of respondents who did not disclose the entity responsible for designating the BSL in cases where the HCBL was not certified suggests that self-assignment is likely a prevalent practice in the country.

A study conducted in Africa by the EDPLN in 2015 revealed that 12 out of 13 HCBLs were certified, with one lab having a glove box within their BSL-2 laboratory, making this question not applicable. Among the certification bodies, most of them were external consultants (9/16, 56.3%), followed by international organizations (2/16, 12.5%), government bodies (1/16, 6.3%), and funders (1/16, 6.3%). Additionally, three laboratories (18.8%) were certified by 'other' bodies²¹¹. The high proportion of external consultants is probably because most African HCBLs receives support from international donors. This situation differs from Brazil, where receiving international funding for HCBLs construction is uncommon.

In addition to the initial certification, which would be mandatory for the operation of an HCBL, it is recommended that periodic re-certifications be conducted, typically on an annual basis. The results obtained in the present study were largely positive regarding this practice, as there were no negative responses to the question, 'Does the lab conduct periodic certifications/qualifications of the equipment/systems?'. However, it is concerning to note that critical equipment/systems are not routinely subjected to testing in several HCBLs. Examples of such equipment/systems include the HVAC system, electric system, access control, sealing system, airlocks, pass-through, EDS, and alarms.

A similar situation was observed in a study conducted in the EU, where only 8/25 (32.0%) HCBLs fully complied with planned preventative maintenance, calibration, and certification records³⁵⁶.

Zaki (2010) advocated that the government, via regulatory bodies, should be able to certify laboratories with the appropriate BSL, and the regulatory bodies must be provided with sufficient stable resources to carry out this function and annually evaluate the laboratories for license renewal³⁶⁰. The US experience with the FSAP is positive in this sense, according to Richards et al. (2014), who stated that oversight improves biosafety¹³. However, in the FSAP context, the annual certification is monitored by the IBCs.

Zaki's intention appears to burden the state apparatus, particularly in countries with many HCBLs. Adopting an approach similar to the US, utilizing IBCs for the annual certification process, could be a more viable option. However, it is crucial to promptly establish regulations for certifying HCBLs in Brazil. These regulations should include specific criteria and clearly define the entities responsible for the initial certification and periodic re-certification. Practices such as self-assignment and certification by the constructor are not acceptable. Failure to establish the essential requirements for HCBLs during the certification process will lead to a lack of standardization of the HCBL concept within the country.

6.2.4.2.7 Construction time and period

Prior to 2000, Brazil witnessed the construction of only three HCBLs: two within universities and one affiliated with the MAPA. It stands in contrast to the US, where numerous such facilities commenced operations in the 1990s¹³.

The findings of this study confirm what other authors have previously stated about a period in the early 2000s when the BFG encouraged the nationwide construction of HCBLs (see Chapter 2.1.6 of the present study). However, it is possible to identify a subsequent cycle of increased HCBL numbers in the country following the COVID-19 pandemic, which is expected to be even more robust than the initial wave. This second cycle includes not only laboratories affiliated with the MS, as observed in the previous wave, but also those affiliated with universities.

The global impact of the COVID-19 pandemic has caused a similar response worldwide, as observed by the authors of the 2023 Global BioLabs report⁵¹, who mentioned a boom in the construction of BSL-4 and BSL-3+ labs as a direct consequence of the COVID-19 pandemic.

Souza & Morel (2021) highlighted the challenging requirement of HCBLs to conduct fundamental research on SARS-CoV-2. They also emphasized the concentration of HCBLs in high-income countries, which allows them to advance the preclinical development of vaccines and antivirals against COVID-19. It is particularly crucial as suitable HCBL platforms for animal experimentation are scarce. The authors noted that in Brazil, laboratories that excelled in generating relevant knowledge during previous epidemics, such as Zika virus, dengue, and chikungunya, had to endure long waiting periods to access HCBLs within their own or collaborating institutions during the COVID-19 pandemic. They argued that investing in the construction of HCBLs facilitates essential health solutions and holds the potential for various economic benefits, including start-ups and licensing new technologies²⁴.

These arguments justify the BFG's initiatives to encourage HCBLs construction in the 2020s, such as the Public Call published by FINEP in July 2020⁷¹, from which 18 projects from 14 institutes were approved to adequate BSL-3 and ABSL-3 facilities, including upgrading BSL-2 and ABSL-2 ones. However, it is crucial for the BFG to simultaneously assess these facilities' biosafety and biosecurity status and be aware of the types of pathogens handled and the nature of the research conducted in each one.

While the construction time for most HCBLs participating in the present study was less than two years, as found by other authors^{97, 352}, it is essential to emphasize that in some instances, this period can be significantly extended due to factors such as:

- a. Laboratory's size, kind and complexity;
- b. Commissioning and certification processes;
- c. Constructor's competence, expertise, and financial health;
- d. Design's quality;
- e. Construction supervision;
- f. Availability of fund resources, etc.

6.2.4.2.8 Companies in charge of design and construction

The results obtained in the present study regarding the companies responsible for HCBLs design and construction raise two concerns: (i) a single company is responsible for approximately half of the design and construction of Brazilian HCBLs; and (ii) the same company typically handles the design, construction, and purported certification of HCBLs.

Regarding the first issue, Hernandez (2008) highlighted that the market for HCBL construction in Brazil is minimal, with few opportunities available. This scarcity of projects poses financial challenges for companies aiming to specialize in this field, resulting in only a few firms able to sustain long-term operations. The author recommends that construction companies also offer specialized maintenance services for HCBLs to generate income when construction projects are not underway. This author emphasizes that some institutes must procure the necessary maintenance services for HCBLs, further exacerbating the challenging scenario⁶⁶.

Despite the second wave of stimulus observed in the present study for HCBLs construction in Brazil since 2020, there is still a concentration of projects in a single company. It is desirable to have multiple companies and healthy competition in all types of businesses, including those closely related to public services, such as HCBL construction. Competition promotes better pricing, a more comprehensive range of technologies, and innovation. The recent revision of Brazil's bidding law³⁶¹ could bring new opportunities for public construction as public managers seek more creative and effective processes.

Once again, improving the legal framework is beneficial for promoting fair competition among companies. As Zhang et al. (2020) stated:

*'standards are the basis and guarantee of biosafety equipment. Because of the wide variety of biosafety equipment, it is necessary to set up technical standards to ensure the quality of products. We should standardize industry behavior and rely on standards to effectively curb the vicious competition from low-price homogeneous products. The market should be promoted by standards to effectively improve the quality of biosafety equipment'*³⁶².

There is an immediate need to establish constructive requirements for HCBLs that consider the essential prerequisites for each BSL, including BSL-3 enhanced facilities. It is essential to foster a basic level of consistency across structures and to provide direction for the design and construction processes.

The absence of regulations addressing constructive requirements may lead to significant shortcomings in the construction, mainly as most HCBLs belong to public institutes. These institutes often make purchasing decisions through bidding processes that prioritize lower prices. Consequently, some companies may offer lower prices to enhance their chances of selection, compromising the essential quality, biosafety, and biosecurity requirements. In this context, the institute may encounter obstacles in penalizing the companies responsible for the construction due to the lack of regulations justifying such punitive actions.

Concerning the practice of having the same company responsible for designing, building, and purported certifying HCBLs, this approach perpetuates design shortcomings, resulting in risks to biosafety and biosecurity. While design and construction might be part of the same bidding process, certification should always be performed by an independent entity to avoid conflicts of interest. The WHO's definition of certification clarifies that a third-party entity should conduct it².

6.2.4.2.9 Funds resources

The BFG's budget was the primary source for developing HCBLs in Brazil. This scenario differs from lower-income countries, such as those in Africa, where the involvement of international organizations is significant. According to a WHO report, 8/13 (61.5%) of the EDPLN BSL-3 laboratories receive financial support from

international bodies²¹¹. Lasley et al. (2023) reported that low-income countries worldwide estimated an average donation of 57% of their laboratory equipment⁶⁰.

The independence of Brazil from international resources in constructing HCBLs is a positive finding, highlighting the strategic importance of these laboratories for the BFG, as indicated in Chapters 2.1.2 and 2.1.5 of the present study.

However, despite the BFG's efforts in providing construction funds, it is necessary to discuss the criteria employed by decision-makers and establish mechanisms to ensure the sustainability of HCBLs. The initial step involves assessing the current number of HCBLs, their purpose, and activities to determine if these laboratories adequately meet national and local demands before approving funds for new HCBL construction. This assessment should follow an interministerial and 'One Health' approach.

A fundamental matter to consider in the context of public policies for biosafety and biosecurity is identifying the financial resources needed for its implementation. After the government assures the initial investments to develop and implement national biosecurity structures and programs, sustained and reliable funding and resources are necessary to maintain them. Between outbreaks and other biosecurity events, continual training and stable funding are required to ensure personnel proficiency, updated equipment, and supplies. Without long-term investments in organizational structures and biosecurity programs, skills and equipment stagnate, and response capabilities degrade, eventually requiring more significant investments during future public health emergencies to bolster atrophied capacity. Effective biosecurity programs require dedicated long-term support instead of one-time emergency resource allocations in response to individual events⁴⁰.

For example, the Thailand government installed BSL-3 facilities in most regional laboratory centers during the HPAI H5N1 outbreaks. However, those laboratories were no longer in operation because of unaffordable maintenance and running costs, except one because of the necessity of providing FMD diagnostic services to member countries in Southeast Asia³⁶³.

The long-term sustainability of the facilities is crucial and should be considered a key criterion for fund approval, particularly for universities. While universities often receive funds from grant providers like FINEP and FAPESP, they may lack sufficient financial resources to cover operational expenses.

Whenever the grant provided is insufficient to cover both the operational costs of an HCBL and the expenses directly related to the research, researchers will find themselves in a dilemma. If they prioritize meeting biosafety and biosecurity requirements, insufficient resources may be left to complete the research. Conversely, if they prioritize the research, there may not be adequate resources to ensure the fulfillment of biosafety and biosecurity requirements. Without regulatory demands and penalties, the temptation to prioritize research over safety becomes more pronounced. As pointed out by Gronvall & Rozo (2015), without nationally recognized biosafety standards and a vested interest in ensuring that research institutions engaged in potentially high-consequence research adhere to these standards, there will be insufficient motivation to allocate the necessary resources for achieving high levels of biosafety within individual laboratories and institutions¹⁶⁰.

Even in high-income countries with well-established laboratory biosafety and biosecurity programs, such as the US, securing financial support for the operation of HCBLs remains a challenge. It is worth mentioning the report published by the US 'Committee on Laboratory Security and Personnel Reliability Assurance Systems for

Laboratories Conducting Research on Biological Select Agents and Toxins,' which stated that:

'Security and compliance procedures called for under the FSAP can be significant, with costs substantially higher than for similar laboratory facilities. Construction of secure laboratories where select agent research will be conducted is often funded by grants specific for that purpose. But select agent laboratories have significant ongoing security and safety sustainment costs that far exceed the indirect costs that grantee institutions receive to cover the costs of facilities, maintenance, and operation. The implications of sustainable funding required to conduct select agent research are troubling. It is not acceptable, either for the institution or for safety and security, to diminish appropriate and necessary risk-based security procedures and resources, regardless of the availability of funding for the facility. The committee urges federal agencies that fund biological select agents and toxins research to establish dedicated funding for ongoing security and compliance responsibilities associated with this type of research. This is an essential obligation, and no facility should operate without appropriate security measures in place. Although this type of funding structure may be unusual for biomedical research laboratories, it is not uncommon for funding those areas of science where central infrastructure plays an important role'¹⁹².

Providing resources for construction without a clear plan for covering operational costs is a common and critical mistake. In Brazil, where financial and human resources are scarce, it is crucial to utilize these resources based on a well-defined strategic governmental plan that establishes priorities and long-term goals related to public health, animal health, biodefense, and agribusiness. Innovations should address HCBLs' high operational costs. For example, cooperation agreements between public institutes could be established to share facilities for specific purposes, or public-private partnerships could be formed with companies interested in conducting research or storing biological assets in a high-biocontainment environment.

This task is complex but necessary, as it is linked to potential threats that can have significant consequences for the country, such as new pandemics (e.g., influenza) or outbreaks that can impact the exportation of goods, like meat, leading to severe economic and social losses (e.g., FMD).

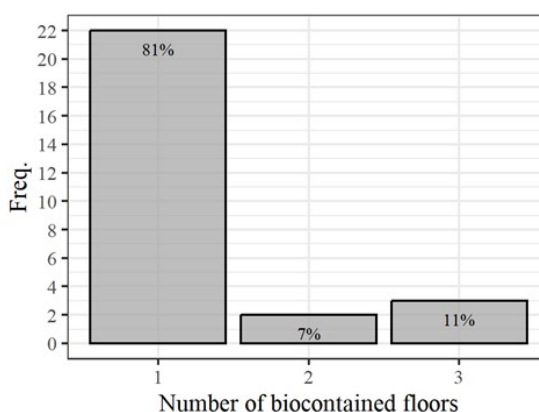
Brazil needs a well-established network of HCBLs, considering various factors such as threats to public health, the importance of agribusiness to the Brazilian gross domestic product, and the natural diversity of ecosystems like the Amazon where emergent viruses can be found³⁸, its regional leadership in Latin America, and other relevant aspects. However, more than constructing new HCBLs is required to create a network. Developing them under a national policy and an interinstitutional strategic plan is necessary.

6.2.5 High-Containment Biological Laboratories general constructive features

6.2.5.1 Results

Table A07 presents various facility features of the Brazilian HCBLs that participated in the survey. Although more than one-third of the HCBLs have multiple floors (37.5%), the majority (46.9%) have only one contained floor (Graph 23).

Graph 23 – Results regarding the number of contained floors of the HCBLs participating in the survey.



All five HCBLs with multiple contained floors reported having filter boxes and EDS within a biocontainment area. Therefore, these variables are closely related to the number of contained floors, as demonstrated in Tables 07 and 08.

Table 07. Correlation analysis between the frequency of HCBLs participating in the survey having their exhaust air filter boxes installed in a contained room and the number of contained floors.

Are the exhaust air filter boxes installed in a contained room?	N	Contained floors					Mann-Whitney test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
No	15	1	1.07	1.00	0.26	2	0.0155
Yes	8	1	1.88	1.50	0.99	3	

Table 08. Correlation analysis between the frequency of HCBLs participating in the survey having their EDS installed in a contained room and the number of contained floors.

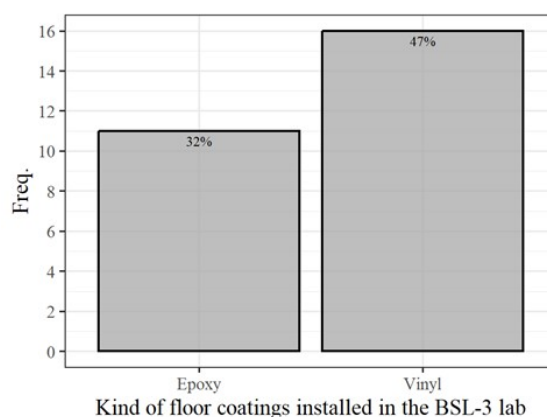
Is the effluent decontamination system installed in a contained area?	N	Contained floors					Mann-Whitney test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
No	10	1	1.00	1.00	0.00	1	0.0057
Yes	8	1	2.00	2.00	0.93	3	

Most HCBLs are connected to a BSL-2 laboratory (21/32, 65.6%). Twenty-one HCBLs (21/32, 65.6%) confirmed that all walls and ceilings are adequately sealed. A few laboratories mentioned having cracks on inner walls (2/32, 6.2%), while only none reported cracks on the floor (1/32, 3.1%) (Table A07).

The percentage of HCBLs with windows facing the outside area (12/32, 37.5%) is slightly lower than those without windows (15/32, 46.9%). Most HCBLs have windows facing the inner area (17/32, 53.1%). Double glass windows are predominantly used. Demonstrably sealed windows have a higher percentage for those facing the inner area (14/17, 82.4%) than those facing the outside area (6/12, 50.0%). None of the HCBLs mentioned having curtains or shutters inside the lab (Table A07).

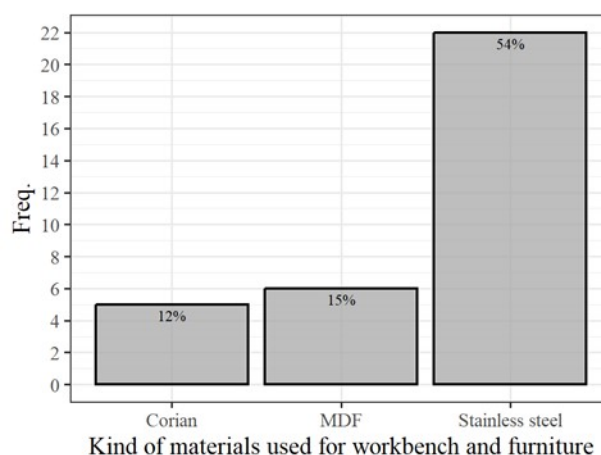
Vinyl and epoxy were the most commonly used floor coatings (Graph 24). Polyurethane and porcelain were mentioned only once.

Graph 24 – Results regarding the kinds of floor coating used by the HCBLs participating in the survey.



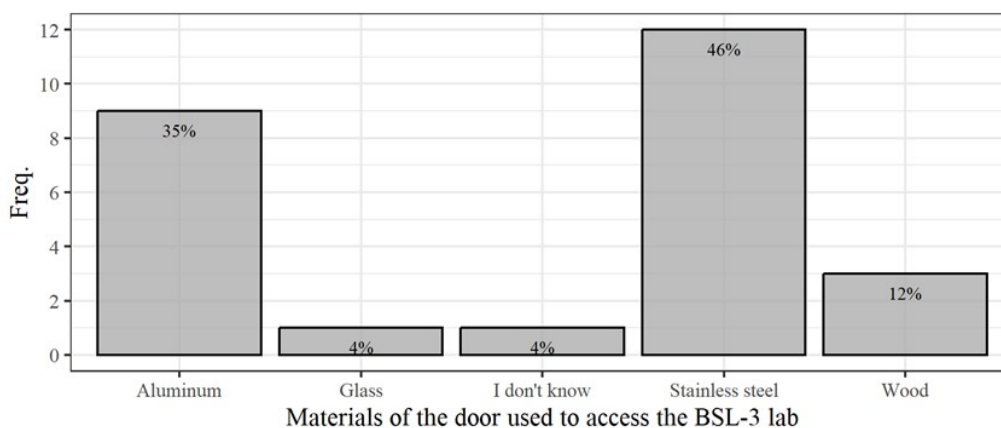
Stainless steel was the primary material for workbenches and furniture, followed by MDF and corian (Graph 25). Granite and wood were mentioned only once.

Graph 25 – Results regarding the kinds of materials used for workbench and furniture by the HCBLs participating in the survey.



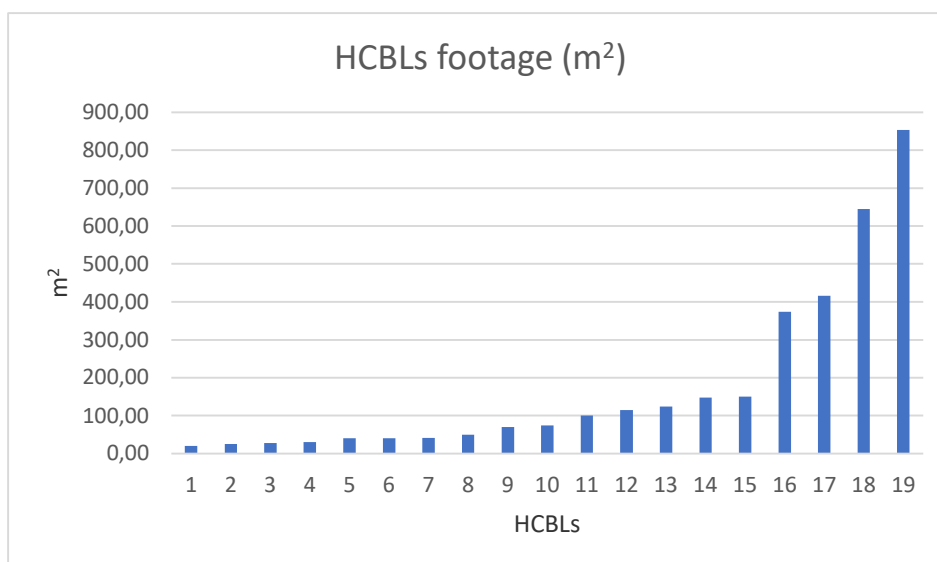
Stainless steel and aluminum are the most common materials used to manufacture the doors, followed by wood and glass (Graph 26).

Graph 26 – Results regarding the kinds of materials used for doors by the HCBLs participating in the survey.



The area of HCBLs varied significantly, ranging from 20 to 10,000.00m². Graph 27 illustrates the area of 19 out of the 23 HCBLs that provided this information. The four largest laboratories were excluded from the graph due to their disproportionate values. Among the HCBLs, eight (8/23, 34.8%) had an area of less than 50m², three (3/23, 13.0%) ranged between 51 and 100m², four (4/23, 17.4%) fell between 101 and 150m², and eight (8/23, 34.8%) had an area exceeding 151m². Out of those with an area greater than 1,000m², three were vaccine manufacturing factories measuring 1,500m², 2,397m², and 10,000m², respectively, while the other is a federal reference laboratory with 2,200m². The average area across all 23 HCBLs was 845m², with a median of 114m². However, excluding the three vaccine manufacturing factories, the average area decreased to 277m², with a median of 87m².

Graph 27 – Results regarding the footage of the HCBLs participating in the survey, excluding the most significant four.



The impact of the HCBLs purpose on their size was calculated by comparing the data from 10 HCBLs with only one purpose. The results indicated strong evidence of differences between the laboratory sizes according to their purpose (Table 09).

Table 09 – Correlation analysis between the purpose of HCBLs participating in the survey and their size.

Purpose	N	Laboratory footage (m ²)					Kruskal-Wallis test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Research	5	25.0	65.9	50.0	39.1	114.5	0.022
Service provider	2	150.0	262.0	262.0	158.4	374.0	
Supply producer	3	1,500.0	4,632.3	2,396.7	4,670.2	10,000.0	

6.2.5.2 Discussion

6.2.5.2.1 'Shell in a shell' principle and air tightness

The 'shell in a shell' principle, also known as the 'box within a box' principle, is commonly employed to contain the spread of microorganisms. Each shell carries a certain probability of failure. According to Behrmann et al. (2007), the outer shell is typically larger than the inner shell, facilitating the distribution and dilution of organisms if the inner containment volume fails. In the event of a single shell failure, the negative pressure cascade should prevent the release of organisms. Should the pressure cascade also fail, a retrograde airflow into the inner shell will impede any release until pressure equilibrium is attained²⁹⁰. Vieira & Salgado (2008) argued that it is desirable to have a BSL-2 laboratory serving as an access zone to the BSL-3 lab, aligning with the 'shell in a shell' principle⁷³. The MS made the same recommendation⁹⁴.

Under this principle, it is preferable to construct the BSL-3 laboratory as an enclosed unit within a BSL-2 laboratory, as observed in most HCBLs that participated in the present survey. In cases where this arrangement is not feasible, direct points of contact with the external environment, such as windows, should be avoided. The windows pose risks to biosafety and biosecurity and should be avoided when facing the outside area. The seven HCBLs that reported no conjugated laboratory in the present study should consider implementing additional biosafety measures to mitigate the risks of potential APELS, especially the only one that stated having windows facing the outside area.

While windows facing the outside area are not recommended, some authors advocate for installing windows facing the inner area to improve the well-being and safety of users, as it serves as an additional means of communication³⁶⁴. Moreover, inner windows can facilitate the observation of experimental animals²⁵⁷. However, Vieira & Salgado (2008) argued that despite these benefits, some workers may feel uncomfortable and distracted when observed through windows. The authors suggested that CCTV monitoring could be a preferable alternative⁷³.

Another aspect to consider is natural illumination. However, Johnson & Casagrande (2016) noted that among various international guidelines, only the UK emphasizes providing access to natural light⁸³.

Few HCBLs participating in the current survey reported not having any windows. While this absence of windows can contribute to biosafety and biosecurity, it may also pose potential risks to the well-being of users. Therefore, it is crucial for critical stakeholders, including users, maintenance staff, and BOs, to discuss the advantages and disadvantages of having windows during the design phase. If the team decides to incorporate windows, it is highly recommended to conduct thorough testing to ensure their tightness.

Although the current survey indicated that there are more HCBLs with windows facing the inner area compared to those with windows facing the outside, paradoxically, a lower proportion of HCBLs with windows facing the outside reported them to be demonstrably sealed, in contrast to a higher percentage for HCBLs with windows facing the inner area. This observation raises concerns.

Brazilian HCBLs should reassess the practice of having windows that face the outside area and acknowledge the significance of having airtight windows that face the inner area while conducting regular periodic testing. Even though most windows are constructed with double glass, this does not guarantee their sealing.

It is worth highlighting that the MS requires all windows installed within an HCBL to be sealed, and ABSL-3 facilities must not have windows⁹⁴. Nevertheless, among the nine HCBLs participating in the survey that reported handling animals, six have windows, and two have windows facing the outside area.

In addition to the windows, it is advisable to ensure that the walls and ceilings of HCBLs are adequately sealed. According to the BMBL:

*'Penetrations in BSL-3 laboratory surfaces should be sealed or capable of being sealed for decontamination purposes. Care should be taken that penetrations in the walls, floors, and ceilings are kept to a minimum and are sight sealed. Verification of the seals is highly recommended but is usually not required for BSL-3 laboratories'*¹.

The Canadian Biosafety Standard states that the airtightness test is mandatory⁸⁰, and the WHO advocates that:

*'Where gaseous disinfection is selected as a heightened control measure for decontamination and waste management, laboratory room/space airtightness will need to be enhanced. This is achieved by sealing all surfaces and/or laboratory penetrations to prevent the escape of hazardous gases'*².

Tearle (2022) emphasized that sealed rooms prevent the release of gaseous, vapor, or mist-based chemical decontaminants while fumigating individual rooms, zones, or the entire containment area. This measure is crucial for safeguarding the health of individuals within the building and maintaining the necessary fumigation conditions³⁶⁵. Furthermore, according to Gmunder et al. (2022), this requirement ensures the assessment of the containment enclosure's tightness in case of pressure cascades or failure of directional inward airflow²⁸⁹.

The data collected in the present study revealed that the walls and ceilings of most HCBLs are demonstrably sealed. This percentage falls short compared to that observed in the EDPLN HCBL, where 100% of the laboratories are sealed²¹¹.

It should be noted that the questionnaire used in the present study did not include a specific question about the type of test employed by HCBLs to assess air tightness. In addition, it can not be ensured that respondents fully understood the term 'demonstrably sealed'.

The only national regulation that addresses this issue is the MAPA ordinance regarding FMD virus handling, which mandates that windows, walls, and ceilings should be confirmed as sealed through a qualitative smoke test²³¹. However, this regulation does not apply to HCBLs that do not handle the FMD virus.

Furthermore, most international guidelines do not specify minimum numerical values for air tightness. Coppens et al. (2019) noted that certain countries, such as Australia and New Zealand, provide numerical guidance in their standards, recommending a maximum air leakage rate of 10–5 m³/Pa, measured at an overpressure of 200Pa³⁶⁶. The pressure decay test, as described in the Canadian Biosafety Standard⁸⁰, and the blower-door test based on ISO 9972:1996, are alternative methods mentioned by Coppens et al. (2019)³⁶⁶.

The absence of comprehensive national regulations is the primary factor contributing to the lack of standardization regarding air tightness requirements for Brazilian HCBLs. The absence of clarity regarding the type of test (qualitative versus quantitative) exemplifies this weakness.

6.2.5.2.2 Contained floors

The results indicated that more than one contained floor on the Brazilian HCBLs is closely related to installing HEPA filter boxes and/or EDS in a contained room. However, the international guidelines and standards have no consensus on that issue.

It is worth quoting Behrmann et al. (2007):

*'The wastewater is one of the most important containment interfaces at the end of a research unit, or an animal or production safety facility. All penetration points of the containment unit are sensitive to potential damage and represent 'weak spots' in the containment shell. Particular attention must be paid to the wastewater inactivation path, when the decontamination device cannot be integrated into the containment. In this case, the decontamination device must be seen as an extension of the containment shell and be subjected to the same safety standards as the containment unit itself. Consequently, adequate safety measures for the wastewater inactivation compartment must be developed during risk assessment and integrated into the safety concept'*²⁹⁰.

The same authors argued that the room containing the wastewater and HEPA filter stations must be contained for biosafety reasons²⁹⁰. Niebel & Meybohm-Röder (2011), referring to BSL-4 facilities, stated that the EDS room must be 'sealable'²⁹¹.

According to Spanish regulations, HEPA filter boxes can be located inside or outside the biocontainment area. However, if they are positioned inside, the ductwork should be designed to place the filtration system as close as possible to the outer boundary, with a maximum distance of 4 meters. If the HEPA filter boxes are situated outside the biocontainment area, the ductwork leading to them should be kept as short as possible, with a maximum length of 4 meters. The same normative states that the EDS should be located in a contained area, apart from the technical rooms⁸².

The BMBL also states that all BSL-3 lab HEPA filters should be located as near as practicable to the laboratory to minimize the length of potentially contaminated ductwork but does not establish parameters related to being inside or

outside the contained area. On the other hand, this manual does not establish requirements concerning the location of the EDS¹.

Gmunder et al. (2022) stated that the HEPA filter system should be installed in the services area above the laboratory, discouraging installation within the containment area due to the challenges in maintaining the filter system. However, if this configuration is implemented, the exhaust air duct from the filter unit to the containment boundary must be airtight²⁸⁹.

In Brazil, apart from the specific regulation for handling FMD virus²³¹, which mandates installing HEPA filter boxes and EDS inside contained areas, there are no specific regulations for other HCBLs. Consequently, as demonstrated in the present study, it is common practice in Brazil to install this equipment outside the biocontainment area. However, HCBLs should adhere to recommendations from international guidelines to mitigate the risk of pathogen release associated with this equipment, regardless of its installation location. Further studies are necessary to thoroughly assess the status and risks posed by such mandatory equipment in Brazilian HCBLs.

6.2.5.2.3 Floors and walls condition

The results obtained in the present study revealed a limited number of laboratories that admitted to having cracks on the inner walls or the floor. These findings indicate that the users' perspectives regarding the study conducted by Vieira & Salgado (2008) have remained the same. According to data gathered by these authors from interviews with representatives of six Brazilian HCBLs, the users' perceptions about surfaces and coatings were consistently satisfactory. It was also observed that mandatory requirements on surfaces and coatings had the highest compliance throughout that research, meeting the expectations in a satisfactory manner⁷³.

Similar results were found in the study by Richards et al. (2014) on US BSL-3 laboratories, where two non-select agent facilities (2/22; 9%) and no select agent facilities (0/20) reported breaks, holes, or open seams in laboratory walls, flooring, or ceiling¹³.

The quality of floors and walls is closely related to the choice of materials. The present study revealed that vinyl is the most commonly used floor coating, followed by epoxy, which aligns with Johnson's (2011) statement during the Workshop 'Biosecurity Challenges of the Global Expansion of High-Containment Biological Laboratories' organized by the US National Academy of Sciences:

*'In BSL-3 labs, seamless welded vinyl flooring and gypsum board walls covered with epoxy paint are often used to bear the weight of BSCs and allow for gas decontamination. More expensive floor and wall materials (poured concrete with troweled epoxy) can be reserved, if needed, for animal rooms where movement of racks and carts would quickly damage walls and floors'*¹⁷.

According to Linster et al. (2020), a single-piece vinyl or rubber covering is ideal for the floor because they are durable and chemical resistant and can withstand an intense cleaning regimen. If this type of flooring is unavailable, care should be taken when sealing the floor to make it impervious to liquids and durable for the intended use. High-quality epoxy floorings can be used for this purpose³⁶⁷. The MS guideline also recommends using epoxy or vinyl to cover the floors of HCBLs⁹⁴.

Vieira (2008) recommended vinyl for areas with low humidity levels and epoxy for areas where water is frequently used³¹³, which aligns with Copps' (2005) assertion that epoxy-covered cement is the most common impervious-to-water, easily cleaned, and sterilized material used in animal facilities²⁸⁴.

However, out of the nine HCBLs in the present study that handle experimental animals, only one uses epoxy floor coating, six use vinyl, and two did not provide this information. Therefore, assessing whether these HCBLs that handle experimental animals and do not use epoxy floor coating must consider adapting their floor coating choice is necessary.

Another aspect to consider in preserving walls and floors is ongoing maintenance. The age of the HCBLs had no impact on the presence of cracks in walls and floors, suggesting that, generally, HCBLs can maintain them in satisfactory condition. However, this finding contradicts other results from this study, such as the financial status of the HCBLs, the lack of maintenance staff, and the difficulties encountered in maintaining their infrastructure. Therefore, further investigations are necessary to assess the actual condition of HCBLs infrastructure, including on-site visits, to verify if the responses provided in this study accurately reflect the current situation.

6.2.5.2.4 Furniture and doors composition

The furniture and door composition reported by most HCBLs participating in the present study is considered suitable. However, some HCBLs utilize materials like MDF and wood, which do not adhere to the international guidelines' recommendations of being impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

While there are limited references regarding specific furniture materials for HCBLs, the NIH Design Requirements Manual specifies that bench tops should be made of epoxy, stainless steel, or phenolic resin⁷⁸. Corian® is a patented solid-surface material that, according to the manufacturer's product overview, consists of a non-porous, homogeneous composition of acrylic resin and alumina trihydrate derived from bauxite³⁶⁸. Although there is limited information on its specific use in HCBLs, it appears to meet the general recommendations.

The absence of curtains or shutters mentioned by HCBLs indicates that most laboratory workers in Brazil know the associated risks, including microbiological and fire hazards, posed by these materials within a laboratory environment.

6.2.5.2.5 High-Containment Biological Laboratories Footage

The proportion of HCBLs with an area of less than 50m² (34.8%) was similar to what was observed in the US. A national survey conducted in 2004 found that 31% of HCBLs had an area of less than 500 sq ft (46m²). However, there was a notable difference regarding facilities with an area greater than 101m², as the present study reported a result of 52.2%. In the US, it was found that 34% of HCBLs had an area larger than 1,001 sq ft (92m²)³⁴⁵.

Another study revealed that the footage of three BSL-3 laboratories belonging to the African EDPLN was 30m², 40m², and 300m²²¹¹.

Considering the high construction and operational costs associated with HCBLs, which are proportional to the size of the facilities, it is not surprising that most HCBLs are relatively small. The size should be appropriate for the intended purpose of the HCBL, highlighting the importance of defining the role and activities to be carried out during the design phase. Oversized laboratories can result in

unnecessary expenses, while undersized units may compromise compliance with biosafety requirements, such as the proper placement of BSCs and areas for donning and doffing PPE.

One of the key factors influencing the size of an HCBL is the location of the EDS or HEPA filter boxes within the containment zone. Among the eight HCBLs participating in the present study with an area greater than 151m², only one stated that both EDS and HEPA filter boxes were not installed in a contained area, and more than 50% had two or three contained floors.

The impact of vaccine manufacturing also played a role in the facility size, as the three companies participating in this study had areas larger than 1,500m². According to MAPA's regulations, it is mandatory to install EDS and HEPA filter boxes in contained areas²³¹, which impacts the HCBLs' size. There is strong evidence that the purpose of the HCBLs impacts its size.

6.2.6 High-Containment Biological Laboratories water and gases supply

6.2.6.1 Results

As shown in Table A08, the public service supplies the potable water of most HCBLs (19/30, 63.3%). Water supply cut-offs are generally infrequent, as 93.4% (28/30) of HCBLs rated their frequency as one or two on a scale of one to five. There was no correlation between the source of potable water and the frequency of water supply cut-offs (Table 10).

Table 10. Correlation analysis between the source of potable water and the frequency of water supply cut-offs in the HCBLs participating in the survey.

Categorical	N	Frequency of water supply cut-offs ^{gg}					Mann-Whitney test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Artesian well	5	1.00	1.20	1.00	0.45	2.00	1.00
Public supply	16	1.00	1.25	1.00	0.58	3.00	

Of the surveyed HCBLs, only six (6/32, 18.8%) reported a draining floor. Additionally, seven HCBLs (7/30, 23.3%) acknowledged the absence of backflow prevention devices in their water and gas pipes. Among the respondents, three HCBLs (3/29, 10.3%) rated the frequency of leaks in their water piping as three or higher on a scale of one to five. In most cases (21/32, 65.6%), most or all pipes and conduits are visible and easily accessible for cleaning purposes (Table A08). The missing questions concerning water supply were from 'dry laboratories' that do not require water usage within their facilities.

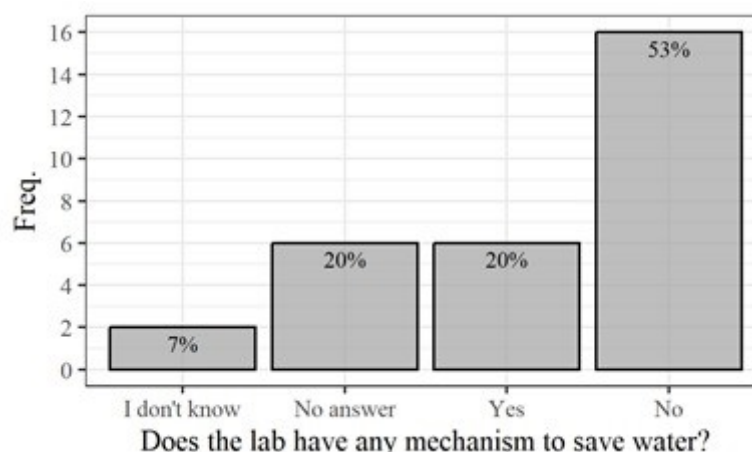
Only a few laboratories (6/30, 20.0%) have implemented water-saving mechanisms (Graph 28). The mentioned mechanisms were as follows:

- Institutional campaigns promoting environmental sustainability;
- Utilization of disposable materials within the HCBL;
- Procurement of small-volume bottles of mineral water for workers' consumption;

^{gg} Scale ranging from 1 (very low) to 5 (very high).

- d. Implementation of a looping cooling system for process tanks to avoid wastage of jacket water from bioreactors;
- e. Integration of the water supply system with another institute's system;
- f. Installation of automatic turn-off systems for sinks used for hand cleaning.

Graph 28 – Results regarding implementing water-saving mechanisms by the HCBLs participating in the survey.



Almost half (15/32, 46.9%) of HCBLs indicated that they have a compressed air supply. Among these, one-third (5/15, 33.3%) confirmed the presence of backflow prevention devices in their compressed air pipes, and 80% (12/15) rated the frequency of compressed air supply shortage as one or two on a scale ranging from one to five. Most HCBLs (19/32, 59.4%) do not store gas cylinders within the laboratory. Among those that do, carbon dioxide was the most commonly used gas, followed by nitrogen and oxygen (Table 09).

6.2.6.2 Discussion

During the preliminary studies about the design and construction of an HCBL, evaluating the availability of essential utilities on the site, including a reliable source of potable water, is imperative⁴⁶.

In Brazil, there are two primary sources of potable water: artesian wells and public water supply. Artesian wells are a more cost-effective long-term solution. Additionally, it may offer a consistent water supply regardless of weather conditions or the availability of public water. However, in the present study, there was no statistical correlation between the source of potable water and the frequency of water supply cut-offs.

Gmunder et al. (2022) advocate for the ideal design of an HCBL to be a dry laboratory, eliminating sinks and showers. In this scenario, any liquid waste generated must be inactivated and discharged through autoclaving or alternative suitable and validated methods²⁸⁹. The WHO supports the concept of a dry BSL-3 laboratory, as water-based HCBLs require substantial engineering controls, resulting in higher costs²¹¹. Vieira (2008) adds that dry HCBLs require less space, which is particularly advantageous when renovating facilities with limited square footage³¹³.

A survey conducted with EDPLN revealed that 4 out of 13 African HCBLs (30.8%) were dry facilities²¹¹. This percentage is higher than the present study, where 6 out of 32 HCBLs (18.8%) reported not generating any liquid effluent, although 20 out of 32 HCBLs (62.5%) lacked floor drains.

The decision regarding the presence of liquid effluent should be based not only on financial aspects but primarily on the activities performed within the HCBL. However, adopting the concept of a dry laboratory can contribute to long-term sustainability. Alternatively, if the HCBL generates liquid effluent, it should be treated as required and according to validated methods. One such requirement is the installation of backflow prevention devices in water pipes²⁸⁹. In the present study, at least 23.3% of the HCBLs lacked these devices, but the actual percentage may be higher as 10 HCBLs did not provide information on this matter.

The frequency of water piping leaks was low in the participating HCBLs. However, approximately half of them did not have visible and easily cleanable pipes and conduits, representing a vulnerability. According to Gunder et al. (2022), it is recommended that lines, cables, and pipes are generally surface-mounted at a distance of at least 2.5 cm in front of the wall to allow cleaning or surface decontamination by hand²⁸⁹.

The WOAHA highlighted that creative and innovative thinking is crucial for improving laboratory sustainability, including mechanisms for water conservation³⁶⁹. Unfortunately, the percentage of HCBLs reporting the existence of such mechanisms was low in the present study, and some of the mentioned mechanisms seemed not to apply to all HCBLs. These results indicate the need for greater attention to this issue in future projects.

The compressed air supply is commonly utilized in HCBLs, particularly for pneumatic sealed doors and specific laboratory equipment. Surprisingly, less than 50% of the participating HCBLs in the present study reported having a compressed air supply. When available, it is advisable to ensure a continuous supply. While the majority of HCBLs reported no issues with their compressed air supply, it is noteworthy that two HCBLs indicated a high frequency of interruptions, which requires attention.

For safety reasons, it is not recommended to have gas cylinders within laboratories⁷⁸, and this recommendation is followed by most of the HCBLs in the present study. If gas cylinders are present, they are most commonly used for storing CO₂, likely due to the usage of CO₂ incubators.

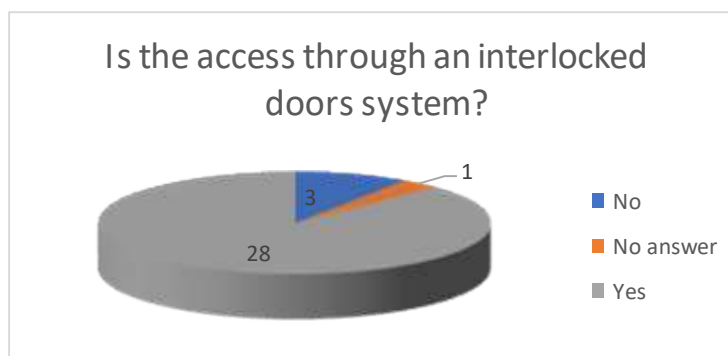
It is important to emphasize that managers must be aware of the risks associated with gas cylinder maintenance and replacement procedures within the HCBL. Special attention should be given to the decontamination procedures for these materials, as well as the training provided to external staff who may need to access the containment zone.

6.2.7 High-Containment Biological Laboratories access control and changing room

6.2.7.1 Results

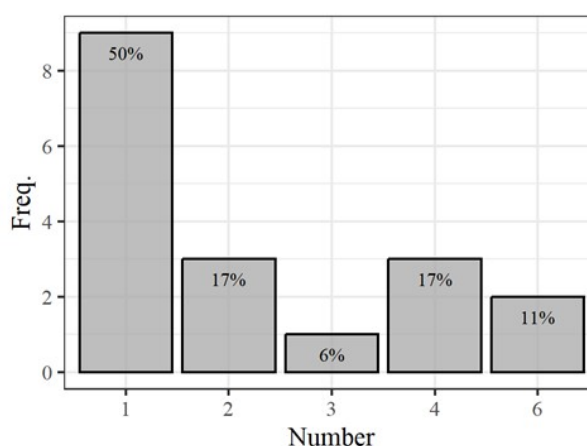
Three HCBLs participating in the present study (3/32, 9.4%) revealed no interlocking door mechanism to access the contained area (Graph 29).

Graph 29 – Results regarding the existence of interlocking door mechanisms for accessing the HCBLs participating in the survey.



Four HCBLs (4/32, 12.5%) do not record access to the laboratory. Two HCBLs (2/32, 6.2%) do not have changing rooms dedicated exclusively to accessing the contained area. Among those who have, most do not have exclusive changing rooms for men and women (18/30, 60.0%), most are divided into clean and dirty areas (19/30, 63.3%), and most have showers (20/30, 66.7%). Among those having showers, most have two showers or less (12/20, 60.0%) (Graph 30), bath water is almost always drained to an EDS (18/20, 90.0%), the air of the bath box is filtered in more than half of them (11/20, 55.0%), the same proportion observed for mandatory bathing when exiting the HCBL (11/20, 55.0%). Among them, bathing is commonly controlled by an automated system (7/11, 63.6%) (Table A10).

Graph 30 – Results regarding the number of showers in the changing room of the HCBLs participating in the survey.



Most HCBLs stated that the changing room footage is suitable (19/29, 65.5%) and that SOPs describing the training procedures for visitors' entrance are present (24/32, 75.0%) (Table A10).

No correlation was observed between the changing room footage's suitability and exclusive changing rooms for men and women (Table 11).

Table 11. Correlation analysis between the suitability of the changing room footage and the existence of exclusive changing rooms for men and women in the HCBLs participating in the survey.

If so, are there exclusive changing rooms for men and women	How do you rate the footage from the changing rooms, considering the current staff?		Fisher's Exact Test
	Insufficient	Suitable	
No	4	14	1.00
Yes	1	4	

Half of the 26 HCBLs that provided some information when asked about how the access to the BSL-3 is done reported that the access is through an anteroom located in a BSL-2 laboratory (13/26, 50.0%). The second most prevalent answer was through a changing room between a BSL-2 and the BSL-3 laboratory (5/26, 19.2%). Two respondents stated that access is done by a changing room accessed directly from the external environment (2/26, 7.7%). In both cases, there is an interlocking door system. Another HCBL reported that the access was through a single door from a BSL-1 laboratory (1/26, 3.8%), but there was no interlocking door system in this case. The remaining five HCBLs informed that the access is through an anteroom or changing room (5/26, 19.2%) but did not inform if these rooms are located in another laboratory or if the access is directly from the external environment.

6.2.7.2 Discussion

6.2.7.2.1 Accessing the High-Containment Biological Laboratories

Access to an HCBL must be granted through interlocked doors to prevent the simultaneous opening of doors between the external corridor and containment areas. This measure minimizes the likelihood of aerosols and particles containing microorganisms escaping^{78, 289}. It is important to note that the MS mandates this requirement for BSL-3 laboratories²²³.

However, nearly 10% of the HCBLs in this study lack this mechanism, marking it a significant concern. Of particular alarm is the case of an HCBL that exclusively relies on a single-entry door from a BSL-1 laboratory. Comparable trends were noted in the EDPLN laboratories, where 1 out of 12 (8.3%) lack an interlocked door system to access the HCBL²¹¹.

By Spanish regulations, access to a contained area should be through a changing room divided into two zones. The containment barrier separates the 'clean' zone (non-contained) from the 'dirty' zone (contained). If a shower is present, it is considered part of the dirty zone, and its exit door serves as the containment barrier⁸². The Canadian Biosafety Standard also acknowledges that the body shower is an extension of the dirty change area and, therefore, falls within the boundaries of the containment barrier⁸⁰.

Regardless of the presence of a shower, it is essential to define the clean and dirty zones clearly. However, more than 15% of the HCBLs in the present study did not observe this definition.

Maintaining the integrity of containment zones relies heavily on the presence of physical and security barriers at entry and exit points. These barriers are crucial in preserving inward airflow and providing designated areas within the containment zone for safely removing and storing contaminated or potentially contaminated PPE behind the barrier⁸⁰. Therefore, it is essential to ensure adequate space in this area to promote the well-being of workers and ensure biosafety compliance by considering the importance of adequate storage and decontamination procedures for PPE⁸².

In this context, the percentage of HCBLs considering the available footage in changing rooms to be sufficient is relatively low. This issue can partly be attributed to the fact that most HCBLs do not have separate changing rooms for men and women. However, there was no statistical correlation between these variables.

According to Spanish regulations, having separate changing rooms for men and women is recommended. In cases where separate rooms are not feasible, the changing room should have a mechanism to indicate occupancy when someone is inside⁸².

One of the two HCBLs that reported not having a dedicated changing room exclusively for BSL-3 access explained that their entrance is through an anteroom connected to a BSL-2 laboratory with interlocked doors. The other HCBL likely follows a similar procedure, as there is also a BSL-2 laboratory adjacent to it. Depending on the risk assessment, an anteroom instead of a changing room can be justified.

The design of the changing room or anteroom used for entering and exiting the containment area should be thoroughly evaluated during the preliminary studies. Factors such as the laboratory's size, activities to be carried out, the expected number of users, the availability of showers, sinks, and toilets, as well as the procedures involved in donning and doffing uniforms and PPE, need to be taken into account. Errors in the design of this area can present significant challenges to the operation of HCBLs.

6.2.7.2.2 Showers

According to Copps (2005), the requirement for body showers upon entering or leaving a containment area is contentious. The author argued that they are crucial when working with animals, as exit showers facilitate the removal of any pathogens present on the body's surface²⁸⁴.

Behrmann et al. (2007) considered showers optional and suggested their availability as safety showers in the dressing airlock. If showers are present, wastewater must be appropriately collected and inactivated²⁹⁰. The Spanish regulation specifies that showers should only be mandatory when the risk assessment deems necessary⁸².

Gmunder et al. (2022) argued that showers can be beneficial when dealing with organisms that are easily transported and can survive for extended periods. Therefore, incorporating a shower at the end of the work shift can be justified based on a risk-based standard. In such cases, the shower can also function as a 'shower airlock,' used as necessary in the airlock area adjacent to the regular transition zone from potentially contaminated to non-contaminated areas²⁸⁹.

Showering is mandatory and should be regulated by an automated system, according to Brazilian regulations for handling the FMD virus²³¹. The high number of Brazilian HCBLs where showering is mandatory and controlled by an automated system is likely a result of this specific regulation being used as a reference, even though the HCBLs do not handle the FMD virus. It highlights the general lack of

comprehensive regulations for HCBLs, leading to the implementation of specific unnecessary rules.

Given that the shower is considered part of the 'dirty' zone and recognizing that the bathwater may carry microorganisms, it is mandatory to treat it, as indicated by nearly all of the HCBLs participating in the present study that have showers within the containment barrier.

In addition to treating the water, filtering the air within the shower zone is necessary. According to Spanish regulations, this area should have an air supply and exhaust system. Alternatively, it is permissible to have a supply system from the clean area to the dirty area, passing through the shower zone, provided the ductwork includes a backflow prevention device⁸². However, the present study revealed that 20% of HCBLs do not treat the air in the shower zone.

When the risk assessment or regulations, such as FMD virus handling, require taking a shower before exiting the HCBL, it is advisable to have a minimum of two showers. It ensures that the HCBL can maintain its operations without disruptions during maintenance activities while ensuring the availability of at least one shower for each gender during regular operations. However, in the present study, out of the eleven participating HCBLs where showers were mandatory, six (54.5%) stated having only one shower.

6.2.7.2.3 Access recording

In a study conducted between 2008 and 2009 involving 235 Brazilian laboratories, ANBio concluded that over 50% of them permitted unauthorized individuals to access their facilities¹⁷. Although referring to laboratories in general, not necessarily HCBLs, this finding starkly contrasts with the results of our current study, which revealed that 78.1% of the HCBLs maintained records of all access, demonstrating the implementation of control measures to prevent unauthorized entry. It is important to emphasize that the MS requires documenting all accesses⁹⁴.

Simonetti (2014) found that 91% of the six Brazilian HCBLs surveyed employed access control⁷⁴. Similar findings were observed in US HCBLs operating under the FSAP, where 85% required individual passwords for laboratory access. However, in the case of HCBLs that do not handle select agents, the percentage decreased to 35%, highlighting the importance of implementing regulations to ensure compliance with this requirement¹³.

Access control in an HCBL is an essential biosecurity measure that should be implemented through an electronic personal identification system, such as a badge system²⁹⁰.

In addition to implementing access control measures, it is highly recommended to provide training to all visitors who enter the HCBL, emphasizing the requirements and precautions to be followed while inside the laboratory. This training is mandatory for laboratories dealing with the FMD virus in Brazil²³¹. Similarly, the Canadian Biosafety Standard mandates that all BSL-2 laboratories or higher should ensure that visitors, maintenance and janitorial staff, contractors, and anyone else who needs temporary access to the containment zone receive appropriate training or are accompanied by authorized personnel, based on their anticipated activities within the containment zone⁸⁰.

However, the present study found that 21.9% of the participating HCBLs did not have an SOP outlining the training procedures for visitor access. It is important to note that the absence of an SOP does not necessarily imply that training and other safety measures are not being conducted. However, having a standardized

procedure is essential to ensure consistent training practices and to establish a method for recording such training activities. It constitutes a fundamental element of the biosafety and biosecurity culture required by HCBLs.

6.2.8 Heating, Ventilation, and Air Conditioning system

6.2.8.1 Results

All HCBLs participating in the present study operate under negative air pressure; however, one HCBL does not use to monitor it (1/32, 3.1%). Two responses were missing regarding the frequency of air pressure meeting the established parameters. One was from the HCBL that does not monitor air pressure, and the second one was from a new HCBL that is not yet operational (Table A11).

The majority of HCBLs stated having a differential air pressure between the rooms. There is strong evidence that this variable is related to the size of the laboratory. The smallest HCBLs tend not to apply differential air pressures (Table 12).

Table 12. Correlation analysis between the size of the HCBLs participating in the survey and the presence of differential air pressures between their rooms.

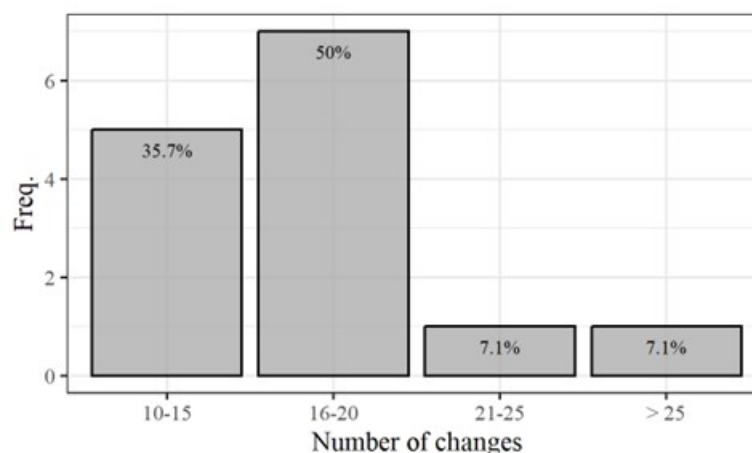
Are there differential air pressures between the BSL-3 rooms?	N	Laboratory footage (m ²)					Mann-Whitney test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
No	4	28.0	592.0	70.0	1,072.5	2,200.0	0.5701
Yes	19	20.0	898.5	124.0	2,287.7	10,000.0	

Most HCBLs (27/32, 84.4%) filter the air supplied. There was some missing information on air filtration. For instance, 5/32 (15.6%) and 17/32 (53.1%) HCBLs did not provide information about the filtration of exhausted air and the decontamination of filters, respectively. The exhausted air filter boxes are predominantly installed outside the biocontainment area (17/32, 53.1%). Most HCBLs (21/31, 67.7%) monitor the efficiency of the filters installed in the air exhaust system Table A12).

Redundancy is more common for exhaust air (19/32, 59.4%) than supply air (12/32, 37.5%). Only one HCBL (1/32, 3.1%) reported lacking sealing dampers. Three HCBLs (3/32, 9.4%) use recirculation of filtered air, while 13/32 (40.6%) did not provide this information. Most laboratories (19/32, 59.4%) rated the security level of the place where the Air Handling Units are located as high or very high. Regarding the company responsible for manufacturing the HVAC system, more responses indicated that it was a national company (9/32, 28.1%) rather than an international one (3/32, 9.4%). Two HCBLs (2/32, 6.2%) reported not monitoring the air temperature inside the HCBL, and 11/31 (35.5%) rated the frequency of the lab air temperature not meeting the established parameters higher than three on a 1 to 5 scale. (Table A13).

While most HCBLs operate with between 10 and 20 air changes per hour, some are reported to operate with more than 25 (Graph 31).

Graph 31 – Results regarding the number of air changes per hour set by the HVAC system of the HCBLs participating in the survey.



For ABSL-3 facilities, this number varied between 15 and 20. This difference was not statistically significant compared to the BSL-3 laboratories (Table 13).

Table 13 – Correlation analysis between the number of air changes per hour set by the HVAC system of the HCBLs participating in the survey and the handling of experimental animals in such facilities.

Does the lab handle animals in high-containment areas?	N	Number of air changes per hour					Mann-Whitney test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
No	10	10	17.3	17.0	5.87	27	0.7198
Yes	4	15	18.3	19.0	2.36	20	

6.2.8.2 Discussion

6.2.8.2.1 Inward airflow

The Canadian Biosafety Standard defines inward airflow as:

‘The air that always flows from areas of lower containment or lower contamination risk to areas of higher containment or higher contamination risk, as the result of a negative air pressure differential within the containment zone created by a ventilation system. Inward airflow protects against the release of airborne pathogens, infectious aerosols, and aerosolized toxins into ‘clean’ areas’⁸⁰.

It represents a fundamental characteristic of an HCBL³⁴⁵. Consequently, it is unsurprising that all HCBLs participating in the present study operate under negative pressure. The same result was observed in EDPLN laboratories²¹¹.

On the contrary, many HCBLs lack differential air pressures between rooms, posing challenges in achieving inward airflow. It is worth noting that the MS guidelines recommend that the airflow in an HCBL should always be unidirectional, flowing from the less contaminated area to the most contaminated one⁹⁴. The

probable explanation for that is related to the size of the HCBL. The smallest HCBLs tend to have only one room, justifying the absence of differential air pressures.

Therefore, managers must gain a deeper understanding of the concepts of inward airflow and pressure differentials. They need to assess whether establishing a unidirectional airflow is adequate or if the facility requires varying air pressures between rooms, taking into account their contamination levels and associated risks.

Maintaining proper air pressure parameters is crucial to ensure laboratory biosafety. According to the NIH, room differential pressure sensors should be installed to monitor the pressure difference between the anteroom, the laboratory, and the corridor or adjoining space. The room pressure differential should be set to maintain a minimum of 2.5 Pa, but in some cases, it can be increased to 12.5 Pa or more⁷⁸. However, Karagül et al. (2021) argued that the pressure difference should never be less than 7.6 Pa under any circumstances, with an ideal pressure difference of 12.5 Pa between clean and contaminated areas³⁹. Other authors have also concurred that a negative pressure differential of 12.5 Pa should be maintained between each pressure zone^{55, 170}. Gmunder et al. (2022) mentioned that pressure differences between 20 and 30 Pa have been proven effective in theory and practice, while pressure differences less than 15 Pa or greater than 60 Pa are not recommended²⁸⁹. The Chinese standard for veterinary vaccine manufacturers similarly defines a minimum of 15 Pa³⁷⁰, while the MS guideline recommends a differential air pressure between rooms ranging from 12.5 to 25 Pa⁹⁴.

Therefore, it was an undesirable finding that one HCBL does not monitor air pressure. Conversely, most HCBLs utilize automatic systems to measure the differential air pressure, which is highly desirable as it enables easy detection and alerts in case of deviations. According to Gmunder et al. (2022), pressure differences should be recorded at least every five seconds²⁸⁹.

It is concerning that 18.8% of the HCBLs rated the frequency of deviation in air pressure, compared to the established parameters, as 3 to 5 on a scale of 1 to 5.

6.2.8.2.2 Air changes per hour

The optimal number of air changes per hour is determined by multiple factors, including humidity, size of the airborne particulates, infective dose, and air turbulence within the room³⁷¹. Other considerations, such as the need for fume hoods, cooling load, pressurization of the area, and elimination of fumes and odors, are also considered when calculating the ventilation rate. Therefore, the infrastructure of the facility and the cooling requirements for the heat generated by equipment are also significant factors affecting the ventilation rate³⁷². Most authors recommend 10-15 air changes per hour, but never fewer than six^{39, 55, 94, 98, 170, 289-290}. However, this number should be higher in ABSL-3 facilities²⁸⁴.

Gmunder et al. (2022) proposed reducing the air exchange rate to six per hour during outside operating hours²⁸⁹. Similar ideas were advocated during the 2012 Workshop '*Biosecurity Challenges of the Global Expansion of High-Containment Biological Laboratories*'. Johnson stated that while HCBLs that work with volatile chemicals may require 10-12 air changes per hour, a minimum of six may be sufficient in other cases. This author added that maintaining some airflow is usually necessary during standby modes to prevent mold, humidity, and condensation inside the laboratory and ducts. In the same event, Chua also suggested considering the possibility of creating a 'sleep mode' for HCBLs with reduced airflow due to the expenses associated with high numbers of air changes per hour¹⁷.

According to the regional office of WHO for Africa, overly high air change rates will lead to far higher electricity costs for no benefit except for the more rapid removal of any aerosol produced from accidents outside primary containment. Air change rates should maintain operators' comfort by reducing heat loads caused by people and equipment, removing odors, and controlling the relative humidity and condensation. It can be achieved by using air change rates of as low as six changes per hour and potentially using carefully designed natural ventilation if sealability is not adopted²⁰⁴.

In the present study, the lowest number of air changes per hour found was 10, while the highest was 27. For ABSL-3 facilities, this number varied between 15 and 20. Although the average number of air changes was higher in ABSL-3 facilities, the difference was statistically insignificant. The findings indicate that most Brazilian HCBLs comply with international guidelines for this parameter but tend to exceed them, suggesting an opportunity to reduce expenses by redefining the setup for this parameter.

Regarding air recirculation, it was suggested that regulators consider allowing 85% of the air to be recirculated with double HEPA filtration. This proposal considers the tropical climate, where it is inefficient to cool and dry air only to pump it back outside¹⁷. However, this topic is controversial. While some argue that recirculating filtered air poses no significant risk, especially when filters are tested as required, others consider it unacceptable. They argue that some HEPA filters may have poor integrity, and even though energy used for heating or cooling the air could be recovered, the air itself should not be recirculated. The MS guidelines state, for example, that the exhaust air must not be recirculated in any part of the building⁹⁴. In contrast, recirculation of HEPA-filtered air from BSCs is currently permitted in BSL-3 labs, which some find acceptable due to additional precautions and potentially more permissive attitudes among lab workers. It is worth noting that there is often a lack of available evidence to accurately characterize and assess risks in these cases¹⁷.

The concept of 'low-cost' HCBL, proposed by Hernandez (2008), predicts that 70% of the air should be recirculated within the laboratory⁶⁶.

The results obtained in the present study indicate that most HCBLs chose not to recirculate the filtered air, although a high percentage of respondents did not provide this information.

Further studies are needed to clarify the risks associated with reducing the air change per hour and the recirculation of filtered air. These studies should consider biosafety aspects and the impact of these measures on the operational costs of HCBLs.

6.2.8.2.3 Position and decontamination of the air filter boxes

According to Gmunder et al. (2022), the HEPA filter system should be installed above the laboratory's service area. Installing it within the containment is not recommended due to the challenges involved in maintaining the filter system. If this alternative is implemented, the exhaust air duct from the filter unit to the containment boundary must be gastight²⁸⁹. The Spanish regulations for BSL-3 facilities also state that the air filter boxes do not necessarily need to be inside the contained area, but both the boxes and the ductwork should be sealed. On the contrary, Brazilian regulations for handling the FMD virus require air filter boxes installed inside a contained area²³¹. The results obtained in the present study revealed that most HCBLs did not install the air filter boxes inside the contained area.

The decontamination of exhausted air filter boxes is a highly critical procedure due to the associated biological risks. Unfortunately, more than 50% of the respondents did not provide information on how they decontaminate the filters when replacing them. Considering the age of the laboratories, it is likely that most of them have never performed this procedure. However, it is concerning that they have not yet established a protocol for doing so. Among those who provided information, the most common decontamination methods were autoclaving, fumigation with formaldehyde, and vaporization with hydrogen peroxide.

Laboratories that use autoclaving should assess the risks involved in transporting the filters from the filter boxes to the autoclave, mainly when the boxes are located outside the contained area. Three of the five HCBLs reported using this method have filter boxes outside the contained area. Similar concerns should be raised regarding the HCBL that mentioned the incineration method. It remains unclear whether a preliminary chemical decontamination is performed as required.

The pros and cons of formaldehyde and hydrogen peroxide will be addressed in Chapter 6.2.11 of the present study. UV is not an acceptable method for decontaminating the filter. Therefore, the HCBL that informed using it should define another method. It was unclear what the respondent meant by the answer 'H₂O₂ and hypochlorite'. It did not get any clarification when he/she was asked about it.

6.2.8.2.4 Redundancy

HEPA filters must be tested annually and after a filter change. In cases where the HEPA filter system lacks redundancy, filter changes and tests should be conducted following the decontamination of the HCBLs as part of regular laboratory maintenance; for systems with redundancy, filter changes, and tests can be carried out during operation after decontaminating the filter system²⁸⁹. Some argued that the routine use of a second set of HEPA filters, originally suggested as a precautionary engineering measure in the 1980s due to the increased risk of filter damage, may no longer be necessary¹⁷. However, in this study, the majority of HCBLs have reported having redundancy in their air exhaust filtration systems, although the proportion (59.4%) was lower than that observed in European HCBLs (76.0%)³⁵⁶.

Several factors must be considered in the risk assessment to support the decision regarding adopting redundancy in equipment and systems related to HVAC, electric systems, etc. These factors include budgetary constraints, the feasibility of suspending HCBL activities for maintenance, the HCBL's location, and the time required to access external maintenance services. Additionally, the size and complexity of the HCBL should also be considered.

6.2.8.2.5 Filtration of the supplying air

In terms of biosafety, the international guidelines do not demand supply-air HEPA-filtration for HCBLs; however, depending on the working program and climate conditions, cleaner supplied air may be demanded^{39, 290}. Cirrota (2004) advocated that using a HEPA system to filter the intake air offers several advantages. It effectively enhances the air quality, extending the lifespan of HEPA filters in both BSCs and exhaust systems³⁷³.

Therefore, each HCBL must evaluate the advantages and disadvantages of implementing a HEPA filter system in the supplied air. Approximately half of the participating HCBLs in this study utilize this technology, less than was observed in European HCBLs³⁵⁶. Further research is required to assess whether this decision

was a collaborative effort between designers and users, considering all the pros and cons, or if the users were not involved in this determination.

6.2.8.2.6 High-Efficiency Particulate Arrestance filters efficiency monitoring

HEPA filters do not need to be changed regularly in HCBLs¹³. Given their high costs, it is advisable to assess their efficiency and replace them only when necessary. This assessment can be performed using manometers with probes installed on both sides of the filters, along with an alarm system that indicates when the efficiency drops below an acceptable level⁸².

In the present study, most HCBLs surveyed reported being able to periodically monitor the efficiency of the filters installed in their air exhaust system. However, further research is needed to determine the specific methods employed for this monitoring and the average filter lifespan.

6.2.8.2.7 Temperature monitoring

According to the Brazilian work regulation on ergonomics, organizations should regulate temperature, air velocity, and humidity levels to ensure optimal thermal comfort in work environments while adhering to the recommended temperature range of 18 to 25°C for air-conditioned spaces³⁷⁴. The MS guidelines for BSL-3 and ABSL-3 laboratories require the temperature within the work areas to be maintained between 19 and 23°C⁹⁴.

In addition to the well-being of workers, air temperature can affect the operation and lifespan of specific equipment, such as freezers and ultra-low freezers. Therefore, it is necessary to monitor the HCBL's temperature, as most participants in the present study use to do. However, the frequency of deviations from the established temperature parameters within the laboratory is relatively high, as reported by around one-third of the HCBLs, rating the frequency higher than three on a 1 to 5 scale.

This finding is comparable to those obtained by Vieira & Salgado (2008) in a survey conducted with six Brazilian HCBLs. Complaints of thermal discomfort have been noted during this study, with users expressing dissatisfaction due to difficulties in adjusting the internal temperature, resulting in inadequate heating or cooling. Maintenance deficiencies were often identified as the cause, leading to a lack of user confidence in the ventilation system⁷³.

6.2.8.2.8 Sealing dampers

Sealing dampers are defined as shut-off valves used to seal supply and exhaust air ductwork to or from a containment zone, allowing for the decontamination of HEPA filters. Automated isolation dampers also protect against backdraft in the event of an HVAC system failure or a reversal of airflow⁸⁰. These dampers are mandatory according to Brazilian regulations for FMD virus handling²³¹.

In the present study, most HCBLs surveyed reported having these devices. However, a high percentage of respondents did not provide this information, indicating a lack of awareness among some users regarding the fundamental features of the HVAC system.

A higher percentage of respondents lacked information for the question, 'Was the HVAC system manufactured nationally?', which makes it difficult to conclude this matter.

6.2.8.2.9 Security

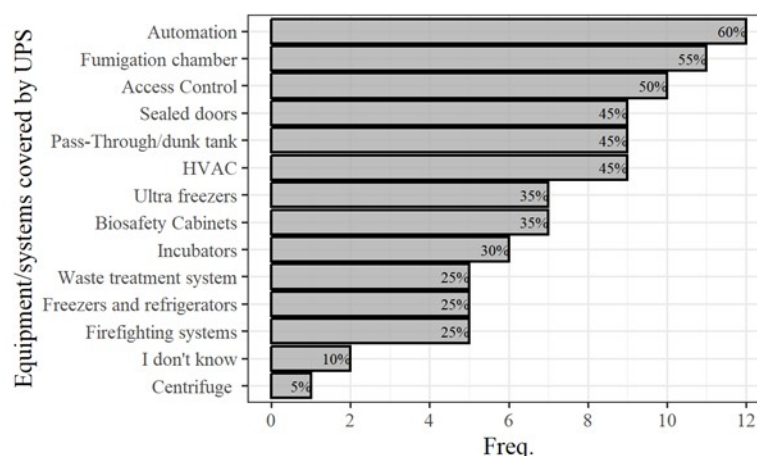
Given the critical importance of the HVAC system in maintaining the biosafety standards of an HCBL, it is imperative to install the Air Handling Units in secure locations to prevent vandalism, sabotage, or any other crimes that may compromise their functionality. The present study's findings revealed that 18.8% of the respondents rated the security level of the installation site for the Air Handling Units as 1 to 3 on a scale of 1 to 5, where 1 represents an entirely insecure location and 5 represents a fully secure location. This outcome can be deemed undesirable, emphasizing the need for improved security measures.

6.2.9 Electrical system

6.2.9.1 Results

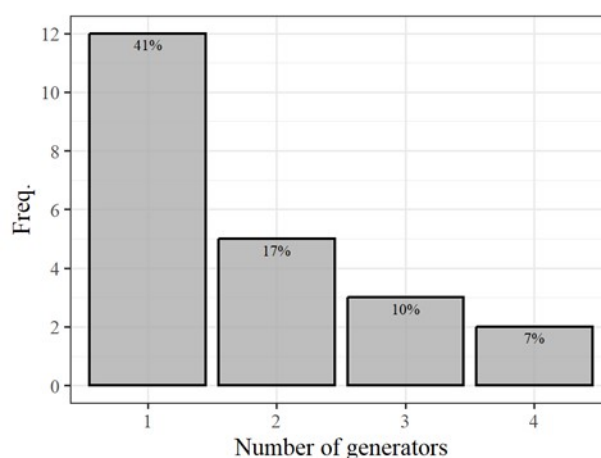
Less than half of the HCBLs in the present study have dedicated power transformers (13/32, 40.6%). The number of transformers in these facilities varied from one to three, except for one facility that reported having nine transformers. The security rating of the installation site for these transformers was considered good or excellent for 60.0% of the respondents. Approximately one-third of the HCBLs experience a current power supply that meets less than 100% of their average power demand (4/14, 28.6%). This percentage increases to 42.9% when considering peak power consumption. Most HCBLs rated the security of the location where the power transformers are installed as 4 or 5 on a scale from 1 to 5 (12/20, 60.0%). Around one-third of the HCBLs do not have a UPS system (10/32, 31.2%). Among those with one, the predominant autonomy period is 30 minutes (6/19, 31.6%) (Table A14). The automation and fumigation chamber were the most common equipment/systems covered by the UPS (Graph 32).

Graph 32 – Results regarding the kinds of equipment/systems covered by the UPS system of the HCBLs participating in the survey.



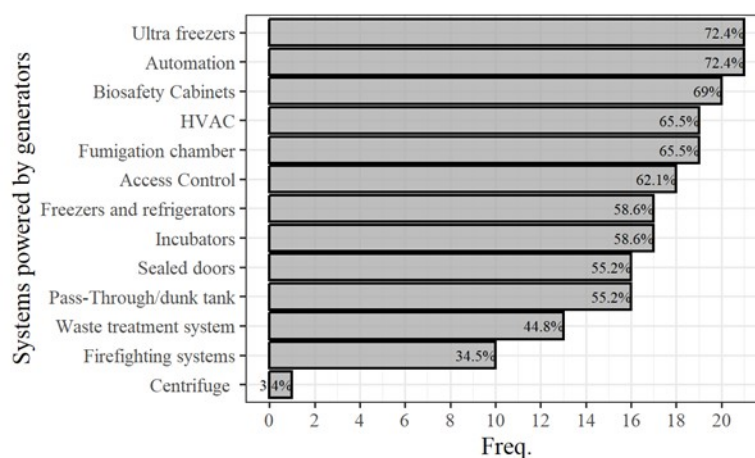
Only one HCBL reported not having a power generator (1/32, 3.1%). Most HCBLs have one generator (12/29, 41.4%), with a maximum of four reported (Graph 33).

Graph 33 – Results regarding the number of power generators installed in the HCBLs participating in the survey.



The most common equipment/systems covered by the power generator were automation and ultra-low freezers (Graph 34).

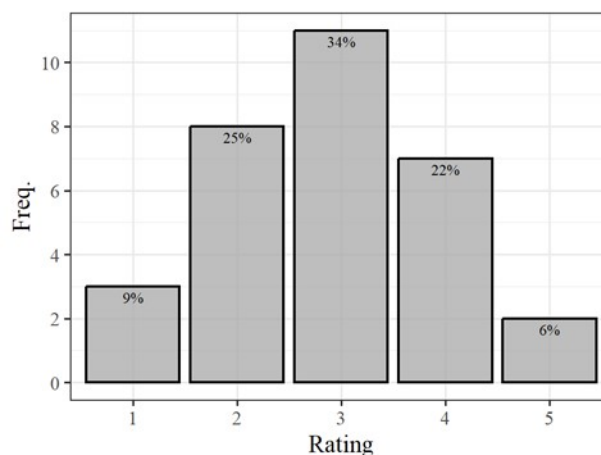
Graph 34 – Results regarding the kinds of equipment/systems covered by power generators in the HCBLs participating in the survey.



Almost all HCBLs have their generators periodically tested by a specialized technical team (27/28, 96.4%), although the frequency of these tests varies widely. Additionally, more than half of the HCBLs have preventive maintenance services for their power generators provided by specialized technical teams (16/29, 55.2%). The security of the location where the power generators are installed was predominantly rated as 4 or 5 on a scale from 1 to 5 (19/30, 63.3%) (Table A15).

The frequency of power outages varies significantly (Graph 35), and only three HCBLs stated having a shallow impact on their activities due to power outages (3/32, 9.4%) (Table A15).

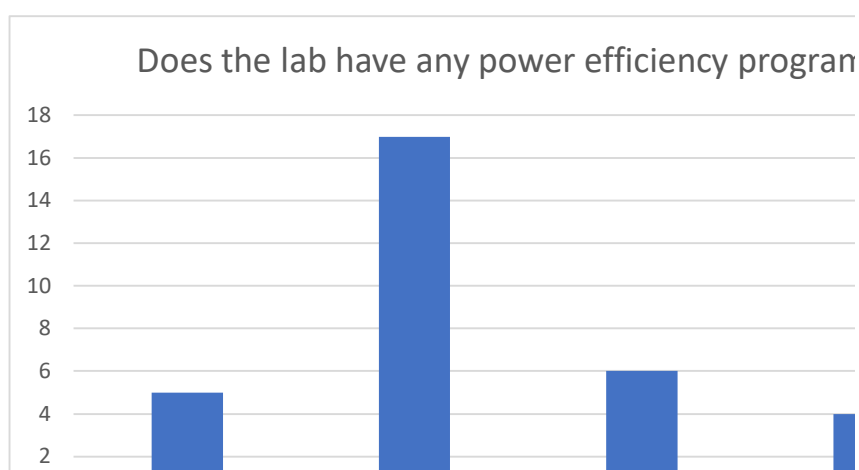
Graph 35 – Results regarding the frequency of energy power failure in the HCBLs participating in the survey^{hh}.



Only four HCBLs stated having any mechanism to save power (4/32, 12.5%) (Graph 36). The methods mentioned were:

- Energy recovery wheel;
- Optimization of HVAC system operation based on demand;
- Implementation of a heat exchange system for cooling liquid effluent;
- Installation of LED (Light Emitting Diode) bulbs.

Graph 36 – Results regarding implementing mechanisms to save electricity by the HCBLs participating in the survey.



6.2.9.2 Discussion

6.2.9.2.1 Transformers

While dedicated power supply lines are only necessary for maximum-contained laboratories, the inadequate or unreliable availability of electrical power poses a significant risk for incidents in HCBLs. It can result in partial breaches or complete failure of systems designed to minimize exposure or release of biological agents².

^{hh} Scale ranging from 1 (very low) to 5 (very high).

It is important to note that HCBLs consume more energy per square foot compared to other commercial buildings. Continuous operation, ventilation requirements, energy-intensive equipment, and water use for steam sterilization contribute to higher energy consumption. Additionally, laboratories with critical research and containment needs often require redundant electrical power systems to ensure fail-safe operations¹.

Therefore, having dedicated power transformers, as observed in 40.6% of the HCBLs in this study, is desirable. Redundancy in power supply enhances biosafety, found in 53.8% out of HCBLs having dedicated power transformers. However, it is crucial to ensure the security of these installations to prevent sabotage or other criminal activities, a concern addressed by most HCBLs.

A significant number of HCBLs reported that their current power supply meets less than 100% of their demand, especially during peak consumption periods, which was a worrisome finding that requires attention.

6.2.9.2.2 Uninterruptible Power Supply and generators

While the BMBL mandates the provision of uninterruptible emergency power only for maximum-biocontainment facilities¹, it is recommended for HCBLs to ensure the ongoing work with microorganisms and their storage, as well as the safe conclusion of work and orderly exit from the HCBL in case of system malfunction or failure^{80, 94, 289}.

The fact that only three HCBLs reported a shallow power failure frequency in the present study highlights the unreliability of the power supply in Brazil. It emphasizes the need for an emergency power supply, as such failures significantly impact HCBL activities.

Emergency power can be provided through a UPS system or a building generator⁸⁰. A survey conducted by FAO and A-PBA of BSL-2 and BSL-3 laboratories in seven countries in the Asia-Pacific region revealed that many labs fall below an acceptable level of functionality for their BSL level, with the lack of generators in areas with unreliable electrical power being a common issue. In contrast, only one HCBL participating in this study lacked a generator. On the other hand, more than one-third of them lacked a UPS, which, when present, typically offers more than 30 minutes of autonomy, as recommended²³¹.

An automatically activated emergency power source should be provided at a minimum for the laboratory exhaust system, alarms, lighting, access controls, BSCs, ventilated cage racks, compressed air for dumpers and door gaskets, fire fighting systems, communication tools, freezers, airlocks, and automation^{1, 231, 289-290}. However, the emergency power supply of some HCBLs participating in the present study does not cover all these systems/equipment. For instance, only 34.5% stated that firefighting systems are covered by generators.

Backup power systems should be tested at least monthly⁴³, followed by the majority of the HCBLs participating in this study, often through the engagement of a specialized third-party team. Like transformers, generators are typically installed in secure locations, as required by MS guidelines⁹⁴.

6.2.9.2.3 Electricity saving mechanisms

The WOA emphasized that creative and open-minded thinking and innovation are vital to improving laboratory sustainability, which will be improved by networking and sharing information and best practices³⁶⁹. As energy power

represents one of the most significant costs in an HCBL, saving electricity is a crucial aspect of sustainability³²⁰.

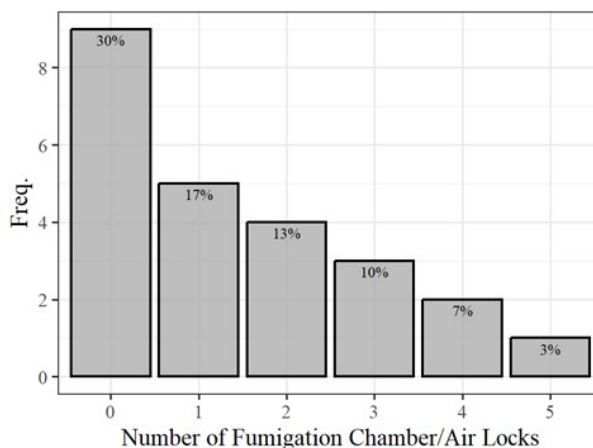
However, the proportion of HCBLs participating in the present study that stated having any power efficiency program was deficient. Some of the initiatives mentioned are feasible for all HCBLs and should be considered in future projects.

6.2.10 Fumigation chambers/Airlock/Pass-through

6.2.10.1 Results

Approximately two-thirds of the HCBLs involved in the current study have fumigation chambers or airlocks for accessing and exiting large volumes (21/32, 65.6%). Such chambers ranged from one to five (Graph 37).

Graph 37 – Results regarding the number of Fumigation chambers/Airlocks installed in the HCBLs participating in the survey.

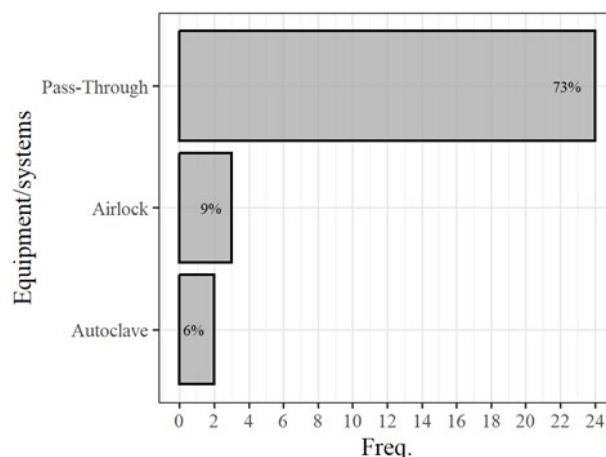


Additional mechanisms mentioned by the participants for transferring large volumes encompassed:

- Single door (n = 4);
- Changing room (n = 4);
- Anteroom (n = 2);
- Autoclave (n = 1).

Most HCBLs utilize a pass-through system to transfer small volumes to and from the contained area (24/32, 75.0%). Other equipment mentioned for this purpose includes airlocks and autoclaves (Graph 38). No HCBL was informed of not having fumigation chambers/airlocks or pass-throughs.

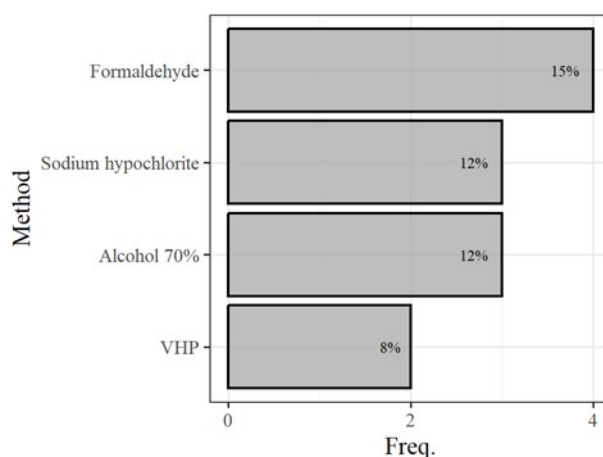
Graph 38 – Results regarding the equipment used to transfer small volumes from and to the HCBLs participating in the survey.



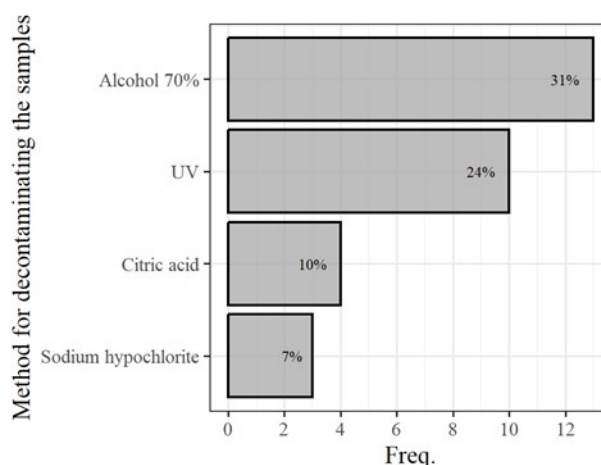
Only one HCBL mentioned the absence of an interlocking door system for fumigation chambers/airlocks (1/21, 4.8%), while two HCBLs lacked this system for the pass-through (2/29, 6.9%). Three HCBLs stated that their fumigation chambers/airlocks were not sealed (3/21, 14.3%). The number of HCBLs that validated the decontamination method was the same as those that did not (7/19, 36.8%). Similarly, 11/28 (39.3%) HCBLs validated the decontamination method for the pass-through, while 10/28 (35.7%) did not. Only one HCBL acknowledged the presence of cracks in their fumigation chamber/airlock (1/22, 68.8%). Additionally, only one HCBL reported that their equipment was manufactured by an international company (1/23, 4.3%) (Table A16).

Formaldehyde fumigation and 70% alcohol were the most used methods to decontaminate the fumigation chambers/airlocks and pass-throughs, respectively. Other methods for decontaminating the fumigation chambers/airlock included sodium hypochlorite, 70% alcohol, vaporized hydrogen peroxide (VHP), and ultraviolet (UV) treatment. For decontaminating the pass-through, other methods mentioned were UV treatment, citric acid, sodium hypochlorite, biguanide + ammonium quaternary, hydrogen peroxide, Lysol, phenol, and surfic (Graphs 39 and 40).

Graph 39 – Results regarding the method applied by the HCBLs participating in the survey to decontaminate their Fumigation chamber/Airlocks.



Graph 40 – Results regarding the method applied by the HCBLs participating in the survey to decontaminate their Pass-throughs.



6.2.10.2 Discussion

The Canadian Biosafety Standard defines pass-through technology as equipment with double-door compartments located on a containment barrier, allowing the safe transfer of materials into and out of the barrier. Such technology includes double-door barrier autoclaves, pass-through chambers, dunk tanks, barrier cage washers, and feed chutes. These systems should incorporate a mechanism that prevents both doors from being opened simultaneously. It can be achieved through visual or audible indicators that signal when doors are opened or closed or other acceptable mechanisms. Keeping at least one door of pass-through technologies on the containment barrier closed ensures containment integrity and prevents the release of regulated materials. Mechanical or electronic door interlocks are the most suitable, as they do not rely on personnel adherence to operational procedures. All penetrations in the containment barrier should be sealed to maintain its integrity⁸⁰.

Gmunder et al. (2022) argued that when doors are opened between two areas with different pressures during regular operation, the pressure difference across the door collapses, and so does the inward airflow. The movement of the door leaf and people generates turbulent air flows in the door opening, thus mixing the air between the two spaces. For this reason, airlocks with interlocking doors must be present at the containment boundary. Combined with a higher air exchange rate, this approach effectively reduces the likelihood of aerosols and particles containing microorganisms escaping from the contained area²⁸⁹.

The Spanish regulation for HCBLs also references pass-through technologies, stating that the equipment should have mechanisms for sterilizing or decontaminating materials transferred from the contained area. These mechanisms should be validated, and the equipment should be sealed, with the air decontaminated or HEPA filtered. Such equipment includes airlocks for large volumes, pass-through systems for small volumes, dunk tanks, and autoclaves⁸².

The Brazilian regulation for FMD virus handling also requires pass-through technology for small and large volumes. It outlines similar requirements to the Spanish regulation, including sealing, an interlocking door system, and a validated method for decontamination²³¹.

The US regulations and the WHO Manual only mandate pass-through technologies for maximum contained laboratories¹⁻². However, Vieira & Salgado

(2008), in their interviews with Brazilian HCBL users, concluded that this device is considered highly necessary for those who do not have it⁷³.

Despite the absence of a Brazilian regulation for specific construction requirements (except for FMD virus handling), most HCBLs participating in this study have implemented pass-through technologies. The majority of them incorporate an interlocking door system. However, only about half of the HCBLs validate their decontamination methods. Additional comments regarding the decontamination method, particularly those using formaldehyde, will be addressed in the next Chapter.

The information regarding the manufacturers indicates that national companies are capable of delivering this technology.

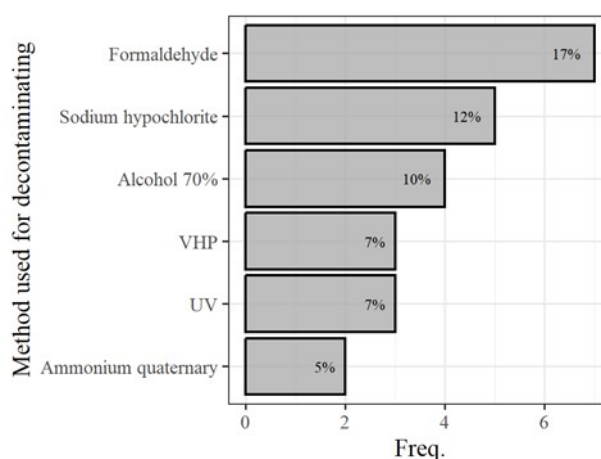
6.2.11 Whole lab decontamination

6.2.11.1 Results

Half of the participating HCBLs in the current study reported implementing a regular decontamination schedule for the entire laboratory (16/32, 50.0%). The frequency of decontamination varied, ranging from daily to annual intervals. The number of HCBLs that validated their decontamination methods (10/30, 33.3%) was similar to those that did not (11/30, 36.7%) (Table A17).

The most used decontamination method was formaldehyde fumigation, followed by sodium hypochlorite, 70% alcohol, VHP, UV light, and ammonium quaternary compounds. Other methods were mentioned only once, such as 0.01% hydrochloric acid, peracetic acid, phenol, and potassium permanganate (Graph 41).

Graph 41 – Results regarding the method applied by the HCBLs participating in the survey to decontaminate the laboratory as a whole.



6.2.11.2 Discussion

Laboratories require regular decontamination through fumigation, surface disinfection, or a combination of both. The main disadvantage of liquid-based methods is ensuring adequate coverage over all contact surfaces for the desired time, especially for large areas and those difficult to access, such as air conditioning system ducts. For this reason, gas-based or vapor-phase methods have been preferred⁹⁴.

The need to decontaminate the whole laboratory arises in various situations, such as responding to emergencies caused by accidental spills of infectious materials, conducting in situ maintenance on contaminated devices or systems,

preventing cross-contamination between animal studies, removing large equipment, changing HEPA filters, or performing periodic maintenance^{55, 291, 365}.

The standard procedure for surface decontamination involves spraying the disinfectant and allowing it to remain for a specified duration, depending on the type of microorganism being handled. The effectiveness of a surface disinfectant depends on the mechanisms of action of its active ingredients against the targeted organisms. The efficacy of disinfectants is influenced by factors such as the population of organisms on surfaces, the concentration of both organisms and disinfectant, the presence of interfering substances (such as organic material), and the duration of contact⁵⁵.

Regarding fumigation methods, currently, there are at least three types of dispersion devices, each based on distinct processes:

- a. Nebulization: droplet size is less than 5 μm ;
- b. Spraying: droplet size ranges from 10 to 50 μm ;
- c. Flash evaporation: the heated biocidal product (e.g., hydrogen peroxide) vaporizes and is drawn by an airstream into the room⁵⁵.

The efficiency of the disinfection process depends on several factors, including the product used, its application method, and the targeted pathogen. Essential factors include the concentration and formulation of the disinfectant, water solubility, pH, type of surface, presence of organic material, temperature, humidity, contact time, and whether mechanical action is employed during application⁵⁵.

In practice, when decontaminating HCBLs, specific steps should be followed. The laboratory should be secured and sealed, and a pre-cleaning step should be performed. Before decontamination, the air handling unit should be configured (either stopped or in bypass mode). The decontamination process consists of multiple phases, including the dispersion and contact of the biocide, followed by room aeration. Biological indicators can be spread throughout the rooms to assess the efficacy of the process⁵⁵.

According to the literature, the most used methods for HCBL decontamination are formaldehyde fumigation, hydrogen peroxide vaporization, and chlorine dioxide gas^{257, 375-377}.

When dealing with an unknown pathogen, using the most virulent pathogen as a standard for disinfection is common practice. A disinfectant containing 0.55% effective chlorine is often selected to disinfect the experimental table and samples, while hydrogen peroxide or peracetic acid is chosen to disinfect the laboratory environment. Although 75% alcohol is commonly used in biosafety laboratories, it is ineffective against all nonlipid viruses²⁷⁵.

Formaldehyde is a widely used but controversial method due to concerns about its toxicity and carcinogenicity. The UK government has recognized the search for alternatives to formaldehyde since 2015. When evaluating alternative methods, factors such as efficacy, usability, reproducibility, reliability, safety, cost, residues, potential damage to the HCBL and its equipment, and the time required for the cycle should be considered^{55, 275, 287, 375, 377-378}.

The ANVISA has recently forbidden the isolated use of formaldehyde as a decontamination method throughout Brazil. Its usage is only permitted when combined with a device registered by ANVISA³⁷⁹.

Hydrogen peroxide has recently gained popularity for room-wide decontamination due to its ability to break down naturally into water and oxygen, leaving no toxic residues and eliminating the need for subsequent cleaning measures^{287, 378}. Henneman et al. (2022) tested a hybrid mixture of vaporous and

aerosolized particles of hydrogen peroxide, concluding that it was a viable option for decontaminating HCBL of all sizes, considering the need for an effective system without residues and improved staff safety³⁷⁷.

Coppens et al. (2019) concluded that validating the decontamination procedure is crucial, as each facility has unique characteristics and may handle different pathogens. A case-by-case approach is necessary, based on proper risk assessment to minimize exposure to pathogens and fumigants. Validation should be considered an essential requirement in the regulatory authorization process before using an HCBL³⁶⁶.

Tearle (2022) supported the application of a risk-based approach for routine decontamination of HCBLs³⁶⁵, while Beswick et al. (2011) emphasized the importance of validating a specific method within the area to be treated, using pathogens commonly handled in that area or effective surrogate challenges. Similarly, evaluating a particular type of fumigation system within the treated area ensures its effectiveness under the existing conditions³⁷⁵.

Pastorino et al. (2017) agreed that validated methods for surface disinfection and laboratory decontamination should be based on risk assessment, followed by testing the efficacy of such products in specific environments and conditions⁵⁵.

However, Behrmann et al. (2007) argued that many experts interpret room fumigation as minimizing the risks of re-entering a hazardous area rather than a standard disinfection measure. This interpretation arises from the challenges encountered in successfully validating all the parameters that influence the effectiveness of the fumigation process, such as agent concentration, agent consumption, penetration of porous materials, and accessibility of cavities²⁹⁰.

Regarding the frequency of decontamination, a study conducted in US HCBLs revealed that select agent facilities decontaminate the entire lab less than biennially or only after an accident. On the other hand, non-select agent facilities are more likely to decontaminate every six months or annually¹³.

It is noteworthy to mention the experimental study by Liu et al. (2020) regarding potential infection risks from exposure to bioaerosols in a BSL-3 facility. Their findings indicated that nearly 70% of bioaerosol particles were deposited on surfaces such as walls and equipment, resulting in a significant risk of LAIs. The authors also observed that over 50% of bioaerosols settled on the walls (22.76%), floor (17.71%), and ceiling (12.03%)³⁸⁰.

The results obtained in the present study demonstrate a variety of procedures and frequencies for whole-lab decontamination in HCBLs. The most common methods mentioned align with the existing literature, except for the chlorine dioxide gas, which no HCBL mentioned. However, the usage of formaldehyde shall be replaced by another method since the BFG forbade it.

Another two concerning findings emerged: the use of specific methods that are not recommended, such as UV light (as discussed in-depth in Chapter 6.2.14.2.3.4), mentioned by three HCBLs, and the lack of validation of the decontamination methods in many HCBLs since only one-third of the HCBLs stated that their methods had been validated. Although the validation process may be complex, most authors advocate for its necessity to ensure biosafety, as mentioned earlier.

The current study's findings corroborate those reported by Simonetti (2014), who conducted interviews with 37 users of Brazilian HCBLs. All participants unanimously agreed on the need to enhance procedures concerning the cleaning and decontamination of the facilities⁷⁴.

6.2.12 Effluent Decontamination System

6.2.12.1 Results

Out of the 32 HCBLs participating in the current study, six stated operating as 'dry' laboratories, meaning they do not generate any liquid effluent (6/32, 18.8%). Among the HCBLs that generate liquid effluent, thermal treatment was the most used decontamination method (17/32, 53.1%). Less than half of the participating HCBLs had validated their decontamination method (12/25, 48.0%). The respondents mentioned a wide range of EDS manufacturers, with only one company being mentioned more than once. The number of HCBLs stating that the EDS was installed within the biocontainment area was the same as those stating it was not (11/26, 42.3%) (Table A18).

Only one HCBL admitted that leaks were commonly observed in the EDS (1/25, 4.0%). Less than one-third of the participating HCBLs had redundancy for the EDS (7/25, 28.0%). Half of the HCBLs reported an EDS maximum capacity of less than 1,000.00 L (6/12, 50.0%). However, a few HCBLs acknowledged that the capacity may not meet the demand (4/25, 16.0%). Around half of the HCBLs conducted periodic qualification tests for the EDS (11/23, 47.8%). The frequency of these tests was typically annual (6/11, 54.5%), but two HCBLs performed them biannually (2/11, 18.2%). Approximately one-third of the HCBLs did not record each EDS cycle (7/23, 30.4%) (Table A18).

6.2.12.2 Discussion

The liquid effluent serves as a potential pathway for releasing pathogens from laboratories. A study conducted in 337 clinical/diagnostic and academic/research laboratories in Pakistan revealed that many students and researchers were directly disposing of high-risk pathogenic samples into the drainage system after completing their experiments. Samples collected from these laboratories contained highly pathogenic organisms such as *Salmonella typhi*, *Mycobacterium tuberculosis*, and Methicillin-resistant *Staphylococcus aureus*. This finding strongly suggests that the pathogens were released into the environment without proper decontamination³⁸¹.

An FMD outbreak severely impacted eight farms in the UK in 2007, resulting in significant economic losses. According to official reports, the outbreak was attributed to a combination of factors, including a damaged and leaking drainage system and inadequate maintenance and inspection protocols for effluent drains¹³⁹.

Since wastewater from cleaning, fire fighting systems, and sinks is a potential source of contamination, it must be retained in the containment area for chemical or thermal inactivation treatment. Alternatively, sinks with a built-in thermal 'inactivation flow through the device' may be applied²⁹⁰.

According to the Canadian Biosafety Standard, an EDS is critical for decontaminating all liquid waste generated in BSL-2 Ag laboratories where prions are handled, BSL-3 laboratories where non-indigenous terrestrial animal pathogens are handled, and all BSL-3 Ag and BSL-4 laboratories⁸⁰.

The BMBL states that enhanced environmental and personal protection may be necessary for BSL-3 laboratories based on risk assessment and applicable local, state, or federal regulations, including installing an EDS¹.

Cote et al. (2021) validated a chemical EDS treatment of BSL-3 and BSL-4 waste effluent using a bleach solution. The authors concluded that the significant disadvantage of the chemical system, in comparison to the thermal one, is the need for effluent treatment capacity. Each run of the chemical EDS required approximately

57 L of bleach for efficacy, and scale-up operations required approximately 1,250 L of bleach per day to accommodate effluent volumes of 11,350 L/day. Using this type of system for large HCBLs would be difficult from a cost and logistical standpoint due to the need to constantly replace bleach containers using a process that could not be automated³⁸². Other disadvantages of the chemical EDS include neutralizing the chemical disinfectant after a treatment cycle and the risk of releasing toxic vapors into the work area or the environment^{94, 382}.

Mourya et al. (2014) mentioned using a solution with a 1:1 (v/v) mixture of 2.5 percent sodium hypochlorite and 0.25N NaOH to treat liquid effluents within an HCBL. Alternatively, the authors suggested autoclaving the liquid effluent at 121°C at 15 psi pressure for 20 min through a specific liquid cycle in the autoclave¹⁷⁰.

Thermal inactivation systems are the most used methods to decontaminate liquid effluent in the HCBLs participating in the current study. However, it was not specified whether autoclave or EDS methods were employed. Similarly, the study did not investigate the disinfectants used for chemical methods. Regardless of the method employed, the most concerning finding was that a significant proportion of HCBLs did not validate the chosen method (10/25, 40%), indicating a high-risk of releasing live pathogens into the drainage system.

The regular performance of EDS qualification tests is as important as validating the methodology, which is not observed by 26.1% of the participants. Among these six HCBLs, only one reported validating the system. Hence, at least five HCBLs do not validate or qualify the system, posing a significant risk of releasing live pathogens.

The frequency of qualification tests is typically annual, as indicated by the results obtained in the present study. However, it may be subject to change based on manufacturer and risk analysis recommendations.

The availability of numerous EDS manufacturers suggests that obtaining this type of technology in the country is a minor concern. However, further studies must assess the various methods and technologies employed more comprehensively.

Installing EDS outside the contained area is generally not a concern, except for HCBLs handling the FMD virus. According to the specific regulations for FMD virus handling, an EDS must be situated in a contained area, segregated, and specifically designated for this purpose, ensuring equivalent biosafety conditions to other contained zones²³¹. However, the HCBLs that chooses to have the EDS outside the contained area must establish risk mitigation procedures, mainly when the system is situated in a separate or open building.

One of the procedures is the detection of leaks. For instance, the EDS of the HCBL, which ranked the observation of leaks as four on a scale of 1 to 5, is installed outside the biocontainment area. This kind of situation represents a severe risk for pathogen release.

Redundancy in EDS may be necessary, as determined by a risk assessment, mainly when the system's capacity is near the daily demand or when the laboratory cannot afford to halt its operations (e.g. when the HCBL is responsible for diagnosing notifiable diseases). The absence of redundancy can result in the suspension of HCBL activities each time the system requires maintenance. However, this appears infrequent based on the responses to the question, 'How often do operational failures occur in the effluent decontamination system?'

Half of the HCBLs participating in the present study reported a maximum capacity of their EDS of less than 1,000 L. Although it was impossible to establish a correlation between the maximum EDS capacity and the frequency at which the

system fails to meet the demand, it is essential to emphasize the importance of conducting comprehensive preliminary studies that can provide the necessary details for an accurate calculation of the EDS capacity.

Recording each decontamination cycle is a fundamental procedure to ensure process traceability⁹⁴, essential for investigating incidents and accidents. However, many HCBLs fail to comply with this fundamental quality assurance requirement.

As observed to HVAC, many responses about the EDS were also marked as 'no answer'. The proportion of these responses ranged from 17% to 34.8%, suggesting that some respondents may have limited knowledge of this topic.

While further studies are required to comprehensively examine the various methods employed by Brazilian HCBLs to decontaminate liquid effluent, the findings from the current study suggest that specific procedures need improvement, particularly those concerning method validation and system qualification. These procedures are crucial to ensure that wastewater does not harbor live pathogens.

6.2.13 Autoclaves

6.2.13.1 Results

Most HCBLs in the present study have only one autoclave (23/32, 71.9%), with the maximum number reported being three. However, most HCBLs believe that the demand for autoclaving is usually or always met (28/31, 90.3%). Four and two HCBLs reported that their border autoclaves do not have an interlocking system (4/31, 12.9%) and lack a bioseal (2/31, 6.5%), respectively. Nearly all HCBLs possess horizontal double-door border autoclaves (30/32, 93.8%). More than half of the HCBLs have validated the cycles for all types of loads (17/31, 54.8%). Less than half of HCBLs decontaminate the output of deaerators and condensates (15/32, 46.9%), but the percentage of HCBLs that did not provide this information was significant (21/32, 65.6%). Most HCBLs make it a practice to record each cycle (25/31, 80.6%) (Table A19).

A single manufacturer supplied one-third of the autoclaves in the HCBLs participating in this study (11/28, 39.3%). Additionally, five HCBLs mentioned using other equipment or systems to decontaminate solid waste (5/32, 15.6%) (Table A19).

The equipment/systems mentioned above were chemical decontamination, formaldehyde fumigation, dry heating, chemical pass-through, and UV light. Two HCBLs mentioned the last two methods.

Most autoclaves in operation in the HCBLs participating in the present study were nationally manufactured (34/38, 85.7%) (Table A20).

6.2.13.2 Discussion

Numerous authors advocate using double-door border (pass-through model) autoclaves as the most suitable option for HCBL^{55, 94, 289-290, 383}. According to the Canadian Biosafety Standards, 'pass-through decontamination technologies on the containment barrier allow material to be decontaminated within the containment barrier and the decontaminated material to be removed from the decontamination technology from outside the containment barrier. This eliminates the possibility of the material becoming contaminated again before they cross the containment barrier'⁸⁰.

The Brazilian General Directives for the Containment Work with Biological Agents recommend the installation of a double-door, single-pass autoclave, with the entrance inside the BSL-3 laboratory and the exit positioned in support area²²³.

All HCBLs participating in the present study reported having at least one double-door border autoclave, except for one that did not provide this information,

indicating widespread adherence to this requirement in Brazil. These results are better than those observed in the African EDPLN, where while all HCBLs have autoclaves, less than 70% have double-door models (9/13, 69.2%)²¹¹.

When double-door border autoclaves are present, they must be equipped with an interlocking door system and a bioseal to ensure the biosafety requirements^{55, 289, 383}. The bioseal, typically made of stainless steel, is fully welded to the outer steam jacket and tightly sealed to the wall surfaces of the secondary barrier. It incorporates a flexible component at the wall to ensure integrity even during differential movement and expansion³⁸³.

While most participating HCBLs in this study reported having interlocking door systems and bioseals, there were some instances where these features were lacking. Furthermore, a significant number of respondents provided "I don't know" answers regarding bioseals, indicating a lack of understanding of this concept among a noteworthy portion of the respondents. This underscores the need to clarify this issue among Brazilian biosafety experts.

Behrmann et al. (2007) argued that the main autoclave in an HCBL should be a pass-through model to simplify waste logistics as much as possible. Additionally, a secondary autoclave, potentially smaller in size, should be available in the laboratory as a backup in case the primary autoclave is undergoing inspection or experiencing technical issues²⁹⁰.

Gmunder et al. (2022) emphasized that installing a redundant autoclave should be evaluated case-by-case. The decision to implement redundancy should consider factors such as the daily accumulation volume of autoclavable material, available space for safe storage, and the availability of validated alternative inactivation methods in case of malfunction or special operations. Handling contaminated materials, equipment, and waste during periods of malfunction, maintenance, or special operations should also be carefully assessed. A second autoclave ensures uninterrupted and potentially reduced BSL-3 operation in case of a failure in one unit²⁸⁹.

The presence of redundant autoclaves is uncommon among the participating HCBLs in this study. The primary reasons are financial constraints, limited space, or even a lack of awareness regarding the risks associated with the absence of backup equipment. This situation significantly impacts the operation of these facilities and can potentially pose a biosafety issue when solid waste is generated within the HCBL and is not properly decontaminated and safely disposed of.

Validation confirms that a method achieves its intended objective and is suitable for its intended purpose, supported by objective evidence. This validation can be accomplished by ensuring that specific conditions are met, such as using biological indicators, chemical integrators, or parametric monitoring devices placed in challenging locations within the load. These measures confirm that a given autoclave cycle effectively decontaminates representative waste loads⁸⁰.

It is the responsibility of institutions to demonstrate the efficacy of the standard programs provided by autoclave suppliers, considering different types of waste loads (e.g., clothes, glassware, liquids, animal carcasses)^{289, 384}. It is essential to consider the impact of these loads' variability in thermal mass and density on the decontamination process. Therefore, individual facilities must develop protocols to handle waste safely³⁸⁵⁻³⁸⁶.

While most participating HCBLs have validated their autoclave cycles, some have not done so for all types of loads. The absence of validation for specific loads

poses a risk, particularly for HCBLs handling animals, as autoclave factory default settings can be inadequate for sterilizing this material³⁸⁵⁻³⁸⁶.

Another recommendation for autoclaves operating with a vacuum system in high-containment environments is to ensure that the exhaust air from the vacuum pump is filtered or inactivated before being released^{55, 290}. Additionally, all condensates produced should be sterilized²⁹⁰.

It is concerning that less than half of the participating HCBLs reported adhering to these recommendations. However, the lack of information provided by many respondents suggests that they may not be aware of this issue. Therefore, further studies are needed to better understand the level of compliance with these requirements.

Approximately 10% of the participating HCBLs admitted to not documenting all autoclavation cycles. This omission hinders tracking and documenting that all decontamination processes were conducted following established procedures.

Regarding alternative methods for decontaminating solid waste, there is a concern that two HCBLs utilized UV light, as observed for the whole lab decontamination. This issue will be discussed in-depth in Chapter 6.2.14.2.3.4.

The results presented in Table A20 indicate that domestic companies can offer this technology nationwide. However, one of the significant suppliers was acquired by a multinational company in 2011, which could potentially lead to changes in this landscape in the coming years.

6.2.14 Biosafety Cabinets

6.2.14.1 Results

The number of BSCs installed in the HCBLs participating in the present study varies significantly, with the most common response being two units (12/32, 37.5%) (Table A21).

There was a strong positive correlation between the number of BSCs and the HCBL footage. The Spearman correlation coefficient registered a value of 0.738, signifying a positive association between the two attributes. The p-value for the test was 0.000058, underscoring the statistical significance of this correlation. The linear regression analysis produced the following outcome: $E(\text{BSCs}) = 1.5343 + 0.9443 \cdot \log(\text{BSL-3 footage})$. With this result, a laboratory measuring 100m² is anticipated to contain 2.8 BSCs. In the case of a 1,000 m² laboratory, the expected number of BSCs would be 5.0.

The percentage of non-functional equipment was relatively low, except for three HCBLs reporting 40 to 60% of BSCs that were not operational. The average of non-functional BSCs was 11.2% (12/107). Most HCBLs indicated that the current location of all BSCs meets the requirements to prevent external interferences (23/32, 71.9%). Only one HCBL does not conduct periodic certification tests for BSCs (1/31, 3.2%). Typically, these tests are performed annually (11/30, 36.7%) or bi-annually (13/30, 43.3%). Only two HCBLs recorded having BSCs with expired certifications (2/31, 6.4%), while 11 HCBLs (11/31, 35.5%) did not respond to this question (Table A21).

Most HCBLs participating in the current survey have an SOP describing using a BSC (25/31, 80.6%). According to almost all respondents, the users always follow the procedure (23/25, 92.0%). None of the HCBLs uses a Bunsen burner inside the BSC. Only two HCBLs admitted handling samples and other potentially infectious materials outside BSCs (2/31, 6.5%) (Table A21).

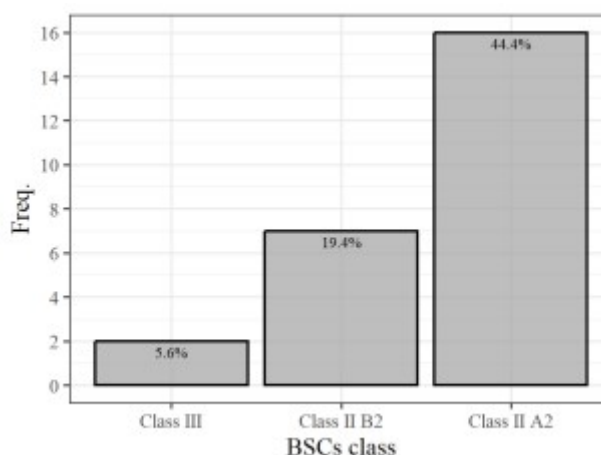
Most of the BSCs in operation in the HCBLs participating in this study were domestically manufactured (20/31, 64.5%) (Table A22).

The most commonly employed method for disinfecting BSCs is applying a 70% Ethanol solution, followed by exposure to UV light. It is worth mentioning that many HCBLs employ more than one method (Table A23).

Regarding monitoring UV light usage, 20 HCBLs provided details on their practices (20/32, 62.5%). Many HCBLs indicated that the lamp power test is part of the BSCs' annual certification (7/32, 21.9%). Others manage UV lamp operating hours either manually (5/32, 15.6%) or automatically (3/32, 9.4%). One HCBL employs BSCs that do not feature UV lamps (1/32, 3.1%), while four HCBLs do not implement UV light usage control (4/32, 12.5%). Some HCBLs employ multiple methods for monitoring UV light usage (Table A24).

The most commonly mentioned BSC class by the respondents was Class II A2 (16/27, 44.4%), followed by Class II B2 (7/36, 19.4%) (Graph 42). Class III was mentioned twice (2/36, 5.6%), while classes II A1 and II A3 were mentioned only once (1/36, 2.8%). When considering only the 23 HCBLs that provided information about the BSC class, the percentage of those with at least one BSC of Class II A2 is 69.6%. The remaining percentages for other classes are: 30.4% for Class II B2, 8.7% for Class III, and 4.4% for classes II A1 and II A3.

Graph 42 – Results regarding the kinds of BSCs installed in the HCBLs participating in the survey.



6.2.14.2 Discussion

6.2.14.2.1 Number and operational status of Biosafety Cabinets

BSCs are one of the most crucial devices in HCBLs since they are the primary means of containment for working safely with infectious microorganisms and prions. They are designed to provide personnel and environmental protection when appropriate practices and procedures are followed^{1, 94}.

The 4th edition of the WHO Laboratory Biosafety Manual has an associate monograph on BSCs and other primary containment devices, emphasizing the relevance of these kinds of equipment. According to the WHO, when properly used and maintained, primary containment devices are highly effective in reducing LAIs since they protect the operator and the environment from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing biological agents²⁹⁵.

While Gassiep et al. (2021) concluded through scientific research that specific tasks involving the handling of *Burkholderia pseudomallei* can be performed safely without a BSC³⁸⁷, handling biological assets inside BSCs is mandatory for HCBLs according to Brazilian regulations^{94, 223, 231}. However, two HCBLs participating in this survey reported handling samples or other potentially infectious materials outside the BSCs. This result shows a slightly higher occurrence than Simonetti's (2014) findings. In that study, it was found that 2.2% (1/45) of professionals working in Brazilian BSL-3 laboratories did not perform all procedures involving biological agents within a BSC⁷⁴.

In a study conducted with African EDPLN laboratories, all HCBLs that handled dangerous pathogens indicated that they are handled in a BSC, with the number of equipment in each facility ranging from one to three²¹¹, meeting the African requirements²¹².

The size of the laboratories influenced the number of BSCs installed in the HCBL participating in the present study. The statistical comparison between these two variables showed a Spearman correlation coefficient of 0.738, indicating a positive relationship (p-value 0.000058). Therefore, as the HCBLs' size increased, the number of BSCs also increased. The linear regression yielded the following result: $E(\text{BSCs}) = 1.5343 + 0.9443 \cdot \log(\text{HCBL footage})$. Based on this result, in a laboratory with 100 m², an expected value of 2.81 BSCs is anticipated, and for a laboratory of 1,000 m², the expected number of BSCs would be 4.99.

Even for smaller HCBLs, it is advisable to have a minimum of two BSCs to prevent disruptions in activities caused by maintenance issues, which was not observed by 15.6% of the participants. It is important to emphasize that, according to the results obtained in the present study, the overall average of non-functional BSCs was 11.2%.

The proportion observed in this study is lower than that reported by the FAO and A-PBA in a survey conducted across seven countries in the Asia-Pacific region, where approximately 30.0% of the tested class II BSCs were found to be poorly designed, dysfunctional, or failed¹⁷.

6.2.14.2.2 Certification of Biosafety Cabinets

More than an adequate number of BSCs is required to handle infectious materials properly. It is necessary to certify that the equipment functions correctly and that users are qualified to operate them. Heckert et al. argued that working with infectious agents in a BSC that has not been positioned correctly in the lab and certified is potentially more dangerous than working on the open bench. Creating a business environment that allows equipment manufacturers, distributors, and scientific service providers to flourish would help neutralize this challenge⁵⁶.

Soolingen et al. (2014) agreed that an inappropriate or malfunctioning BSCs can create a false sense of protection, posing greater risks than the absence of such equipment⁹⁶.

The operational integrity of a BSC should be validated before being put into service and after undergoing repairs or relocation. It is recommended that each BSC undergoes testing and certification at least once a year to ensure continued and appropriate operation^{1, 295, 388}.

The certification process includes testing the integrity of HEPA filters, mapping downflow velocity, measuring intake airflow, assessing velocity at the front of the cabinet opening, evaluating ventilation rate, and conducting airflow smoke

pattern tests and alarm assessments²⁹⁴⁻²⁹⁵. This service should be done by suitably trained and certified staff.

The findings of this study indicate that periodic certification of BSCs is a common practice among Brazilian HCBLs, which contrasts with the results reported by Zerwekh et al. (2004), who surveyed 190 BSL-2 laboratories linked to the University of Texas Health Science Center at Houston to assess compliance with recommended biosafety guidelines. These authors found that the most frequent violation cited was failing to ensure BSCs certification (31/150 violations detected, 20.6%)³⁸⁹. Similar results were obtained by Lasley et al. (2023). Their study revealed that 18.0% of Class II A2 BSCs worldwide are poorly maintained. The percentage increases to 40.0% in the Americas⁶⁰. This significant discrepancy may be attributed to the study's lack of explicit focus on HCBLs.

Surprisingly, biannual certification was more prevalent than annual certification in the present study. It may be attributed to regulations governing the handling of the FMD virus, which require BSC filters to be tested every six months²³¹.

Further studies are required to provide scientific, evidence-based recommendations regarding the optimal frequency for BSC certification tests.

6.2.14.2.3 Proper use of Biosafety Cabinets

While BSCs are extremely important, concerns related to their usage and maintenance were identified as significant issues during a site assessment conducted by the FAO in 20 BSL-2 and BSL-3 laboratories across nine countries in Southeast Asia⁸³.

Another study conducted in 25 BSL-3 laboratories in 18 EU countries concluded that only 11 (44.0%) were in full compliance with biorisk management requirements related to the usage and maintenance of BSCs³⁵⁶.

Even with up-to-date certification tests, it is necessary to ensure that users operate the equipment correctly. In this regard, the current survey assessed four aspects: the equipment's location, the presence of SOPs for user training, the use of Bunsen burners, and the BSC cleaning procedures.

6.2.14.2.3.1 Biosafety Cabinets location

Various studies have reported that motion, including the movement of the laboratory door, walking past the cabinet, and removing products rapidly and adversely affects the protective effects of BSCs. The airflow pattern and degree of air turbulence in the room also affect performance³⁸⁸.

According to the self-assessment, none of the HCBLs considered the location of the BSCs inadequate. However, four participants did not provide this information, and another three stated that they could not provide an answer. Further studies that include visits to the facilities are necessary to validate the present study's findings.

6.2.14.2.3.2 Standard Operating Procedures for Biosafety Cabinets users training

In a study conducted in 133 laboratories in Singapore, the improper use of BSCs was one of the most common findings during audits (56/133, 42.1%). According to the authors, laboratory users do not understand the proper use of BSCs, such as blocking the inlet grills. They must be made aware of avoiding clutter inside the BSCs during operation. It was noted that many used BSCs as a storage

facility by keeping laboratory apparatus and stuff not required for experiments. There were no clean, dirty, and working zones being practiced³⁹⁰.

Training individuals using SOPs that outline the proper usage of BSCs is necessary to prevent such mistakes. The results obtained in the current survey indicate that the use of SOPs is a commonly adopted practice in Brazilian HCBLs.

6.2.14.2.3.3 Bunsen burners

The WHO, among many other authors, advise against using Bunsen burners inside BSCs due to the potential airflow disruption and damage to filters caused by the generated heat. Instead, an electric micro incinerator is allowed, but disposable sterile transfer loops are preferred²⁹⁵. Notably, none of the participating HBLCs in the current study reported using Bunsen burners inside BSCs.

6.2.14.2.3.4 Biosafety Cabinets cleaning procedures

It is recommended to clean the work surface, interior walls, and window using one of the following options: 70% ethanol, a 1:100 dilution of sodium hypochlorite, or any other disinfectant chosen by the investigator that meets the activity's requirements. If bleach is utilized, a second wiping with sterile water is necessary to eliminate residual chlorine, as it may corrode stainless steel surfaces over time¹.

Likewise, all materials and containers placed inside the cabinet should be wiped with 70% ethanol or an appropriate disinfectant, as determined by the laboratory's needs, to minimize the introduction of contaminants into the BSC environment. This simple step significantly reduces the introduction of mold spores, consequently minimizing culture contamination¹.

Among the HBCLs participating in the present survey, the most mentioned cleaning method was 70% ethanol, followed by UV light. However, it is essential for HBCLs utilizing UV light to be aware that it is not recommended as the sole sterilization method for the device⁹⁴. If UV light is used, it must be accompanied by regular cleaning every week to remove any dust or dirt that may diminish the germicidal effectiveness of the light. They should be positioned and kept at a distance up to 30 cm from the operating area. Furthermore, it is crucial to check the intensity of the UV light during device re-certification to ensure that the emission is at an appropriate level. This step guarantees that the UV light functions optimally and maintains its desired germicidal properties^{94, 295}.

Memarzadeh (2021), reviewing the evidence for utilizing UV light to disinfect air and surfaces, emphasized that its germicidal effectiveness is influenced by the presence of organic matter, wavelength, type of suspension, temperature, humidity, type of microorganism, and UV intensity, which is affected by distance and tube cleanliness. The effectiveness of UV lights also depends on the exposure time and the ability of the UV light to reach the microorganisms in folds, crevices, and undersurfaces of materials and surfaces where the UV light cannot reach. Furthermore, because of the potential health risks, UV light disinfection must only occur when rooms are unoccupied to avoid injury to occupants³⁹¹.

Further studies are needed to determine Brazilian HCBL users' awareness of the UV light limitations and requirements in the disinfectant process of BSCs, pass-throughs, airlocks, and surfaces since this is a prevalent method in the country. For instance, in a study conducted with 14 laboratory professionals in Pakistan, 88.9% of the participants said they could operate BSCs, but 77.8% recognized they needed to be aware of the pitfalls of UV light utilization¹⁰⁴.

6.2.14.2.4 Biosafety Cabinets Classification

There are three classes of BSCs:

- a. Class I: open-fronted enclosures that draw an inward airflow across the work surface through the front opening. The air passes upwards through a HEPA filter before being exhausted. They provide personnel and environmental protection but do not offer product protection for materials located in the work area;
- b. Class II: open-fronted enclosures, similar to Class I BSC, in which air enters the cabinet through the front opening to provide operator protection. The inward air bypasses the work area by being pulled through the front grille and underneath the workspace. Then, it travels through a plenum to the BSC blower, which is either exhausted through a HEPA filter or directed into the work area after passing through a separate downflow HEPA filter. Some of the now-clean air passes over the work surface as a downward flow of air to protect work materials from contaminated room air and is then recirculated through the front and rear vents. The surplus air in the plenum is not recirculated but passes through a separate HEPA filter and is exhausted. The National Sanitary Foundation (NSF) standard defines five types of Class II cabinets: A1, A2, B1, B2, and C1. Each type uses different mechanisms for the intake, recirculation, and exhaust of air to achieve the inward and downward airflow combination;
- c. Class III: a closed, sealed, negative-pressure enclosure to which HEPA-filtered air is supplied; this air then passes through another HEPA filter on extraction. The enclosure is sealed to ensure safe gaseous decontamination. Operators access the working area using integrated gloves or gauntlets. Class III BSCs are typically fitted with a pass box or dunk tank to facilitate the movement of potentially contaminated work materials in and out of the cabinet. An autoclave may also be attached for waste management, mainly in facilities with a maximum-containment cabinet line. A sealable and detachable front window may be fitted to allow the occasional movement of large equipment into and out of the cabinet after fumigation. These devices offer the highest degree of operator protection as the user is physically separated from the device interior²⁹⁵.

The BSC class II is recommended when working with infectious agents in diagnostic and research laboratories, as it provides product, personnel, and environmental protection^{94, 388}.

In a survey conducted in Pakistan, a descriptive questionnaire was used to record data about the types and usage of BSCs in 30 laboratories. Among these facilities, 63.6% had BSC class II A2, 39.9% had BSC class II B2, 4.5% had BSC class III, and only 0.9% had BSC class I cabinet. The authors argued that the high frequency of class II A might be explained by the more straightforward installation, running procedures, and maintenance compared with class II B cabinets, which require infrastructure, secondary containment support, and dedicated exhaust systems³⁸⁸.

These results are slightly like those obtained from the Brazilian HCBLs, especially considering the percentage of BSCs class II A2 (63.6% in Pakistan versus 69.6% in Brazil).

Simonetti (2014) also surveyed the classification of BSCs in Brazilian HCBLs and found that 72% of the laboratories had BSCs classified as class II. However, the author did not investigate the specific types of BSCs. In the current study, the percentage of BSC classified as class II, regardless of the type, was slightly higher (78.4%). It indicates a potential trend of replacing BSCs class III with class II, as the

percentage of class III devices reported by Simonetti (17.0%)⁷⁴ was higher than observed in the present survey (8.7%).

Further studies are necessary to determine if BSCs installed in Brazilian HCBLs are chosen according to a risk assessment and considering the pros and cons of each class.

6.2.14.2.5 Biosafety Cabinets manufacturers

Acquiring national BSCs can be challenging in many parts of the world. However, due to their size and weight, transportation fees can often match the purchase price, doubling the cost of each unit⁵⁶. Fortunately, the present study's findings demonstrated that this technology can be provided domestically. Despite this, it is noteworthy that approximately one-third of HCBLs still prefer to source BSCs from other countries. Further investigation is needed to explore the reasons behind this decision.

6.2.15 Other equipment

6.2.15.1 Results

Among the HCBLs participating in the present study, six do not have an ultra-low freezer installed within their facility (6/32, 18.8%). For those with ultra-low freezers, the number varied from one to seven. While most have one ultra-low freezer installed (10/32, 31.2%), only five HCBLs (5/31, 16.1%) reported that the number of refrigerators, freezers, and ultra-low freezers does not meet their demand. Most HCBLs do not have a dedicated room for refrigerators, freezers, and ultra-low freezers (Table A25).

Laboratories that confirmed the adequacy of the current number of refrigerators and ultra-low freezers had an average size of 942.2 m², exceeding those labs that responded negatively by 152.2 m². However, there was no significant difference between the groups when evaluating laboratory size (Table 14).

Table 14. Correlation analysis between the adequacy of the number of refrigerators, freezers, and ultra-low freezers installed in the HCBLs participating in the survey and the laboratory footage.

Does the current number of refrigerators, freezers, and ultra-freezers meet the needs of the BSL-3 lab?	N	Laboratory footage (m ²)					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
No	3	20.0	790.0	150.0	1,222.8	2,200.0	1.000
Yes	18	25.0	942.2	119.3	2,345.8	10,000.0	

In contrast, the square footage of the HCBLs significantly influenced the presence of a dedicated room for refrigerators, freezers, and ultra-low freezers (Table 15) and also in having at least two ultra-low freezers installed within the HCBL (Table 16).

Table 15. Correlation analysis between the existence of a dedicated room for refrigerators, freezers, and ultra-low freezers in the HCBLs participating in the survey and the laboratory footage.

Does the BSL-3 lab have a dedicated room for refrigerators, freezers, and ultra-freezers?	N	Laboratory footage (m ²)					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
No	17	20.0	331.9	74.0	606.6	2,200.0	0.01375
Yes	4	374.0	3,406.1	1,625.1	4,479.9	10,000.0	

Table 16. Correlation analysis between the number of ultra-low freezers installed in the HCBLs participating in the survey and the laboratory footage.

How many ultra-freezers are currently installed in the BSL-3 lab?	N	Laboratory footage (m ²)					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
0 - 1	11	25.0	61.1	41.0	41.1	150.0	0.0003
2 - 7	11	100.0	1,704.3	644.8	2,870.27	10,000.0	

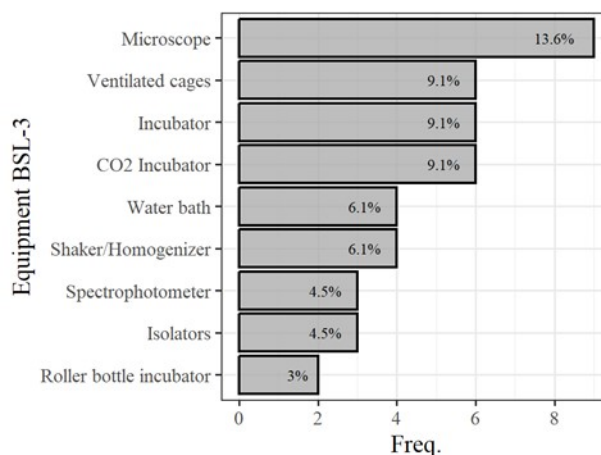
Most HCBLs do not have any non-functional ultra-low freezers. The overall percentage of non-functional ultra-low freezers was 13.2% (7/53). Slightly less than half of the HCBLs have all their refrigerators, freezers, and ultra-low freezers suitable for laboratory use (15/32, 46.9%). Most HCBLs monitor the temperature inside such devices (26/32, 81.3%), and the majority do not have a dedicated room to install them (22/32, 68.8%) (Table A25).

Most of the ultra-low freezers currently in use in the HCBLs participating in this study were manufactured overseas (17/23, 73.9%) (Table A26).

Most HCBLs in the present study have installed centrifuges (27/32, 84.4%). However, four do not use sealed rotors or cups (4/27, 14.8%). Among the HCBLs with a centrifuge, approximately 40% do not have an SOP stating its decontamination procedure (10/27, 37.0%). However, among those that do have this SOP, 75% (12/16) reported that the procedures are consistently followed by the users (Table A27).

Regarding possessing other relevant equipment, the most common answer was a microscope, followed by ventilated cages, incubators, and CO₂ incubators (Graph 43). The following devices were mentioned once: flow cytometer, freeze dryer, GeneXpert®, gradient generator, immunofluorescence microscope, mycobacterial growth indicator tube, real-time PCR, sonicator, tissue digester, tissue laser pipettes, ultrafiltration system, and vacuum pump.

Graph 43 – Results regarding other equipment installed in the HCBLs participating in the survey.



6.2.15.2 Discussion

6.2.15.2.1 Freezers, ultra-low freezers, and refrigerators

Whether materials are stored for a short or long duration, their storage must comply with containment regulations and adhere to the appropriate BSL. Activities carried out in an HCBL should be tailored to the available capacity of the containment facilities rather than compromising best practices due to space constraints. For instance, viable high-risk materials should always be stored within an HCBL until properly inactivated and deemed safe for transfer outside, although limited space may sometimes pose challenges. Breaches in both biosafety and biosecurity best practices need to be promptly addressed¹⁷⁸.

Therefore, freezers and fridges of all standard temperature levels (0°C, -20°C, and -80°C) must be available as a standard in biological laboratories in each containment area to avoid unnecessary pass-in/pass-out procedures²⁹⁰.

It was observed that six HCBLs participating in the current study did not have an ultra-low freezer installed inside their BSL-3 laboratory, a factor closely associated with their size.

Among them, only two (2/6, 33.3%) reported that the existing number of refrigerators, freezers, and ultra-low freezers falls short of meeting their laboratory requirements. It indicates that the other four HCBLs do not consider owning an ultra-low freezer essential. Nonetheless, it remains crucial for HCBLs to evaluate the risks associated with the absence of these equipment pieces, which are commonly utilized for storing live pathogens. Additionally, they should identify the primary reasons for their unavailability, such as space constraints, financial limitations, or lack of demand.

Less than half of the HCBLs participating in the current study reported that all their refrigerator, freezer, and ultra-low freezer models are specifically designed for laboratory use. Considering that these specialized models generally exhibit superior temperature maintenance capabilities, HCBLs should consider replacing household devices. These findings align with those of Simonetti (2014), where 78% of interviewed Brazilian HCBL managers emphasized the necessity of acquiring new equipment, including ultra-low freezers⁷⁴.

The overall percentage of non-functional ultra-low freezers (13.2%) was very similar to that of BSCs (11.2%). Further studies are required to evaluate the underlying reasons and examine these findings associated financial and technical implications.

Monitoring the temperature of equipment such as refrigerators, freezers, and ultra-low freezers is crucial to ensure the integrity of stored materials. This practice is carried out by 81.2% of the surveyed HCBLs, indicating that it is a standard procedure.

Separate rooms for equipment support and storage should be considered. The heat load from equipment such as low-temperature freezers is more easily handled if grouped where additional focused cooling, such as fan coil units, can be applied³⁹². This recommendation is also crucial for addressing biosecurity concerns, as facility design enables restricted and monitored access to specific laboratory zones while providing secure storage rooms for hazardous agents⁷⁶.

However, only 12.5% of the HCBLs participating in the current study adhere to this recommendation, likely due to the limited space within their laboratories.

In cases where dedicated rooms are not feasible, it is recommended for HCBLs to provide padlocks for the equipment where high-risk biological assets are stored¹³.

The percentage of domestically manufactured ultra-low freezers (26.1%) is notably lower compared to other essential equipment such as autoclaves (85.7%) and BSCs (64.5%). This indicates that the country still relies heavily on the external market to meet the demand for this technology.

6.2.15.2.2 Centrifuge and other equipment

The most concerning findings regarding centrifuges are the non-compliance of four HCBLs with the literature^{1, 226, 290} and MS requirement to use sealed rotors or tubes⁹⁴, and the notable proportion of HCBLs that reported not having an SOP in place to define the decontamination procedure for this equipment.

According to a study by the WHO in African EDPLN laboratories, all 13 HCBLs surveyed confirmed that the centrifuge buckets and rotors are sealed to prevent the release of infectious materials²¹¹.

It is worth noting that only one HCBL mentioned having a real-time PCR machine installed within the BSL-3 laboratory. Since this was an open-ended question, most respondents may not recall including this equipment in their responses. Further studies are required to investigate the laboratory methods and technologies commonly employed in Brazilian HCBLs, as it was not a primary objective of the current research.

6.2.16 Equipment operational failures and quality of external services

6.2.16.1 Results

The quality of external services provided for the maintenance and calibration of various equipment/systems was assessed using a 1-5 scale, as depicted in Table A28. The EDS system had the highest proportion of unanswered questions (6/22, 27.3%), followed by ultra-low freezers (6/25, 24.0%). Services related to BSCs received the most positive evaluations (22/31, 70.9% of ratings between 4 and 5), closely followed by Fumigation Chamber/Airlocks (13/19, 68.4% of ratings between 4 and 5). On the other hand, services related to autoclaves received the lowest evaluations (9/30, 30.3% of ratings between 1 and 3).

Although the service quality was better in the Southeast region for all kinds of equipment/systems assessed, the difference was not statistically significant, as demonstrated in Tables 17 to 22.

Table 17. Correlation analysis between the quality of services provided for HVAC systems installed in the HCBLs participating in the survey located in the Southeast region and the HCBLs in other regions.

Region	N	HVAC systems services' quality rating					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Southeast	18	2.0	4.06	4.00	0.87	5.00	0.8721
Others	7	3.0	4.14	4.00	0.90	5.00	

Table 18. Correlation analysis between the quality of services provided for Fumigation Chamber/Airlocks installed in the HCBLs participating in the survey located in the Southeast region and the HCBLs in other regions.

Region	N	Fumigation Chamber/Airlocks services' quality rating					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Southeast	9	2.00	4.22	4.00	0.97	5.00	0.4594
Others	7	2.00	3.86	4.00	1.07	5.00	

Table 19. Correlation analysis between the quality of services provided for EDS installed in the HCBLs participating in the survey located in the Southeast region and the HCBLs in other regions.

Region	N	EDS services' quality rating					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Southeast	9	3.00	4.44	5.00	0.73	5.00	0.2550
Others	7	2.00	3.86	4.00	1.07	5.00	

Table 20. Correlation analysis between the quality of services provided for autoclaves installed in the HCBLs participating in the survey located in the Southeast region and the HCBLs in other regions.

Region	N	Autoclaves services' quality rating					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Southeast	17	2.00	4.12	4.00	0.93	5.00	0.1358
Others	9	2.00	3.44	3.00	1.13	5.00	

Table 21. Correlation analysis between the quality of services provided for BSCs installed in the HCBLs participating in the survey located in the Southeast region and the HCBLs in other regions.

Region	N	BSCs services' quality rating					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Southeast	17	2.00	4.47	5.00	0.87	5.00	0.9491
Others	9	2.00	4.33	5.00	1.12	5.00	

Table 22. Correlation analysis between the quality of services provided for ultra-low freezers installed in the HCBLs participating in the survey located in the Southeast region and the HCBLs in other regions.

Region	N	Ultra-low freezers services' quality rating					Mann-Whitney (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Southeast	13	1.00	4.08	4.00	1.19	5.00	0.1259
Others	6	2.00	3.33	4.00	1.03	4.00	

The frequency of operational failures for specific equipment/systems was also assessed on a scale of 1-5, as shown in Table A29. The EDS system had the highest proportion of unanswered questions (8/23, 34.8%). Autoclaves exhibited the highest frequency of operational failures (10/31, 32.3% of ratings between 3 and 5), while the Fumigation Chamber/Airlocks had the lowest frequency (15/20, 75.0% of ratings between 1 and 2), closely followed by BSCs (23/31, 74.2% of ratings between 1 and 2).

6.2.16.2 Discussion

The relatively high proportion of respondents who did not provide answers to questions regarding the EDS system suggests a potential lack of knowledge regarding the operation and maintenance of this critical system. It highlights the importance of capacity-building initiatives for HCBL managers and BOs, focusing on this issue.

Autoclaves received the most unfavorable evaluation concerning the quality of external services and operational failures, indicating a strong correlation between these aspects. This finding underscores the pressing need for a general enhancement in the services provided for this type of equipment across the country, which aligns with the observations made by Simonetti (2014). In his study, 51.4% (19/37) of professionals working in Brazilian HCBLs who were interviewed expressed dissatisfaction with the long-term performance of their autoclaves⁷⁴.

The WOAHL conducted an equipment management and sustainability survey involving 182 countries between May and August 2019, focusing on central veterinary laboratories. The results indicated that the primary reported barriers to maintenance included costly services, inadequate budget allocation, and a lack of local service providers. Notably, 69% of the laboratories expressed satisfaction with local service providers, as opposed to 58% with international service providers⁶⁰.

Another study explicitly involving EDPLN laboratories found that approximately half of their HCBLs rely on international support to maintain BSCs and autoclaves²¹¹.

Therefore, the situation in Brazil is comparatively better. Although the level of satisfaction with the local service providers is comparable to the results obtained by the WOAHL's study, technical assistance for the primary equipment and systems used in HCBLs is most readily available within the country. However, it is important to consider a limitation of the current study, which is the potential bias associated with the expectation that the HCBLs participating have, on average, a better scenario than those that did not participate (see Chapter 6.7).

The proportion of BSCs (16.1%) and ultra-low freezers (18.2%) that are out of service in Brazilian HCBLs falls within an intermediate range when compared to the results obtained from the WOAHL's survey for different laboratory equipment in

countries with different income levels. According to this study, on a global scale and considering all types of equipment, 10.8% (7,394 out of 68,455) were reported as out of service. Laboratories in low and lower-middle-income countries reported a higher percentage, with 25.8% (390 out of 1,512) of their equipment being out of service⁶⁰.

It is crucial to consider the WOA's recommendations to address sustainability challenges through improved equipment maintenance and calibration, which include:

- a. Check annual operating budgets for equipment maintenance and calibration resources;
- b. Offer calibration or preventive and corrective maintenance services to neighboring laboratories;
- c. Perform preventative maintenance on prioritized equipment without delay;
- d. Train staff on proper preventative maintenance of prioritized equipment;
- e. Have prioritized equipment calibrated without delay;
- f. Have prioritized equipment repaired without delay;
- g. Perform equipment inventory review⁶⁰.

Lasley et al. (2023) also emphasized that inadequate equipment maintenance and calibration pose significant threats to the safety, security, business continuity, quality, accuracy, and timeliness of laboratory results. According to these authors, novel strategies are required to ensure the laboratory's sustainability. It includes fostering public-private partnerships where local businesses and expertise are supported through subsidies, incentives, or agreements aimed at promoting the development of local service provision rather than relying solely on in-house expertise. Moreover, addressing the waste of valuable resources should involve innovative and pragmatic solutions. It entails encouraging manufacturers, researchers, innovators, and engineers in their efforts to design equipment that is not only effective but also more sustainable and appropriately sized for use in low-resource settings⁶⁰.

6.2.17 Laboratory animals

6.2.17.1 Results

Nine HCBLs participating in the current study reported handling animals (9/32, 28.1%). Almost all these HCBLs have dedicated rooms for activities involving animals (8/9, 88.9%), with one HCBL not answering this question. Most HCBLs do not handle different species simultaneously in the same room (6/9, 66.7%). Regarding the presence of CCTV systems in the animal rooms, one-third of the respondents reported having such a system (3/9, 33.3%), another one-third reported not having it (3/9, 33.3%), and the remaining one-third did not provide this information (3/9, 33.3%). Ventilated cages are the most common device used as a primary barrier (5/8, 62.5%), followed by isolators (2/8, 25.0%). Only two manufacturers were mentioned for ventilated cages and isolators. Five HCBLs (5/9, 55.6%) mentioned a national company, while one HCBL (1/9, 11.1%) mentioned an international one. Half of the HCBLs reported periodically certifying the ventilated racks and isolators (4/8, 50.0%). Six HCBLs reported using BSCs for necropsies (6/9, 66.7%), while the remaining three HCBLs did not provide this information (Table A30).

Most workers in the HCBLs participating in the present study have received formal training on animal welfare (6/9, 66.7%). Only two HCBLs reported conducting specific risk assessments for using laboratory animals (2/8, 25.0%). All respondents reported that their institute has an IACUC, including two HCBLs that do not handle

animals in high containment (11/11, 100.0%). However, one HCBL is not registered with the National Council for Animal Use Control (CONCEA). Most HCBLs reported that the conditions of space, temperature, humidity, light cycles, acoustics, watering, and feeding meet the animal welfare requirements for the species they handle (7/9, 77.8%). Autoclavation was the primary method used to decontaminate animal carcasses (6/8, 75.0%), but less than half of the respondents reported that this method was validated (3/7, 42.9%). The frequency of incidents and accidents involving animals appears to be very low, as none of the HCBLs rated it as three or higher on a scale of 1 to 5 (Table A31).

None of the HCBLs participating in the present study handle arthropods. One HCBL did not provide information about the animal species handled. Among the other eight HCBLs, only one does not handle mice (*Mus musculus*). The other species mentioned were *Cricetinae*, *Rattus norvegicus*, *Cavia porcellus*, *Gallus gallus*, and *Zebrafish* (Table A32).

6.2.17.2 Discussion

As stated in Chapter 2.13, the utilization of animals in high-containment facilities necessitates the implementation of additional safety measures^{103, 283}, highly skilled personnel^{29, 285}, and a dedicated risk assessment^{277, 285}. Whenever feasible, the use of ventilated cages or isolators is recommended²⁸⁴.

The findings from the present study indicate that less than one-third of the HCBLs included in the study engage in animal handling within the containment zone. Small rodents, particularly mice, are the primary experimental models utilized. This situation contrasts with observations in the US, where a study conducted in 2005 revealed that 59.6% of the BSL-3 laboratories (146/245) carried out animal-related activities. Additionally, the study found that while research involving small animals was the most prevalent (44.9%), other species such as insects/arthropods (8.6%), large animals (3.3%), and non-human primates (2.9%) were also handled³⁴⁵.

Enclosed cage systems with negative pressure and independent HEPA filtration to prevent cross-contamination and increase containment of infectious agents are relatively easy to implement for small rodents. The cages can be changed in a biosafety cabinet, or other contained aseptic field, thereby maintaining primary containment relatively easily³⁹³.

On the other hand, handling larger animals within zones where the rooms act as the primary barriers presents additional challenges. These challenges are associated with increased risks of exposure to the workers and the environment and issues concerning animal welfare. Consequently, it necessitates the implementation of additional biosafety and biosecurity procedures³⁹³.

The lack of high-containment facilities able to handle studies with other species beyond small rodents represents a limitation to research development. It contributes to the technological dependence on other countries. For instance, certain infectious diseases in Brazil that have significant implications for public health necessitate studies on ticks (such as spotted fever and Lyme disease), mosquitoes (including dengue, Zika virus, chikungunya, and yellow fever), and non-human primates (like yellow fever and monkeypox). Furthermore, certain animal diseases that affect public health or result in high economic impact require ABSL-3Ag facilities to conduct activities related to diagnosis, research, and supplies production. These facilities are essential for species such as pigs (e.g., AFS and CSF), horses (e.g., glanders, West Nile virus, and equine encephalitis), goats and sheep (e.g., brucellosis and scrapie), cattle (e.g., FMD, bovine spongiform encephalopathy,

brucellosis, and tuberculosis), and poultry (e.g., avian influenza and Newcastle disease).

The present study's findings indicate that most HCBLs that engage in activities involving experimental animals meet specific requirements associated with animal handling. These requirements include the utilization of dedicated rooms maintained in appropriate environmental conditions, the use of ventilated cages or isolators as primary barrier devices, the usage of BSCs for necropsies, the provision of worker training on animal welfare, and the establishment of an IACUC.

However, it is worth noting that certain HCBLs need to adjust their procedures regarding certifying ventilated cages and isolators, developing risk assessments focused on animal handling, and validating autoclaving for animal carcasses. As Cascardo et al. (2020) demonstrated, the autoclave primary factory programmed cycles are inefficient in decontaminating animal carcasses and bed waste³⁸⁶.

The widespread use of domestically manufactured ventilated cages and isolators by almost all HCBLs indicates the availability of this technology within the country. However, it is concerning that only one national company was mentioned in their production.

In a study by Jirkof & Schmutz (2019) on compliance with safety regulations in Swiss academic and state-run experimental ABSL-2 and ABSL-3 facilities, the interviewers rarely mentioned accidents or incidents. The authors suggested that factors contributing to enhanced compliance included management commitment, adequate equipment, risk awareness, and practical training. However, opportunities for improvement were also identified, particularly in the participative process of rule design and communication procedures¹³⁴.

Similarly, the present study also observed low reported accidents and incidents in animal facilities. Nonetheless, further research is necessary to ascertain whether the limited number of recorded accidents and incidents accurately reflects the actual situation or if there is underreporting due to the absence of practical reporting tools.

CCTV is not mandatory but can prove beneficial in certain situations. For instance, some HCBLs employ it to prevent the need to enforce the 'no one works alone' rule within the contained area. Instead of having two individuals inside the laboratory, one person can be stationed outside nearby, equipped to monitor the laboratory. If the person on the outside detects a safety or security issue, they can request additional assistance or potentially enter the laboratory. While CCTV systems are often associated with physical security, they can also be integrated into personnel assurance programs to oversee activities in HCBLs. However, even if not continuous, real-time monitoring is costly and necessitates trained personnel capable of identifying illicit activities or signs of distress within the laboratory. Archived recordings hold value for investigative purposes, but the retention period can vary widely, from a few weeks to a year, depending on the laboratory, which can limit their effectiveness in investigations after an incident has occurred or been alleged¹⁹².

Hence, while the 'General Directives for the Containment Work with Biological Agents' by MS stipulates the necessity of having at least two personnel inside the BSL-3 laboratory²³³, it is important to note that this requirement doesn't align with international guidelines universally^{1-2, 7, 82}. Instead, each laboratory should evaluate the applicability of this rule based on their risk assessments and available resources, including the CCTV system.

6.2.18 Uniforms and Personal Protective Equipment

6.2.18.1 Results

Out of all the HCBLs participating in the current study, only one reported not having uniforms available exclusively for use in the BSL-3 lab (1/32, 3.1%). In most HCBLs, the quantity and quality of uniforms were deemed suitable (22/28, 78.6%). Autoclaving was the sole method mentioned for decontaminating the uniforms (20/28, 71.4%), although some HCBLs did not provide this information (8/28, 28.6%) (Table A33).

Twenty HCBLs confirmed that the provided PPE is suitable for all activities conducted in the BSL-3 zone (20/32, 62.5%), while eight HCBLs did not provide this information (8/32, 25.0%). The quantity of PPE available in the BSL-3 laboratory was consistently sufficient according to 83.9% of respondents (26/31). Four HCBLs admitted to reusing disposable PPE (4/32, 12.5%). Most HCBLs have SOPs in place to ensure proper use of PPE (26/31, 83.9%). Compliance with these procedures by the workers in these HCBLs is generally high, as 96.2% of respondents (25/26) rated their compliance as 4 or 5 on a 1-5 scale (Table A34).

6.2.18.2 Discussion

6.2.18.2.1 Uniforms

HCBL workers shall wear protective clothing with a solid front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing must be decontaminated before being laundered¹. Where personal clothing and footwear are worn in a high-containment area, they should be fully covered by PPE⁸⁰.

Disposable PPE is likely being used by the HCBLs, which reported a lack of dedicated uniforms for exclusive use in the BSL-3 lab. However, removing personal clothing and footwear and donning dedicated clothes and footwear prevents the contamination of personal clothing and footwear and the spread of contamination outside the containment barrier⁸⁰.

Notably, four HCBLs have reported issues regarding uniform quality and/or quantity. The costs of these items are minimal compared to other expenses in an HCBL. Therefore, encountering problems with uniform supply may indicate severe financial and management issues, suggesting that these institutes should assess their capacity to manage an HCBL without posing risks to the users and the environment.

The missing questions were associated with new facilities not yet in operation when the questionnaire was filled in.

6.2.18.2.2 Personal Protective Equipment

Ensuring the provision of appropriate uniforms and PPE is a fundamental requirement that all HCBLs must adhere to. The selection of PPE within an HCBL should be determined based on a risk assessment. Commonly recommended PPE includes a double pair of gloves, safety glasses or face shields, N95 masks or full-face masks (such as the Powered Air Purifying Respirator – PAPR), and shoe covers or dedicated footwear¹. While all these items are essential, gloves and respiratory protection are particularly critical²⁸⁴.

Regarding respiratory protection, Richards et al. (2014) compared US HCBL registered in the FSAP program with laboratories that do not participate in this program, finding that in select agent laboratories, workers use N95 respirators (8/19, 42%), N100 respirators (1/19, 5%), or PAPRs (10/19, 53%) with either a full hood or

a face shield. In contrast, non-select agent workers used only N95 respirators (14/18, 78%) and PAPRs (4/18, 22%)¹³.

Despite its significance, laboratory workers worldwide have overlooked the correct utilization of PPE. Of 337 laboratories assessed for biorisk management practices in Pakistan in 2019, PPE usage was unsatisfactory in 95.0% of them³⁸¹. In another study conducted in EU from 2016 to 2017, it was found that only 48.0% (12/25) of the HBSL-3 facilities fully adhered to the biorisk management requirements for PPE³⁵⁶.

According to the document 'Overview of Biosafety and Biosecurity in High-containment Labs in Brazil: A Report of the Brazilian Biosafety Association', approximately 55.0% of reported accidents in HCBLs in Brazil were attributed to a lack or improper fit of PPE¹⁷.

Simonetti (2014) also highlighted issues related to the use of PPE in Brazilian HCBLs, specifically mentioning problems concerning the quality and suitability of PPE in terms of quantity. Consequently, some professionals had to reuse disposable PPE or use inappropriate models for their tasks. According to this author, 44.0% of the interviewed workers reported reusing disposable gowns⁷⁴.

The results obtained in the present study were better than those mentioned above. However, the higher number of non-responses regarding the suitability of PPE types, compared to their quantity, suggests that some respondents were uncertain about the appropriate PPE to use. This information and the proportion of HCBLs that reuse disposable PPE emphasize the need for focused capacity training on PPE usage. Further studies are necessary to evaluate the adequacy of the PPE currently used in Brazilian HCBLs, particularly for respiratory protection.

While most HCBLs reported having SOPs that guide the proper use of PPE, which users largely adhere to, further studies are necessary to evaluate whether the content of these SOPs, especially regarding the kind of PPE recommended, aligns with national and international requirements.

6.2.19 Human resources

6.2.19.1 Results

The number of users of the HCBLs participating in the present study varied from two to 30, with an average of 9.71. Their average training hours focused on biosafety and biosecurity varied from eight to 60, with a mean of 22.2 hours per year. Most HCBLs reported having an SOP that outlines the capacity-building pathway for BSL-3 users (24/30, 80.0%), which they always or frequently follow (20/25, 80.0%). According to 90.0% of the respondents (27/30), the level of worker awareness regarding biosafety procedures is high or very high (Table A35).

Only eight HCBLs participating in the present study do not allow cleaning staff access to the BSL-3 laboratory (8/30, 26.7%). Among those that allow access, most provide training on biosafety issues for these workers (15/19, 78.9%). Most HCBLs do not grant IT staff access to the BSL-3 laboratory (16/27, 59.3%). Among those that do allow access, only two do not provide training on biosafety issues for these workers (2/11, 18.2%). Approximately half of the HCBLs do not have an engineer with biosafety expertise in their workforce (16/31, 51.6%). Still, the majority of them have a permanent maintenance team (19/32, 59.4%) that is primarily trained in biosafety issues (15/20, 75.0%) (Table A36).

A permanent maintenance team is less common in public universities than in other public institutes, as shown in Table 23.

Table 23. Correlation analysis between the existence of a permanent maintenance team in the HCBLs participating in the current survey affiliated with universities and in the HCBLs affiliated with other bodies.

Governmental body	Does the laboratory have a permanent maintenance team with biosafety expertise in its workforce?		Fisher's Exact Test
	No	Yes	
University	6	3	0.0308
Others	3	13	

Most HCBLs in the present study reported having a BO (21/31, 67.7%). According to most HCBLs, this position allows the independence of those responsible for implementing the program of work (15/23, 65.2%). However, most of these professionals are dedicated to more than just the position of BO (13/23, 56.5%). Biochemistry Pharmaceutical was the most common background of the BO (7/23, 30.4%), followed by Veterinary Medicine (5/23, 21.7%). Most of them were trained on biosafety issues before starting their activities related to this position (17/23, 73.9%), and their average refresher training hours focused on biosafety and biosecurity varied from zero to 40, with a mean of 13.6 hours per year (Table A37).

6.2.19.2 Discussion

6.2.19.2.1 Number of High-Containment Biological Laboratories users

Interestingly, the results concerning the HCBL users were the same as those obtained from EDPLN laboratories, where the number also ranged from two to 30²¹¹. In a survey conducted by ABSA with 41 HCBL' BOs, it was also revealed that most BSL-3 laboratories have fewer than 26 employees¹⁴².

The average number of users in the current study was nearly 10, which can be deemed high, as HCBsL should be utilized solely for specific tasks and by well-trained people. HCBLs must assess whether all authorized individuals require access to the contained area.

6.2.19.2.2 High-Containment Biological Laboratories user's training and level of awareness on biosafety

The average amount of training hours is relatively low, as 22.2 hours falls short of three full business days and is less than the average duration of a symposium or workshop. Chapter 2.17 of this thesis emphasizes the significance of capacity-building programs for HCBL workers, which include annual refresher sessions. While no specific rule stipulates a minimum number of hours for refresher training, ISO 35001 recommends consistently providing refresher biorisk training¹¹. The Canadian Biosafety Standard Manual states that:

'A training needs assessment can be used when initially developing a training program to determine key components of the training program, including objectives, content, target groups, implementation strategy, and retraining cycles. It can also be used to assess an existing training program against its established objectives to determine whether any gaps or deficiencies exist in the program. As training needs can vary according to personnel responsibilities and experience, separate assessments can be performed for different target

*groups. Assessments also determine any need for additional training and the frequency of refresher training. The need for refresher training can change over time, based on the annual review process, personnel competence and experience, incidents, or changes in the biosafety program or biosecurity plan*⁸⁰.

Refresher training does not necessarily need to be as extensive as the initial training a person receives but should at least consist of an overview of containment, biosafety, security, and incident response and include changes to the entity's plans³¹⁰.

In this regard, it is essential to highlight the significant role of SB3 in offering capacity-building opportunities to HCBL workers in Brazil. Additionally, it would be beneficial if specific national reference laboratories, such as those associated with FIOCRUZ or LFDA networks, could serve as references for biorisk management, thereby strengthening their role as training providers.

A local mentorship program is another valuable tool that can be utilized as a training method, as advocated by Norlock et al. (2021). According to these authors, local mentorship in biosafety and biosecurity offers mentees culturally and regionally relevant professional development opportunities where technical concepts are discussed within national and regional systems and legislation frameworks. Mentors are described as experienced biosafety and biosecurity professionals with a at least three to five years of professional expertise³⁹⁴. Brazil boasts numerous qualified professionals capable of fulfilling this role, particularly within the national reference laboratories. Their knowledge and experience should not be underestimated.

Another approach that Brazilian authorities could assess is the provision of introductory online training courses for HCBL users like those implemented in Singapore. According to Tun & Wah (2016), an online basic biosafety program was developed, including biosafety concepts, legislation requirements, good microbiological practices, and emergency procedures. All workers in biological laboratories are expected to have completed the course and the tests before being allowed into an HCBL. Developing such basic competency is the basis of introducing a national biosafety training program, known as the 'Biosafety Competency Framework', by the Work Development Agency with the strong endorsement of the Ministry of Manpower and the Ministry of Health. The strength of such a program will be standardized or harmonized practices in this area and promote mutual recognition of competency of laboratory users from various institutions. 'Biosafety Passport' will be awarded upon completion of the course. The passport holder will be well qualified and recognized as a competent worker in any biological laboratory in Singapore³⁹⁰.

While the majority of HCBLs participating in this study reported having an SOP that outlines the capacity-building pathway for BSL-3 users and claimed adherence to the procedures, additional research is necessary to determine if the content of these SOPs meets the requirements mentioned above.

Similarly, additional studies should be conducted to validate the extensive awareness of HCBL workers regarding biosafety procedures. This assessment was not the primary objective of the present study, and the results were derived from self-assessment. Specific tools designed to evaluate the biosafety culture of an organization can be utilized, such as the one developed by the 'International Working Group on Strengthening the Culture of Biosafety, Biosecurity, and Responsible Conduct in the Life Sciences'³⁹⁵.

Furthermore, conducting studies that specifically investigate non-compliance in biosafety facilities could provide valuable insights into the level of awareness among workers in Brazilian HCBLs regarding biosafety procedures. For example, a study by Jirkof & Schmutz (2019) sought to gather information about the factors influencing compliance with biosafety rules in Swiss academic and state-run ABSL-2 and ABSL-3 facilities. According to their findings, factors perceived to increase compliance were management commitment, adequate equipment, risk awareness, and practical training¹³⁴.

A report released by the US National Science and Technology Council emphasized the importance of developing evidence-based laboratory biorisk management, including human resources management. In this context, the authors recommended the following research areas that could be instrumental in addressing factors hindering a culture of compliance in the workforce:

- a. Identification of evidence-supported practices for personnel management, including sociology and psychology studies surrounding workplace behaviors that support or impede a positive biosafety and biosecurity management culture;
- b. Identification of factors in optimal design of biorisk management review processes;
- c. Identification of the appropriate resources that produce a thriving biorisk management culture, including funds; administrative staff to reduce burden; incentivization and award programs; customized, frequent, career-long training; and routine proficiency evaluations;
- d. Identification of the impediments and their solutions to building a proactive biorisk management culture, including reasons for non-compliance, metrics for a successful biorisk management program, and tailored techniques to improve biorisk management programs;
- e. Description of practical approaches to biosafety training, biorisk communication, and transparency, including understanding the relationship between various approaches to biorisk teaching or managing in areas like accountability, workforce state of mind, or fear of reprisal and the number or severity of biological incidents³⁹⁶.

6.2.19.2.3 High-Containment Biological Laboratories support staff

The access of cleaning and IT staff to HCBLs should be determined based on the risk assessment, considering the laboratory's size and the nature of activities conducted within it. Whenever possible, their access should be avoided. However, they should receive training on biosafety protocols if their access is permitted.

Based on the present study's findings, there appears to be a prevalent culture among Brazilian HCBLs regarding the necessity of allowing cleaning staff to access high-containment areas, as only less than one-third of the respondents reported denying such access. The percentage of respondents indicating that cleaning staff has access to the contained area (46.6%) is comparable to the result obtained by Simonetti (2014) in Brazilian HCBLs (48%)⁷⁴. However, in contrast to this author, the current study found that 78.9% of the cleaning staff have received biosafety training, a lower proportion than Simonetti's result (93%)⁷⁴.

According to Johnson & Casagrande (2016), Australian and New Zealand regulations include entry by people involved with cleaning and repairs until potentially contaminated laboratory surfaces have been decontaminated and authorization is obtained from the laboratory supervisor or the BO⁸³.

Therefore, HCBLs should critically evaluate, while elaborating on the risk assessment, whether access to cleaning staff is truly necessary.

IT staff access was less frequent than cleaning staff; nevertheless, two HCBLs reported that the IT workers accessing the high-containment area were not adequately trained. It is important to note that these two HCBLs were not the same ones that reported such an issue with the cleaning staff. The IT infrastructure for HCBLs should be designed so that all maintenance can be conducted from outside the containment area.

Regarding the maintenance staff, Salerno & Gaudioso (2015) argued that depending on the complexity of a facility, it is vital to ensure that all necessary trades are covered. Most large facilities employ at least one electrician and one mechanical engineer. In some cases, employing specialists in HVAC and plumbing/wastewater treatment systems may be necessary. A staff member should have expertise in building management systems if the facility is particularly complex. If a facility does not have maintenance personnel on staff, outside contractors can be used⁷⁶.

The MS guidelines require an engineering professional for HCBLs. According to the document entitled: 'Biocontainment: the risk management in environments of high biological containment NB3 and NBA3', the engineering professional should ensure that the facility's engineering systems are in optimal condition for monitoring, maintenance, operation, and functionality. They must lead a technical team capable of implementing their guidelines for predictive, preventive, and corrective maintenance measures on the facilities, focusing on emergency operating procedures. It guarantees the seamless operation of the engineering systems forming the laboratory's secondary barrier⁹⁴.

However, most HCBLs do not follow this requirement. The number of HCBLs reporting a permanent maintenance staff was higher than those with an engineer.

For laboratories without permanent maintenance staff, preventive and corrective services are usually outsourced to third-party companies, who provide the services on-demand through contracts. Further studies are necessary to assess the feasibility and sustainability of such contracts for HCBLs, particularly in emergencies.

Once again, it was reported that some workers accessing HCBLs did not receive proper training on biosafety issues.

6.2.19.2.4 High-Containment Biological Laboratories Biosafety Officers

The MS regulation requires the designation of a skilled professional to implement the institutional biosafety policies within the HCBLs. The regulation does not explicitly define the title of this position¹⁰. On the other hand, the MAPA's regulation for FMD virus handling defines this role as the 'biosafety supervisor'²³¹. For this study, this professional is called a 'biosafety officer' (BO).

The APHL developed a competency-based BO description template³⁹⁷, and Denmark's National Biosecurity Agency listed some of the BO's duties related to biosecurity, including:

- a. Ensuring a solid biosecurity culture at the facility;
- b. Training personnel at the facility in relevant aspects of biosecurity;
- c. Ensuring that all biosecurity procedures are correctly followed;
- d. Being aware of irregularities and suspicious behavior;
- e. Maintaining an up-to-date list of controlled inventories;
- f. Reporting the purchase, sale, or handover of controlled materials;
- g. Reporting any biosecurity problems or incidents;
- h. Staying informed of new biosecurity trends and threats²⁰⁵.

Chapter 2.6 of the present study discusses the significance and role of the BOs, emphasizing that all HCBLs must designate a BO to implement and uphold

their biological risk management program. It is thus surprising that over 25% of the HCBLs participating in this study acknowledged not having appointed a BO. Furthermore, it is concerning that more than half of the HCBLs reported that this employee is only partially dedicated to activities related to biorisk management.

The situation is considerably more concerning than observed in the EDPLN laboratories, where all but one laboratory (12/13, 92.3%) reported having designated BOs²¹¹. Similarly, 51 out of 55 US laboratories (92.7%) affiliated with the APHL reported employing a full-time BO³⁹⁸.

In contrast, the situation in the Broader Middle East and North Africa (BMENA) region was even more alarming. An assessment of the availability of skilled biosafety professionals or biorisk managers was conducted through an online survey involving representatives from 23 laboratories. This assessment revealed that 40.0% of them considered the availability of such professionals to be poor, while only 10.0% considered it excellent³⁹⁹.

The present study's findings underscore the urgent need for a comprehensive legal framework that encompasses HCBLs beyond the jurisdiction of the MS. Additionally, there is a pressing requirement to strengthen oversight mechanisms. Notably, among the eight HCBLs lacking a BO, two belong to the Health State Secretariat network and, therefore, should comply with the MS regulation, which mandates the presence of a BO¹⁰.

Subramaniam (2014) noted that formal courses to ensure the BO's competencies are limited in Malaysia. Furthermore, there is no standardized curriculum even at the international level, as the biorisk spectrum of each organization varies depending on its scope of activities. In Malaysia, the appointment of a BO has been predominantly based on work experiences, as there is no established procurement process within the employment scheme¹⁷¹.

In Brazil, the situation is comparable, which may explain the diversity in the academic backgrounds of the BOs found in this study. The two primary academic backgrounds observed (Biochemistry Pharmaceutical and Veterinary Medicine) align with the basic qualification requirements stated by CWA 16335 – Biosafety Professional Competence. According to this document, a biosafety professional should have an education in life sciences or other relevant fields (e.g., safety, industrial hygiene, engineering), supplemented by appropriate levels of biology¹⁶⁷.

The average number of hours of refresher training on biosafety issues offered to the BOs found in this survey was surprisingly low (13.6 hours per year). It is especially concerning, given the availability of numerous webinars that are provided at no cost. Therefore, there is a pressing need not only to convince the institute's leadership about the importance of investing financial resources in refresher training for the BOs, including participation in international workshops, but also for the BOs themselves to take a more proactive approach in seeking capacity-building opportunities with limited or no costs.

Moreover, federal authorities, particularly those associated with MS, MAPA, MD, and MCTI, could develop a national program aimed at enhancing the competencies of BOs. The SB3 could support this program and establish a minimal curriculum and mandatory training courses. Offering these courses online would facilitate recording information about professionals who have completed the basic program, ultimately generating a national database of BOs.

Taking such measures will elevate biosafety knowledge and practices among BOs and contribute to a more robust and cohesive national biosafety framework.

6.2.20 Occupational Health

6.2.20.1 Results

In the present study, most participating HCBLs reported including risk assessment pathway issues related to the health of BSL-3 users (24/31, 77.4%). However, approximately one-third of the respondents admitted that their institute does not provide periodic medical exams for the employees (10/31, 32.3%). More than half of those that provide such exams include specific tests related to the activities carried out within the HCBL (10/18, 55.6%). Less than half of the HCBLs have a first-aid station (15/32, 46.9%). When it comes to the average time spent by workers within the BSL-3 laboratory, the most common response was four hours (13/31, 41.9%). However, three HCBLs reported that workers stayed there for eight hours (3/31, 9.7%). The number of HCBLs that have not defined a maximum period of stay for users within the BSL-3 lab (13/31, 41.9%) was almost the same as those who have defined one (14/31, 45.2%). Typically, this period is four hours (8/12, 66.7%) (Table A38).

In the present study, the majority of participating HCBLs do not allow their users to drink water (24/32, 75.0%) or have food (27/32, 84.4%) within the facility (Table A38). The procedures implemented by HCBLs that permit water and food consumption within the controlled zone to prevent worker contamination encompassed:

- a. Utilization of single-use mineral water cups instead of a drinking fountain;
- b. Consumption in a controlled, clean, or isolated area, such as the changing room;
- c. Before consuming food or water, workers should remove gloves, masks, and other PPE, as well as their lab coats, and disinfect their hands.

Few HCBLs conduct surveys to assess their users' well-being (4/32, 12.9%). Only three respondents reported that the HCBL design considered accessibility requirements (3/32, 9.4%). Less than half of the HCBLs reported having an emergency shower within the BSL-3 laboratory (13/32, 40.6%), while half had emergency eye-washing facilities (16/32, 50.0%) (Table A38).

6.2.20.2 Discussion

Chapter 2.13 of this thesis underscores the importance of managing occupational health programs in HCBLs, focusing on identifying and addressing users' specific health risks. While most HCBLs participating in this study reported assessing these specific risks, it is apparent that certain procedures do not adequately support the well-being of workers. For instance, over one-third of HCBLs lack periodic medical exams for their staff. This finding closely aligns with observations made by Richards et al. (2014) in US HCBLs, where only 12 out of 20 (60.0%) of the laboratories handling select agents and 8 out of 22 (36.4%) of those handling non-select agents collected baseline and annual follow-up serum samples from scientific staff¹³.

Furthermore, even when exams are conducted, many HCBLs do not include tests related to the specific activities within the HCBL. This scenario is unfavorable, but it is an improvement compared to the results obtained by Simonetti (2014). In that study, only 31% of the participating HCBLs conducted annual medical exams for their workers, and a mere 5% of the institutes wholly adhered to NR n. 32 regulations⁷⁴.

Although this regulation applies to all healthcare services, certain governmental institutes do not adhere to it, and there is a lack of inspection or sanctions for non-compliant behavior in governmental bodies.

Nearly all HCBLs are affiliated with public services, so they must comply with NR n. 32²⁴⁰, requiring the development of a PGR that addresses specific biological risks and outlines an action plan to mitigate them. This action plan may include additional medical exams, vaccinations, serological tests, and pulmonary function tests. Serological monitoring offers the advantage of detecting subclinical LAs.

It is worth noting that the MS document 'Biocontainment: the risk management in environments of high biological containment NB3 and NBA3' emphasizes the importance of conducting psychological and physiological monitoring for HCBL workers⁹⁴.

Another mitigation action that could be outlined in the PGR is establishing a maximum period of stay for users within the BSL-3 lab. While fewer than 50% of the HCBLs participating in this study have defined such a limit, only four HCBLs acknowledged that their workers tend to spend more than four hours per day within the HCBL. However, since many HCBLs did not provide this specific information, further studies are necessary to validate the findings of this survey and delve deeper into the impact of working within an HCBL on mental health. Remarkably, no national study addressing this critical issue has been identified so far.

According to Simonetti (2014), taking into consideration the conditions, regulations, and work practices in an HCBL, which include restrictions on eating, using the restroom, using cell phones, touching one's face, and using PPE, it is recommended that professionals dedicate no more than four hours per day. Excessive working hours, the mental well-being of the professionals, fatigue, and workplace stress should be considered when evaluating their performance, as they can compromise the execution and safety of the procedures they undertake⁷⁴.

Lin et al. (2020) agreed that limiting each entry time to the laboratory to a maximum of four hours contributes to reducing the likelihood of accidents caused by personnel fatigue in HCBLs²⁷⁵.

Working within an HCBL entails certain restrictions, such as limitations on water intake and meals, as revealed in the current survey, which can result in a high level of dissatisfaction. However, only a limited number of HCBLs conduct surveys to assess the well-being of their workers. Such surveys can offer valuable insights to managers, aiding them in making decisions to enhance worker satisfaction, thereby potentially contributing to mitigating biological risks.

A first aid station near the HCBL appears feasible in many cases, as demonstrated in the present study. Nevertheless, the PGR of an HCBL needs to incorporate procedures related to first aid in the event of accidents involving biological threats. It includes specifying the designated health unit for first aid in case the institute does not have one.

The results obtained in the present study concerning emergency showers and eye-washing devices within the BSL-3 lab were unexpected, especially regarding eye-washing. While specific standards do not require the installation of emergency showers within the HCBL (one standard even contraindicated it²⁸⁹), most of them do specify the necessity of eye-washing facilities. Notably, Brazilian regulations mandate both installations, as indicated in Chart 06.

When these devices are connected to the hydraulic system, the effluent must be directed to the EDS (Effluent Decontamination System). Dry laboratories, on the other hand, should utilize portable devices.

Chart 06 – Requirement of emergency showers and eye washing for BSL-3 laboratories according to international standards.

Standard	Emergency eye washing	Emergency showers
BMBL ¹	An eyewash station is readily available in the laboratory.	Emergency showers are readily available, easily accessible, and appropriately maintained (only for ABSL-3 facilities).
Canadian Biosafety Handbook ⁷	Emergency eyewash and shower equipment, where required, provide on-the-spot treatment to flush out, dilute, and remove any infectious material or toxins that have contaminated the eyes, face, or body. The need for and placement of emergency eyewash stations and showers within a containment zone is based on an assessment of the activities performed and provincial/territorial occupational health and safety legislation.	
WHO Laboratory Biosafety Manual ²	If eyewash stations with piped water are to be used, these should also be checked regularly for correct functioning.	There is no specific recommendation.
EU Directive 2000/54 ²⁰³	There is no specific recommendation.	
Recommendation on Structural and Technical Safety Measures in BSL-3 Laboratories ²⁸⁹	As a rule, self-contained eyewashers, without a water connection, according to DIN EN 15154, are suitable as emergency devices. It must be determined in advance in the risk assessment.	Emergency safety shower stations with water connections in the work area and the airlocks should be avoided as far as possible based on the risk assessment. The use of emergency safety showers to shower off biologically contaminated persons is contraindicated.
UNE 171400-1 ⁸²	An eyewash station and emergency shower must be provided. It could be either portable or connected to the hydraulic system. These devices should be easily accessible and strategically installed in locations that do not interfere with laboratory activities.	
Australian SSBA Standards ⁴⁰⁰	There is no specific recommendation.	
General Directives for the Containment Work with Biological Agents ²²³	Equipment such as a shower and emergency eyewash must be readily available in the containment areas and adjacent to the laboratory area.	
Africa CDC framework ²¹²	Emergency eyewash and shower equipment are to be provided by containment zone activities.	

Only two HCBLs reported complying with accessibility requirements, but whether these requirements include access to the containment zone was not assessed. No scientific publication regarding the feasibility of adapting an HCBL structure to accommodate workers with different types of disabilities was found in Brazil or other countries. Therefore, further studies are needed to address this question.

6.2.21 Communication tools

6.2.21.1 Results

In the present study, most participating HCBLs do not permit their users to bring their cell phones into the BSL-3 laboratory (23/32, 71.9%). The most common communication tools used within the HCBL are the telephone (22/30, 68.8%) and the internet (21/30, 65.6%). Of the 29 HCBLs that provided information about communication tools, 31.0% (9/29) reported having only one tool. Among these, the most common tool was the telephone (4/9, 44.4%), followed by the internet and

intercom (2/9, 22.2%), and whiteboard (1/9, 11.1%). Most respondents expressed satisfaction with the quality of the internet service, with 80.9% (17/21) rating it as 4 or 5 on a scale of 1 to 5 (Table A39).

6.2.21.2 Discussion

Shurtleff et al. (2012) addressed communication issues related to maximum-containment laboratories and emphasized the significance of effective communication among coworkers inside and outside the laboratory to ensure successful performance. The authors highlighted various communication tools available, such as telephones, visual information marquees, visible and audible alarms, fax machines, and computers, which can aid in daily and emergency communication²⁵⁷.

Although that study primarily focused on maximum-containment laboratories, some concepts also apply to HCBLs. For example, Gmuder et al. (2022) advocated that HCBLs should have at least two independent and redundant communication systems²⁸⁹. Additionally, Vieira (2008) stressed the importance of installing intercoms in anterooms, changing rooms, and other areas where users may become trapped in the event of door malfunctions³¹³.

McCall & Hardcastle (2014) argued that the layers of protection in an HCBL can limit effective communication methods among users and staff outside the containment area. Despite the importance of instant and secure communication tools, HCBL work often requires coordinated efforts between workers inside the containment zone and support staff outside the area⁴⁰¹.

The results obtained in the present study showed a notable contrast to those obtained by Simonetti (2014). In that study, the telephone/intercom was present in 78% of the surveyed HCBLs, while the internet was present in 7%, mimicry in 6%, and 9% lacked any communication tool⁷⁴. Although the telephone remained the most prevalent communication tool in the current study, the internet was nearly as common. Furthermore, the percentage of HCBLs without any communication tool was significantly lower than the previous findings.

Taking cell phones into the containment area is forbidden according to the MS requirements⁹⁴, as they can be a source of contamination not only to the worker but also to their family and others who may come into contact with the device. The presence of alternative communication tools, such as the internet and telephone, makes using cell phones unnecessary within the BSL-3 area. Therefore, the three HCBLs that reported permitting cell phone usage inside the containment area shall review their procedures.

Surprisingly, over one-third of the surveyed HCBLs reported having only one communication tool, which contradicts Gmuder et al.'s (2022) recommendation of having at least two independent and redundant communication systems²⁸⁹.

When using the internet, it is crucial to ensure its quality, as highlighted by the majority of the HCBLs. However, it is concerning that one HCBL, which solely relies on the internet as its communication tool, gave one of the lowest ratings for the quality of this service.

6.2.22 Biosecurity

6.2.22.1 Results

Most HCBLs participating in the present study considered the frequency of biosafety-related incidents to be low or very low (29/32, 90.6%), as well as the risk of an external-sourced criminal event occurring in the BSL-3 lab (29/32, 90.6%). Only

one respondent reported theft or stealing materials from the BSL-3 laboratory (1/24, 4.2%). The same HCBL has been sabotaged (1/32, 3.1%). No HCBL reported a high-risk of sabotage (Table A40).

Most HCBLs have a guard service (25/32, 78.1%), which is primarily available 24 hours a day (21/25, 84.0%), including weekends and holidays (24/25, 96.0%). Only four HCBLs reported having any cooperation agreement established with security authorities (4/32, 12.5%) (Table A41).

Most HCBLs reported that all rooms where high-risk biological agents are stored have their own access control (22/32, 68.8%). Among those who do not have this feature, only one stated that the equipment where high-risk biological agents are stored is kept locked (1/6, 16.7%). Most HCBLs reported having an inventory for biological agents (20/32, 62.5%) and an SOP for their transportation (24/32, 75.0%). In almost all cases, the SOP is always followed (20/22, 90.9%). Only a few HCBLs reported that transferring biological assets is frequent or highly frequent (5/23, 21.7%). Less than half of the HCBLs reported having workers certified according to the International Air Transport Association (IATA) and to the Brazilian National Civil Aviation Agency (*Agência Nacional de Aviação Civil* – ANAC) rules (13/32, 40.6%), but for three of them, the certification is expired (Table A42).

Most HCBLs participating in the present study reported having a protocol to evaluate the adequacy of receiving external visits (21/32, 65.6%). Still, few HCBLs have a complete protocol implemented for evaluating criminal records and behavioral profiles before hiring workers who will access the BSL-3 lab (3/32, 9.4%). Most HCBLs rated the security level of the data generated and stored in the BSL-3 lab as high or very high (20/28, 71.4%), and only two have reported incidents involving the security of these data (2/25, 78.1%) (Table A43).

CCTV was the most common additional security barrier surrounding the HCBL (Table A44).

6.2.22.2 Discussion

6.2.22.2.1 Biosecurity incidents

Coelho (2017) argued that the MS regulations and guidelines should be updated to incorporate concepts and guidelines related to biosecurity. According to this author, despite the risk of a biological incident in Brazil being lower than in the US and the EU, the investment made by the country to prevent such incidents related to biosecurity is insufficient, especially considering the severe consequences of an event like that¹⁸.

Similarly, Astuto-Gribble et al. (2009), in an online survey involving 765 life scientists from 81 countries in Asia, Eastern Europe, Latin America, and the Middle East, concluded that biosecurity practices are inconsistently employed worldwide. However, when asked about the likelihood that their laboratory could be involved in or affected by the theft of samples with the intent to harm by either an employee or an outsider, the overwhelming majority of respondents claimed this was a remote possibility³⁰⁷, which aligns with the results obtained in the present study.

Although bioterrorism is not considered a significant threat in Brazil^{18, 154}, urban violence related to drug trafficking and other crimes can directly or indirectly affect the functioning of HCBLs, as observed at FIOCRUZ⁴⁰² and LFDA-SP⁴⁰³. In a survey conducted by ANBio involving 235 laboratory workers in Brazil, approximately one-third of respondents reported a risk related to theft or misuse of organisms by unauthorized persons. In the same study, over half of the participants indicated that their institutions do not provide biosecurity training¹⁷.

Therefore, despite the scarce incidents related to biosecurity reported by the HCBLs participating in the present study and the relatively low risks associated with theft, stealing, and sabotage, basic measures to avoid such issues, including biocrimesⁱⁱ, must be implemented by all HCBL.

A laboratory biosecurity program's main components are physical security, personnel security, material control and accountability, transport security, information security, and program management¹⁸⁰. These components should be evaluated by a risk assessment approach, utilizing tools like those provided by the National Institute for Public Health and the Environment in the Netherlands⁴⁰⁴.

6.2.22.2.2 Guard services

The need to provide guard services should be taken into account during a biosecurity risk assessment, considering factors such as the level of violence in the neighborhood, the number of employees, the laboratory's size, the presence of physical security barriers, electronic surveillance, historical incident records in the institute, and other relevant aspects. While this may not be a legal requirement, it should be adopted when the risk assessment concludes that it is necessary. The fact that most HCBLs participating in the present study have guard services is not necessarily linked to biosecurity; instead, it is likely a common practice related to property security adopted by various public and private companies.

6.2.22.2.3 Inventory, transportation, and control of biological assets

Keeping an updated inventory of biological assets and controlling their use and transfer is one of the fundamental aspects of biorisk management in an HCBL. According to the WHO:

*'A comprehensive programme of accountability is necessary to establish adequate control of at-risk biological agents, and to discourage theft and/or misuse. Procedures that can be used to achieve this include compilation of a detailed inventory, including description of the biological agent(s), its quantities, storage location and use, the person responsible, documentation of internal and external transfers, and an inactivation and/or disposal of the materials. A periodic review is recommended and any discrepancies should be investigated and resolved'*².

The MS document 'Biocontainment: the risk management in environments of high biological containment NB3 and NBA3' states that managers are required to implement control and accountability systems for biological material, encompassing the following elements:

- a. Creation of inventories detailing the biological materials within their collections;
- b. Maintenance of records and daily updated backups to track the location of the biological material;
- c. Identification of individuals with access to or custody of the biological material⁹⁴.

The managers are responsible for controlling and overseeing the biological material held within various security areas, including those posing low or negligible biosecurity risk⁹⁴.

ⁱⁱ Biocrime can be defined as the use of a disease-causing agent or toxin to kill, debilitate, or cause panic for a specific individual or a limited group of individuals, motivated by personal reasons such as revenge, jealousy, or the desire for monetary gain by extortion¹⁴⁸

Therefore, considering the potentially severe consequences of losing or stealing high-risk pathogens or toxins, access to the rooms and devices where they are stored should be strictly restricted.

It was evident that four HCBLs participating in the present survey lacked proper adherence to this requirement. They reported not having specific access control measures for the rooms containing high-risk biological assets, and their storage equipment has been unlocked. Even more concerning, three of these four HCBLs admitted to not maintaining proper inventories of their materials, further exacerbating the criticality of the situation.

Similar concerns have been raised in studies conducted in other regions. Gaudioso & Zemlo (2007) reported that only 54% of over 300 surveyed life scientists indicated that their laboratories maintained an inventory of biological assets³⁵⁰. Meanwhile, Appelt et al. (2020) determined that 56% of the EU BSL-3 laboratories they studied fully complied with pathogen accountability requirements³⁵⁶.

Coelho (2017) advocated that the BFG should make it mandatory for all institutes to report the possession of high-risk pathogens, thus enabling the establishment of a centralized inventory. According to this author, it is likely that some of these microorganisms are being maintained in laboratories that do not have the appropriate biosafety levels¹⁸.

It is encouraging to note that most HCBLs surveyed in the current study reported having SOPs outlining the procedures for transferring biological assets despite the limited common practice. Further studies are necessary to assess whether the content of the SOPs adheres to the national regulations and to determine the frequency of transfers conducted by non-authorized staff.

A cause for concern arises as most HCBLs lack personnel trained under IATA's and ANAC's regulations. The MS guideline 'Biocontainment: the risk management in environments of high biological containment NB3 and NBA3' highlights that to adhere to the correct protocols for transporting potentially contaminated biological materials, it is essential for the professionals engaged in this task to possess knowledge of the requirements outlined in both national and international regulations, to be qualified for the proper management of these agents, and to be well-versed in containment, packaging, labeling, documentation, and transportation procedures⁹⁴.

It is worth noting that the book 'An Efficient and Practical Approach to Biosecurity' includes a Chapter dedicated to procedures related to the inventory control of biological assets. This Chapter can serve as a valuable reference for HCBLs looking to enhance their practices²⁰⁵.

6.2.22.2.4 Personnel background assessment

The NSABB has released a document to enhance personnel reliability and strengthen the culture of responsibility. This guidance recommends the following practices for responsible hiring:

- a. Willingness on the part of current and former employers to provide candid references;
- b. Thorough checking of references for prospective employees, as well as a review of available past performance evaluations;
- c. Rigorous, biosecurity-minded review of credentials and professional status of prospective employees, as well as a check of any possible criminal history;

- d. Periodic performance review for all laboratory personnel that addresses, among other topics, the responsible conduct of research, adherence to biosecurity policies and practices, and practices that contribute to a culture of responsibility;
- e. Clear articulation and documentation of employment conditions and expectations regarding trust, integrity, and reliability²⁸⁰.

The CDC/USDA Suitability Assessment Program Guidance assists entities in developing a site-specific suitability assessment program for individuals with access to high-risk pathogens, including short-term training visitors. According to this document, an entity may use the campus police department, the public safety office, or even a private investigator to obtain criminal records of personnel accessing high-risk pathogens⁴⁰⁵.

Appelt et al. (2020) found that less than half of the European BSL-3 laboratories surveyed fully complied with personnel suitability and reliability (10/25, 40.0%), highlighting the necessity to enhance adherence to this requirement in those countries³⁵⁶.

The findings from this study indicate that although most HCBLs have a protocol for assessing the suitability of external visits, there is a lack of culture in evaluating criminal records and behavioral profiles when hiring personnel who will have access to the HCBL. Implementing this procedure should be considered in the biosecurity risk assessment.

6.2.22.2.5 Data safety

Murch et al. (2018) proposed the term 'Cyberbiosecurity', addressing the vulnerabilities to unwanted surveillance, intrusions, and malicious and harmful activities which can occur within or at the interfaces of comingled life and medical sciences, cyber, cyber-physical, supply chain, and infrastructure systems, and developing and instituting measures to prevent, protect against, mitigate, investigate and attribute such threats as it pertains to security, competitiveness, and resilience. The authors argued that experts in biosecurity and cybersecurity are generally unaware of each other's domains, expertise, perspectives, priorities, and the potential mutually supportive opportunities that could lead to positive outcomes⁴⁰⁶.

Peccoud et al. (2018) added that DNA (Deoxyribonucleic Acid) sequencing, synthesis, manipulation, and storage are increasingly digitized, so there are more ways than ever for nefarious agents inside and outside of the community, to compromise security. To mitigate these risks, the culture of the life sciences community needs to shift from one of blind trust to one of heightened awareness. Those focused on biological processes and production need to develop a broader perspective, which includes a detailed understanding of cyber-physical threats. Once individuals within the community are aware of cyberbiosecurity risks, they can implement safeguards within their work environments and work with regulators to develop policies to prevent cyberbiosecurity breaches⁴⁰⁷.

Even though the majority of HCBLs participating in the present study believe that the data generated and stored are adequately safeguarded, and only two HCBLs reported incidents related to this issue, further research is necessary to assess the level of awareness among BSL-3 users concerning cyberbiosecurity concepts and principles. Additionally, it is essential to understand better the risks involved and how they have been addressed within the operational HCBLs in Brazil. Such studies should contribute to strengthening and enforcing national regulations concerning cyberbiosecurity.

6.2.22.2.6 Additional security barriers

Salerno & Gaudioso (2015) highlighted that access to the site of a research facility that works with highly infectious diseases may be restricted to only authorized personnel. The facility may be secured with perimeter fencing, gates that require card access, and video monitoring of all entries⁷⁶.

However, some authors have found that many laboratories worldwide neglect these measures. Astuto-Gribble et al. (2009) applied a questionnaire to 765 scientists, healthcare professionals, laboratory staff, and physicians from Asia, Eastern Europe, Latin America, and the Middle East between 2005 and 2007. The authors conclude that physical security to reduce the risk of unauthorized access to a laboratory is relatively weak in nearly every region. The most widespread biosecurity measures include locked laboratory doors, locked building doors, and locked cabinets. A smaller proportion of respondents use more sophisticated physical security measures. Video monitors, sensors and alarms, and self-closing doors are rarely used³⁰⁷.

In a more recent study involving 337 clinical, diagnostic, academic, and research laboratories in Pakistan, the researchers concluded that biosecurity was inadequate in most laboratories. While some had installed locks and security cameras, most cameras were non-functional and lacked monitoring. Additionally, only some labs had fences and grills on windows. Remarkably, no labs had intrusion alarms, making them easily accessible to anyone. The satisfaction level in terms of biosecurity, as per WHO requirements, was shallow across all assessed parameters, including graded physical protection (16%), capacity to react to an invasion (16%), personnel training (23%), and security awareness (37%)³⁸¹.

A study evaluating biorisk management systems in 25 European HCBLs achieved better results. Appelt et al. (2020) found that 64.0% (16/25) of the BSL-3 laboratories surveyed fully complied with the physical security measures in place³⁵⁶.

Gaudioso & Zemlo (2007), studying biosecurity practices in Asian countries, concluded that at least half of the respondents' laboratories always have a guard posted at the buildings entrance, lighted buildings at night, and locked cabinets. According to these authors, laboratories in developing countries, in contrast to those located in advanced and emerging countries, tend to have personnel-dependent security measures such as guards and patrols. More sophisticated physical protection measures, such as intrusion sensors, alarms, and video monitors, are less commonplace³⁵⁰.

The current study revealed that additional security barriers are uncommon in Brazilian HCBLs, except for CCTV systems, which are present in 51.7% of the laboratories that answered this question.

6.2.23 Genetic Modified Organisms

6.2.23.1 Results

Most HCBLs participating in the survey reported not working with GMOs (25/32, 78.1%). Among the six HCBLs that work with GMOs, five (5/6, 83.3%) conduct a specific risk assessment for this purpose and possess a Biosafety Quality Certificate. The remaining HCBLs mentioned that they were unsure how to respond to both questions (Table A45).

There was no statistically significant correlation between the handling of GMOs and facility certification (Table 24).

Table 24. Correlation analysis between handling GMOs and the certification of the HCBLs participating in the survey.

Does the lab work with GMOs?	Was the lab certified?		Fisher's Exact Test
	No	Yes	
No	9	10	0.6609
Yes	2	4	

Similarly, no significant correlation was found between the handling of GMOs and the presence of an IBC (Table 25).

Table 25. Correlation analysis between handling GMOs and the existence of an IBC in the HCBLs participating in the survey.

Does the lab work with GMOs?	Does the lab have an IBC?		Fisher's Exact Test
	No	Yes	
No	6	17	1.0000
Yes	1	5	

6.2.23.2 Discussion

As Chapter 2.10 of this thesis emphasizes, the current national legislation primarily focuses on regulating GMOs, leaving other aspects related to laboratory biosafety and biosecurity insufficiently covered. Therefore, the statement made by Macedo during the 'Biosecurity Challenges of the Global Expansion of High-Containment Biological Laboratories Workshop' still accurately reflects the situation in Brazil: 'There is no oversight or evaluative mechanism for laboratories, high-containment or otherwise, that are established at public or private universities for research activities with biological agents (other than GMOs)'¹⁷.

The findings from the present study indicate that most HCBLs in Brazil do not work with GMOs. Consequently, it can be inferred that most HCBLs not affiliated with MS, MAPA, MCTI, or MD operate without regulation. It emphasizes the critical need for a comprehensive legal framework to oversee all HCBLs nationwide.

Some HCBLs harbor a misconception that the oversight of all biosafety and biosecurity aspects is overlooked when dealing with GMOs, per the mechanisms established by Law n. 11,105/2005²¹⁸. However, CTNBio Resolution n. 18/2018 clarifies that the biosafety assessment conducted per these legal requirements exclusively applies to the scope associated with GMOs²²⁰.

During the development of regulations, one crucial aspect to consider is the role of IBCs. Simonetti (2014) reported that in his study of six HCBLs, all had an IBC. However, these committees usually focus on GMOs, neglecting other critical biosafety issues in the process⁷⁴.

According to a report by the NSABB, numerous institutions in the US have chosen to assign their IBCs the responsibility of reviewing recombinant DNA research and other biohazardous research. The reason behind this decision is the need for overlapping expertise in biosafety and biosecurity considerations, leading some IBCs to effectively take on the additional responsibility of evaluating protocols for biosecurity aspects as well²⁸⁰. A similar approach was taken in Taiwan, where IBCs developed verification regulations for storing and transferring RG 3 and 4 pathogens²⁰⁹.

Hence, the establishment of legislation must be accompanied by enforcement mechanisms and detailed regulations specifying how to comply, such as defining the certification process and the roles of IBCs.

6.2.24 Risk assessment

6.2.24.1 Results

Slightly more than half of the HCBLs participating in the present study reported having SOPs defining how to conduct a risk assessment (18/31, 58.1%). Among them, only one did not do the risk assessment, and two HCBLs that do not have an SOP have done the risk assessment. Therefore, 19 HCBLs reported doing the risk assessment (19/31, 61.3%). Only two do not review this document periodically (2/16, 12.5%). This question did not apply to the other three HCBLs to be launched. Numerous professionals were mentioned as being in charge of elaborating the risk assessment, but the most common position was the head of the biorisk management department (5/18, 56.2%). Most HCBLs reported that the person in charge of the risk assessment elaboration received specific training for this purpose (13/19, 68.4%) and demonstrated satisfaction with the quality of the document (17/19, 89.5%) (Table A46).

Three HCBLs reported that the professional responsible for conducting the risk assessment received internal training on how to do so. Additionally, some HCBLs mentioned receiving external training, citing the following institutions:

- a. CDC (n = 3);
- b. USP;
- c. MS;
- d. Kansas State University;
- e. International Society for Pharmaceutical Engineering.

6.2.24.2 Discussion

Careful risk assessment requires a balanced approach that uses available data yet refrains from selecting more stringent safeguards that might impose unnecessary burdens and expense and increase the risk of non-compliance with safety standards²⁷⁹. The risk assessment must be specific to handle each pathogen in an HCBL⁴⁰⁸.

According to the guidelines set by the MS, conducting a risk assessment for each necessary analysis or diagnostic procedure is mandatory. This assessment includes discussions and determinations regarding the appropriate level of containment required to handle the respective samples. Additionally, it involves determining the BSL and making well-informed decisions for effective risk management²²³.

Chapter 2.3 of this thesis emphasizes the crucial significance of risk assessment and provides examples of tools that can be utilized for its implementation. Despite the importance of this procedure from the initial design phase of the HCBL to its operational stage, the results obtained from the current survey revealed that at least one-third of the HCBLs still needed to incorporate it into their routine practices. This lack of a culture surrounding risk assessment is also present in other countries.

Astuto-Gribble et al. (2009) surveyed bioscience research and biosafety and biosecurity practices in Asia, Eastern Europe, Latin America, and the Middle East. Their study highlighted a trend in the risk perception data. Respondents from higher-income countries collectively perceived significantly greater risk than those from

lower-income countries, suggesting a possible indication of better awareness and risk assessment practices in the former group³⁰⁷.

More recently, Sarwar & Vijayan (2021) conducted a training needs analysis in Pakistan's laboratories, revealing that those facilities were neglecting the risk assessment process. The authors emphasized the critical importance of the risk assessment process and how overlooking its correct implementation can significantly impact laboratory safety, consequently undermining the confidence of those involved. They also argued that developing a risk assessment as part of a biorisk management system should not be seen as a one-time task; rather, it must be continually strengthened to ensure the establishment and execution of a comprehensive and sustainable biorisk management system¹⁰⁴.

A study found that almost two-thirds of British scientists used their organization's approved form for risk assessments — which is mandated by the nation's Health and Safety Executive — compared with only one-quarter of Americans. Instead, more than half of US scientists said they assessed risk informally⁴⁰⁹. Salerno & Gaudioso (2015) stated that biorisk management is not yet ingrained in the bioscience mindset of the US, as only a minority of the biosafety community conducts substantive risk assessments, and even fewer comprehensively document those assessments⁷⁶.

A more favorable situation was observed in laboratories affiliated with EDPLN and APHL. In the case of the EDPLN laboratories, out of the 13 surveyed, 10 (76.9%) indicated that they had access to written risk assessments. Among these, three (30.0%) employed a combination of personnel to approve risk assessments, while others relied on the IBC (2/10, 20.0%), laboratory scientists (1/10, 10.0%), PIs (1/10, 10.0%), or laboratory manager (3/10, 30.0%) as the sole approver. All the laboratories reported having SOPs or other detailed procedures available²¹¹. Regarding the APHL laboratories, 52 out of 55 surveyed laboratories (94.5%) reported that they performed risk assessments³⁹⁸.

The WHO emphasizes the importance of clearly defining the professional responsible for risk assessment and identifying those who should assist them. For a comprehensive and practical risk assessment, input from laboratory personnel who thoroughly understand the processes and procedures within the scope of the work being assessed is essential. The risk assessment team may comprise various individuals, including but not limited to PIs, laboratory and quality managers, laboratory technicians, and BOs. Furthermore, the WHO highlights the significance of involving laboratory and organizational leadership in risk assessment. This involvement can be direct participation in the risk assessment team or communication with the team. Such engagement is crucial in establishing organizational support for and ensuring the sustainability of a biosafety management program⁹⁰.

Therefore, while the present study revealed that positions equivalent to BOs were most mentioned as being in charge of the risk assessment, as expected, further studies are needed to ascertain whether this task was supported by other professionals following a team approach, as recommended by the WHO.

Given the complexity of the risk assessment procedure and the need for support from a multidisciplinary team, the individual in charge of this task must undergo appropriate training on risk assessment tools, as Sarwar & Vijayan (2021) emphasized. In their study, after conducting a training needs analysis, participants were asked about their confidence in managing biosafety and biosecurity risks in the laboratory, and 88.9% expressed confidence. However, the responses to the

questions posed by the researchers revealed that their knowledge needed to be improved to develop and implement a biorisk management system effectively¹⁰⁴.

Therefore, it is concerning to note that among the HCBLs in the current study that claimed to have conducted risk assessments, 21.1% did not provide specific training to the professionals responsible for the task. It raises doubts about the quality of these assessments despite the respondents' high confidence level. Therefore, further studies are necessary to verify the actual accuracy and effectiveness of these risk assessments.

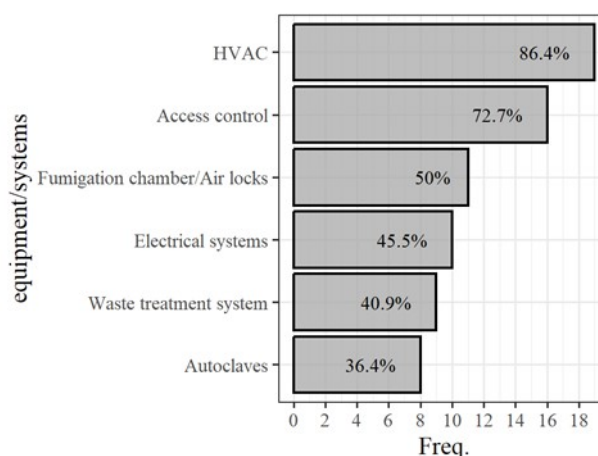
6.2.25 Automation

6.2.25.1 Results

Most HCBLs participating in the present study reported employing an automation system for monitoring and controlling their equipment and systems (22/32, 68.8%). Among these, 68.2% rated the software quality as good or excellent (15/22), and 86.4% mentioned having audible or visual alerts in case of any deviation from critical parameters (19/22). Three HCBLs stated that their automation system could be operated by only one person (3/22, 13.6%), while one HCBL mentioned outsourcing the operation to a third-party company (1/22, 4.5%). Most HCBLs can back up the data generated by their automation system (14/22, 63.6%), but the frequency of this procedure was not provided by the majority of them (8/14, 57.1%). Additionally, less than half of the HCBLs reported having software to record data related to preventive maintenance plans (13/31, 41.9%) (Table A47).

The HVAC system was the most commonly monitored by the automation system, followed by the access control (Graph 44). The firefighting system was mentioned once.

Graph 44 – Results regarding the kinds of equipment/systems covered by an automation system in the HCBLs participating in the survey.



6.2.25.2 Discussion

6.2.25.2.1 Automation system

Gmunder et al. (2022) stated that alarms or fault messages must safeguard failures, faults, and malfunctions in structural-technical systems that can result in personal injury and environmental or property damage. Furthermore, building automation must be suitable and flexibly adaptable for HCBL control, regulation, and monitoring. Various suitable products are available on the market and must be adaptable to the conditions. When planning HCBL, the corresponding requirements must be defined early. The authors emphasized that there are no standards or norms

for the design, execution, and testing of building automation and alarm systems for HCBLs. The building automation system should continuously record the parameters over time²⁸⁹.

Audible and visual alarms for high-priority malfunctions must always be triggered in the containment and outside at all entrances. The alarm is forwarded via suitable systems to the responsible persons and the control center. Gmunder et al. (2022) recommended that at least the following systems should be alarm-supported:

- a. Firefighting system;
- b. HVAC: failure or significant deviation of pressure control or volume flow rate, ventilation control and regulation components, and heating or cooling system failure, loading of filter systems;
- c. Mains power failure;
- d. Interlocked doors;
- e. Communication systems;
- f. Emergency call and monitoring systems;
- g. EDS;
- h. Access control;
- i. Other systems and equipment according to a risk assessment: e.g., BSCs and other laboratory equipment, such as freezers²⁸⁹.

The guidelines provided by the MS for HCBLs also emphasize the importance of implementing an automation system, particularly for controlling the HVAC system, as it requires continuous monitoring and strict adherence to the parameters outlined in the design. An alarm-equipped device should be installed to monitor airflow and promptly alert laboratory personnel and engineering staff of any deviations from the established parameters. Digital electronic controls should be the preferred choice for managing ventilation systems unless their use is impractical due to the project's small scale, operational and maintenance complexities, installation location, or other valid reasons⁹⁴.

Despite the recommendations above, five HCBLs participating in the present survey have not installed an automation system, and another five did not provide this information, totaling almost one-third of the sample, which raises concerns. Among those with an automation system, the majority of the systems work adequately, except for two HCBLs that mentioned a lack of alarms and for those without a backup routine.

The MS guidelines require that the supervisory system be designed to provide real-time readings with data recording and archiving capabilities. The data must always be readily accessible for consultation and report generation⁹⁴. Therefore, it is advisable to create a backup of the data generated by the automation system to ensure the tracking of all relevant information regarding the functioning of the monitored equipment and systems. This information can be valuable during investigations of incidents and accidents and during inspections.

It is also essential to establish an appropriate frequency for the backup, ideally performed at least daily, as reported by most HCBLs that provided such information.

The four HCBLs, where only one or two people can operate the automation system, should consider providing training to additional workers in case of their absence. Furthermore, the HCBLs that relies on a third-party company to operate the system should assess whether this approach results in a loss of autonomy.

Regarding the equipment and systems covered by the automation system, it is no surprise that HVAC was the most commonly mentioned by the respondents,

given its critical importance in an HCBL. On the other hand, the EDS system was expected to be less prevalent than the HCBL, considering that many HCBLs do not have an EDS. Similarly, autoclaves were less commonly mentioned since most of this equipment already has its automation system and alarms. The questionnaire did not list the firefighting system as a choice; thus, the only HCBL that mentioned it included this information under the 'Others' category. It is possible that other HCBLs also have the firefighting system monitored by the automation system, but the respondents did not specify it.

It was expected that a higher number of HCBLs would have their electrical systems monitored by automation systems, given their crucial role in an HCBL.

6.2.25.2.2 Preventive maintenance software

Salerno & Gaudio (2015) emphasized the crucial role of thorough planning in the maintenance program in an HCBL. It can be achieved through two approaches: utilizing specialized maintenance software, commercially available or locally developed with existing resources, or employing a hardcopy or file card system containing all the necessary information. The authors argued that commercial software systems allow the creation of maintenance tasks that align with the overall facility's maintenance plan. Activities can be scheduled based on recommended maintenance intervals, generating order forms for each task with specific due dates. These forms can be signed and reported upon completion of work, ensuring comprehensive documentation and accounting for all maintenance activities. Similar principles can be incorporated into non-electronic systems, although challenges may arise regarding time management and work scheduling, as automatic notifications are not feasible⁷⁶.

The MS guidelines also underscore the significance of establishing a well-defined maintenance plan for critical equipment. The plan must be easily accessible to maintenance staff and equipment users⁹⁴.

Therefore, although utilizing software for recording preventive maintenance plans and tasks performed is not mandatory, it can be considered a beneficial practice in HCBLs and an improvement that could be implemented by more than half of the surveyed HCBLs.

6.2.26 Financial management and sustainability

6.2.26.1 Results

Only six HCBLs responded to the questions regarding maintenance costs, investment expenditures, and purchasing expenses to operate the HCBL. The annual maintenance costs ranged from R\$ 47,000.00^{jj} to R\$ 1,000,000.00^{kk}, with an average of R\$ 363,386.67^{ll}. Similarly, the expenses for purchasing supplies varied from R\$ 40,000.00^{mm} to R\$ 704,809.00ⁿⁿ, with an average of R\$ 303,319.50^{oo}, while the investment expenditures ranged from zero to R\$ 4,500,000.00^{pp} (Table A48).

^{jj} Equivalent to USD 9,251.24, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{kk} Equivalent to USD 196,834.89, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{ll} Equivalent to USD 71,527.18, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{mm} Equivalent to USD 7,873.40, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

None of the HCBLs participating in the present study reported having their operations suspended in the last 12 months due to a lack of funds for acquiring supplies. Only one HCBL experienced a suspension of operations due to insufficient funds for equipment and systems maintenance. The respondent's financial condition ratings exhibited considerable variation. Specifically, 19.4% (6/31) stated that financial resources are always or frequently insufficient, while 38.7% (12/31) indicated that they are always or frequently sufficient (Table A49). There was no statistical evidence that the financial condition of public HCBLs is influenced by their affiliation (Table 26).

Table 26. Correlation analysis between the affiliation of the HCBLs participating in the survey and their financial status rating^{qq}.

Governmental body	N	Financial status rating					Kruskal-Wallis test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
MAPA	5	1.00	2.00	2.00	1.22	4.00	0.1146
MS	6	3.00	3.83	4.00	0.75	5.00	
University	6	1.00	3.00	3.00	1.41	5.00	
Others	3	3.00	3.67	4.00	0.58	4.00	

Classifying the laboratory as public or private/hybrid significantly impacted the financial status rating (Table 27).

Table 27. Correlation analysis between the linkage of the HCBLs participating in the survey (public or private/hybrid) and their financial status³⁵.

Lab linkage	N	Financial status rating					Mann-Whitney test (p-value)
		Min	Mean	Median	Standard Deviation	Max	
Private/Hybrid	4	4.00	4.50	4.50	0.58	5.00	0.033
Public	19	1.00	3.05	3.00	1.27	5.00	

Fifteen HCBLs participating in the current study responded to questions regarding the sources of funds for covering BSL-3 operating costs and investment

ⁿⁿ Equivalent to USD 138,731.01, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{oo} Equivalent to USD 59,703.86, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{pp} Equivalent to USD 885,757.03, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{qq} Scale ranging from 1 (very bad) to 5 (excellent).

expenses (15/32, 46.9%). Concerning operating costs, the most frequently mentioned answers were research grants and BFG funding (9/15, 60.0%), followed by resources obtained internally by the institute (7/15, 46.7%). The results for investment expenses were similar, with slightly fewer mentions of the BFG (Table A49). It is worth noting that respondents were allowed to provide multiple answers to these questions.

Four HCBLs participating in the present study reported suspending activities at some point due to a lack of demand (4/32, 17.4%). Among them, three (3/4, 75.0%) rated the frequency as low, while the remaining rated it as moderate. Additionally, more than half of the HCBLs reported a lack of partnerships with other institutes to utilize the HCBL (17/32, 53.1%). Most HCBLs anticipate an increase in their demands for the coming years (21/31, 67.7%), and seven HCBLs stated that they currently have unmet demands due to the absence of a higher-level biocontainment structure (7/32, 21.9%) (Table A49).

Some unmet demands listed by the respondents were:

- a. Development of vaccines against COVID-19 and other diseases;
- b. Efficacy testing in COVID-19 vaccines;
- c. Diagnosis of equine and swine influenza;
- d. Diagnosis of glanders;
- e. Production of antigens and antisera for HPAI and Newcastle disease;
- f. In vivo research with mice (pre-clinical studies);
- g. In vivo research with non-human primates;
- h. In vivo research with cattle on tuberculosis pathophysiology;
- i. Other tuberculosis-related studies;
- j. Viral neutralization tests in vaccinated animals;
- k. Testing viricidal products;
- l. Research involving phages.

6.2.26.2 Discussion

Chapter 2.1.5 of the present study emphasizes the high costs for construction and, mainly, for the maintenance of HCBLs and the challenges these expenses pose to their long-term sustainability. Astuto-Gribble et al. (2009) surveyed bioscience research, biosafety, and biosecurity practices in Asia, Eastern Europe, Latin America, and the Middle East. Their findings indicated that respondents identified a shortage of funding and other resources as the foremost barrier preventing laboratories from implementing suitable biosafety and biosecurity measures across all regions³⁰⁷.

Likewise, a WHO report on the status of EDPLN BSL-3 laboratories in certain countries within the African region concluded that consistent funding remains an ongoing concern. Numerous laboratories rely on external funding, either partially or entirely. Ensuring enduring financial support is an ongoing endeavor²¹¹.

Even in high-income countries like the US, some HCBLs face financial issues. Rao & Bordelon (2019) stated that the costs of maintaining and upgrading physical security infrastructure to remain compliant with FSAP were a critical factor in the decision of many academic institutions and public health laboratories to shut down their select agent programs⁸⁶. Inadequate funding for maintenance was identified as one of the underlying factors behind the release of the FMD virus from an HCBL in Pirbright, UK¹³⁹.

Abad (2018) used the acronym SAFER to illustrate that HCBLs in all countries, but particularly in low- and medium-income countries, must be:

- a. Sustainable: laboratory operation can be maintained independently over a long-term period;
- b. Affordable: the facility should not depend on external aid for core functionality;
- c. Functional: the laboratory staff is safer than currently through lifelong education and active training in the same facility;
- d. Effective: the laboratory is devoted to the most suitable research application or diagnostic activities for such specific environment or region;
- e. Realistic: the laboratory facility is an answer to questions or services that the country or the region³⁴¹.

The omission of sustainability planning, specifically the estimation of maintenance expenses while designing a new HCBL, represents one of the foremost factors contributing to the closure of HCBLs in Latin America³²⁰.

It is worth reminding the WOA's definition for laboratory sustainability: 'a system of laboratories that together continuously deliver specialised services in a manner which is efficient, timely, accurate, consistent, secure, and safe; is in line with international standards and best practices; is provided at an acceptable cost; responds to clients' needs across sectors (public and/or private), and benefits One Health goals and the overall One Health system'⁶⁰.

Heckert et al. (2011) highlighted that maintenance budgets are often overlooked during the initial facility planning, design, and budgeting process, with few resources allocated for long-term maintenance, creating a false sense of confidence in the containment provided by the building systems. As maintenance costs are considered a burden, service contracts are typically awarded to the lowest bidder; this may result in poor facility and equipment performance. Additionally, the lack of equipment, reagents, and support services presents a significant challenge to biosafety and biosecurity capacity in low-resource countries⁵⁶.

According to a USG Accountability Office (GAO) report, HCBL sustainability is especially challenging in academic laboratories, which rely heavily on research grant funding to sustain their infrastructure maintenance and research programs. Consequently, these two endeavors might vie for the limited funds available⁴³.

Some possible actions to promote sustainability include:

- a. Adopting a relative-risk approach that prioritizes biosafety and biosecurity conditions to enhance safety, security, and sustainability within specific settings⁵³. Recognizing that these practices are not isolated techniques or universal solutions that can be selected from a standardized catalog and applied uniformly is essential. Instead, they should be examined within the unique historical contexts and contingencies of each situation⁴¹⁰. For example, when considering the procedures and infrastructure necessary for safe work with *Mycobacterium tuberculosis*, it becomes evident that these requirements may vary based on specific protocols. In this context, the MEC must establish criteria for laboratories within the EBSEH that handle this microorganism. Importantly, this does not necessarily mandate the use of a BSL-3 facility. Depending on the results of a comprehensive risk assessment, a BSL-2 laboratory with specific enhancements may suffice⁹⁵. Conversely, the MAPA has defined specific criteria for working with the FMD virus, necessitating the use of a BSL-3 enhanced facility²³¹;
- b. Establishing, by the federal agencies, a distinct funding category to ensure continuous and sustained support for facilities engaged in research involving high-risk pathogens¹⁹²;
- c. Developing the national capability to build and maintain HCBLs. By using locally available materials, methods, and equipment wherever possible, the resulting facility

will be less expensive to maintain and will be more likely to be kept in good running order because skilled service personnel and materials for repairs and changes will be readily available⁷⁶;

d. Facilitating coordinated training programs among HCBLs associated with various organizations⁵⁸. The SB3 could play a relevant role in this sense;

e. Understanding very well all operating costs and resources⁷⁶;

f. Promoting evidence-based laboratory biorisk management can be facilitated through government-wide coordination and mechanisms for sharing sustainable biorisk management practices³⁹⁶.

Regarding the last topic, examples of research areas that can contribute to improving the biorisk management sustainability include:

a. Development and demonstration of validated pathogen inactivation, decontamination, destruction, and sterilization procedures;

b. Development of evidence-based protocols for laboratory and field techniques, including sample collection, transport, or waste management, with scientific instruments and biotechnology tools;

c. Identification of risks and mitigation options for pathogen exposure associated with laboratory and field-based instruments, including modern and automated instruments; validation of pathogen surrogates, that can be worked with safely and securely in lower containment settings; evidence to inform pathogen-specific biorisk assessment and management options (e.g., environmental survival, exposure routes, pathogenicity); identification of metabolic, immunological, or other early indicators of exposure;

d. Development of cost-benefit analysis for engineering controls;

e. Study of PPE specifications for primary and secondary containment³⁹⁶.

Regarding the understanding of operating costs and resources, only a limited number of HCBLs participating in the present study furnished details regarding their financial management. Several potential reasons for this are as follows:

a. The respondent lacked access to this particular category of information;

b. The information was deemed confidential;

c. The uppermost echelons of the organization exclusively oversee such information, thus remaining inaccessible at the laboratory level;

d. The structure of financial management information did not facilitate addressing these specific inquiries.

The UPMC Center for Health Security faced the same difficulty gathering budgetary information while interviewing 21 international experts on biosafety and biosecurity. The authors argued that the lack of funding information could be due to biosafety being incorporated into larger budgets and not 'called out' as a separate item. Additionally, the biosafety funding may be spread across several governmental agencies rather than a single budget item¹⁶⁸.

Given that most of the respondents in the present study occupy decision-making positions within HCBLs, there was a reasonable expectation that they would be capable of providing such information. Therefore, it was frustrating getting this information from only six HCBLs. The technical staff should exhibit increased interest and awareness regarding financial management information, given their potential to provide pertinent insights. Subsequent investigations are necessary to assess the makeup of the staff accountable for the financial management of HCBLs and to gain insight into the mechanisms employed to make strategic decisions regarding the distribution of financial resources across various types of institutes.

Information regarding maintenance costs for HCBLs remains limited within the existing literature. One of the few available sources of information is a study by Bruzek (2013), referencing insights from Dr. John Jack Murphy, the Director of Boston University Medical Center's National Emerging Infectious Diseases Laboratories (NEIDL). According to this reference, maintenance costs for a BSL-3 laboratory can reach up to USD 2,000/m²/year in the US⁵⁹.

By examining the average size of HCBLs contributing data to the present study, we calculated an average maintenance cost of R\$ 1,206.46/m²/year^{rr} in Brazil. Notably, this calculation was derived from data collected from merely six HCBLs, and the information regarding maintenance costs was self-reported. As a result, it is imperative to conduct further research to obtain a more precise estimation.

The average maintenance costs slightly exceeded the average expenses associated with supply purchasing. It further underscores the earlier assertion about the sustainability challenges confronting HCBLs. Among these challenges, one pertains to investment costs, encompassing acquiring new equipment, systems, and necessary infrastructure updates.

The findings exhibited substantial variation across the six HCBLs that contributed this information, with a standard deviation of R\$ 1,789,253.10/year^{ss}. The absence of financial resources to maintain infrastructure emerges as a fundamental factor, potentially contributing to certain HCBLs ceasing their operation as BSL-3 facilities, as informed by one HCBL participating in the present study that experienced a suspension of operations due to insufficient funds for equipment and systems maintenance.

Considering the data collected regarding the outputs of Brazilian HCBLs (refer to Chapter 6.2.4.1 of this study), estimating the cost of each assay performed in these facilities becomes feasible. This estimation took into account the average expenses for supplies procurement (R\$ 303,319.50/year^{tt}), the average maintenance cost (R\$ 1,206.46/m²/year^{uu}), the typical facility size (277m², as outlined in Chapter 6.2.5.1), and the mean number of assays performed annually (8,858). As a result, the estimated average cost per assay conducted in an HCBL is R\$ 71.97^{vv}. It is crucial to approach this estimate cautiously due to the diversity of methodologies employed in the laboratories, each with its respective costs and complexity. It is also crucial to note that the expenses associated with human resources were not included in this calculation.

A significant variation in the financial status among the HCBLs participants was observed, contrasting with the findings of Richards et al. (2014). Their study, which involved interviews with 359 BSL-3 users in the US, reported that most facilities received sufficient funding¹³.

The primary sources of financial resources in the present study are research grants and the BFG, emphasizing the imperative to devise innovative solutions for

^{rr} Equivalent to USD 237.47/m²/year, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{ss} Equivalent to USD 352,187.44/year, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{tt} Equivalent to USD 59,703.86/year, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{uu} Equivalent to USD 237.47/m²/year, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{vv} Equivalent to USD 14.17, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

enhancing sustainability. It is essential to consider that these funds can become highly constrained during specific periods, presenting challenges in managing maintenance costs effectively. A favorable observation that can be deduced from the data regarding the sources of financial resources is that the maintenance costs of HCBLs do not appear to rely on international donors, as observed in Africa²¹¹.

The WOAHA published a document with conclusions and recommendations to enhance laboratory sustainability. Some of them are apply to HCBLs, such as:

- a. Laboratory facilities (including their infrastructure, engineering, and flexibility of design) must be 'fit for purpose' and thus adapted to the local context and risks;
- b. Sustainability of laboratory biosafety and biosecurity, quality management, and business continuity are inextricably linked;
- c. Sustainability will be improved by networking and sharing of information and best practices at all levels (local, national, regional, and international);
- d. The adoption of risk-based and evidence-based approaches will make a positive contribution to sustainable laboratory biosafety and biosecurity, especially in low-income countries;
- e. Creative and open-minded thinking and innovation are crucial to improving laboratory sustainability;
- f. Business models for sustainable laboratories and capacity building benefit from utilizing private-public-partnership models, which engage private sector users and suppliers³⁶⁹.

Concerning the last point, various authors emphasized that promoting entrepreneurial grants and seeking alternative funding sources is highly recommended to develop a diversified investment model^{37, 57, 290}.

In line with the UK Biological Security Strategy (2023), apart from fostering private-public partnerships, consolidating various cross-government initiatives addressing biological risks into a unified strategic framework can lead to a more efficient and effective response⁵⁴. However, in numerous countries, there is limited collaboration between government departments responsible for delivering animal health services and those overseeing human health matters³⁷.

Furthermore, collaborating with other nations within the same geographic region can also significantly contribute to advancing the sustainability of HCBLs³⁹⁹. In this regard, an effort was made in 1998 to establish a functional network of laboratories involving Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay. The primary objective of this initiative was to obtain precise and high-quality laboratory results pertaining to new, emerging, and re-emerging infections prevalent in the region. Initially, the focus was on infectious diseases such as influenza, antimicrobial resistance, particularly in the context of tuberculosis, acute diarrhea, and hantavirus⁴¹¹. Regrettably, this endeavor failed to materialize. Unfortunately, it is not uncommon to witness the proposal of promising initiatives that remain unrealized due to various factors, including limited financial resources, political shifts, and a lack of effective leadership, among others.

An illustrative success case in the region was the establishment of the South American Network of Diagnostic Laboratories for Avian Influenza and Newcastle Disease (RESUDIA) in 2014⁴¹². It is a prominent network led by the LFDA-SP, a WOAHA's reference laboratory in avian influenza and Newcastle disease.

In the current study, our primary focus was to examine the prevalence of partnerships that share BSL-3 facilities among different institutions, thereby enhancing their long-term viability. Unfortunately, our findings indicate that this practice is not widely adopted among Brazilian HCBLs.

Most HCBLs in the present study anticipate increased demand in the coming years but without requiring higher-level biosafety facilities. Further studies are needed to address the future demands for HCBLs in Brazil and assess their alignment with the current demand. Nevertheless, it is essential to consider optimization strategies for HCBL utilization, such as conducting research in diagnostic laboratories during periods of low demand and utilizing research laboratories for diagnostic testing during peak demand, as recommended in the 'Biosecurity Challenges of the Global Expansion of High-biocontainment Biological Laboratories Workshop'¹⁷.

6.2.27 Biorisk management system and other regulatory issues

6.2.27.1 Results

Of the HCBLs participating in the current survey, the majority have an IBC (23/32, 71.9%). Similarly, an equal number of HCBLs reported having an established documentation structure for the biorisk management, while a slightly lower percentage of HCBLs have developed their own Biosafety Manual (21/32, 65.5%). Among HCBLs with a documentation structure for managing biorisk, responses regarding the level of awareness were divided into 'unknown' (13/31, 41.9%) and 'highly satisfactory' (17/31, 54.9%, falling between high and very high). Furthermore, most HCBLs reported having an emergency contingency plan (21/32, 65.6%) (Table A50).

Among the HCBLs participating in the current survey, the majority have SOPs for recording accidents (28/32, 87.5%). The average number of accidents reported by these HCBLs ranged from zero to 15 per year. The overall accident rate was 1.5 per year for the entire laboratory and 0.7 per year for the BSL-3 facility. None of the HCBLs reported any LAIs due to these accidents (Table A51).

Among the HCBLs that participated in the current survey, most conduct periodic internal audits focusing on biosafety aspects (19/31, 61.3%), typically annually (13/19, 68.4%). Half of the HCBLs reported undergoing an external audit (12/24, 50.0%) (Table A52). Only one (1/12, 8.3%) did not carry out internal audits. The following organizations were identified as responsible for conducting the external audits: MS (n = 4), MAPA (n = 4), FAO (n = 2), CTNBio (n = 1), Butantan Institute (n = 1), Vital Brazil Institute (n = 1), and CDC (n = 1). One participant mentioned that a third-party company carried out the audit.

There was no statistically significant correlation between receiving external audits and the affiliation of the public HCBL (Table 28).

Table 28. Correlation analysis between the affiliation of the HCBLs participating in the survey and the reception of external audits.

Governmental body	Has the laboratory already received an external audit addressing biosafety aspects?		Fisher's Exact Test
	No	Yes	
Agriculture State Secretariat	1	0	0.6752
Health State Secretariat	2	2	
MAPA	3	2	
MCTI	1	0	
MS	1	4	
University	2	2	

None of the HCBLs reported abstaining from employing methods aligned with legislative or referenced guidelines/standards. Nevertheless, fewer than half of them (15/31, 48.4%) consistently apply such methods across their entire scope. Responses regarding method accreditation demonstrated substantial variation. Only five HCBLs (5/29, 17.2%) had accreditation for all their methods.

There was a correlation between the utilization of accredited methods and methods compliant with regulations and standards, depending on the laboratory's purpose. Laboratories engaged solely in research or research and teaching often do not employ these methods, as shown in Table 29 and Table 30.

Table 29 – Correlation analysis between the purpose of the HCBLs participating in the survey and the application of methods supported by legislation or reference guidelines/standards.

Are the methods applied in the lab supported by legislation or reference guidelines/standards?	HCBLs purpose		Fisher's Exact Test
	Research or Teaching	Others	
I don't know, or no answer	6	4	0.0401
Yes	4	17	

Table 30 – Correlation analysis between the purpose of the HCBLs participating in the survey and the application of accredited methods.

Are the methods applied in the lab accredited by an accreditation body?	HCBL purpose		Fisher's Exact Test
	Research or Teaching	Others	
I don't know, or No answer	6	5	0.009
No	2	5	
Yes	0	11	

Less than half of the participants (13/32, 40.6%) voiced concerns about deficiencies in the legal framework governing HCBLs operations, even though more than one-third of them did not provide specific information on this issue (11/32, 34.4%) (Table A53).

The gaps described by the participants were:

- Absence of well-defined constructive requirements;
- Lack of certification bodies and certification criteria;
- The regulatory scope is limited to GMOs and FMD virus handling;
- Lack of periodic inspections;
- Outdated regulatory framework;
- Absence of a PNBB;
- Absence of defined capacity-building processes and minimum competency criteria for BSL-3 users within the regulatory framework;
- Necessity to regulate high-containment activities by establishing defined processes, conducting associated risk analyses, and specifying required mitigation and control measures.

Most HCBLs have implemented a waste management plan (26/32, 81.2%) and have established contracts with specialized companies for solid waste disposal (29/32, 90.6%) (Table A53).

Eleven HCBLs (11/32, 34.4%) acknowledged that they do not comply with their legal obligations to the fire department, and 12 do not possess a fire detection system within the BSL-3 lab (12/32, 37.5%). Over half of the HCBLs have a fire brigade in place (17/32, 53.1%). Among these, six (6/17, 35.3%) mentioned that all or most BSL-3 users are involved in the fire brigade (Table A53).

6.2.27.2 Discussion

6.2.27.2.1 Institutional Biosafety Committee and biorisk management system documentation

The percentage of HCBLs included in the current study with a biosafety manual closely aligns with the findings reported by Simonetti (2014). In a survey encompassing nine Brazilian HCBLs, this author identified six (66.7%) having a biosafety manual⁷⁴. Gaudioso & Zemlo (2007) obtained similar results in a survey with 300 Asia-based life scientists. Approximately 25% of those working in BSL-3 and BSL-4 facilities indicated that they did not have a biosafety manual, and 25-33% lacked procedures for training their staff on biosafety protocols³⁵⁰.

The MS legal framework mandates that the Brazilian Public Health Laboratories designate an IBC directly linked to high-level management. Furthermore, the same regulation requires these laboratories to possess a Biosafety Manual outlining their policies managing biorisk¹⁰.

Kimman et al. (2008) noted that individuals involved in laboratory accidents often hold unfavorable opinions about safety programs, take excessive risks, work at an accelerated pace, and exhibit lower awareness of the infectious risks associated with the agents they handle⁹⁸. It underscores the importance of an effective biorisk management system in preventing LAI.

Despite the regulatory requirement, three out of the seven HCBLs without an IBC are Public Health Laboratories, and five out of the eight HCBLs without their own Biosafety Manual also fall into the category of Public Health Laboratories. Thus, it becomes evident that having a regulatory framework in place is essential but insufficient. Additional enforcement measures, such as inspections and sanctions, are necessary.

According to Astuto-Gribble et al. (2009), organizations employ various methods to manage their biosafety and biosecurity programs. The most commonly utilized methods include a biosafety operation manual, an IBC, biosafety training procedures, and a laboratory management plan³⁰⁷.

As articulated in Chapter 6.2.23, the role of an IBC should extend beyond the evaluation of activities involving GMOs. Schniederberend et al. (2019), drawing from their experience in establishing an IBC at Southern Connecticut State University (SCSU), advocate that the primary objective of an IBC is to assist the institution in ensuring a safe environment for its employees, students, and the general public. An IBC, along with its associated policies, necessitates the development of a written risk assessment and risk management plan for obtaining authorization to conduct research involving biohazards or regulated biological materials. This process allows subject matter experts within the IBC to confirm the adequacy and suitability of proposed protective measures. The IBC also fosters an ongoing relationship with the PI conducting the research through requirements such as reporting incidents or issues and periodic renewal or reapproval of registrations⁴¹³.

The collaborative efforts of the IBC are essential, and it should cooperate with other committees, such as the Environmental Health and Safety Office, to conduct site visits before and after the project initiation. These visits assess the facility and work practices, ensuring PPE and containment controls are appropriately utilized. The IBC's review process also verifies the documentation of appropriate training and competency for new laboratory personnel. Additionally, site visits encompass the inspection of emergency equipment, including eye washes, emergency showers, and fire extinguishers, as well as the confirmation of the proper functioning of chemical fume hoods and BSCs. Physical safety aspects are also assessed, covering safe egress, basic electrical safety, eliminating trip hazards, and properly storing gas cylinders. Effective communication among the IBC and other research compliance committees, such as the IACUC, is paramount. In cases where researchers seek approval from multiple compliance committees for their studies, the IBC can facilitate the exchange of concerns related to projects involving biohazards or other potentially infectious materials. One strategy to promote open communication among institutional committees is to encourage cross-membership, where a member of the IBC may also serve on the IACUC and so on. Another approach is establishing a research oversight committee comprising chairs or members from the IBC and other relevant committees⁴¹³.

Zaki (2010) incorporated the establishment of occupational health programs as one of the responsibilities of an IBC³⁶⁰.

While the recommendation regarding the collaboration between IBCs and other compliance committees holds value, the practical mechanisms for implementation may vary from one institution to another. These variations can be attributed to factors such as institute size, the nature of activities conducted, workload, organizational chart, and more⁴¹⁴.

The findings from the current survey concerning the structural aspects of biorisk management documents and the awareness level among HCBL users regarding this documentation are satisfactory. However, as the results stem from a self-administered survey, additional research is warranted to comprehensively evaluate the knowledge and awareness levels among Brazilian HCBLs staff regarding biosafety issues. These subsequent studies should assess whether the biorisk management framework is adequate and aligns with international standards. It is worth noting that in the present study, we only inquired about the presence of biorisk management documentation without delving into its completeness.

One aspect that could be explored in further studies is the implementation of Key Performance Indicators (KPIs) by HCBLs to evaluate the effectiveness of their biorisk management systems continually. Per Salerno and Gaudio (2015)⁷⁶, employing activity indicators can assist in recognizing both top-down and bottom-up achievements and areas of improvement. The authors highlighted that, although biorisk management performance measurements are rarely executed, success in establishing indicators and metrics and measuring positive performance will breed further success and upbeat performance. This positive performance will reinforce risk reduction strategies and support a safe and secure workplace. They outlined the following steps associated with introducing biorisk management indicators:

- a. Identify the critical issues of concern;
- b. Define outcome indicators and metrics;
- c. Define activity indicators and metrics;
- d. Collect data and report indicator results;
- e. Act on findings from performance indicators;

f. Evaluate and refine performance indicators⁷⁶.

The WHO Laboratory Biosafety Manual (2020) includes an associated monograph that describes the fundamental elements of a biosafety program⁴¹⁴. Among these elements, emergency and incident response planning stands out as it equips organizations to take appropriate actions in the event of a spill, exposure, power failure, fire, or other emergencies. The regulations from the MS also mandate that Public Health Laboratories possess plans and procedures for handling emergencies and contingencies¹⁰. The ISO 35001 also highlights the relevance of establishing an emergency response and contingency planning in biological laboratories¹¹.

Therefore, given the significance of this topic, the results obtained in the present survey can be deemed unsatisfactory, as more than one-third of the HCBLs indicated that they either lacked such a plan or did not provide this information.

However, these results are not as unsatisfactory as those obtained by Appelt et al. (2020). Their assessment of biorisk management systems in EU HCBLs found that only seven out of the 25 surveyed BSL-3 laboratories (28.0%) fully complied with emergency provisions, plans, and responses³⁵⁶.

6.2.27.2.2 Accidents recording

Recording accidents is also considered a foundational element according to the WHO Manual⁴¹⁴ and is required by the Brazilian regulation. The MS Ordinance mandates that the laboratory maintain comprehensive records of its occurrences to ensure traceability and facilitate the formulation of preventive and corrective measures during accidents or incidents. These records should be archived under the requirements stipulated by current legislation. Additionally, the regulation related to occupational health for employees working in health services determines that whenever an accident involving biological risks occurs, whether or not it results in the worker's absence, a work accident report must be generated²⁴⁰.

The proportion of HCBLs participating in the present study with an SOP detailing accident documentation procedure is significantly higher than that of HCBLs reporting having an accident record form (65%) in a prior study conducted by Simonetti (2014)⁷⁴. Nevertheless, it is worth noting that the average annual accident rate observed in the current study was surprisingly low, except for two laboratories that documented eight and 15 accidents, respectively. When specifically examining the BSL-3 facility, the accident rate is even lower, with no HCBL reporting morbidity resulting from LAIs.

Based on the literature review presented in Chapter 2.4 of this study, particularly the findings reported by Vogler et al. (2014), it appears evident that laboratory accidents in Brazil are significantly underreported¹⁴¹.

This same issue was identified during a workshop organized by the US NRC on 'Anticipating Biosecurity Challenges of the Global Expansion of High-Containment Biological Laboratories' in 2011. According to this report, while some accidents in HCBLs are documented in master's and doctoral theses and Brazilian journals, there is no regular system for reporting incidents that would enable the production of a comprehensive report. As a result, the available data needs to be more accurate in the number of cases. The same report emphasized that no designated authority is responsible for investigating these accidents, except when GMOs are involved¹⁷.

Underreporting incidents is a global concern. According to Vogler et al. (2014), there is a general lack of information regarding the types and frequency of incidents in HCBLs, especially concerning lower-consequence or minor incidents¹⁴¹.

In a study conducted by Siengsan-Lamont & Blacksell (2018) examining the occurrence of LAIs in the Asia-Pacific region, the reporting of LAIs was more prevalent in high-income countries, where laboratories were more likely to adhere to international biosafety standards¹¹⁹.

Wurtz et al. (2016), studying LAIs in BSL-3 and BSL-4 laboratories around the world, mainly located in the EU and the US, concluded that there is no way to quantify the actual number of people involved in this kind of accident or to know the precise risks for workers because there is no systematic reporting system. The authors found that only four of the 23 surveyed laboratories reported 15 LAIs caused by four different pathogenic organisms: *Mycobacterium tuberculosis* (ten cases), *Coxiella burnetii* (two cases), *Brucella melitensis* (two cases), and foamy virus (one case). Most of the LAIs (73%) occurred in a BSL-3 laboratory. Laboratory technicians were most commonly infected (87% of the cases), while in only 7% and 6 % of the cases, respectively, the infected person was an animal caretaker or a researcher. No deaths were reported. The authors concluded that LAIs have been infrequent in recent years, and human errors represent a high percentage of the cases. According to them, LAIs have been infrequent with highly pathogenic microorganisms and rare in recent years due to improved working conditions, mainly the biosecurity measures implemented⁹³.

Access to reliable and detailed information on accident occurrences in Brazilian HCBLs is necessary to determine whether the conclusions reached by Wurtz et al. (2016)⁹³ regarding the infrequent incidence of LAIs apply to the Brazilian context.

Manheim (2020) conducted a survey involving 60 BOs, primarily located in the US, Canada, or New Zealand. The objective of this survey was to gain a deeper insight into the reporting of laboratory accidents. The survey results revealed that internal oversight was nearly universal, with 58 out of 60 respondents (97%) reporting incidents to one or more entities, such as a BO, IBC, or a similar dedicated committee, as well as upper management. However, external reporting was generally less common. Among the 41 respondents responsible for BSL-3 laboratories, external reporting was more prevalent, with 14 respondents (34%) sending reports to national agencies and 12 respondents (29%) reporting to state or local agencies. The majority of the BOs (82%) agreed that workers understood the importance of reporting for their safety. Nonetheless, external reporting remained relatively uncommon overall¹⁴².

In another study aiming to assess social and organizational factors affecting biosafety compliance in seven Swiss academic and state-run ABSL-2 and ABSL-3 facilities, nearly half of the interviewees stated that they suspected some co-workers of not being willing to share information on errors and violations. Another concern was that only more significant, and therefore 'visible', violations might be reported, while minor errors and violations without visible consequences might remain unreported¹³⁴.

Additional research is essential to identify the root causes of the accidents underreporting in Brazil despite regulatory requirements. Some authors, for example, suggest that concerns about potential damage to the institute's reputation could contribute to the underreporting of accidents^{145, 168}.

Urgent measures are needed to establish mechanisms that motivate laboratory workers, BOs, and occupational health staff to report accidents and incidents and share this information with other HCBLs, including corrective and

preventive actions. It is closely tied to the establishment of a biosafety culture, along with a culture of transparency.

Rocha (2011) argued that publicly known incidents and accidents can instigate institutional and individual learning attitudes. Emphasizing events linked to non-compliance with biosafety and biosecurity guidelines can foster the development of health and ecological awareness, facilitating the transition from a culture of blame and punishment for errors to one that encourages shared responsibilities and a culture of safety⁶⁵.

In this sense, the initiative of the LFDA network to standardize the accident notification form and create an indicator for measuring accident occurrences, allowing the sharing of information among the various laboratories in this network, is commendable.

6.2.27.2.3 Audits

Many authors emphasized the benefits of periodic audits and inspections in HCLBs^{3, 98, 114, 415}.

Chapter 2.8 of this study states that numerous countries adopt audits to oversee HCBLs. According to the WHO, internal and external audits and inspections take part in the review and improvement step for a biosafety program, actively identifying hazards, deficiencies, or areas needing improvement. They contribute to the prevention of incidents with and exposures to biological agents⁴¹⁴.

The audits should be conducted periodically, as the absence of routine inspections hampers the ability to identify whether individual safety lapses, which may seem like isolated incidents, actually indicate systemic weaknesses¹⁶⁴. It is also hoped that periodic inspections by external experts will contribute to the establishment of a biosafety culture¹²⁷.

The MS regulation requires that Public Health Laboratories conduct internal audits of their operations periodically, preferably annually, following a predetermined schedule and established procedures. The BO is responsible for planning and conducting these audits per schedule. These audits should be performed by trained and qualified professionals whenever resources permit¹⁰.

Coelho (2017) advocated that it is imperative to establish external and independent audits to examine laboratories housing high-risk biological agents in Brazil. The author highlighted that internal audits within HCBLs should not substitute external and independent audits, as the latter is vital to ensure the credibility of the inspection system¹⁸.

The most relevant findings of the audits and incident reports should be communicated to the high-level managers of the institutes. It will enable them to take appropriate measures to enhance the management of hazardous biological agents in HCBLs and ensure the safety and security of personnel working in these facilities¹⁶⁴.

External audits are even more crucial in universities, private companies, and research centers not affiliated with the MS, MCTI, MAPA, or MD, as they may lack oversight from any public authority. As noted by Subramaniam (2014), while scientists in many laboratories are acutely aware of the challenges associated with advancing scientific innovation, utilizing sophisticated technologies, and staying current with rapid developments in science, the urgency surrounding biosafety and biosecurity culture and awareness has not been appropriately recognized¹⁷¹.

Gaudioso & Astuto-Gribble (2009) emphasized that, while the bioscience community hasn't historically encountered government oversight restricting the

location or personnel conducting their work, HCBLs may become subject to inspections¹⁵⁹.

Despite the significance, advantages, and regulatory requirements associated with this topic, many HCBLs reported not conducting routine internal or external audits focusing on biosafety aspects. The MS showed the highest rate of receiving external audits, but the difference from other governmental bodies was not statistically significant.

These findings align with those reported in African EDPLN laboratories, where 76.9% (10/13) of the HCBLs indicated regular inspections or audits, with 53.8% and 46.1% undergoing internal and external audits, respectively²¹¹.

While additional research is necessary to uncover the root causes behind the low participation of Brazilian HCBLs in audit programs, some potential reasons may include:

- a. Absence of government oversight and enforcement of MS and other regulations;
- b. Absence of a BO or an IBC, typically responsible for internal audits;
- c. Insufficient personnel with training in conducting audits and inspections;
- d. Inadequate availability of checklists and other tools to streamline audit processes;
- e. Absence of a quality system and a biosafety management culture.

It is essential to emphasize that the audits must encompass biosecurity issues¹⁷⁸. In this regard, the initiative by the MAPA to invite representatives from the ABIN to participate in COMBIOLAB activities is commendable.

6.2.27.2.4 Other regulatory issues

Integrating biosafety and biosecurity security requirements into certification processes of well-established quality management systems such as Good Laboratory Practice and ISO standards, like ISO 17025, could assist organizations in addressing these challenges¹⁷¹.

Hou et al. (2019) emphasized that the core principles of implementing a quality management system encompass transparency, traceability, adaptability, and responsiveness since the documentation system is a dynamic entity that continually evolves through the input and experiences of the staff. The authors underscored the significance of HCBLs seeking accreditation under a quality management system, even without a specific standard for accrediting these laboratories on biosafety and biosecurity¹⁷².

Perkins et al. (2019) agreed that laboratory quality management may be a way to start a discussion about safety and biosecurity¹⁶⁶.

There are several benefits to initiating the implementation of a biorisk management system in an HCBL that has previously been accredited under ISO 17025, such as taking advantage of the following:

- a. Well-defined policies and procedures for documenting non-conformities;
- b. Presence of an information management system for recording inventories, accidents, incidents, equipment management plans, preventive and corrective actions, and more;
- c. An institutional culture that embraces the PDCA process, emphasizing continuous improvement;
- d. Established procedure for document generation and control;
- e. Policies and procedures for the procurement of services and supplies;
- f. Familiarity among staff with internal and external audit processes;
- g. Policies and procedures regarding facility accommodations and environmental conditions;

- h. Policies and procedures for equipment management and control;
- i. Established procedures for conducting management system reviews.

Chapters 6.3 and 6.4 of this study addressed the gaps within the Brazilian regulatory framework. Given several glaring gaps, it was surprising that a quarter of the respondents answered that they do not perceive any deficiencies in the current regulations.

The prevalence of waste management plans in most HCBLs may be attributed to the comprehensive regulations on this matter, associated sanctions, and increasing awareness in Brazil concerning environmental preservation. As emphasized by Cascardo et al. (2020), ensuring safe and reliable biological waste management in BSL-3 facilities is a legal requirement and a social responsibility³⁸⁶.

Conversely, the findings concerning fire safety policies were disconcerting. The absence of inspections and a lack of awareness on this matter could be the underlying factors contributing to the negligence observed in numerous HCBLs.

6.2.28 Main maintenance challenges faced

6.2.28.1 Results

The most common answers to the question 'What are the primary maintenance challenges faced in the BSL-3 laboratory?' are listed in Table A54. Fourteen HCBLs did not respond to this question (14/32, 43.8%). Among them, two cited that the laboratory is too new.

6.2.28.2 Discussion

It is possible to group the main challenges into the following topics:

- a. Financial resources;
- b. HVAC system;
- c. Maintenance staff;
- d. Service procurement.

Chapter 2.1.5 of this study emphasizes the importance of financial management for the sustainability of HCBLs, given the high costs associated with their operation and maintenance. As mentioned in Chapter 6.1.2, a lack of financial resources is one of the primary reasons some HCBLs in Brazil have been adapted to operate as BSL-2 laboratories. The data collected in this survey indicates that other laboratories currently operating as BSL-3 facilities may, unfortunately, face a similar fate.

The HVAC system is one of the most critical components in an HCBL, as it is responsible for air filtration and maintaining air pressure and temperature within specified parameters. Adjusting the unidirectional airflow and pressure cascade can be challenging, depending on the facility's size and layout. Therefore, it is unsurprising that this system was identified in this study as one of the most challenging aspects of HCBL maintenance. This finding is closely associated with the frequency of air pressure and temperature deviations, as discussed in Chapters 6.2.8.2.1 and 6.2.8.2.7 of this study.

The challenges related to maintenance personnel are linked to the limited presence of engineers with biosafety expertise in the workforce and the absence of a permanent maintenance team within a low percentage of HCBL. This issue is discussed in detail in Chapter 6.2.19.2.3 of this study. One contributing factor to the shortage of qualified personnel is the inadequate availability of training courses and postgraduate programs addressing this subject, as highlighted in Chapter 2.17 of this study.

The constraints in service procurement, notably the sluggishness within the purchasing process, are more prevalent in HCBLs connected to public services, mainly due to the bureaucratic hurdles associated with procurement regulations. Managers of public laboratories may already be aware of the risks associated with HCBL operations. As a result, several management initiatives should be implemented to ensure the continual availability of critical equipment, its parts, and essential services. These initiatives encompass:

- a. Comprehensive acquisition planning;
- b. Prioritization of acquisitions associated with HCBLs;
- c. Identification of critical equipment components for stock maintenance;
- d. Effective inventory management for critical equipment parts;
- e. Utilization of software or other automated tools for preventive maintenance service tracking;
- f. Streamlining communication among administrative, maintenance, and technical staff, including BOs, Pls, and other stakeholders;
- g. Ensuring high-level decision-makers are aware of the need for timely financial resource allocation;
- h. Assessing the suitability of establishing a periodic 'shutdown' maintenance strategy;
- i. Establishing a network for sharing information about service providers.

Furthermore, HCBL managers should consider shifting from a reactive maintenance approach to a reliability-centered maintenance strategy. This approach amalgamates principles from reactive, preventive, and predictive maintenance methodologies to assess the entire system comprehensively. It discerns varying degrees of risk, acknowledging that the importance of equipment may vary and that not all equipment failures threaten safety and security. Its key advantage lies in recognizing that while constant monitoring is critical for some equipment, others require less scrutiny. This maintenance strategy is the most robust option for integrating into a biorisk management system. It considers the specific equipment usage within the overarching system and adapts maintenance strategies accordingly based on this new wealth of data⁷⁶.

The primary maintenance challenges listed by participants in the current study mirror some key constraints and limitations identified in other sections of the questionnaire, such as the inadequacy of financial and human resources. This inadequacy threatens the effective management of HCBLs, including the maintenance of equipment and critical systems.

6.2.29 Main biorisk management challenges faced

6.2.29.1 Results

The most common answers to the question 'What are the primary biorisk management challenges faced in the BSL-3 laboratory?' are listed in Table A55. Fifteen HCBLs did not respond to this question (15/32, 46.9%). Among them, two cited that the laboratory is too new.

6.2.29.2 Discussion

The main challenges identified in the present study align with the findings of global authors^{46, 55, 57-58, 171, 176, 389}, and from Brazilian researchers^{18, 20, 74, 316}. They can be categorized into the following topics:

- a. Financial resources;
- b. Human resources, including capacity-building and biosafety culture;

- c. Management issues;
- d. Structural concerns.

The challenges related to financial resources have been discussed in the previous Chapter and other sections of the present study.

Chapter 2.17 of the present study emphasizes the importance of having well-trained personnel working in HCBLs. Numerous authors have underscored the necessity of enhancing biosafety culture in such facilities, as non-compliance issues are primarily related to practices rather than structural deficiencies^{4, 93, 98, 134, 141, 389}. Hence, it is imperative to consider investment in capacity-building programs for HCBL users in Brazil as a national priority, as emphasized in Chapter 6.2.19.2.2 of the present study.

It is worth noting that the shortage of human resources was mentioned as frequently as the lack of financial resources. These two issues are likely closely interconnected. The lack of human resources was noted in the technical sector and administrative and support functions, resulting in an overwhelming workload and deficiencies in biorisk management. Consequently, this vulnerability could lead to accidents and expose high-risk biological materials.

Management issues encompass challenges in the bidding process, budgetary planning constraints, service procurement difficulties, and the need for well-trained managers experienced in dealing with issues associated with a highly complex facility like an HCBL. Consequently, the findings of this study underscore the importance of offering capacity-building opportunities to managers, including exchanging experiences with peers, which is just as crucial as training the technical staff.

Although the structural concerns were mentioned by a person working in an old building, it is essential to highlight that even new facilities may face structural problems due to design and construction failures. In this sense, Chapter 2.15 of the present study stressed the role and importance of the commissioning and periodic certification of HCBLs.

6.3 Comparative analysis between Brazilian, the United States, and Canadian legal framework

6.3.1 Results

Chart 07 summarizes the primary legislation and guidelines on biosafety and biosecurity in the US, Canada, and Brazil.

Chart 07 – Primary legislation and guidelines relating to biosafety and biosecurity in the US, Canada, and Brazil.

Subject	Brazil	The US	Canada
Select Agent Program	-	7 CFR Part 331 ¹⁸⁹ 9 CFR Part 121 ¹⁹⁰ 42 CFR Part 73 ¹⁹¹	Canadian Biosafety Standard (2022) – SSBA program ⁸⁰
Laboratory Biosafety and Biosecurity and Good Laboratory Practice	Law 11,105/2005 ²¹⁸ Decree 5,591/2005 ²¹⁹ Ordinance 3,204/2010 – Ministry of Health ¹⁰ General Guidelines for Work in Containment with Biological Agents (2010) – Ministry of Health ²²³ Normative 5/2012 – Ministry of Agriculture ²³¹	21 CFR 58 ⁴¹⁶ NIH Guidelines (2019) ³⁷² BMBL (2020) ¹	Human Pathogens and Toxins Act ¹⁹⁶ Human Pathogens and Toxins Regulations ¹⁹⁷ Canadian Biosafety Standard (2022) ⁸⁰ Canadian Biosafety Guideline – Pathogen Risk Assessment (2018) ²⁰⁰ Canadian Biosafety Guideline – Conducting a Biosecurity Risk (2018) ¹⁰⁰
Constructive requirements	-	ARS Facilities Design Standards (2002) ⁷⁹ NIH Design Requirements Manual (2016) ⁷⁸	Canadian Biosafety Handbook (2016) ⁷ Containment Standards for Facilities Handling Plant Pests (2014) ⁸⁵ Containment Standards for Facilities Handling Aquatic Animal Pathogens (2013) ²⁰¹
Risk Group Classification	Ordinance 3,398/2021 - Ministry of Health ⁹¹	NIH Guidelines (2019) ³⁷²	Human Pathogens and Toxins Act ¹⁹⁶ https://health.canada.ca/en/epathogen ²²⁸ https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html ²²⁷
Dual-use research	-	P3CO policy ⁴¹⁷ NRC Responsible research with biological select agents and toxins (2009) ¹⁹² NIH Tools for the Identification, Assessment, Management, and Responsible Communication of Dual-use Research of Concern (2014) ⁴¹⁸	Plan for Administrative Oversight for Pathogens and Toxins in a Research Setting - Required Elements and Guidance ⁴¹⁹
Transportation of biological agents	Resolution RDC 20/2014 – ANVISA/Ministry of Health ²³⁵ General Guidelines for Work in Containment with Biological Agents (2010) – Ministry of Health ²²³ Supplementary instruction 175-004D approved by Ordinance 4,497/SPO/2021 – ANAC/Ministry of Transportation ²³⁶ Dangerous Goods Regulations – IATA ⁴²⁰	49 CFR 171-180 ⁴²¹ 42 CFR 71.54 ⁴²² USDA Guideline 1125 ⁴²³ 7 CFR Part 331 ¹⁸⁹ 9 CFR Part 121 ¹⁹⁰ 42 CFR Part 73 ¹⁹¹ Dangerous Goods Regulations – IATA ⁴²⁰	Transportation of Dangerous Goods Act/1992 and Regulations ⁴²⁴ Dangerous Goods Regulations – IATA ⁴²⁰
Export/Import of biological agents	Normative 29/2010 – Ministry of Agriculture ⁴²⁵ Law 13,123/2015 ⁴²⁶ Decree 8,772/2016 ⁴²⁷ Resolution RDC 208/2018 – ANVISA/Ministry of Health ⁴²⁸	15 CFR 738 ⁴²⁹ , 742 ⁴³⁰ , 745 ⁴³¹ , and 774 ⁴³²	Human Pathogens and Toxins Act ¹⁹⁶ Human Pathogens and Toxins Regulations ¹⁹⁷ Health of Animals Act ¹⁹⁸ Health of Animals Regulations ¹⁹⁹
Bioterrorism preparedness and iodefense	Decree 77,374/1976 ²³² Law 9,112/1995 ²³³ Decree 2,977/1999 ⁴³³ Decree 7,722/2012 ¹⁵⁸ Decree 9,573/2018 ¹⁵ Decree 10,569/2020 ⁴³⁴ Decree 11,200/2022 ¹⁶ Normative 2,312/2023 ⁴³⁵	Public Law 107-56 (2001) ⁴³⁶ Public Law 107-188 (2002) ⁴³⁷ Homeland Security Presidential Directive/HSPD-9 (2004) ⁴³⁸ National Biodefense Strategy and Implementation Plan (2022) ¹⁹⁵	Plan for Administrative Oversight for Pathogens and Toxins in a Research Setting - Required Elements and Guidance ⁴¹⁹ Biological Weapons Convention Meeting of States Parties November 2021 – National Statement of Canada ⁴³⁹ Federal/Provincial/Territorial Public Health Response Plan for Biological Events
Waste Treatment	Law 12,305/2010 ²³⁷ Resolution CONAMA 430/2011 – Ministry of Environment ²³⁸ Resolution RDC 222/2018 – ANVISA/Ministry of Health ²³⁹	NIH Guidelines (2019) ³⁷² BMBL (2020) ¹ 29 CFR 1910.1030 ⁴⁴⁰	Canadian Biosafety Standard (2022) ⁸⁰
Occupational Health	Ordinance 485/2005, approving the Regulatory Standard 32 – Ministry of Employment ²⁴⁰	29 CFR 1910.120 ⁴⁴¹ , 29 CFR 1910.132 ⁴⁴² , 29 CFR 1910.133 ⁴⁴³ , 29 CFR 1910.134 ⁴⁴⁴ , 29 CFR 1910.136 ⁴⁴⁵ , 29 CFR 1910.138 ⁴⁴⁶ , 29 CFR 1910.1030 ⁴⁴⁰ , 29 CFR 1910.1200 ⁴⁴⁷ , 29 CFR 1910.1450 ⁴⁴⁸	Canada Occupational Health and Safety Regulations (SOR/86-304) ⁴⁴⁹
Animal Care	Law 11,794/2008 ²⁴¹	Guide for the Care and Use of Laboratory Animals (2011) ⁴⁵⁰ Guide for the Care and Use of Agricultural Animals in Research and Teaching (2020) ⁴⁵¹	Provinces' legislations

6.3.2 Discussion

Some topics, such as transportation, import and export of biological agents, waste treatment, occupational health, and animal care, are well addressed in all scenarios.

Conversely, each regulatory framework has its limitations. For instance, the Canadian regulation led to excessive facilities involved in the oversight process. At the same time, HCBLs that do not handle biological select agents and toxins are omitted according to the US FSAP. Other criticisms from the scientific community regarding the US FSAP are the regulatory burden on researchers and institutions, primarily related to the accurate inventory of individual vials containing biological select agents and toxins, the red tape to transfer biological materials, and the requirement to develop and maintain a skilled workforce⁵⁸.

Brazilian regulations should be updated and improved, taking advantage of experiences from other countries that could be adapted to the national context to strengthen laboratory biosafety and biosecurity measures, standardizing definitions and requirements, minimizing the risks of laboratory-acquired infections and other accidents that could result potentially lead to the release of high-risk pathogens with severe implications for public and animal health. The main gaps are detailed in Chart 08.

Chart 08 – Opportunities to improve the Brazilian legal framework on laboratory biosafety and biosecurity.

Element	Legislation			Main Gaps	Possible models from other regulatory frameworks
	Type/Number	Source	Coverage		
The National Biosafety Policy	Law 11,105/2005 ²¹⁸	National Congress	Overall	Limited to GMOs Biosecurity is not addressed	Canadian Human Pathogens and Toxins Regulations ¹⁹⁷ US FSAP ³³² Australian SSBA Regulatory Scheme ⁴⁰⁰
Biocontainment Laboratories Overseeing	Ordinance 3,204/2010 and complimentary guideline ¹⁰	MS	Public Health Laboratories	Out of Date Limited to Public Health Laboratories Little emphasis on biosecurity Technical staff capacity-building is not addressed Little emphasis on the IBC's competencies	US BMBL ¹ Australian SSBA Regulatory Scheme ⁴⁰⁰ Canadian Biosafety Standard ⁸⁰ Canadian Biosafety Handbook ⁷ Canadian Biosafety Guideline – Pathogen Risk Assessment ²⁰⁰ Canadian Biosafety Guideline – Conducting a Biosecurity Risk ¹⁰⁰ EU Directive 2000/54/EC ²⁰³
Risk Group Classification	Ordinance 3,398/2021 ⁹¹	MS	Overall	Limited to human pathogens Toxins are omitted	Canadian pathogen safety data sheet ²²⁷
Veterinary Biocontainment Laboratories	Normative 5/2012 ²³¹	MAPA	FMD virus handling	Out of Date Limited to FMD virus	US BMBL ¹ Australian SSBA Regulatory Scheme ⁴⁰⁰ Canadian Biosafety Standard ⁸⁰ EU Directive 2000/54/EC ²⁰³
Biodefense	Decree 2,977/1999 ⁴³³ and correlated legislation	Presidency of Republic	Overall	DURC is omitted	US P3CO policy ²¹⁷ US Policy for Institutional Oversight of DURC ²¹⁵
National Safety Plan for Critical Facilities	Decree 11,200/2022 ¹⁶	Presidency of Republic	Overall	Regulation for biosafety and biosecurity is needed	US BMBL ¹ Australian SSBA Regulatory Scheme ⁴⁰⁰ Canadian Biosafety Standard ⁸⁰ Canadian Biosafety Handbook ⁷ EU Directive 2000/54/EC ²⁰³ ISO 35001:2019 ¹¹

The IEGBBR could offer support in this context, as its primary objective is to serve as a platform for exchanging knowledge and expertise related to the current biosafety and biosecurity oversight challenges posed by human and zoonotic animal pathogens. One of the key aim of this group is to enhance global biosafety and biosecurity efforts by sharing insights and best practices drawn from their respective regulatory systems on a worldwide scale. In pursuit of this goal, the IEGBBR develops reference tools and materials that apply to all nations, including those in developing countries²⁶⁵.

The WHO “Guidance on implementing regulatory requirements for biosafety and biosecurity in biomedical laboratories”¹² could be another powerful tool that the BFG can use during this regulatory improvement process (see Chapter 6.4).

The regulatory improvement process should actively involve the scientific community and civil society throughout the entire process. Establishing a balanced legal framework that ensures effective oversight without overwhelming institutions or discouraging research on high-risk pathogens is essential.

In this sense, the US ‘Committee on Laboratory Security and Personnel Reliability Assurance Systems for Laboratories Conducting Research on Biological Select Agents and Toxins’ alerted: ‘While many current policies and practices are effective, some actions suggested to enhance security are not likely to make select agent research more secure, just more difficult to conduct; this may yield the opposite result: that overall security will be diminished, not strengthened.’¹⁹²

Considering that the BFG is preparing a security plan and strategic actions for Laboratory Biosafety and Biosecurity, the time is suitable to address crucial gaps, especially the lack of HCBL oversight. In this sense, establishing a national biological laboratory monitoring program must address the following questions⁴⁵².

6.3.2.1 Who should do the monitoring? Is there critical infrastructure support for a monitoring program?

The centralization of laboratory biosafety management actions presents a primary challenge, which involves clearly defining the roles of each entity involved in the matter and establishing a hierarchical structure. Effective leadership, both in formal roles and in practical terms, is crucial to ensure proper coordination. Actions aimed at promoting cooperation in biosafety laboratories can be incorporated into an existing Brazilian framework, such as the General Coordination of Sensitive Goods, an administrative unit integrated into the structure of the Special Advisory on International Affairs within the MCTI, or the Secretariat of Defense Affairs and National Security of the GSI/PR, or within any relevant Ministry of Defense structure related to chemical, biological, radiological, and nuclear threats.

Nonetheless, in most countries, this responsibility typically falls under the respective Ministry of Health, the best alternative for the Brazilian scenario. The MS creates a security plan and strategic actions for laboratory biosafety and biosecurity. However, a multidisciplinary and interinstitutional approach should be considered, as there are biosafety experts within the MS and other government bodies such as MAPA, MCTI, MD, Federal Police, ABIN, and public universities. Representatives from these organizations have the potential to provide valuable contributions to the MS in developing the regulatory framework and can also actively participate as inspectors in the monitoring program.

Pompeu (2014) argued that Brazil’s technical capabilities are currently dispersed and fragmented across various agencies, including ABIN, Federal Police, MCTI, MD, and MS. However, what needs to be added is a coordinating entity with

the authority to harmonize actions in response to deliberate uses of biological agents within the national borders. Likewise, the country lacks a national protocol for managing biological incidents. Achieving these necessary adjustments will be challenging without establishing a conducive political environment. Hence, there is a pressing need to institute a National Policy for the Prevention and Response to Biological Incidents. This policy would delineate guidelines and establish protocols for the involvement and actions of various national, civilian, and military entities responsible for biosecurity and bioprotection in the country¹⁵⁴.

6.3.2.2 What should be the program focus, and who should be monitored?

According to the WHO, how regulatory frameworks are applied to laboratory operations varies significantly across countries. While some countries are highly regulated and have detailed legislation on biosafety and biosecurity, including established networks of stakeholders with well-defined responsibilities and processes, other countries lack regulatory guidance on laboratory biosafety and biosecurity¹².

Given the challenges countries might face because of limited resources, emerging diseases, and the misuse of advanced technologies, controlling biological risks at the national level may require context-specific consideration to devise the most appropriate approach for a country¹².

In general terms, three common approaches can be used by national authorities to assess risks and apply regulatory frameworks: activity-based, list-based, risk or hazard group-based. Each approach uses a classification system to group either the biological agent or the work being performed with them into categories to which different regulations can be applied. Many countries use a combination of these approaches to appropriately address nationally identified risks and cover the various activities where biological agents may be handled in different sectors that may extend beyond public health to other sectors¹².

Licensing regulations help ensure the government monitors and controls activities involving hazardous biological agents and toxins. Licensing enables a government to have a database of who is holding or working with which controlled agents or toxins and for what purpose. These regulations will require any legal or governmental entity or individual engaged in the development, acquisition, manufacture, possession, transfer, storage, or use of controlled agents or toxins to obtain a license from the relevant government authority⁴⁵³.

As seen in many other countries, the US FSAP and Australian SSBA programs are based on microorganisms that can be used as bioweapons or of potential agricultural and trade impact. However, the list of biological select agents and toxins varies between them¹⁵⁹. A limitation of this approach is that not all HCBLs are included in the monitoring program. The Canadian legal framework has no limitations as it covers all RG 2 or higher pathogens. However, to prevent excessive burden on the system, the monitoring criteria vary for different RGs; for example, facilities and personnel that handle SSBA are subjected to more stringent requirements.

Millet et al. (2023) argued that taxonomic lists of pathogens have historically served as a foundational element in biosecurity programs. However, they contend that numerous other tools now enable the consideration of pathogen functions intended for control. The authors emphasized the need for more adaptable forms of risk management and ultimately concluded that taxonomic lists should be one of many tools at the disposal, given the emergence of more effective tools tailored to

modern biotechnology's demands, such as sequence databases focused on potential concerns⁴⁵⁴.

Given that bioterrorism is not a great concern to the Brazilian context^{18, 154} (although this threat should not be underestimated, given the report of the most important agrobioterrorism in the modern ages with suspicious over a worker from a reference institution who possessed sufficient knowledge to understand the potential risk of that action¹⁵¹), an approach that could be feasible is focusing the program on critical infrastructures, including the HCBLs themselves, instead of specific biological agents. Creating a comprehensive list of such facilities and understanding their activities is crucial in comprehending risks and the overall landscape and establishing criteria and oversight procedures.

In this strategy, the number of facilities to be registered and monitored would be higher than those that only handle pathogens that could be used as bioweapons. However, the frequency and kind of inspection should be defined according to a risk assessment, considering the risk group of microorganisms stored, laboratory purpose and scope, volume of samples received and supplies produced (e.g., vaccines and reagents), methods and equipment in use, complaints recorded by employees or customers, facility's age and size, location, usage of animal testing models, and so on. Ideally, the program would require that new or remodeled facilities be inspected before launching.

The BFG should define whether participation in the program would be voluntary. If so, the benefits should be attractive, for example, restricting public grants to registered laboratories only.

Considering the successful experience of the UK in having the monitoring program centralized in one body and considering that the Brazilian MS was designated to elaborate the national safety plan and strategic actions for laboratories¹⁶, this body could create a working group with representatives from other ministries, especially MAPA, MCTI, and MD to establish a central line of authority for the monitoring process.

This recommendation is consistent with one of the conclusions outlined in a report published by the Verification Research, Training, and Information Centre (VERTIC) titled 'National and Regional Strategies to Enhance Legislation on Biosafety and Biosecurity in Central Asia':

*'Most of the partner countries have several ministries and agencies which have an interest in biosafety and biosecurity-related legislation. Whilst the MS features most frequently in the surveys and matrices, there is generally a need for central coordination on biosafety and biosecurity in the partner countries'*¹⁸⁶.

In China, the Ministry of Health oversees human health research, laboratory and experiment approvals, and biosafety oversight. Meanwhile, the Ministry of Agriculture handles animal research and health matters, along with laboratory and experiment approvals, following a similar model to that observed in the US, where the responsibilities related to the FSAP are shared between APHIS and CDC⁴⁹.

The number of professionals and the amount of time they would dedicate to the program should be addressed. Taking this as a reference, the CDC had 51 inspector positions for FSAP in 2017, whereas APHIS had 11 at that time⁵⁰.

The organization overseeing the program should also offer technical assistance and guidance to registered entities. This support entails organizing workshops, designating a primary point of contact for each institute, creating a

repository of frequently asked questions on the program's website, and delivering technical presentations at various meetings and conferences¹⁹².

6.3.2.3 How extensive should the monitoring be? What standards and requirements should be used?

Although on-site inspections are usually more comprehensive, one of the lessons learned during the COVID-19 pandemic was the benefit of remote work. Owing to travel restrictions, FSAP implemented hybrid inspections, including on-site (focused on laboratory and facility reviews) and remote inspections (focused on document review). US FSAP will continue to refine the hybrid inspection process and expects this will become the primary method of inspecting facilities in the future. Of the 206 inspections conducted in 2021, 19 were on-site, 106 were remote, and 81 were hybrid, with an overall average length of 3 business days¹⁹³. The extensive monitoring will depend on its aims (assessing compliance, initial registration, periodic regular inspections, etc.), the facility's complexity, and inspectors' availability. An inspection's success depends on the inspectors' expertise, training, and ability to follow the audit schedule. Another aspect to be addressed is the availability and source of financial resources. In the fiscal year 2016, for instance, the CDC's budget to manage its component of the FSAP was about USD 21 million, and APHIs' was about USD 5.5 million⁵⁰. It is essential to highlight that the number of entities registered in FSAP is more than two times the estimated number of self-declared BSL-3 laboratories in Brazil — no more than 100²⁰. The BFG should consider creating a specific budget source linked, for example, to MS or MCTI to meet the costs related to regulatory oversight.

Inspections must be based on objective criteria and comprehensive checklists; which Brazilian legislation does not establish well (except for FMD vaccine manufacturers). Although the country has no guidelines and checklists, international references may be an acceptable alternative. The questionnaire developed in the present study can be adapted as a checklist to assess HCBLs. Other references can be used according to the audit or inspection goals^{81, 170, 185, 203, 211, 395, 455}.

Another possible approach is focusing the monitoring program on evaluating the laboratories' risk assessment instead of emphasizing specific biological agents and toxins or structural features. The BFG could take advantage of ISO 35001¹¹, incorporating it into the national legal framework and requiring the laboratories to follow it. The benefit of this approach is addressing the facilities and research specificities, which can demand higher or lower biosafety and biosecurity requirements beyond those considered in the traditional four laboratory BSLs⁷⁶. In contrast, this approach requires a high level of risk assessment training for auditors and those being audited. Standardizing the monitoring process and avoiding subjective assessments would be more difficult.

Rodgers et al. (2021) agreed that ISO 35001 can help build confidence that participating labs are taking proper precautionary measures. However, countries must agree on a third-party entity to systematically validate and certify compliance to implement this standard at an international level. The authors also highlighted that ISO 35001 must not be a substitute for national and international legislation or regulation¹⁷⁴.

Another challenge would be defining criteria to decide which institutions should participate in the oversight program.

6.3.2.4 What are the consequences if a laboratory does not meet the requirements or is unwilling to comply?

US FSAP may refer entities to the HHS Office of Inspector General (OIG) or APHIS Investigative and Enforcement Services (IES) for serious non-compliance for further investigation and possible civil monetary penalties. The HHS can levy civil money penalties (for an individual up to USD 250,000 and an entity up to USD 500,000 for each violation) or recommend criminal enforcement. Since 2015, three to seven entities have been referred to either HHS or APHIS each year, except for 2019 and 2020, when only one entity was referred. In 2021, no entity was referred. FSAP notifies the FBI of any security-related issue identified and any report of a loss of a select agent. The FBI investigates the incidents to determine whether there is a criminal threat¹⁹³.

The UK, Canada, France, and Switzerland can pursue criminal prosecution in response to severe violations of their laws or regulations governing high- and maximum-containment laboratories. In Canada, penalties for severe violations can include up to 10 years in prison⁵⁰.

Brazilian authorities should define whether the monitoring process would focus on punitive sanctions (once compatible with the broader legal framework) or an educational approach (focused on providing capacity-building tools and guidance rather than punishments), assessing the pros and cons of each strategy. It is essential to highlight that depending on the requirements, restrictions, burden, and sanctions forced by the regulations, the cost and effort to operate HCBLs can culminate in the discouragement of researchers from studying relevant infectious diseases, as observed in France, where many scientists have redirected their research to the study of attenuated strains that are not listed as controlled microorganisms³².

6.4 The Brazilian scenario in the face of the *WHO Guidance on implementing regulatory requirements for biosafety and biosecurity in biomedical laboratories*

6.4.1 Introduction

The WHO document titled 'Guidance on Implementing Regulatory Requirements for Biosafety and Biosecurity in Biomedical Laboratories - A Stepwise Approach'¹² emphasizes the pivotal role biological laboratories can play in enhancing a nation's biological risk management capabilities and fostering a culture of responsibility. It underscores that the management of biological risks typically commences at the national level, with countries enacting laws and regulations that delineate the specific control measures necessary for laboratory authorization. Moreover, most nations with such regulatory frameworks have also established monitoring or oversight mechanisms to ensure compliance with national laws and regulations by laboratories.

This Chapter is dedicated to assessing Brazil's adherence to the seven steps recommended by the WHO in its guidance document.

6.4.2 Results and Discussion

6.4.2.1 Step 1: Mobilize national commitment and resources for the development and implementation of a national biosafety and biosecurity policy

The enactment of Law 11,105/2005²¹⁸ marked the integration of the Cartagena Protocol on Biosafety into the Convention on Biological Diversity, giving rise to a National Biosafety Law tailored to the specific needs of Brazil²²². This legislation established comprehensive biosafety regulations and protocols for overseeing activities exclusively related to GMOs. It also led to the formation of the National Council for Biosafety, a restructuring of the National Technical Committee for Biosafety, and the formulation of the PNB. However, it is worth noting that the scope of this law is primarily confined to GMOs, making it insufficiently broad in its efforts to promote biosafety and biosecurity across Brazil.

Notwithstanding these limitations, it is imperative to acknowledge that the importance of biosafety and biosecurity has been steadily growing within the BFG, even preceding the emergence of the COVID-19 pandemic. In 2018, the BFG elevated this subject to a strategic priority area managed by the CREDEN under the auspices of the GSI/PR, along with transport, water, finances, communications, energy, and borders²⁴³.

The CREDEN defines strategic issues as facilities, services, and goods whose disruption or destruction could inflict severe social consequences and critical harm to the economy, public policy, public safety, and international relations²⁴⁴. The GSI/PR has recently established several TGs dedicated to biosafety and biosecurity.

The TG on Critical Structures Safety is responsible for identifying, surveying, and evaluating threats and vulnerabilities related to biosafety and biosecurity. The TG on National Biosafety and Biosecurity Policy was tasked with defining and shaping this pivotal policy. The TG on BSL-4 Laboratory has been established to develop a proposal for constructing a Brazilian BSL-4 laboratory²⁴⁵.

These TGs comprised representatives from various governmental entities, in addition to the Presidency of the Republic, including the Ministries of Justice, International Affairs, Environment, MD, MAPA, MCTI, and MS. While these TGs align with WHO recommendations, it is crucial to recognize the absence of representation from state and municipal levels, as well as non-governmental organizations.

6.4.2.2 Step 2: Conduct a national evaluation and surveys

Conducting a literature review has enabled us to identify several critical studies about Brazil's biosafety and biosecurity landscape, as summarized in Chart 04 (see Chapter 2.17 of the present thesis). While these studies are undeniably relevant, it is worth noting that most of them consist of official documents released by the MS or professionals closely associated with this body. More research papers need to be published in specialized international journals. Further investigations must be conducted and disseminated through international journal outlets.

Another noteworthy area for improvement in the existing literature is the lack of studies addressing the current status of HCBLs operating in Brazil. More comprehensive studies should examine various facets, including infrastructure, human resources (including training programs), operational scope (encompassing pathogens handled and methodologies employed), national and international collaborations, information management, and financial aspects, among other relevant considerations.

Creating graduate programs dedicated to laboratory biosafety and biosecurity can accelerate technical and scientific advancements in this field. In this regard, it's crucial to emphasize the 'Academic Cooperation Program in National Defense' (PROCAD-DEFESA/CAPES)³²³, which has launched a specific call for an innovative project centered around Biosafety Management and Governance. The Applied Biochemistry Graduate Program at UFV began offering this program in 2020, and this thesis serves as one of the results of the scholarly efforts within it.

6.4.2.3 Step 3: Establish national institutions and operational mechanisms and develop best-fitting regulations

A prior study compared the regulatory frameworks for biosafety and biosecurity in various countries, including the US, EU, Canada, Australia, Singapore, and Brazil¹⁹. According to this review, the Brazilian legislation exhibited the most notable gaps, although the validity of these findings may be subject to debate. The study's authors did not identify specific regulations about some issues in Brazil, such as Pathogen Work – Animals; Waste Management; Dual-use Items; GMO Transboundary Movement – Cartagena Protocol Implementation; Animal Health – Notifiable Diseases and Emergency Measures. However, it is essential to recognize that regulations exist for all these areas in Brazil. Therefore, it is imperative to gain a deeper understanding of the criteria employed by the authors in their assessment.

Brazil boasts an array of regulations related to biosafety and biosecurity, as elucidated in Chart 07. Furthermore, the ABNT has established multiple committees tasked with reviewing or formulating standards in biosafety and biosecurity, as elaborated upon in Chapter 2.10 of this study.

Nevertheless, despite these commendable initiatives, the national regulatory framework exhibits certain deficiencies in biosafety and biosecurity, including:

- a. The absence of a centralized national authority responsible for overseeing and coordinating biosafety and biosecurity matters within the BFG, setting criteria for HCBL certification, and conducting facility inspections;
- b. The lack of standardized criteria for risk analysis;
- c. The absence of templates for reporting accidents and incidents;
- d. The deficiency in building requirements tailored for HCBLs;
- e. The absence of a standardized pathogen classification system based on risk groups.

In summary, the imperative for harmonizing biosafety and biosecurity regulations is unmistakable, particularly within a 'One Health' approach.

6.4.2.4 Step 4: Strengthen expertise to support the implementation of a suitable regulatory system

In recent years, concerted efforts from national commissions, universities, research institutes, scientific associations, and various governmental and non-governmental organizations have significantly bolstered the culture of biosafety and biosecurity in Brazil. These collaborative initiatives have also enhanced the expertise of professionals engaged in establishing a robust regulatory framework.

Several noteworthy initiatives have contributed to the accumulation of knowledge in biosafety and biosecurity. These include workshops organized by the MS under the theme 'Building the National Biosafety and Biosecurity Policy: Strategic Health Actions' in 2017 and 2018. Additionally, the BioGib Workshop in 2018, SB3 Webinars in 2020 and 2021, and IVBW Workshop in 2017 have played pivotal roles in knowledge generation. Furthermore, several Brazilian universities, such as UFV, UFF, UFG, USP, and UNICAMP, have offered undergraduate courses on biosafety. Notably, many of these events have featured participation from international experts, a vital component in fortifying local technical expertise.

The BFG's decision to establish multidisciplinary TGs dedicated to biosafety and biosecurity in 2020²⁴⁵ represented a significant impetus for advancing technical proficiency in this domain. One notable outcome of this initiative was the 'International Workshop – National Maximum-containment Laboratory,' organized by the BSL-4 Laboratory TG under the support of the MCTI. This historic event attracted members from other TGs. It featured speakers from esteemed international public and private organizations such as FAO, WHO, CDC, USDA, NIH, UTMB, AMexBio, Merrick Company, Perkins & Will, the Chemical, Biological, Radiological, Nuclear, and Explosives Resilience Strategy for Canada (CBRNE), the P4 Jean Mérieux-Inserm Laboratory (France), CCRD-WSP, and the Robert Koch Institute (Germany)³²⁰.

In 2022, the MCTI launched the Public Call n. CNPq/MCTI-FNDCT 29/2022 to offer strategic training for HCBL workers. The funding could be allocated to overseas Ph.D. scholarships and to provide training courses nationwide^{ww}.

However, despite these commendable strides, no 'National Training Program' is dedicated to biosafety and biosecurity. In an ideal scenario, a centralized body responsible for coordinating biosafety and biosecurity efforts in the country would oversee its design and implementation. Such a body could regularly offer standardized courses tailored to the unique characteristics and requirements of the country on a regular basis. It would include online introductory courses targeted at biosafety officers. Centralized training provision also facilitates the creation of a national register of certified professionals, contingent upon the successful completion of an evaluation test after the course. Additionally, it would foster establishing a national network, enabling the exchange of information and promoting collaborative inter-institutional initiatives.

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at:

http://memoria2.cnpq.br/web/guest/chamadas-publicas?p_p_id=resultadosportlet_WAR_resultadoscnpqportlet_INSTANCE_0ZaM&filtro=abertas&detalha=chamadaDivulgada&idDivulgacao=10931. Accessed on: December 27, 2023.

6.4.2.5 Step 5: Implement and enforce regulations

As delineated in step 3, substantial deficiencies exist within the Brazilian legal framework regarding biosafety and biosecurity. The formulation and enhancement of this framework hinge upon the elaboration of the PNBB, which is currently in progress. Consequently, a comprehensive assessment of these gaps and developing strategies to align the legal framework more effectively will become feasible shortly after the policy's publication. Such an evaluation could be conducted by a newly established TG operating within the purview of CREDEN. Chart 09 provides an overview of potential strategies this TG could employ.

Chart 09. Possible strategies to review and adapt the Brazilian legal framework on biosafety and biosecurity.

Step	Strategies
Designation of a TG	An inter-institutional and multidisciplinary TG into the CREDEN domain may be created to identify gaps in the Brazilian legal framework on biosafety and biosecurity.
Information gathering	Conducting a comparative study considering other countries' legal frameworks on biosafety and biosecurity.
	Identifying overlap and contradiction between the regulations released by different governmental bodies.
Activities development	Internalization of international reference bodies (WHO, FAO, UN, WOA, and ISO, among others) recommendations and compliance evaluation of agreements established through multilateral conventions and protocols.
	Creating a unified group risk classification for human, animal, and vegetal pathogens.
	Standardization of criteria for qualification/verification/certification of critical equipment like autoclaves, BSCs, pass-through, airlocks, etc.
	Standardization of risk analysis methodology.
Releasing	Creating a BFG Committee with broad representativeness and competence to release standards, guidelines, and ordinances.
	Regular revision of the legal framework, standards, and guidelines, as well as the communication tools.
Monitoring	Creating an inter-institutional and multidisciplinary Technical Commission (similar to the COMBIOLAB), to oversee the high-containment laboratories in operation through a standardized evaluation process.
	Defining and implementing a high-containment laboratory certification process.

The future legal framework should encompass the following key components:

- a. Foundational Standards: This includes the establishment of essential standards encompassing professional vocabulary, terminology, explanations, classifications, and identifications;
- b. Biosafety Laboratory Planning and Design Standards: This entails formulating standards governing the planning and design of diverse laboratory types, such as veterinary, microbiology, and medical. It also encompasses guidelines for the architectural planning and design of laboratory buildings, including considerations for functional aspects like heating, ventilation, and air conditioning systems;
- c. Biosafety Laboratory Construction Standards: This section should encompass construction technology standards for laboratories, as well as building standards for functional architecture, including heating, ventilation, and air conditioning systems;
- d. Standards for Acceptance, Certification, and Evaluation: These standards are crucial for assessing biosafety facilities. They should encompass procedures for building acceptance, laboratory accreditation, and technical evaluations;

- e. Operation and Management Standards: This category encompasses various aspects, including quality management, environmental considerations, risk management, pollutant control, waste management, work environment regulations, occupational safety guidelines, and worker hygiene standards;
- f. Device and Technology Standards: This section should define standards for laboratory equipment, applications, and operational procedures;
- g. Scientific Research Standards: These standards should address scientific research protocols, including those related to sterilization, equipment operation, and research involving biology, plants, and animals;
- h. Competency Verification and Training Standards: This component should cover the evaluation of competence and training requirements, including the operation and management of laboratories, dissemination of standards, and training programs;
- i. Repair and Maintenance Standards: These standards should outline guidelines for the upkeep and maintenance of laboratory facilities, including HVAC, technical equipment, and sterilization procedures²⁰⁷.

The BFG must conduct a legal analysis to determine the types of requirements that should be specified in laws, ordinances, and non-binding instruments. Non-binding instruments have the advantage of being quicker and simpler to implement than binding agreements. They also offer more flexible mechanisms for updates and amendments, making them particularly suitable for certain guidelines⁴⁵⁶.

Several countries significantly increased their investments in laboratory biosafety and biosecurity, including legislative enhancements, following serious incidents that triggered internal crises. For instance, the criminal dissemination of *Bacillus anthracis* by mailing in the US (2001)¹⁸⁷, the cases of SARS-associated LAIs in China (2003), Singapore (2003), and Taiwan (2003), as well as the accidental release of the FMD virus from an HCBL in the UK (2007)^{49, 127} prompted these investments. While the establishment of the PNBB is an ongoing process, there is a pressing need to expedite its development and implementation of a comprehensive legal framework in Brazil. Hopefully, a severe event with catastrophic consequences will not be necessary to motivate national authorities to accelerate regulatory progress.

In this regard, civil society should exert pressure on the BFG to expedite the enhancement of regulatory aspects about the operation of HCBLs, and the SB3 and National Biosafety Commissions, affiliated with various ministries, could play a crucial role in catalyzing this process.

Chapter 2.8.2 of this study outlines essential questions that need addressing during the development of measures to strengthen the regulatory framework, particularly concerning the implementation of oversight mechanisms.

6.4.2.6 Step 6: Establish national information exchange networks and international partnerships

Coelho (2017) advocated that there is a pressing need for an integrated system of prevention, preparation, and response to chemical, biological, radiological, and nuclear events in Brazil. The current lack of uniformity in every action, every actor, and every system has led to a fragmented landscape of state measures that require optimization. According to this author, the National Biosafety Commissions of different ministries should incorporate representatives from each other as consultative members. Additionally, representatives from security agencies, such as

ABIN, should be included to promote intersectoral integration to prevent and respond to biological events¹⁸.

Despite some sporadic initiatives, like the establishment of COMBIOLAB and CREDEN's TGs, there is a distinct absence of a coordinated effort to facilitate the formation of a 'National HCBL Network' in Brazil. Such a network is necessary to determine the number of HCBLs in operation, under construction, or undergoing renovation within the nation.

In a prior survey focused on HCBL operating in Brazil, MAFRA underscored the potential for inaccuracies in the biosafety level classification of some listed laboratories. The author has acknowledged that some facilities may no longer operate under high-containment conditions. Furthermore, certain facilities may be operational without proper registration, hindering swift traceability through online searches²⁰.

Coelho (2017) also asserted that the BFG needs to gain knowledge regarding the total count of HCBLs operating within the country and the pathogens handled within them¹⁸.

In the absence of an official network, there exist no mechanisms for overseeing information related to operational facilities, incidents, and accidents, the transport of biological agents, stored pathogens, diagnostic methods in use, financial resources allocation, ongoing research, immunobiological production, sample reception volume, general operational capacity, and so forth¹⁴³.

A well-structured and responsive information system is necessary to develop a national strategy to identify structural vulnerabilities, partnership opportunities, training requirements, and gaps in disease diagnostics and research of government interest. In summary, the government needs to allocate public resources efficiently.

Additionally, there needs to be more clarity with international reference bodies due to challenges in understanding each laboratory or network's organizational structures, competencies, and spheres of influence. Consequently, establishing partnerships and participation in support programs becomes more daunting.

Centralized governance has enabled certain countries, such as Ethiopia and Uganda, to enhance the management of laboratory systems and networks. These countries have reported updated national policies and strategic plans and allocated budget lines specifically earmarked for laboratory functions and regulations²³.

A recent program developed in Thailand aimed to enhance biosafety and biosecurity measures within government veterinary diagnostic and research laboratories. Despite established national policies on laboratory biosafety and biosecurity, significant challenges persisted, including the harmonization and enforcement of these policies, particularly at the regional level, and the engagement of personnel in implementing biosafety and biosecurity measures. The authors emphasized the need for consistent biosafety policies, allocated resources, and ongoing training to foster sustainable biosafety and biosecurity at the national level³⁶³.

6.4.2.7 Step 7: Review performance and adaptability to the national context and evolving risks

After the release of the PNBB, the BFG should establish a mechanism to evaluate its implementation following the PDCA cycle. As mentioned, practical evaluation and continuous improvement can only occur after establishing a

centralized structure with authority to enforce the legal framework across various BFG bodies.

This centralized structure should employ management tools to identify threats and weaknesses and the actions required to address them. It should also formulate an action plan to enhance the national biosafety and biosecurity infrastructure in alignment with the country's specific needs, BFG strategies, and available financial resources.

To illustrate a successful centralized initiative, we can refer to recent Public Calls issued by the FINEP in 2020⁷¹ and 2021⁷², which aimed to provide financial support for selected projects focused on expanding BSL-3 laboratories and animal facilities. Such initiatives are always welcomed, particularly in the context of a pandemic. However, the BFG could achieve its objectives more efficiently if the PNBB had already been established and implemented.

Having more laboratories is only sometimes the best approach. The decision to allocate resources for remodeling, constructing new laboratories, or expanding the capacity of existing facilities should be preceded by a thorough risk analysis and scenario assessment⁴⁶.

Further studies are required to establish a correlation between the presence and operation of HCBL in various regions of the country and the incidence or threat of human and animal diseases that necessitate the presence of HCBL in those specific areas.

6.4.3 The post-New Public Management and public policies targeted to laboratory biosafety and biosecurity

Given the significant costs and risks associated with handling hazardous pathogens, the central government should be responsible for managing and supervising laboratory biosafety and biosecurity. Even during an economic crisis, when governments typically implement austerity measures to reduce public expenditures, it is unwise to delegate tasks such as diagnosing emerging diseases, researching high-risk pathogens, and inspecting HCBLs. These activities require a continuous and robust governmental presence to support the 'One Health' and 'Biodefense' paradigms. It holds even in a new model of public governance known as *post-New Public Management* (post-NPM). In this model, there is a shift from the state's traditional active and managerial role to that of an activator and collaborator. However, as emphasized in Chapter 2.8.1 of this study, strategic services and those with a high specificity level should be directly provided directly by the government¹⁸⁴.

In this context, Cavalcante (2017) has distilled trends in public management principles and guidelines characteristic of the post-NPM era¹⁸³. This information has been incorporated in the present study to explore potential applications in public policies to enhance laboratory biosafety and biosecurity, as outlined in the third column of Chart 10.

Chart 10. Patterns of the post-NPM described by Cavalcante (2017)¹⁸³ exploring potential applications of public policies targeted at laboratory biosafety and biosecurity.

Principles and guidelines	Meanings	Possibilities of application to public policies targeted to laboratory biosafety and biosecurity
Collaboration and partnerships	Collaborative process and partnerships in multiple ways.	Establish partnerships with private companies and research institutions to conduct research that necessitates HCBL.
Networking	Establishment of networks to supply public services.	Create a 'National HCBL Network' under the leadership of the BFG.
A holistic view of public management	Assumption of public services supplied in an integrated and broad perspective way (cohesive and coherent).	Operate the 'National HCBL Network' within a 'One Health' framework, involving various BFG bodies such as MS, MAPA, MD, MCTI, and others.
Accountability and responsiveness	Enhancement of the accountability processes and improvement of the capacity to respond to society's demands.	Enhance transparency and information dissemination related to the operation of HCBLs, including risk analysis, contingency plans, scope, budget, protocols, partnerships, and more.
Social Commitment	Expansion of the channels of social participation and encouragement of the involvement of the society in the public management.	Encourage participation of stakeholders within the 'National HCBL Network' in open scientific associations like SB3.
Leadership	Relevance of the leader role in public management, especially in entrepreneurial initiatives.	Provide international training opportunities to enhance the capabilities of BOs and HCBL managers.
Coordination and control	Strengthening the government's capabilities to coordinate and control actions to deliver services with coherence and cohesion.	Centralize the management of the 'National HCBL Network' within a single federal entity.
<i>E-government</i> , information technology, and communication	Continuous incorporation of information technology to improve the transparency of government actions, as well as the access and involvement of the citizens with the public management.	Develop a web portal for the 'National HCBL Network,' offering information accessible to the general public and specialized audiences.
Strengthening bureaucracy	Qualification and valorization of civil servants, enabling public service to be more efficient, multidisciplinary, and able to respond to society.	Define training objectives for various professions in public HCBLs and explore the feasibility of establishing specific careers dedicated to these facilities.

6.5 Comparative analysis between Brazilian High-Containment Biological Laboratories and laboratories affiliated with Research Alliance for Veterinary Science and Biodefense BSL-3 Network

6.5.1 Introduction

Leveraging the internship at GHRC/TAMU, the same questionnaire administered to Brazilian HCBLs was also applied to some HCBLs affiliated with the RAV3N network.

This network is a collaborative initiative between the USDA NBAF (National Bio- and Agro-Defense Facility) and GHRC/TAMU. It represents a cooperative effort involving various US universities and high and maximum-biocontainment laboratories under the purview of the USG. Its primary aim is to develop strategic and coordinated approaches for leveraging the collective large-animal biocontainment infrastructure and research capabilities. This collaborative effort seeks to enhance bio-surveillance, diagnostics, and the development of countermeasures against high-consequence pathogens of veterinary and zoonotic significance. The network comprises 18 member organizations, 17 located in the US and one in Canada²⁷⁰.

Seven HCBLs filled in the questionnaire, as listed in Chart 11. Those who agreed to participate were provided with a Free Consent Agreement Form to demonstrate the commitment to maintaining the confidentiality of the information provided.

Chart 11. List of RAV3N HCBLs participating in the survey.

HCBL	Laboratory affiliation	Location
Global Health Research Complex (GHRC)	TAMU	College Station, Texas
National Animal Disease Center (NADC)	USDA	Ames, Iowa
Plant Animal Agrosecurity Research (PAAR)	Ohio State University	Wooster, Ohio
BSL-3E Lab	University of Texas Medical Branch	Galveston, Texas
School of Medicine BSL-3 Lab	TAMU	College Station, Texas
School of Veterinary Medicine BSL-3 Lab	TAMU	College Station, Texas
Office of Texas State Chemist	TAMU	College Station, Texas

6.5.2 Results and Discussion

It is crucial to emphasize that the answers obtained from RAV3N should not be generalized to the broader US context, as this data represents a specific sample of laboratories.

Table 31 displays responses with a high positive correlation (p-value = 1.000), while Table 32 presents responses with statistically significant differences (p-value $\leq$ 0.005) when comparing the two scenarios (Brazilian HCBL vs. RAV3N HCBL). The p-value was adjusted in both tables after considering the error caused by the non-response rate, as shown in Chart 05.

Table 31. Compilation of strongly positive correlations identified in the comparative analysis between the Brazilian HCBLs and RAV3N Laboratories.

Question	Answer	Brazilian HCBL (%)	RAV3N HCBL (%)	Error (%)	p-value
Does the lab belong to any international network?	Yes	15.6	14.3	-7.1	1.000
Are the current activities the same as those foreseen in the design?	Yes	93.3	100.0	0	1.000
Was the lab commissioned?	Yes	85.7	100.0	-3.3	1.000
Are there currently cracks on the inner walls?	No	93.5	100.0	-0.5	1.000
Is the access to the BSL-3 lab through a mechanism that includes interlocking doors?	Yes	90.3	100.0	-0.5	1.000
Is there a changing room dedicated exclusively to the BSL-3 lab access?	Yes	93.8	100.0	0	1.000
Is the air pressure automatically and/or manually monitored?	Yes	96.9	100.0	0	1.000
How do you rate how often the lab air pressure does not fulfill the established parameters?	Scale 1 to 5	Median 2.0	Median 2.0	-2.3	1.000
Does the lab periodically monitor the efficiency of the filters installed in the air exhaust system?	Yes	87.5	85.7	-4.2	1.000
Is the air exhaust system redundant?	Yes	82.6	85.7	-5.4	1.000
Does the HVAC system have sealing dampers?	Yes	95.2	100.0	-7.1	1.000
Was the HVAC system nationally manufactured?	Yes	64.3	75.0	-4.3	1.000
Is the air temperature automatically and/or manually monitored?	Yes	93.3	100.0	-1.0	1.000
Is the BSL-3 lab covered by power generators?	Yes	96.7	100.0	-1.0	1.000
Do the equipment or systems that transfer small volumes to and from the BSL-3 lab have an interlocking door system?	Yes	92.9	100.0	-0.3	1.000
Has the decontaminating method of the equipment or systems used for transferring small volumes to and from the BSL-3 lab been validated?	Yes	52.4	66.7	-2.8	1.000
Does the number and capacity of autoclaves meet the lab demands?	Yes	93.3	100.0	0	1.000
If there are border autoclaves, do they have bioseal?	Yes	91.7	100.0	-1.6	1.000
Does the lab keep records of each autoclaving cycle?	Yes	89.3	100.0	-1.6	1.000
Are carried out periodically certification tests for BSCs?	Yes	96.3	100.0	-2.2	1.000
Is there an SOP for training users on the proper use of BSCs?	Yes	89.3	100.0	-1.6	1.000
Are samples and other potentially infectious materials handled exclusively in BSCs?	Yes	91.3	100.0	-2.3	1.000
Is the temperature of refrigerators, freezers, and ultra-low freezers automatically and/or manually monitored?	Yes	86.7	83.3	1.3	1.000
Are there centrifuges installed in the BSL-3 lab?	Yes	90.0	100.0	-1.0	1.000
Is the recommended protocol to decontaminate centrifuges followed?	Yes	85.7	83.3	-2.7	1.000
Are different species handled in the same room at the same time?	No	75.0	60.0	1.5	1.000
If equipment such as ventilated racks and isolators are used, are they certified periodically?	Yes	50.0	60.0	0	1.000

Question	Answer	Brazilian HCBL (%)	RAV3N HCBL (%)	Error (%)	p-value
Have the technicians who handle the animals received formal training on the concepts of animal welfare?	Yes	85.7	100.0	-6.2	1.000
Does the room where the animals are kept have a CCTV system enabling continuous monitoring?	Yes	50.0	50.0	-10.0	1.000
Are uniforms available for exclusive use in the BSL-3 lab?	Yes	96.7	100.0	-1.0	1.000
Is there a SOP containing guidance on the proper use of PPE?	Yes	92.9	100.0	-1.6	1.000
Have the IT staff received training on biosafety issues?	Yes	71.4	100.0	-8.6	1.000
Has the BO received training on biosafety issues before starting their activities related to this position?	Yes	89.5	100.0	-0.7	1.000
Is food intake allowed in the BSL-3 lab?	No	90.0	100.0	-1.0	1.000
Are the BSL-3 users allowed to take their cell phones in the BSL-3 lab?	No	88.5	85.7	-3.3	1.000
Has the lab defined a maximum period of stay for users within the BSL-3 lab?	Yes	48.1	42.9	-2.2	1.000
Is any survey carried out to assess the well-being of the professionals working in the BSL-3 lab?	Yes	17.4	16.7	-2.3	1.000
Has there ever been a reported theft or stealing of materials from the BSL-3 lab?	No	95.2	100.0	0.4	1.000
Has the lab ever been sabotaged?	No	96.0	100.0	-1.4	1.000
Is the guard service provided on weekends and holidays?	Yes	96.2	100.0	0	1.000
Do the rooms where high-risk biological agents are stored have their own access control?	Yes	75.9	85.7	-1.5	1.000
Have there already been reported incidents involving the security of the data generated/stored in the BSL-3 lab?	No	91.3	100.0	1.2	1.000
Does the automation system have audible or visual alerts in case of deviation from critical parameters?	Yes	90.9	100.0	-0.8	1.000
Does the lab have software for recording data related to preventive maintenance plans?	Yes	65.0	57.1	-7.5	1.000
Has the operation of the BSL-3 lab been suspended in the last 12 months due to a lack of funds for equipment and/or systems maintenance?	No	95.0	100.0	-2.6	1.000
Have the activities of the BSL-3 lab been suspended at some point due to a lack of demands?	No	82.6	83.3	2.6	1.000

Table 32. Compilation of statistically significant differences results identified in the comparative analysis between the Brazilian HCBLs and RAV3N Laboratories.

Question	Answer	Brazilian HCBL (%)	RAV3N HCBL (%)	Error (%)	p-value
What's the BSL-3 lab workspace footage (m ²)?	Median	50	256	0.7	0.038
Is the BSL-3 lab conjugated to BSL-2 and or BSL-1 labs?	Yes	76.7	14.3	-1.0	0.004
Are there exclusive changing rooms for men and women?	Yes	25.0	85.7	-3.7	0.007
Is bath water drained to a waste treatment system?	Yes	94.7	57.1	-1.0	0.047
Are there filters installed in the air supply?	Yes	100.0	55.6	-2.6	0.002
How do you decontaminate the filters when replacing them?	Autoclave	33.3	0.0	-13.9	0.042
	Formaldehyde	26.7	71.4		
	H ₂ O ₂ and hypochlorite	6.7	0.0		
	Incineration	6.7	0.0		
	UV	6.7	0.0		
	VHP	20.0	0.0		
	Chlorine dioxide	0.0	28.6		
How many air changes per hour are provided in the BSL-3 rooms?	Median	18	10	-4.3	0.018
How do you rate how often the power electricity is cut off, determining the need to put the UPS and power generators into operation?	Scale 1 to 5	Median 3	Median 2	-0.5	0.049
Has the decontaminating method for the whole laboratory been validated?	Yes	47.6	100.0	-6.1	0.023
How do you rate how often leaks are observed in the EDS?	Scale 1 to 5	Median 1	Median 2	-5.3	0.014
How many BSCs are currently installed in the BSL-3 lab?	Median	2	9	1.7	0.032
How many ultra-freezers are currently installed in the BSL-3 lab?	Median	1	3	0.8	0.048

Question	Answer	Brazilian HCBL (%)	RAV3N HCBL (%)	Error (%)	p-value
Which equipment/system does the lab have to transfer small volumes from and to the contained area	Airlock	10.3	9.1	-2.1	<0.001
	Autoclave	6.9	9.1		
	Pass-through	82.8	9.1		
	Dunk tunk	0.0	27.3		
	None	0.0	36.4		
What is the method used to decontaminate the lab as a whole?	Alcohol 70%	14.3	20.0	-10.1	0.001
	Ammonium quaternary	7.1	0.0		
	Formaldehyde	25.0	0.0		
	Hydrochloric acid 0,01%	3.6	0.0		
	Peracetic acid	3.6	0.0		
	Phenol	3.6	0.0		
	Potassium permanganate	3.6	0.0		
	Sodium hypochlorite	17.9	0.0		
	UV	10.7	0.0		
	VHP	10.7	33.3		
	Chlorine dioxide	0.0	13.3		
	Microchem	0.0	6.7		
	Virkon	0.0	20.0		
	iHP	0	6.7		
Does the lab handle animals in high-containment areas?	Yes	29.0	85.7	-0.5	0.010
What is the approximate workload of the biosafety and biosecurity capacity-building training?	Median	18	40	-0.5	0.024
How many cleaning professionals are currently allowed to access the BSL-3 lab?	Median	1	0	-5.2	0.007
How do you rate the frequency of biosafety-related incidents in your institution?	Median	1	2	1.7	0.005
Is there any cooperation agreement established with security authorities?	Yes	16.7	100.0	-4.6	<0.001
Is the equipment where high-risk biological agents are stored locked?	Yes	20.0	100.0	-4.5	0.015
Is there a protocol for evaluating criminal records and behavioral profiles before hiring workers who will access the BSL-3 lab?	Yes	25.0	100.0	-2.1	0.001
How do you rate how often high-risk biological agents are removed from the BSL-3 to be transported to another lab?	Scale 1 to 5	Median 1	Median 2	1.1	0.015

Question	Answer	Brazilian HCBL (%)	RAV3N HCBL (%)	Error (%)	p-value
Does the lab work with genetic engineering, like DNA recombination methods?	Yes	19.4	85.7	-0.5	0.002
Are the methods applied in the lab supported by legislation or reference guidelines/standards?	Yes	100.0	57.1	-6.1	0.011
Are there additional security barriers surrounding the BSL-3 facility?	CCTV	40.5	42.9	-1.5	0.006
	Electric fence	2.7	0.0		
	Presence sensor	8.1	7.1		
	Regular fence	10.8	14.3		
	Wrought iron fence	5.4	7.1		
	Bollards	0.0	28.6		
	No	32.4	0.0		
Is the lab up to date with all its legal obligations with the fire department?	Yes	45.0	100.0	-8.0	0.022
Which is the fire detection system inside the BSL-3 lab?	Heat detector	4.2	54.5	-2.1	<0.001
	Smoke detector	45.8	45.5		
	None	50.0	0.0		

The limitations of this comparative study include:

- A limited number of HCBLs participated from RAV3N;
- Typical responses were consistently observed across all four TAMU laboratories, particularly in areas related to biorisk management, owing to their shared BO;
- Both scenarios relied solely on self-reported survey data;
- Different regulatory frameworks applied to the two scenarios;
- Different economic contexts applied to the two countries;
- Different culture on biosafety and biosecurity;
- Different level of knowledge from the respondents.

Despite these limitations, the following findings appear to be relevant and worthy of in-depth exploration in future studies:

- Size of BSL-3 labs: the Brazilian HCBLs have smaller facilities, which may be attributed to the volume of activities required and the associated construction and operational costs;
- Low participation in international networks: insufficient involvement in international networks appears to be a shared weakness in both scenarios;
- Location of HEPA filter boxes and EDS in a contained area: this concept appears to be more prevalent in Brazilian HCBL;
- Presence of pass-throughs for small volume transfer: this practice appears to be more commonly adopted in Brazilian HCBL;
- Validation of decontamination methods: it appears that the validation of decontamination methods is more consistently practiced in the RAV3N HCBL;
- Research with animals: a smaller number of Brazilian HCBL engage in live animal research compared to the RAV3N scenario;

- g. Capacity building: capacity building emerges as one of the most significant differences between the two scenarios, with more favorable outcomes observed in RAV3N HCBL;
- h. Occupational health: the procedures for the promotion of occupational health also emerge as one of the most notable distinctions between the two scenarios, with RAV3N HCBL demonstrating superior outcomes;
- i. Accident and incident report: although the number of accidents and incidents is higher among RAV3N laboratories, there may be underreporting among Brazilian HCBL;
- j. Inventory control: inventory control is notably different between the two scenarios, with better practices observed in RAV3N HCBL, probably due to the US FSAP requirements;
- k. Firefighting regulations: this is a critical topic in Brazilian HCBL;
- l. Legal framework for HCBL registration and monitoring: while most HCBLs in the RAV3N scenario participate in the US FSAP program, Brazil has no equivalent regulations.

6.6 Developing a website application as a tool for maintaining information on the High-Containment Biological Laboratories operational in Brazil

6.6.1 Introduction

Information about HCBLs in Brazil is scarce, even among those affiliated with governmental bodies. The absence of reliable and up-to-date information represents a critical vulnerability for decision-makers, including health, agricultural, and defense authorities. Regardless of the laboratory biosafety policy adopted by the BFG, having timely information on HCBLs is essential for enforcing the legal requirements.

As stated in Chapter 2.18 of this study, the 'Global BioLabs' is an initiative of a group of researchers aiming to gather information about the world's maximum-biocontainment laboratory. As a result of their efforts, an open-access website was developed, allowing anyone to view the locations of BSL-4 laboratories on a world map and access additional information such as addresses, affiliations, footage, research focus, operational status, and website links⁵¹.

This project aims to enhance the tool by incorporating information about BSL-3 enhanced laboratories. However, collecting such data on a global scale presents challenges. Nonetheless, we have developed a proposal to structure a similar tool, building upon the data gathered during the current study, with a focus on the Brazilian context.

6.6.2 Results and Discussion

The interactive interface 'BrazilBioLabs' has been developed to deliver an intuitive user experience. The visualization of HCBLs allows filtering by laboratory BSL and state where the facility is located, providing users with a clear and informative spatial representation on the interactive map.

Furthermore, a bar chart has been included, illustrating the distribution of laboratories across different states, categorized by BSL, as depicted in Figures 02-04.

Figure 02. Screenshot of the tool 'BrazilBioLabs' showing the HCBLs location nationwide by their type.



Figure 03. Screenshot of the tool 'BrazilBiolabs' highlighting the HCBLs located in the São Paulo State.

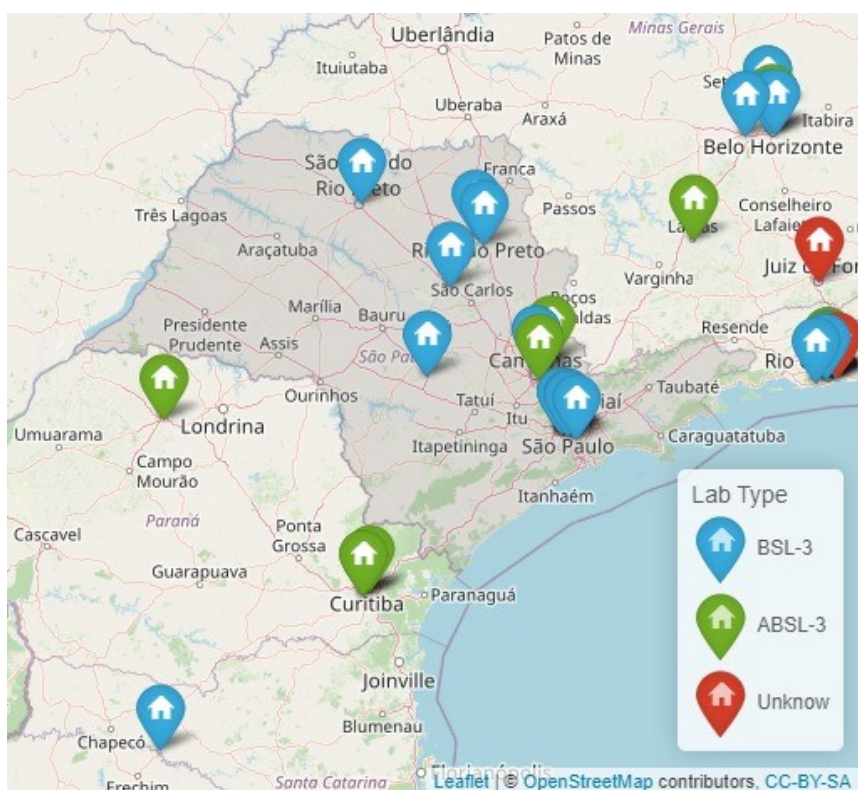
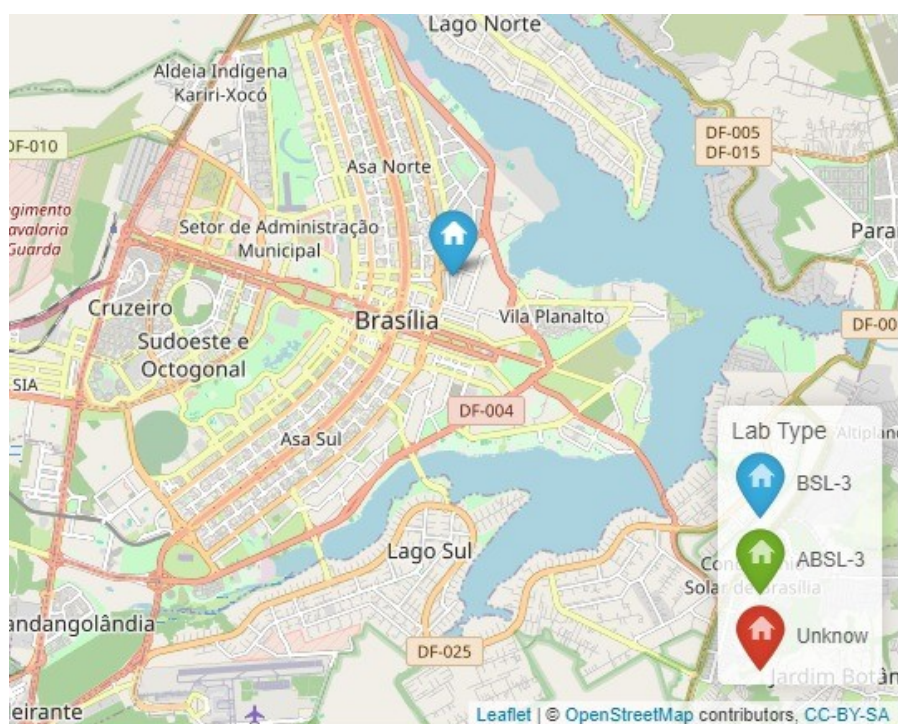


Figure 04. Screenshot of the tool 'BrazilBiolabs' highlighting the specific location of an HCBL.



The initial database was established based on the data collected in the present study. Therefore, for those HCBLs who did not complete the questionnaire, it was not possible to distinguish between BSL-3 and ABSL-3. These laboratories were categorized as 'Unknown'.

The registration process for this tool with the National Institute of Industrial Property (INPI – *Instituto Nacional de Propriedade Industrial*) is currently in progress. Once registration is complete, the tool can be strategically utilized by various authorities, including those in National Defense, Health, Agriculture, Science and Technology, and Education, among others. These authorities will have the capability to input and manage sensitive information on the platform, including comprehensive details related to conducted research, available laboratory methodologies, manipulated microorganisms, and information about responsible managers and researchers.

Upon establishing an oversight process, potentially involving the registration of all HCBLs operating within the country, the application is ready to be updated with information furnished by the competent authority. It ensures that the platform remains current and reflects the prevailing operational landscape of HCBLs nationwide.

In contrast to the 'Global BioLabs', the aim of the 'BrazilBioLabs' is not to provide open access to the general public, given the sensitive nature of the information to be provided. Potentially, basic information may be made publicly available by defining different levels of access.

This concern pertains to the concepts of biosecurity and cyberbiosecurity. While open-access offers the advantage of providing information to the general public in accordance with the principle of transparency, we cannot ignore the potential misuse of the same information by criminals, including those associated with terrorist groups, as outlined in Chapter 2.7 of this thesis.

Here are some examples of potential future applications for this tool:

- a. Health and agricultural authorities could utilize the tool to identify experts specializing in specific pathogens and diseases during sanitary threats or outbreaks in certain regions of the country;
- b. The BFG could establish networks to address specific issues, involving experts from various institutes, particularly considering the 'One Health' approach;
- c. Federal inspectors could utilize the tool to investigate complaints or suspicious activities;
- d. The information provided, including the number and location of HCBLs, as well as the pathogens handled and methodologies available in the laboratories, could prove invaluable during the development of the PNBB and its regulation. This data can help determine the demands of the BFG;
- e. During severe crises such as strikes, power outages, floods, extreme environmental events, civil unrest, or the inoperability of critical infrastructure, federal authorities may promptly assess whether HCBLs could be impacted and the possible consequences;
- f. Supporting agencies that fund research projects and other activities could use the tool to conduct a preliminary assessment of the biocontainment features of a specific facility when reviewing a proposal;
- g. Enabling government entities to review and develop approaches and strategies.

In conclusion, creating the interactive interface 'BrazilBioLabs' represents a significant achievement in delivering an intuitive and informative user experience. This initiative is forward-looking, promoting transparency, collaboration, and

heightened awareness in biosafety, biosecurity, intelligence, one health, and national defense.

6.7 Limitations of the present study

This research, while extensive, encounters several limitations that merit consideration for interpreting the findings and guiding future inquiries:

1. **Self-Reporting Bias:** The reliance on self-reported data introduces potential biases, including the possibility of misunderstanding questions, reluctance to provide negative responses, or inaccuracies due to a lack of detailed knowledge. Such biases can impact the precision and reliability of the collected data, as noted by other authors who have worked with surveys involving biosafety and biosecurity. They observed a tendency among respondents to conceal weaknesses and vulnerabilities.^{34, 211}

2. **Expertise and Understanding of Respondents:** Assuming responses were provided by biosafety experts, the study acknowledges the possibility that not all participants possessed a comprehensive understanding of critical biosafety and biosecurity concepts. This variability in expertise could impact the accuracy of responses related to specific technical aspects. Therefore, we cannot overlook the potential for conceptual misunderstandings of specific terms such as commissioning, certification, and system validations.

3. **Non-Response and Missing Data:** The extensive length of the survey contributed to a relatively high rate of non-responses and skipped questions, which could potentially skew the results and limit the completeness of the dataset. Some respondents may have been reluctant to diligently cross-check responses or seek input from experts in specific fields, such as engineering and finance staff.

4. **Generalizability of Findings:** The study's findings, based on a specific sample of Brazilian HCBLs, may not fully represent the broader national context due to the sample size and the self-selection of participating laboratories. Considering that many laboratories abstained from participating in this study due to a lack of BOs or other personnel capable of providing answers, it is expected that the conditions in such facilities are, on average, worse than those reported in the present study.

5. **Use of a non-standardized questionnaire.** The author developed the questionnaire, making it an outcome of the present study. However, comparing most of the collected data with previous studies that employed a standardized checklist, such as the WHO's LAT³⁰¹, was not feasible.

In light of these limitations, the study's conclusions should be interpreted with caution, recognizing the potential for overestimation of compliance and underreporting of challenges within Brazilian HCBLs. Future research should aim to address these limitations through on-site evaluations, third-party verifications, and the development of more concise and targeted survey instruments. Additionally, engaging a broader spectrum of HCBLs, including those not represented in this study, will be crucial for developing a more comprehensive understanding of the biosafety and biosecurity landscape in Brazil.

7. CONCLUSIONS

This study systematically explores the current state and challenges facing HCBLs in Brazil, providing critical insights and actionable recommendations for enhancing biosafety and biosecurity practices. The findings underscore a significant need for an authoritative national policy and strategic oversight to govern the establishment, operation, and maintenance of HCBLs across the country. Key conclusions drawn from the research include:

1. Urgent need for a national biosafety and biosecurity framework and database: The lack of a unified policy framework and comprehensive data on HCBLs, particularly those affiliated with EBSEH, private companies, and universities, significantly impedes the country's ability to effectively manage biocontainment facilities. Establishing a PNBB is imperative to address these gaps and ensure a coordinated approach to managing biological risks.
2. Strategic planning for HCBLs: Brazil must adopt a strategic plan to evaluate and optimize the distribution and functionality of HCBLs. This plan should consider the geographical distribution of the laboratories, their research focus, and their contribution to public and animal health, preventing redundancy and ensuring efficient use of resources. Additionally, it is crucial to consider the necessity and role of multifunctional BSL-4 and ABSL-3Ag laboratories in the country to address vulnerabilities stemming from the absence of such facilities for public and animal health.
3. Regulatory and oversight mechanisms: The study reveals a critical need for a centralized oversight mechanism for HCBLs, similar to those in place in the US and Canada, which also encompasses DURC monitoring. Such a mechanism would standardize biosafety and biosecurity practices, facilitate resource sharing, and enhance the national culture of safety and security.
4. Financial sustainability and operational challenges: Many HCBLs face financial and operational challenges that compromise their sustainability. Addressing these issues requires identifying long-term funding sources, improving infrastructure and maintenance practices, and ensuring that HCBLs operate within their intended biosafety levels. In this regard, managers and BOs must possess a more comprehensive understanding of and interest in financial and maintenance matters, particularly concerning HVAC and EDS systems. The validation of decontamination methods is an issue that requires more attention and improvement.
5. Capacity building and biosafety and biosecurity culture: Enhancing the biosafety and biosecurity culture and knowledge among HCBL personnel is essential. This involves establishing national training programs, promoting adherence to international standards, and fostering a culture of continuous improvement and accountability. HCBLs must be encouraged to implement biorisk management systems, with a focus on risk assessment. It is important to emphasize the need to refine the roles of BOs and IBCs. Mechanisms to incentivize and enforce the notification of accidents and incidents should be established. An urgent need exists to strengthen the culture of biosecurity in light of the high levels of urban violence and crime across the country.
6. Legal and regulatory reforms: The study advocates for comprehensive legal and regulatory reforms to establish clear standards for the design, construction, commissioning, certification, operation, and decommissioning of HCBLs. These reforms should align with international guidelines and be adaptable to the evolving landscape of biological research and biosecurity threats.

7. National and international collaboration: Strengthening collaboration within the national HCBL network and with international partners is crucial for sharing best practices, resources, and expertise. This collaboration will enhance Brazil's capacity to address biosecurity challenges effectively and contribute to global health security. It is desirable to identify laboratories that can serve as national references for biosafety and biosecurity.

8. Strengthening services provided by national companies: It is crucial to encourage the participation of more domestic companies in the design, construction, commissioning, certification, and maintenance of Brazilian HCBLs to disseminate the expertise associated with this market across the country. Design companies should be aware that the most significant design deficiencies identified were associated with EDS, HVAC systems, and airlocks/pass-throughs. Additionally, autoclaves received the most unfavorable evaluation regarding the quality of external services. Domestic companies may consider implementing water and electricity-saving mechanisms in future projects. The concept of a dry HCBL should always be taken into consideration during the preliminary studies and risk assessments. It is concerning that Brazil continues to rely heavily on the external market for ultra-low freezers.

8. FINAL CONSIDERATIONS

According to the official documents and literature review, the current study was the most comprehensive study on the Brazilian HCBLs.

The presented results provide a comprehensive overview of the current state of Brazilian HCBLs, including their number, scope, affiliation, geographical distribution, infrastructure, equipment and maintenance, biosafety and biosecurity management, financial status and sustainability, human resources, use of laboratory animals, as well as their primary strengths, weaknesses, and challenges.

These results raise concerns about the vulnerabilities of certain facilities, which could serve as an alert to both Brazilian society and authorities, emphasizing the urgent need to enhance control measures. Furthermore, managers, BOs, PIs, and other users of HCBLs must be aware of the risks associated with their work. The enhancement and widespread promotion of a biosafety and biosecurity culture nationwide, grounded in a scientific approach, are crucial to avoiding a false sense of safety and overconfidence while working with high-risk pathogens.

Most of the identified vulnerabilities could be mitigated by developing a national regulatory framework to address laboratory biosafety and biosecurity.

Enhancing the Brazilian legal framework on laboratory biosafety and biosecurity is necessary and urgent. By strengthening the regulatory framework, the risks of LAIs would be minimized, as well as the occurrence of accidents that could lead to the release of high-risk pathogens with significant implications for public and animal health.

It will also be necessary to define if the country prefers adopting a biological select agents and toxins program, such as the US FSAP or Australian SSBA, or a more comprehensive approach, including all HCBLs in the monitoring process, as established by the Canadian legal framework. Regardless, it is advisable to emphasize the relevance of risk assessment in biorisk management and the adoption of ISO 35001:2019, or another international guideline, as a national standard.

Compliance with regulations and international recommendations on laboratory biosafety and biosecurity is essential and should encompass all stages of an HCBL's lifecycle, including design, construction, certification, operation, and decommissioning.

The increasing private research on infectious diseases worldwide, including Brazil, and the plan to build a laboratory with the characteristics of a maximum-containment facility in the country reinforce the need to prove reliability to the international community. Having a more comprehensive regulation is an appropriate step for that. Taking advantage of the lessons learned and regulations applied in other countries can be helpful if adjusted to the national context and priorities.

This approach should involve the scientific community and civil society throughout the process. Establishing a balanced legal framework that ensures effective oversight without overwhelming institutions or discouraging research on high-risk pathogens is essential.

Besides the regulation improvement, other actions are necessary to strengthen the laboratory biosafety and biosecurity culture in Brazil, like supporting education, technological development, innovation, and research on this topic, coordinating a 'National Training Program', creating and overseeing an 'HCBL Network', designing an action plan to adequate the national infrastructure, identifying needs about the amount and biosafety levels of the official laboratories and the scope of their activities, defining a mechanism to certify HCBLs, and coordinating partnership initiatives at the national and international level.

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APPENDIX A – Tables generated from the questionnaire

Table A01 – Results regarding the affiliation of the HCBLs participating in the survey.

Variable	Values	Freq. (% of Valid) ^{yy}	Valid	Missing
Laboratory affiliation	Hybrid Private Public (Federal) Public (State)	2 (6.2%) 3 (9.4%) 19 (59.4%) 8 (25.0%)	32 (100.0%)	0 (0.0%)
In the case of a public or hybrid lab, please inform the governmental body to which it is linked	Agriculture State Secretariat Health State Secretariat MAPA MCTI MS University (Federal) University (State)	1 (3.4%) 4 (13.8%) 5 (17.2%) 1 (3.4%) 6 (20.7%) 7 (24.1%) 5 (17.2%)	29 (90.6%)	3 (9.4%)

^{yy} During the presentation of the study's results, the term 'missing' was used to indicate answers that were not applicable. The term 'valid' was applied to the total number of participants (n = 32), excluding the 'missing' responses. The frequency values represent only the 'valid' answers.

Table A02 – Results regarding the profile of the respondents of the survey.

Variable	Values	Freq. (% of Valid)	Valid	Missing
Job title	Professor/Researcher	15 (46.9%)	32 (100.0%)	0 (0.0%)
	Federal Inspector	3 (9.4%)		
	Biosafety Officer	2 (6.3%)		
	Pharmacist	2 (6.3%)		
	Technologist	2 (6.3%)		
	Others	8 (25.0%)		
Role in the HCBL	Head of the lab	16 50.0%)	32 (100.0%)	0 (0.0%)
	Biosafety Officer	7 (21.9%)		
	Manager	6 (18.8%)		
	Engineer	1 (3.1%)		
	Researcher	1 (3.1%)		
	Veterinarian	1 (3.1%)		

Table A03 – Results regarding the networking and coverage of the HCBLs participating in the survey.

Variable	Values	Freq. (% of Valid)	Valid	Missing
Does the lab belong to any national network?	No Yes	19 (59.4%) 13 (40.6%)	32 (100.0%)	0 (0.0%)
If so, please inform us which network.	EMBRAPA CGAL/SDA/MAPA RNLSP/CGLAB/SVS/MS	2 (15.4%) 3 (23.1%) 8 (61.5%)	13 (40.6%)	19 (59.4%)
Does the lab belong to any international network?	No Yes	27 (84.4%) 5 (15.6%)	32 (100.0%)	0 (0.0%)
What's the lab coverage (please consider the widest)?	International Municipal National No answer State	7 (21.9%) 1 (3.1%) 13 (40.6%) 3 (9.4%) 8 (25.0%)	32 (100.0%)	0 (0.0%)

Table A04 – Results regarding the yearly average number of assays conducted by the HCBLs participating in the survey.

HCBL	Assays carried out
1	240
2	20,000
3	7,346
4	20,000
5	1,000
6	6,941
7	22,000
8	9,800
9	700
10	3,000
11	6,415
Mean	8,858
Median	6,941

Table A05 – Results regarding the design and construction features of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How much was the design? (R\$)	10,000.00 ^{zz} 220,500.00 ^{aaa} 75,000.00 ^{bbb} Confidential information I don't know No answer The cost of the design was included in the construction cost	1 (3.1%) 1 (3.1%) 1 (3.1%) 1 (3.1%) 12 (37.5%) 13 (40.6%) 3 (9.4%)	32 (100.0%)	0 (0.0%)
Are the current activities the same as those foreseen in the design?	Most activities are not Yes, most of them are Yes, totally	2 (6.7%) 9 (30.0%) 19 (63.3%)	30 (93.8%)	2 (6.2%)
Mark the most appropriate response to the kind of construction:	Expansion or remodeling New lab No answer	12 (37.5%) 19 (59.4%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
Does the building include other facilities besides the BSL-3 lab?	I don't know No No answer Yes	1 (3.1%) 10 (31.2%) 9 (28.1%) 12 (37.5%)	32 (100.0%)	0 (0.0%)
Does the lab periodically carry out certifications/qualifications of the equipment/systems?	I don't know No answer Yes	1 (3.3%) 3 (10.0%) 26 (86.7%)	30 (93.8%)	2 (6.2%)
In your opinion, does the design have relevant shortcomings?	I don't know No No answer Yes	4 (12.5%) 12 (37.5%) 5 (15.6%) 11 (34.4%)	32 (100.0%)	0 (0.0%)

^{zz} Equivalent to USD 1,968.35, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{aaa} Equivalent to USD 43,402.09, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

^{bbb} Equivalent to USD 14,762.62, as per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

Table A06 – Results regarding the construction cost of the HCBLs participating in the survey.

Laboratory	Cost of construction (R\$)	Cost of construction (USD) ^{ccc}	New lab or expansion/remodeling?	Period of construction	Does the value reported include other facilities besides the BSL-3 lab?	BSL-3 footage (m ²)?	Average cost (R\$/m ²)	Average cost (USD) ⁴⁸
A	30,000,000.00	5,905,046.85	New lab	2011-2020	Yes	374.00	80,213.90	15,788.89
B	1,000,000.00	196,834.89	Expansion/remodeling	2007-2007	No	100.00	10,000.00	1,968.35
C	400,000.00	78,733.96	Expansion/remodeling	2017-2018	No	40.00	10,000.00	1,968.35
D	11,995,623.07	2,361,157.21	New lab	2009-2015	Yes	114.49	104,774.42	20,623.26
E	3,000,000.00	590,504.68	New lab	2008-2016	Yes	124.00	24,193.55	4,762.13
F	2,000,000.00	393,669.79	New lab	1997-1999	Yes	20.00	100,000.00	19,683.49
G	50,000,000.00	9,841,744.74	New lab	2007-2008	Yes	2,396.71	20,861.93	4,106.36
H	1,787,100.00	351,763.64	New lab	2020-2023	No	28.00	63,825.00	12,562.99
I	700,000.00	137,784.43	Expansion/remodeling	2021-2022	No	70.00	10,000.00	1,968.35
J	41,000,000.00	8,070,230.69	New lab	2007-2009	Yes	10,000.00	4,100.00	807.02
K	8,843,369.20	1,740,683.65	New lab	2005-2010	Yes	2,200.00	4,019.71	791.22
L	7,500,000.00	1,476,261.71	Expansion/remodeling	2000-2003	Yes	644.82	11,631.15	2,289.42
M	1,500,000.00	295,252.34	Expansion/remodeling	2013-2014	Yes	41.00	36,585.37	7,201.28
N	1,400,000.00	275,568.85	Expansion/remodeling	2021-2022	Yes	30.00	46,666.67	9,185.63
O	800,000.00	157,467.92	Expansion/remodeling	2022-2022	No	40.00	20,000.00	3,936.70
P	1,200,000.00	236,201.87	New lab	2022-2022	No	74.00	16,216.22	3,191.92

^{ccc} As per the currency conversion tool provided by the Brazilian Central Bank (<https://cdn-www.bcb.gov.br/conversao>). Reference data applied: March 31, 2023.

Table A07 – Results regarding general constructive features of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Number of floors (including non-contained floors)	1 2 3 4 No answer	15 (46.9%) 4 (12.5%) 7 (21.9%) 1 (3.1%) 5 (15.6%)	32 (100.0%)	0 (0.0%)
Is the BSL-3 lab conjugated to BSL-2 and or BSL-1 labs?	No No answer Yes (BSL-1) Yes (BSL-2 and BSL-1) Yes (BSL-2)	7 (21.9%) 2 (6.2%) 2 (6.2%) 4 (12.5%) 17 (53.1%)	32 (100.0%)	0 (0.0%)
Are the walls and ceiling demonstrably sealed?	I don't know No Partially Yes	6 (18.8%) 3 (9.4%) 2 (6.2%) 21 (65.6%)	32 (100.0%)	0 (0.0%)
Are there windows in the BSL-3 facing the outside area?	No No answer Yes	15 (46.9%) 5 (15.6%) 12 (37.5%)	32 (100.0%)	0 (0.0%)
If so, please mark the kind of windows	Double armored glass Double glass	4 (33.3%) 8 (66.7%)	12 (37.5%)	20 (62.5%)
Are these windows demonstrably sealed?	I don't know No No answer Yes	2 (16.7%) 3 (25.0%) 1 (8.3%) 6 (50.0%)	12 (37.5%)	20 (62.5%)
Are there windows in the BSL-3 facing the inner area?	No No answer Yes	10 (31.2%) 5 (15.6%) 17 (53.1%)	32 (100.0%)	0 (0.0%)
If so, please mark the kind of windows.	Double armored glass Double glass I don't know Single armored glass	2 (11.8%) 13 (76.5%) 1 (5.9%) 1 (5.9%)	17 (53.1%)	15 (46.9%)
Are these windows demonstrably sealed?	I don't know No Yes	1 (5.9%) 2 (11.8%) 14 (82.4%)	17 (53.1%)	15 (46.9%)
Are there curtains or shutters inside the lab?	No No answer	24 (75.0%) 8 (25.0%)	32 (100.0%)	0 (0.0%)
Are there currently cracks on the inner walls?	No No answer Yes	29 (90.6%) 1 (3.1%) 2 (6.2%)	32 (100.0%)	0 (0.0%)
Are there currently cracks on the floor?	No No answer	29 (90.6%) 2 (6.2%) 1 (3.1%)	32 (100.0%)	0 (0.0%)

Table A08 – Results regarding the water supply for the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
What's the laboratory's source of potable water?	Artesian well No answer Public supply	5 (16.7%) 6 (20.0%) 19 (63.3%)	30 (93.8%)	2 (6.2%)
How do you rate how often the water supply is cut off?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	20 (66.7%) 8 (26.7%) 2 (6.7%) 0 (0.0%) 0 (0.0%) 0 (0.0%)	30 (93.8%)	2 (6.2%)
Does the lab have any mechanism to save water?	I don't know No No answer Yes	2 (6.7%) 16 (53.3%) 6 (20.0%) 6 (20.0%)	30 (93.8%)	2 (6.2%)
Are there floor drains inside the lab?	I don't know No No answer Yes	1 (3.1%) 20 (62.5%) 5 (15.6%) 6 (18.8%)	32 (100.0%)	0 (0.0%)
Are there backflow prevention devices in the water and gas pipes?	I don't know No No answer Yes	5 (16.7%) 7 (23.3%) 5 (16.7%) 13 (43.3%)	30 (93.8%)	2 (6.2%)
How do you rate how often leaks are observed in the water piping?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	24 (82.8%) 2 (6.9%) 1 (3.4%) 1 (3.4%) 1 (3.4%) 0 (0.0%)	29 (90.6%)	3 (9.4%)
Are the pipes and conduits visible and easily cleanable?	I don't know Most of them are Most of them are not No No answer Yes, all of them	1 (3.1%) 4 (12.5%) 3 (9.4%) 5 (15.6%) 2 (6.2%) 17 (53.1%)	32 (100.0%)	0 (0.0%)

Table A09 – Results regarding the gas supply for the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Is there a compressed air supply?	I don't know No No answer Yes	1 (3.1%) 10 (31.2%) 6 (18.8%) 15 (46.9%)	32 (100.0%)	0 (0.0%)
How do you rate how often the compressed air supply is cut off?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	8 (53.3%) 4 (26.7%) 0 (0.0%) 1 (6.7%) 1 (6.7%) 1 (6.7%)	15 (46.9%)	17 (53.1%)
Are there gas cylinders located inside the lab?	No No answer Yes	19 (59.4%) 6 (18.8%) 7 (21.9%)	32 (100.0%)	0 (0.0%)
If so, what kind of gas is stored in these cylinders?	CO ₂ N ₂ O ₂	5 (71.4%) 1 (14.3%) 1 (14.3%)	7 (21.9%)	25 (78.1%)

Table A10 – Results regarding the access control and changing room of the HCBLs participating in the survey HCBL.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab keep records of each access to the BSL-3 area?	No answer No, the lab does not record access Yes, the lab records access manually, but only for visits Yes, the lab records any access electronically Yes, the lab records any access manually Yes, the lab records any access manually and electronically	2 (6.2%) 4 (12.5%) 1 (3.1%) 18 (56.2%) 4 (12.5%) 3 (9.4%)	32 (100.0%)	0 (0.0%)
Is there a changing room dedicated exclusively to the BSL-3 access?	No Yes	2 (6.2%) 30 (93.8%)	32 (100.0%)	0 (0.0%)
If so, are there exclusive changing rooms for men and women?	No No answer Yes	18 (60.0%) 6 (20.0%) 6 (20.0%)	30 (93.8%)	2 (6.2%)
Are the changing rooms divided into clean and dirty areas?	No No answer Yes	5 (16.7%) 6 (20.0%) 19 (63.3%)	30 (93.8%)	2 (6.2%)
Are the changing rooms provided with a shower?	No No answer Yes	8 (26.7%) 2 (6.7%) 20 (66.7%)	30 (93.8%)	2 (6.2%)
If so, is bathing mandatory?	No No answer Yes, to exit	7 (35.0%) 2 (10.0%) 11 (55.0%)	20 (62.5%)	12 (37.5%)
Does an automated system control mandatory bathing?	No Yes	4 (36.4%) 7 (63.6%)	11 (34.4%)	21 (65.6%)
Is bath water drained to a waste treatment system?	I don't know No Yes	1 (5.0%) 1 (5.0%) 18 (90.0%)	20 (62.5%)	12 (37.5%)
Is the air of the bath box filtered (to decontaminate aerosols generated during bathing)?	I don't know No No answer Yes	2 (10.0%) 4 (20.0%) 3 (15.0%) 11 (55.0%)	20 (62.5%)	12 (37.5%)
Considering the current staff, how do you rate the footage from the changing rooms?	Insufficient No answer Suitable	5 (17.2%) 5 (17.2%) 19 (65.5%)	29 (90.6%)	3 (9.4%)
Is there an SOP describing the training procedures for visitors' entrance?	No No answer Yes	7 (21.9%) 1 (3.1%) 24 (75.0%)	32 (100.0%)	0 (0.0%)

Table A11 – Results regarding the internal air pressure of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Is the lab air pressure negative in comparison to the outside environment?	Yes	32 (100.0%)	32 (100.0%)	0 (0.0%)
Are there differential air pressures between the BSL-3 rooms?	No Yes	8 (25.0%) 24 (75.0%)	32 (100.0%)	0 (0.0%)
Please mark the most appropriate answer related to the air pressure monitoring	The air pressure is automatically monitored The air pressure is manually and automatically monitored The air pressure is manually monitored The air pressure is not monitored	15 (46.9%) 11 (34.4%) 5 (15.6%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
How do you rate how often the lab air pressure does not fulfill the established parameters?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	12 (40.0%) 8 (26.7%) 3 (10.0%) 1 (3.3%) 2 (6.7%) 4 (13.3%)	30 (93.8%)	2 (6.2%)

Table A12 – Results regarding the air filtration of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Are there filters installed in the air supply?	I don't know No answer Yes	2 (6.2%) 3 (9.4%) 27 (84.4%)	32 (100.0%)	0 (0.0%)
If so, please describe the kind of filters installed.	HEPA No answer Non-HEPA	14 (51.9%) 8 (29.6%) 5 (18.5%)	27 (84.4%)	5 (15.6%)
Are there filters installed in the air exhaustion?	I don't know No answer Yes	1 (3.1%) 4 (12.5%) 27 (84.4%)	32 (100.0%)	0 (0.0%)
If so, please describe the kind of filters installed	HEPA No answer	22 (81.5%) 5 (18.5%)	27 (84.4%)	5 (15.6%)
Is the air exhaust filtration system redundant?	I don't know No Yes	5 (18.5%) 4 (14.8%) 18 (66.7%)	27 (84.4%)	5 (15.6%)
How do you decontaminate the filters when replacing them?	Autoclave Formaldehyde H ₂ O ₂ and hypochlorite I don't know Incineration No answer UV VHP	5 (15.6%) 4 (12.5%) 1 (3.1%) 1 (3.1%) 1 (3.1%) 16 (50.0%) 1 (3.1%) 3 (9.4%)	32 (100.0%)	0 (0.0%)
Does the lab periodically monitor the efficiency of the filters installed in the air exhaust system?	I don't know No No answer Yes	3 (9.7%) 3 (9.7%) 4 (12.9%) 21 (67.7%)	31 (96.9%)	1 (3.1%)
Are the exhaust air filter boxes installed in a contained room?	I don't know No No answer Yes	3 (9.4%) 17 (53.1%) 2 (6.2%) 10 (31.2%)	32 (100.0%)	0 (0.0%)

Table A13 – Results regarding additional information on the HVAC system of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Is the air exhaust system redundant?	I don't know No No answer Yes	7 (21.9%) 4 (12.5%) 2 (6.2%) 19 (59.4%)	32 (100.0%)	0 (0.0%)
Does the HVAC system have sealing dampers?	I don't know No No answer Yes	5 (15.6%) 1 (3.1%) 6 (18.8%) 20 (62.5%)	32 (100.0%)	0 (0.0%)
Does the HVAC system permit air recirculation? If so, what is the percentage of the recirculated air?	100% 75% I don't know No No answer	2 (6.2%) 1 (3.1%) 3 (9.4%) 16 (50.0%) 10 (31.2%)	32 (100.0%)	0 (0.0%)
How do you rate the security level of the place where the Air Handling Units are installed?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	1 (3.1%) 2 (6.2%) 3 (9.4%) 9 (28.1%) 10 (31.2%) 7 (21.9%)	32 (100.0%)	0 (0.0%)
Was the HVAC system nationally manufactured?	I don't know No No answer Partially Yes	10 (31.2%) 3 (9.4%) 8 (25.0%) 2 (6.2%) 9 (28.1%)	32 (100.0%)	0 (0.0%)
Please mark the most appropriate response related to the air temperature monitoring:	No answer The air temperature is automatically monitored The air temperature is manually and automatically monitored The air temperature is manually monitored The air temperature is not monitored	2 (6.2%) 17 (53.1%) 5 (15.6%) 6 (18.8%) 2 (6.2%)	32 (100.0%)	0 (0.0%)
How do you rate how often the lab air temperature does not fulfill the established parameters?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	8 (25.8%) 6 (19.4%) 5 (16.1%) 5 (16.1%) 1 (3.2%) 6 (19.4%)	31 (96.9%)	1 (3.1%)

Table A14 – Results regarding power transformers and UPS installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the BSL-3 have dedicated power transformers?	I don't know No No answer Yes	3 (10.0%) 9 (30.0%) 5 (16.7%) 13 (43.3%)	32 (100.0%)	0 (0.0%)
If so, how many?	1 2 3 9 No answer	4 (30.8%) 3 (23.1%) 3 (23.1%) 1 (7.7%) 2 (15.4%)	13 (40.6%)	19 (59.4%)
What's the average percentage of the lab demand met by the current power supply capacity?	Between 100% and 150% I don't know Less than 100% More than 200% No answer	1 (7.1%) 3 (21.4%) 4 (28.6%) 2 (14.3%) 4 (28.6%)	14 (43.8%)	18 (56.2%)
What's the percentage of the lab demand met by the current power supply capacity during the peak of consumption?	Between 150% and 200% I don't know Less than 100% No answer	2 (14.3%) 2 (14.3%) 6 (42.9%) 4 (28.6%)	14 (43.8%)	18 (56.2%)
How do you rate the security level of the place where the power transformers are installed?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	1 (5.0%) 0 (0.0%) 3 (15.0%) 3 (15.0%) 9 (45.0%) 4 (20.0%)	20 (62.5%)	12 (37.5%)
Is the BSL-3 lab covered by a UPS system?	I don't know No No answer Yes	1 (3.1%) 10 (31.2%) 3 (9.4%) 18 (56.2%)	32 (100.0%)	0 (0.0%)
If so, how long is its autonomy? (minutes)	10 30 35 60 I don't know No answer	1 (5.3%) 6 (31.6%) 1 (5.3%) 1 (5.3%) 3 (15.8%) 7 (36.8%)	19 (59.4%)	13 (40.6%)

Table A15 – Results regarding power generators installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Are power generators tested periodically by a specialized technical team?	I don't know Yes	1 (3.6%) 27 (96.4%)	28 (87.5%)	4 (12.5%)
If so, what is the frequency of the test?	Annually Biannually Bimonthly Monthly More than once per month No answer Occasionally Quarterly	1 (3.7%) 3 (11.1%) 3 (11.1%) 5 (18.5%) 6 (22.2%) 6 (22.2%) 1 (3.7%) 2 (7.4%)	27 (84.4%)	5 (15.6%)
Is there currently a preventive maintenance service for the power generators provided by a specialized technical team?	I don't know No No answer Yes	1 (3.4%) 6 (20.7%) 6 (20.7%) 16 (55.2%)	29 (90.6%)	3 (9.4%)
How do you rate the security level of the place where the power generators are installed?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 1 (3.3%) 2 (6.7%) 6 (20.0%) 13 (43.3%) 8 (26.7%)	30 (93.8%)	2 (6.2%)
How do you rate how often there is any impact on the activities carried out by the lab when there is power electricity cut off?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	3 (9.4%) 8 (25.0%) 11 (34.4%) 7 (21.9%) 2 (6.2%) 1 (3.1%)	32 (100.0%)	0 (0.0%)

Table A16 – Results regarding Fumigation chambers/Airlocks/pass-throughs installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Do the Fumigation Chamber/Airlocks have an interlocking door system?	No No answer Yes	1 (4.8%) 1 (4.8%) 19 (90.5%)	21 (65.6%)	11 (34.4%)
Are the Fumigation Chamber/Airlocks sealed?	I don't know No Yes	1 (4.8%) 3 (14.3%) 17 (81.0%)	21 (65.6%)	11 (34.4%)
Has the decontaminating method been validated?	I don't know No No answer Yes	4 (21.1%) 7 (36.8%) 1 (5.3%) 7 (36.8%)	19 (59.4%)	13 (40.6%)
Do the Fumigation Chamber/Airlocks have their own system for inflating and exhausting the air?	I don't know No No answer Yes	2 (9.1%) 5 (22.7%) 7 (31.8%) 8 (36.4%)	22 (68.8%)	10 (31.2%)
Are there cracks inside the Fumigation Chamber/Airlocks?	I don't know No No answer Yes	1 (4.5%) 14 (63.6%) 6 (27.3%) 1 (4.5%)	22 (68.8%)	10 (31.2%)
Are the Fumigation Chamber/Airlocks nationally manufactured?	I don't know No No answer Yes	4 (17.4%) 1 (4.3%) 8 (34.8%) 10 (43.5%)	23 (71.9%)	9 (28.1%)
Does the pass-through have an interlocking door system?	No answer No Yes	2 (6.9%) 1 (3.4%) 26 (89.7%)	29 (90.6%)	3 (9.4%)
Has the pass-through decontaminating method been validated?	I don't know No No answer Yes	5 (17.9%) 10 (35.7%) 2 (7.1%) 11 (39.3%)	28 (87.5%)	4 (12.5%)

Table A17 – Results regarding the decontamination methods of the HCBLs participating in the current survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Is there a set periodicity for the decontamination of the laboratory as a whole?	I don't know No No answer Yes	3 (9.4%) 10 (31.2%) 3 (9.4%) 16 (50.0%)	32 (100.0%)	0 (0.0%)
If so, what is the frequency?	Annually Biannually Daily Every time the lab is used More than once per semester No answer	6 (37.5%) 2 (12.5%) 1 (6.2%) 1 (6.2%) 5 (31.2%) 1 (6.2%)	16 (50.0%)	16 (50.0%)
Has the decontaminating method been validated?	I don't know No No answer Yes	3 (10.0%) 11 (36.7%) 6 (20.0%) 10 (33.3%)	30 (93.8%)	2 (6.2%)

Table A18 – Results regarding the EDS installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
What method is used for decontaminating the effluent generated in the BSL-3 lab?	Chemical No answer There is no effluent Thermal Thermal, Chemical Thermo-Chemical	5 (15.6%) 1 (3.1%) 6 (18.8%) 17 (53.1%) 1 (3.1%) 2 (6.2%)	32 (100.0%)	0 (0.0%)
Has that method been validated?	I don't know No No answer Yes	1 (4.0%) 10 (40.0%) 2 (8.0%) 12 (48.0%)	25 (78.1%)	7 (21.9%)
Please inform us of the EDS manufacturer.	A B C D E F I don't know No answer	1 (3.8%) 1 (3.8%) 3 (11.5%) 1 (3.8%) 2 (7.7%) 1 (3.8%) 1 (3.8%) 16 (61.5%)	26 (81.2%)	6 (18.8%)
Is the effluent decontamination system installed in a contained area?	No No answer Yes	11 (42.3%) 4 (15.4%) 11 (42.3%)	26 (81.2%)	6 (18.8%)
How do you rate how often leaks are observed in the effluent decontamination system?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	15 (60.0%) 1 (4.0%) 0 (0.0%) 1 (4.0%) 0 (0.0%) 8 (32.0%)	25 (78.1%)	7 (21.9%)
Is there redundancy in the effluent decontamination system?	No No answer Yes	10 (40.0%) 8 (32.0%) 7 (28.0%)	25 (78.1%)	7 (21.9%)
What is the maximum capacity of the effluent decontamination system? (L)	50-1000 1001-10000 10001-30000 > 30000	6 (50.0%) 4 (33.3%) 1 (8.3%) 1 (8.3%)	12 (37.5%)	20 (62.5%)
How do you rate how often that capacity meets the lab demand?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 4 (16.0%) 0 (0.0%) 0 (0.0%) 15 (60.0%) 6 (24.0%)	25 (78.1%)	7 (21.9%)
Are qualification tests for the effluent decontamination system carried out periodically?	I don't know No No answer Yes	2 (8.7%) 6 (26.1%) 4 (17.4%) 11 (47.8%)	23 (71.9%)	9 (28.1%)
If so, what is the frequency of the test?	Annually Biannually No answer	6 (54.5%) 2 (18.2%) 3 (27.3%)	11 (34.4%)	21 (65.6%)
Does the lab keep records of each effluent decontamination cycle?	No No answer Yes	7 (30.4%) 6 (26.1%) 10 (43.5%)	23 (71.9%)	9 (28.1%)

Table A19 – Results regarding the autoclaves installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How many autoclaves are there in the BSL-3 lab?	1 2 3 No answer	23 (71.9%) 5 (15.6%) 3 (9.4%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
Does the number and capacity of autoclaves meet the lab demands?	No answer No, usually Yes, always Yes, usually	1 (3.2%) 2 (6.5%) 17 (54.8%) 11 (35.5%)	31 (96.9%)	1 (3.1%)
What kinds of autoclaves are present in the BSL-3 lab?	Horizontal double-door border Vertical double-door border No answer	30 (93.8%) 1 (3.2%) 1 (3.2%)	32 (100.0%)	0 (0.0%)
If there are border autoclaves, do their doors have an interlocking system?	I don't know No No answer Yes	1 (3.2%) 4 (12.9%) 1 (3.2%) 25 (80.6%)	31 (96.9%)	1 (3.1%)
If there are border autoclaves, do they have bioseal?	I don't know No No answer Yes	6 (19.4%) 2 (6.5%) 1 (3.2%) 22 (71.0%)	31 (96.9%)	1 (3.1%)
Have the autoclave's cycles been validated for the different types of loads?	I don't know No No answer Yes, but not for all kinds of loads Yes, for all kinds of loads	3 (9.7%) 3 (9.7%) 3 (9.7%) 5 (16.1%) 17 (54.8%)	31 (96.9%)	1 (3.1%)
Is the output of deaerators and condensates decontaminated?	I don't know No No answer Yes	3 (9.4%) 5 (15.6%) 9 (28.1%) 15 (46.9%)	32 (100.0%)	0 (0.0%)
Does the lab keep records of each autoclaving cycle?	No No answer Yes	3 (9.7%) 3 (9.7%) 25 (80.6%)	31 (96.9%)	1 (3.1%)
In addition to autoclaves, does the BSL-3 lab have other equipment/systems for decontaminating solid waste?	No No answer Yes	26 (81.2%) 1 (3.1%) 5 (15.6%)	32 (100.0%)	0 (0.0%)

Table A20 – Results regarding the place of manufacturing of the autoclaves installed in the HCBLs participating in the survey.

Company	Place of manufacturing	
	Brazil	Other countries
A	11 (39.3%)	
B	7 (25.0%)	
C	3 (10.7%)	
D	3 (10.7%)	
E		2 (7.1%)
F		1 (3.6%)
G		1 (3.6%)
Total	34 (85.7%)	4 (14.3%)

Table A21 – Results regarding the BSCs installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How many BSCs are currently installed in the BSL-3 lab?	1 2 3 4 5 6 7 9 10 No answer	5 (15.6%) 12 (37.5%) 4 (12.5%) 3 (9.4%) 3 (9.4%) 1 (3.1%) 1 (3.1%) 1 (3.1%) 1 (3.1%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
How many installed BSCs are currently out of use (in maintenance, with an expired certification, or for any other reason)? (%)	0 14 20 40 50 60 No answer	24 (77.4%) 1 (3.2%) 1 (3.2%) 1 (3.2%) 1 (3.2%) 1 (3.2%) 2 (6.5%)	31 (96.9%)	1 (3.1%)
Does the current location of BSCs meet the requirements to avoid external interferences, like from the HVAC system, door openings, etc.?	I don't know No answer Yes, always Yes, usually	3 (9.4%) 4 (12.5%) 23 (71.9%) 2 (6.2%)	32 (100.0%)	0 (0.0%)
Are carried out periodically certification tests for BSCs?	I don't know No No answer Yes	1 (3.2%) 1 (3.2%) 3 (9.7%) 26 (83.9%)	31 (96.9%)	1 (3.1%)
If so, what is the frequency of the tests?	Annually Biannually I don't know No answer	11 (36.7%) 13 (43.3%) 1 (3.3%) 5 (16.7%)	30 (93.8%)	2 (6.2%)
Among the currently installed BSCs, how many are with the certification expired? (%)	0 20 50 No answer	18 (58.1%) 1 (3.2%) 1 (3.2%) 11 (35.5%)	31 (96.9%)	1 (3.1%)
Is there an SOP for training users on the proper use of BSCs?	No No answer Yes	3 (9.7%) 3 (9.7%) 25 (80.6%)	31 (96.9%)	1 (3.1%)
If so, do the technicians follow the recommended protocols?	Yes, always Yes, usually	23 (92.0%) 2 (8.0%)	25 (78.1%)	7 (21.9%)
Does the lab use a Bunsen burner inside the BSCs?	No No answer	29 (96.7%) 1 (3.3%)	30 (93.8%)	2 (6.2%)
Are samples and other potentially infectious materials handled exclusively in BSCs?	No No answer Yes	2 (6.5%) 8 (25.8%) 21 (67.7%)	31 (96.9%)	1 (3.1%)

Table A22 – Results regarding the place of manufacturing of the BSCs installed in the HCBLs participating in the survey.

Company	Place of manufacturing	
	Brazil	Other countries
A	11 (35.5%)	
B		6 (19.4%)
C	6 (19.4%)	
D		3 (9.7%)
E	2 (6.5%)	
F		1 (3.2%)
G		1 (3.2%)
H	1 (3.2%)	
Total	20 (64.5%)	11 (35.5%)

Table A23 – Results regarding the cleaning procedures of the BSCs installed in the HCBLs participating in the survey.

Procedures mentioned	Number of HCBL
Ethanol 70%	17
UV light	12
Sodium hypochlorite	4
Disinfectant selected according to the pathogen	4
Citric acid	2
Detergent	2
Iodized alcohol	1
Lysol	1

Table A24 – Results regarding the UV light usage controlling methods of the BSCs installed in the HCBLs participating in the survey.

Method	Number of HCBL
Lamp power testing is carried out during the annual certification by a specialized company	7
Manual control of the UV light's operating hours	5
Automatic control of the UV light's operating hours by the BSC itself	3
Microbiological test	3
There is no kind of control	4
There is no UV light in the BSC	1
No answer	12

Table A25 – Results regarding the freezers, ultra-low freezers, and refrigerators installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How many ultra-low freezers are currently installed in the BSL-3 lab?	0 1 2 3 4 5 7 No answer	6 (18.8%) 10 (31.2%) 6 (18.8%) 1 (3.1%) 4 (12.5%) 1 (3.1%) 1 (3.1%) 3 (9.4%)	32 (100.0%)	0 (0.0%)
How many installed ultra-low freezers are currently out of use (in maintenance or for any other reason)? (%)	0 14 75 100 No answer	17 (77.3%) 1 (4.5%) 1 (4.5%) 2 (9.1%) 1 (4.5%)	22 (68.8%)	10 (31.2%)
Does the current number of refrigerators, freezers, and ultra-low freezers meet the needs of the BSL-3 lab?	No No answer Yes	5 (16.1%) 4 (12.9%) 22 (71.0%)	31 (96.9%)	1 (3.1%)
Are the refrigerators, freezers, and ultra-low freezer models suitable for laboratory use, or are they household equipment?	All equipment is suitable for laboratory use All of them are household equipment Most are household equipment Most equipment is suitable for laboratory use No answer	15 (46.9%) 1 (3.1%) 2 (6.2%) 12 (37.5%) 2 (6.2%)	32 (100.0%)	0 (0.0%)
Please mark the most appropriate response related to the equipment temperature monitoring:	No answer The equipment temperature is automatically monitored The equipment temperature is manually monitored The equipment temperature is not monitored	2 (6.2%) 9 (28.1%) 17 (53.1%) 4 (12.5%)	32 (100.0%)	0 (0.0%)
Does the BSL-3 lab have a dedicated room for refrigerators, freezers, and ultra-low freezers?	No No answer Yes	22 (68.8%) 6 (18.8%) 4 (12.5%)	32 (100.0%)	0 (0.0%)

Table A26 – Results regarding the place of manufacturing of the ultra-low freezers installed in the HCBLs participating in the survey.

Company	Place of manufacturing	
	Brazil	Other countries
A		7 (30.4%)
B	4 (17.4%)	
C		4 (17.4%)
D	2 (8.7%)	
E		2 (8.7%)
F		1 (4.3%)
G		1 (4.3%)
H		1 (4.3%)
I		1 (4.3%)
Total	6/23 (26.1%)	17/23 (73.9%)

Table A27 – Results regarding the centrifuges installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Are there centrifuges installed in the BSL-3 lab?	No No answer Yes	3 (9.4%) 2 (6.2%) 27 (84.4%)	32 (100.0%)	0 (0.0%)
If so, are the rotors or cups sealed?	I don't know No Yes	1 (3.7%) 4 (14.8%) 22 (81.5%)	27 (84.4%)	5 (15.6%)
Is there an SOP about the periodic decontamination of centrifuges?	I don't know No Yes	1 (3.7%) 10 (37.0%) 16 (59.3%)	27 (84.4%)	5 (15.6%)
If so, is the recommended decontamination protocol followed?	No answer Yes, always Yes, usually	2 (12.5%) 12 (75.0%) 2 (12.5%)	16 (50.0%)	16 (50.0%)

Table A28 – Results regarding assessing the quality of external services for specific systems/equipment installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How do you rate the quality of the external services provided to the HVAC system maintenance/certification?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 1 (3.2%) 5 (16.1%) 10 (32.3%) 9 (29.0%) 6 (19.4%)	31 (96.9%)	1 (3.1%)
How do you rate the quality of the external services provided to the Fumigation Chamber/Airlocks maintenance?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 2 (10.5%) 1 (5.3%) 7 (36.8%) 6 (31.6%) 3 (15.8%)	19 (59.4%)	13 (40.6%)
How do you rate the quality of the external services provided to the effluent decontamination system maintenance/qualification?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 1 (4.5%) 2 (9.1%) 6 (27.3%) 7 (31.8%) 6 (27.3%)	22 (68.8%)	10 (31.2%)
How do you rate the quality of the external services provided to the autoclave maintenance?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 3 (10.0%) 6 (20.0%) 8 (26.7%) 9 (30.0%) 4 (13.3%)	30 (93.8%)	2 (6.2%)
How do you rate the quality of the external services provided to the BSCc maintenance/certification?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 2 (6.5%) 2 (6.5%) 5 (16.1%) 17 (54.8%) 5 (16.1%)	31 (96.9%)	1 (3.1%)
How do you rate the quality of the external services provided to ultra-low freezers?	1 2 3 4 5 No answer	1 (4.0%) 2 (8.0%) 2 (8.0%) 8 (32.0%) 6 (24.0%) 6 (24.0%)	25 (78.1%)	7 (21.9%)

Table A29 – Results regarding the frequency of operational failures of specific systems/equipment installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How do you rate how often operational failures are observed in the Fumigation Chamber/Airlocks?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	12 (60.0%) 3 (15.0%) 1 (5.0%) 2 (10.0%) 1 (5.0%) 1 (5.0%)	20 (62.5%)	12 (37.5%)
How do you rate how often operational failures are observed in the effluent decontamination system?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	11 (47.8%) 3 (13.0%) 0 (0.0%) 1 (4.3%) 0 (0.0%) 0 (0.0%)	23 (71.9%)	9 (28.1%)
How do you rate how often operational failures are observed in the autoclaves?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	8 (25.8%) 9 (29.0%) 8 (25.8%) 2 (6.5%) 0 (0.0%) 4 (12.9%)	31 (96.9%)	1 (3.1%)
How do you rate how often operational failures are observed in the BSCs?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	17 (54.8%) 6 (19.4%) 1 (3.2%) 0 (0.0%) 1 (3.2%) 6 (19.4%)	31 (96.9%)	1 (3.1%)

Table A30 – Results regarding using and handling laboratory animals in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab handle animals in high-containment areas?	No No answer Yes	22 (68.8%) 1 (3.1%) 9 (28.1%)	32 (100.0%)	0 (0.0%)
Are different species handled in the same room at the same time?	No No answer Yes	6 (66.7%) 1 (11.1%) 2 (22.2%)	9 (28.1%)	23 (71.9%)
Are the rooms used for handling animals used exclusively for that purpose?	No answer Yes, all of them	1 (11.1%) 8 (88.9%)	9 (28.1%)	23 (71.9%)
Does the room where the animals are kept have a CCTV system enabling continuous monitoring?	I don't know No No answer Yes	1 (11.1%) 3 (33.3%) 2 (22.2%) 3 (33.3%)	9 (28.1%)	23 (71.9%)
Which equipment is used as a primary barrier?	Isolators No answer Ventilated cages	2 (25.0%) 1 (12.5%) 5 (62.5%)	8 (25.0%)	24 (75.0%)
Please inform us of the manufacturer of the racks and isolators	Company A (National) Company B (International) No answer	5 (55.6%) 1 (11.1%) 3 (33.3%)	9 (28.1%)	23 (71.9%)
Are the ventilated racks and isolators certified periodically?	No Yes	4 (50.0%) 4 (50.0%)	8 (25.0%)	24 (75.0%)
If so, how often is the certification?	Annually Biannually No answer	2 (50.0%) 1 (25.0%) 1 (25.0%)	4 (12.5%)	28 (87.5%)
If animal necropsies are performed, where is the procedure carried out?	BSCs No answer	6 (66.7%) 3 (33.3%)	9 (28.1%)	23 (71.9%)

Table A31 – Results regarding risk assessment, animal welfare, decontamination methods, and reporting of accidents and incidents related to handling laboratory animals in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Have the technicians who handle the animals received formal training on the concepts of animal welfare?	No No answer Yes	1 (11.1%) 2 (22.2%) 6 (66.7%)	9 (28.1%)	23 (71.9%)
Is specific risk assessment carried out for the use of laboratory animals?	I don't know No No answer Yes	1 (12.5%) 3 (37.5%) 2 (25.0%) 2 (25.0%)	8 (25.0%)	24 (75.0%)
Does the lab have an IACUC officially constituted?	Yes	11 (100.0%)	11 (34.4%)	21 (65.6%)
If so, is the Committee registered in the CONCEA	No No answer Yes	1 (11.1%) 2 (22.2%) 6 (66.7%)	9 (28.1%)	23 (71.9%)
Do the conditions of space, temperature, humidity, light cycles, acoustics, watering, and feeding meet animal welfare requirements for the handled species?	No answer Yes	2 (22.2%) 7 (77.8%)	9 (28.1%)	23 (71.9%)
What is the method used for the decontamination of animal carcasses?	Autoclave No answer	6 (75.0%) 2 (25.0%)	8 (25.0%)	24 (75.0%)
Has the method reported in the previous response been validated?	I don't know No No answer Yes	1 (14.3%) 2 (28.6%) 1 (14.3%) 3 (42.9%)	7 (21.9%)	25 (78.1%)
How often are there incidents or accidents involving the handling of live animals?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	5 (62.5%) 2 (25.0%) 0 (0.0%) 0 (0.0%) 0 (0.0%) 1 (12.5%)	8 (25.0%)	24 (75.0%)

Table A32 – Results regarding species of laboratory animals handled in the HCBLs participating in the survey.

Species	Freq	%
<i>Mus musculus</i>	7	77.8%
<i>Cricetinae</i>	2	22.2%
<i>Rattus norvergicus</i>	2	22.2%
<i>Cavia porcellus</i>	1	11.1%
<i>Gallus gallus</i>	1	11.1%
<i>Zebrafish</i>	1	11.1%
No answer	1	11.1%

Table A33 – Results regarding the utilization of uniforms in the HCBLs participating in the current survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Are uniforms available for exclusive use in the BSL-3 lab?	No No answer Yes	1 (3.1%) 2 (6.2%) 29 (90.6%)	32 (100.0%)	0 (0.0%)
Is the uniform suitable (quantity and quality) for all BSL-3 users?	No No answer Yes (both quantity and quality) Yes (just quantity)	1 (3.6%) 2 (7.1%) 22 (78.6%) 3 (10.7%)	28 (87.5%)	4 (12.5%)
What is the decontamination method?	Autoclave No answer	20 (71.4%) 8 (28.6%)	28 (87.5%)	4 (12.5%)

Table A34 – Results regarding the utilization of PPE in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Are the kinds of PPE provided suitable for all activities in the BSL-3 lab?	No answer Yes, always Yes, usually	8 (25.0%) 20 (62.5%) 4 (12.5%)	32 (100.0%)	0 (0.0%)
About the quantity of PPE available in the laboratory:	Are always sufficient Are usually sufficient No answer	26 (83.9%) 3 (9.7%) 2 (6.5%)	31 (96.9%)	1 (3.1%)
Does the lab use to reuse disposable PPE?	No answer No, never Yes, usually	2 (6.2%) 26 (81.2%) 4 (12.5%)	32 (100.0%)	0 (0.0%)
Is there a SOP containing guidance on the proper use of PPE?	No No answer Yes	2 (6.5%) 3 (9.7%) 26 (83.9%)	31 (96.9%)	1 (3.1%)
If so, how do you rate how often BSL-3 users follow the procedures?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 0 (0.0%) 0 (0.0%) 6 (23.1%) 19 (73.1%) 1 (3.8%)	26 (81.2%)	6 (18.8%)

Table A35 – Results regarding human resources (training and biosafety culture) in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How many people currently perform activities in the BSL-3 lab?	Minimum Average Median Maximum Standard Deviation No answer	2 9.71 9 30 7.0 3 (12.5%)	27 (84.4%)	5 (15.6%)
What is the approximate workload of the biosafety and biosecurity capacity-building training (considering theory and practice)?	Minimum Average Median Maximum Standard Deviation No answer	8 hours/year 22.2 hours/year 18 hours/year 60 hours/year 16.4 hours/year 7 (30.4%)	23 (71.9%)	9 (28.1%)
Is there an SOP containing the capacity-building pathway for BSL-3 users, emphasizing the biosafety and biosecurity issues?	No No answer Yes	5 (16.7%) 1 (3.3%) 24 (80.0%)	30 (93.8%)	2 (6.2%)
If so, how do you rate how often the procedures are followed?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 0 (0.0%) 3 (12.0%) 4 (16.0%) 16 (64.0%) 2 (8.0%)	25 (78.1%)	7 (21.9%)
How do you rate the level of awareness of technicians concerning biosafety procedures?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 0 (0.0%) 1 (3.3%) 11 (36.7%) 16 (53.3%) 2 (6.7%)	30 (93.8%)	2 (6.2%)

Table A36 – Results regarding human resources (support staff) in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How many cleaning professionals are currently allowed to access the BSL-3 lab?	0 1 2 3 5 No answer	8 (26.7%) 7 (23.3%) 5 (16.7%) 1 (3.3%) 1 (3.3%) 8 (26.7%)	30 (93.8%)	2 (6.2%)
Have the cleaning staff received training on biosafety issues?	No No answer Yes	2 (10.5%) 2 (10.5%) 15 (78.9%)	19 (59.4%)	13 (40.6%)
How many IT professionals are currently allowed to access the BSL-3 lab?	0 1 2 No answer	16 (59.3%) 4 (14.8%) 2 (7.4%) 5 (18.5%)	27 (84.4%)	5 (15.6%)
Have the IT staff received training on biosafety issues?	No No answer Yes	2 (18.2%) 4 (36.4%) 5 (45.5%)	11 (34.4%)	21 (65.6%)
Does the laboratory have an engineer with biosafety expertise in its workforce?	I don't know No No answer Yes	2 (6.5%) 16 (51.6%) 3 (9.7%) 10 (32.3%)	31 (96.9%)	1 (3.1%)
Does the laboratory have a permanent maintenance team with biosafety expertise in its workforce?	I don't know No No answer Yes	1 (3.1%) 9 (28.1%) 3 (9.4%) 19 (59.4%)	32 (100.0%)	0 (0.0%)
Have the maintenance team received training on biosafety issues?	I don't know No Yes	2 (10.0%) 3 (15.0%) 15 (75.0%)	20 (62.5%)	12 (37.5%)

Table A37 – Results regarding human resources (biosafety officers) in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab have a biosafety officer?	No No answer Yes	8 (25.8%) 2 (6.5%) 21 (67.7%)	31 (96.9%)	1 (3.1%)
If so, does their position allow independence of those responsible for implementing the program of work?	I don't know No No answer Yes	2 (8.7%) 3 (13.0%) 3 (13.0%) 15 (65.2%)	23 (71.9%)	9 (28.1%)
Does the biosafety officer dedicate their time exclusively to this activity?	No No answer Yes	13 (56.5%) 3 (13.0%) 7 (30.4%)	23 (71.9%)	9 (28.1%)
What is the biosafety officer's academic background?	Biochemistry Pharmaceutical Biology No answer Nurse Process Manager Veterinary Medicine	7 (30.4%) 1 (4.3%) 8 (34.8%) 1 (4.3%) 1 (4.3%) 5 (21.7%)	23 (71.9%)	9 (28.1%)
Has the biosafety officer received training on biosafety issues before starting their activities related to this position?	I don't know No No answer Yes	1 (4.3%) 2 (8.7%) 3 (13.0%) 17 (73.9%)	23 (71.9%)	9 (28.1%)
How many hours of refresher training on biosafety issues does the biosafety officer receive on an annual basis (on average)?	Minimum Average Median Maximum Standard Deviation I don't know No answer	0 13.6 12.0 40 14.0 1 (4.5%) 11 (50.0%)	22 (68.8%)	10 (31.3%)

Table A38 – Results regarding occupational health in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab include issues related to the BSL-3 users' health in its risk assessment pathway?	No No answer Yes	5 (16.1%) 2 (6.5%) 24 (77.4%)	31 (96.9%)	1 (3.1%)
Does the lab provide periodic medical exams for its employees?	No No answer Yes	10 (32.3%) 3 (9.7%) 18 (58.1%)	31 (96.9%)	1 (3.1%)
If so, does the medical exam include specific tests related to the activities carried out in the BSL-3 lab?	I don't know No Yes	2 (11.1%) 6 (33.3%) 10 (55.6%)	18 (56.2%)	14 (43.8%)
Does the lab have a first aid station?	I don't know No No answer Yes	1 (3.1%) 14 (43.8%) 2 (6.2%) 15 (46.9%)	32 (100.0%)	0 (0.0%)
How many hours per day, on average, do users stay within the BSL-3 lab?	3 4 6 8 No answer	4 (12.9%) 13 (41.9%) 1 (3.2%) 3 (9.7%) 10 (32.3%)	31 (96.9%)	1 (3.1%)
Has the lab defined a maximum period of stay for users within the BSL-3 lab?	I don't know No No answer Yes	1 (3.2%) 14 (45.2%) 3 (9.7%) 13 (41.9%)	31 (96.9%)	1 (3.1%)
If so, what is the given period?	4 5 6 No answer	8 (66.7%) 1 (8.3%) 1 (8.3%) 2 (16.7%)	12 (37.5%)	20 (62.5%)
Are the BSL-3 users allowed to drink water in the BSL-3 lab?	No No answer Yes	24 (75.0%) 3 (9.4%) 5 (15.6%)	32 (100.0%)	0 (0.0%)
Is food intake allowed in the BSL-3 lab?	No No answer Yes	27 (84.4%) 2 (6.2%) 3 (9.4%)	32 (100.0%)	0 (0.0%)
Is any survey carried out to assess the well-being of the professionals working in the BSL-3 lab?	I don't know No No answer Yes	1 (3.2%) 19 (61.3%) 7 (22.6%) 4 (12.9%)	31 (96.9%)	1 (3.1%)
If so, what is the frequency of the survey?	Annually Biannually No answer	1 (25.0%) 2 (50.0%) 1 (25.0%)	4 (12.5%)	28 (87.5%)
Was the lab designed considering accessibility requirements?	I don't know No No answer Yes	3 (9.4%) 26 (81.2%) 1 (3.1%) 2 (6.2%)	32 (100.0%)	0 (0.0%)
Does the lab have an emergency shower within the BSL-3 lab?	No No answer Yes	15 (46.9%) 4 (12.5%) 13 (40.6%)	32 (100.0%)	0 (0.0%)
Does the lab have emergency eye washing within the BSL-3 lab?	No No answer Yes	13 (40.6%) 3 (9.4%) 16 (50.0%)	32 (100.0%)	0 (0.0%)

Table A39 – Results regarding communication tools in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Are the BSL-3 users allowed to take their cell phones in the BSL-3 lab?	No No answer Yes	23 (71.9%) 6 (18.8%) 3 (9.4%)	32 (100.0%)	0 (0.0%)
Which communication tools are present in the BSL-3 lab?	Intercom Internet No answer None Radio Telephone Whiteboard	8 (25.0%) 21 (65.6%) 2 (6.3%) 1 (3.1%) 6 (18.8%) 22 (68.8%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
If internet service is provided in the BSL-3 lab, how do you rate the quality of this service?	1 (very bad) 2 (bad) 3 (medium) 4 (good) 5 (very good) No answer	0 (0.0%) 2 (9.5%) 2 (9.5%) 9 (42.9%) 8 (38.1%) 0 (0.0%)	21 (65.6%)	11 (34.4%)

Table A40 – Results regarding incidents in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
How do you rate the frequency of biosafety-related incidents in your institution (considering not only the BSL-3 lab)?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	25 (78.1%) 4 (12.5%) 1 (3.1%) 1 (3.1%) 0 (0.0%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
How do you rate the risk of an external-sourced criminal event occurring in the BSL-3 lab?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	24 (75.0%) 5 (15.6%) 2 (6.2%) 0 (0.0%) 0 (0.0%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
Has there ever been a report of theft or stealing of materials from the BSL-3 lab?	No No answer Yes	20 (83.3%) 3 (12.5%) 1 (4.2%)	24 (75.0%)	8 (25.0%)
Has the lab ever been sabotaged?	No No answer Yes	24 (75.0%) 7 (21.9%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
How do you rate the risk of sabotage in your institution?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	18 (56.2%) 4 (12.5%) 2 (6.2%) 0 (0.0%) 0 (0.0%) 8 (25.0%)	32 (100.0%)	0 (0.0%)

Table A41 – Results regarding guard services in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab have a guard service?	No No answer Yes	5 (15.6%) 2 (6.2%) 25 (78.1%)	32 (100.0%)	0 (0.0%)
If so, how many hours per day is the service provided?	10h 24h No answer	1 (4.0%) 21 (84.0%) 4 (16.0%)	25 (78.1%)	7 (21.9%)
Is the service provided on weekends and holidays?	No Yes	1 (4.0%) 24 (96.0%)	25 (78.1%)	7 (21.9%)
Is any cooperation agreement established with security authorities such as military service, federal police, local police, FBI, etc.?	I don't know No No answer Yes	4 (12.5%) 20 (62.5%) 4 (12.5%) 4 (12.5%)	32 (100.0%)	0 (0.0%)

Table A42 – Results regarding inventory, transportation, and control of biological assets in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Do the rooms where high-risk biological agents are stored have their own access control?	No No answer Yes, all of them Yes, some of them	6 (18.8%) 3 (9.4%) 22 (68.8%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
If not, is the equipment where high-risk biological agents are stored locked?	No No answer Yes	4 (66.7%) 1 (16.7%) 1 (16.7%)	6 (18.8%)	26 (81.3%)
Is there inventory and records of all transportation of high-risk biological agents?	I don't know No No answer Partially Yes	1 (3.1%) 5 (15.6%) 3 (9.4%) 3 (9.4%) 20 (62.5%)	32 (100.0%)	0 (0.0%)
Is there an SOP for transporting high-risk biological agents?	No No answer Yes	5 (15.6%) 3 (9.4%) 24 (75.0%)	32 (100.0%)	0 (0.0%)
If so, how do you rate how often BSL-3 users follow the procedures?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 0 (0.0%) 1 (4.5%) 1 (4.5%) 20 (90.9%) 0 (0.0%)	22 (68.8%)	10 (31.2%)
How do you rate how often high-risk biological agents are transported to another lab?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	15 (65.2%) 3 (13.0%) 0 (0.0%) 2 (8.7%) 1 (4.3%) 2 (8.7%)	23 (71.9%)	9 (28.1%)
Does the lab have workers certified according to the transportation rules for biological materials (IATA/ANAC)?	I don't know No No answer Yes	2 (6.2%) 15 (46.9%) 2 (6.2%) 13 (40.6%)	32 (100.0%)	0 (0.0%)
If so, is the certificate valid at this moment?	No No answer Yes	3 (23.1%) 1 (7.7%) 9 (69.2%)	13 (40.6%)	19 (59.4%)

Table A43 – Results regarding personnel background assessment and data safety in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Is there a protocol to evaluate the adequacy of receiving external visits?	No No answer Yes	8 (25.0%) 3 (9.4%) 21 (65.6%)	32 (100.0%)	0 (0.0%)
Is there a protocol for evaluating criminal records and behavioral profiles before hiring workers who will access the BSL-3 lab?	I don't know No No answer Partially Yes	1 (3.1%) 21 (65.6%) 3 (9.4%) 4 (12.5%) 3 (9.4%)	32 (100.0%)	0 (0.0%)
How do you rate the security level of the data generated/stored in the BSL-3 lab?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	2 (7.1%) 0 (0.0%) 3 (10.7%) 10 (35.7%) 10 (35.7%) 3 (10.7%)	28 (87.5%)	4 (12.5%)
Have there already been reported incidents involving the security of the data generated/stored in the BSL-3 lab?	I don't know No No answer Yes	1 (4.0%) 21 (84.0%) 1 (4.0%) 2 (8.0%)	25 (78.1%)	7 (21.9%)

Table A44 – Results regarding additional security barriers surrounding the BSL-3 facility in the HCBLs participating in the survey.

Security barrier	Freq	% ^{ddd}
CCTV	15	51.7%
Regular fence	4	13.8%
Presence sensor	3	10.3%
Wrought iron fence	2	6.9%
Electric fence	1	3.5%
None	12	41.4%

^{ddd} Considering 29 respondents

Table A45 – Results regarding GMO handling in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab work with genetic engineering, like DNA recombination methods?	No No answer Yes	25 (78.1%) 1 (3.1%) 6 (18.8%)	32 (100.0%)	0 (0.0%)
If so, does the lab carry out specific risk assessments for this purpose?	I don't know Yes	1 (16.7%) 5 (83.3%)	6 (18.8%)	26 (81.2%)
Does the laboratory have a Biosafety Quality Certificate (CQB)?	I don't know Yes	1 (16.7%) 5 (83.3%)	6 (18.8%)	26 (81.2%)

Table A46 – Results regarding the realization of risk assessment by the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Is there an SOP defining how to do a risk assessment for the activities carried out in the BSL-3 lab?	I don't know No No answer Yes	2 (6.5%) 8 (25.8%) 3 (9.7%) 18 (58.1%)	31 (96.9%)	1 (3.1%)
Has the risk assessment been done for all activities in the BSL-3 lab?	I don't know No No answer Yes	2 (6.5%) 8 (25.8%) 2 (6.5%) 19 (61.3%)	31 (96.9%)	1 (3.1%)
If so, is the risk assessment periodically reviewed?	I don't know No No answer Yes	1 (6.2%) 2 (12.5%) 2 (12.5%) 11 (68.8%)	16 (50.0%)	16 (50.0%)
What is the position of the professional in charge of the risk assessment elaboration?	Expert Head of the laboratory Head of the biorisk management departament Health security officer I don't know Member of the IBC No answer Researcher	1 (5.6%) 3 (16.7%) 5 (27.8%) 1 (5.6%) 2 (11.1%) 1 (5.6%) 3 (16.7%) 2 (11.1%)	18 (56.2%)	14 (43.8%)
Has the person responsible for the risk assessment elaboration received specific training?	I don't know No Yes	2 (10.5%) 4 (21.1%) 13 (68.4%)	19 (59.4%)	13 (40.6%)
How do you rate the quality of the risk assessment carried out?	1 (very bad) 2 (bad) 3 (medium) 4 (good) 5 (very good) No answer	0 (0.0%) 1 (5.3%) 0 (0.0%) 5 (26.3%) 12 (63.2%) 1 (5.3%)	19 (59.4%)	13 (40.6%)

Table A47 – Results regarding the automation systems installed in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab have an automation system for monitoring and/or controlling equipment and systems?	No No answer Yes	5 (15.6%) 5 (15.6%) 22 (68.8%)	32 (100.0%)	0 (0.0%)
How do you rate the quality of the automation software?	1 (very bad) 2 (bad) 3 (medium) 4 (good) 5 (very good) No answer	0 (0.0%) 0 (0.0%) 4 (18.2%) 10 (45.5%) 5 (22.7%) 3 (13.6%)	22 (68.8%)	10 (31.2%)
Does the automation system have audibled or visual alerts in case of deviation from critical parameters?	No No answer Yes	2 (9.1%) 1 (4.5%) 19 (86.4%)	22 (68.8%)	10 (31.2%)
How many employees are currently able to operate the automation system?	1 2 3 4 5 6 7 8 No answer Third part company	3 (13.6%) 1 (4.5%) 4 (18.2%) 2 (9.1%) 1 (4.5%) 2 (9.1%) 1 (4.5%) 1 (4.5%) 6 (27.3%) 1 (4.5%)	22 (68.8%)	10 (31.2%)
Are the data generated by the automation system being backed up?	I don't know No No answer Yes	3 (13.6%) 4 (18.2%) 1 (4.5%) 14 (63.6%)	22 (68.8%)	10 (31.2%)
If so, what is the frequency of the backup?	Biannually Daily More than once/day No answer Weekly	1 (7.1%) 3 (21.4%) 1 (7.1%) 8 (57.1%) 1 (7.1%)	14 (43.8%)	18 (56.2%)
Does the lab have software for recording data related to preventive maintenance plans?	I don't know No No answer Yes	1 (3.2%) 7 (22.6%) 10 (32.3%) 13 (41.9%)	31 (96.9%)	1 (3.1%)

Table A48 – Results regarding the annual costs of the HCBLs participating in the survey.

	What was the approximate value for maintenance expenses related to the BSL-3 lab in the previous year?
Mean	363,386.67
Std. Dev	374,445.00
Min	47,000.00
Median	252,660.00
Max	1,000,000.00
Valid Answers	6
	What was the approximate value for purchasing supplies destined for the BSL-3 lab the previous year?
Mean	303,319.50
Std. Dev	274,718.73
Min	40,000.00
Median	262,554.00
Max	704,809.00
Valid Answers	6
	What were the total investment expenditures in the previous years destined for the BSL-3 lab?
Mean	866,666.67
Std. Dev	1,789,253.10
Min	0.00
Median	75,000.00
Max	4,500,000.00
Valid Answers	6

Table A49 – Results regarding the sustainability of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Has the operation of the BSL-3 lab been suspended in the last 12 months due to a lack of funds to acquire supplies?	No No answer	20 (87.0%) 3 (13.0%)	23 (71.9%)	9 (28.1%)
Has the operation of the BSL-3 lab been suspended in the last 12 months due to a lack of funds for equipment and/or systems maintenance?	No No answer Yes	19 (82.6%) 3 (13.0%) 1 (4.3%)	23 (71.9%)	9 (28.1%)
How do you rate the financial condition of the laboratory, explicitly considering the BSL-3 management?	1 (very bad) 2 (bad) 3 (medium) 4 (good) 5 (very good) No answer	3 (9.7%) 3 (9.7%) 5 (16.1%) 8 (25.8%) 4 (12.9%) 8 (25.8%)	31 (96.9%)	1 (3.1%)
What are the sources of funds to cover the BSL-3 operating costs?	Research grants BFG The institute itself No answer Confidential information	9 (28.1%) 9 (28.1%) 7 (21.9%) 17 (53.1%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
What are the sources of funds to cover the BSL-3 investment expenses?	Research grants BFG The institute itself No answer Confidential information	9 (28.1%) 7 (21.9%) 8 (25.0%) 17 (53.1%) 1 (3.1%)	32 (100.0%)	0 (0.0%)
Have the activities of the BSL-3 lab been suspended at some point due to a lack of demands?	No Yes	19 (82.6%) 4 (17.4%)	23 (71.9%)	9 (28.1%)
If so, how often the activities are suspended for this reason	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 3 (75.0%) 1 (25.0%) 0 (0.0%) 0 (0.0%) 0 (0.0%)	4 (12.5%)	28 (87.5%)
Does the institution currently have partnerships with others to use the BSL-3 lab?	I don't know No No answer Yes	1 (3.1%) 17 (53.1%) 3 (9.4%) 11 (34.4%)	32 (100.0%)	0 (0.0%)
How do you predict the volume of laboratory activities for the coming years compared to the current demand?	Greater Lower No answer Similar	21 (67.7%) 3 (9.7%) 5 (16.1%) 2 (6.5%)	31 (96.9%)	1 (3.1%)
Are there demands currently unmet due to a lack of a higher-level biocontainment structure?	I don't know No No answer Yes	3 (9.4%) 19 (59.4%) 3 (9.4%) 7 (21.9%)	32 (100.0%)	0 (0.0%)

Table A50 – Results regarding the biorisk management system of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab have an IBC?	No No answer Yes	7 (21.9%) 2 (6.2%) 23 (71.9%)	32 (100.0%)	0 (0.0%)
Does the lab have its own Biosafety Manual?	I don't know No No answer Yes	1 (3.1%) 8 (25.0%) 2 (6.2%) 21 (65.6%)	32 (100.0%)	0 (0.0%)
Does the lab have a documental structure for managing biorisks?	I don't know No No answer Yes	2 (6.2%) 4 (12.5%) 3 (9.4%) 23 (71.9%)	32 (100.0%)	0 (0.0%)
If so, how do you rate the level of awareness that BSL-3 users have of this documentation?	1 (very low) 2 (low) 3 (medium) 4 (high) 5 (very high) No answer	0 (0.0%) 0 (0.0%) 1 (3.2%) 7 (22.6%) 10 (32.3%) 13 (41.9%)	31 (96.9%)	1 (3.1%)
Does the lab have a documented contingency plan for emergencies?	I don't know No No answer Yes	3 (9.4%) 5 (15.6%) 3 (9.4%) 21 (65.6%)	32 (100.0%)	0 (0.0%)

Table A51 – Results regarding accidents recorded by the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Is there a SOP for recording accidents?	No No answer Yes	2 (6.2%) 2 (6.2%) 28 (87.5%)	32 (100.0%)	0 (0.0%)
How many accidents per year are recorded, on average, considering the whole lab?	0 0,5 1 3 8 15 No answer	11 (35.5%) 1 (3.2%) 7 (22.6%) 1 (3.2%) 1 (3.2%) 1 (3.2%) 9 (29.0%)	31 (96.9%)	1 (3.1%)
Did any of these accidents lead to morbidity or mortality due to laboratory-acquired infection?	No No answer	11 (55.0%) 9 (45.0%)	20 (62.5%)	12 (37.5%)
How many accidents are recorded, on average, per year, considering just the BSL-3 lab?	0 0,5 1 10 No answer	15 (62.5%) 1 (4.2%) 3 (12.5%) 1 (4.2%) 4 (16.7%)	24 (75.0%)	8 (25.0%)
Did any of these accidents lead to morbidity or mortality due to laboratory-acquired infection?	No No answer	6 (66.7%) 3 (33.3%)	9 (28.1%)	23 (71.9%)

Table A52 – Results regarding the audits carried out in the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Does the lab carry out periodic internal audits addressing biosafety aspects?	No No answer Yes	9 (29.0%) 3 (9.7%) 19 (61.3%)	31 (96.9%)	1 (3.1%)
If so, how often are the internal audits carried out?	Annually Biannually I don't know No answer	13 (68.4%) 4 (21.1%) 1 (5.3%) 1 (5.3%)	19 (59.4%)	13 (40.6%)
Has the laboratory already received an external audit addressing biosafety aspects?	No No answer Yes	11 (45.8%) 1 (4.2%) 12 (50.0%)	24 (75.0%)	8 (25.0%)

Table A53 – Results regarding numerous regulatory issues related to the operation of the HCBLs participating in the survey.

Variable	Stats / Values	Freq. (% of Valid)	Valid	Missing
Are the methods applied in the lab supported by legislation or reference guidelines/standards?	I don't know No answer Yes, always Yes, usually	1 (3.2%) 9 (29.0%) 15 (48.4%) 6 (19.4%)	31 (96.9%)	1 (3.1%)
Are the methods applied in the lab accredited by an accreditation body?	I don't know No answer No, never No, usually Yes, always Yes, usually	3 (10.3%) 8 (27.6%) 5 (17.2%) 2 (6.9%) 5 (17.2%) 6 (20.7%)	29 (90.6%)	3 (9.4%)
In your opinion, are there gaps in the regulation/legislation of the activities carried out by the BSL-3 labs?	I don't know No No answer Yes	7 (21.9%) 8 (25.0%) 4 (12.5%) 13 (40.6%)	32 (100.0%)	0 (0.0%)
Does the lab have a waste management plan?	I don't know No No answer Yes	1 (3.1%) 4 (12.5%) 1 (3.1%) 26 (81.2%)	32 (100.0%)	0 (0.0%)
Does the lab have a contract with a specialized company for solid waste disposal?	No No answer Yes	1 (3.1%) 2 (6.2%) 29 (90.6%)	32 (100.0%)	0 (0.0%)
Is the lab up to date with all its legal obligations with the fire department?	I don't know No No answer Yes	7 (21.9%) 11 (34.4%) 5 (15.6%) 9 (28.1%)	32 (100.0%)	0 (0.0%)
Which is the fire detection system inside the BSL-3 lab?	Heat detector I don't know No answer None Smoke detector	1 (3.1%) 5 (15.6%) 3 (9.4%) 12 (37.5%) 11 (34.4%)	32 (100.0%)	0 (0.0%)
Does the lab have a fire brigade?	I don't know No No answer Yes	1 (3.1%) 11 (34.4%) 3 (9.4%) 17 (53.1%)	32 (100.0%)	0 (0.0%)
If so, are the BSL-3 users brigade members?	I don't know Most of them are Most of them are not No No answer Yes, all of them	2 (11.8%) 4 (23.5%) 4 (23.5%) 2 (11.8%) 3 (17.6%) 2 (11.8%)	17 (53.1%)	15 (46.9%)

Table A54 – List of the primary maintenance challenges faced by the HCBLs participating in the current survey.

Challenge faced	Frequency of similar manifestations
Lack of financial resources	6
The need for corrective maintenance in the HVAC system	4
Difficulty in maintaining the air temperature within the specified parameters	2
Lack of qualified personnel	2
The need for corrective maintenance in autoclaves	2
The need for corrective maintenance in sealed doors	2
The need for corrective maintenance in EDS	2
The need for corrective maintenance in the automation system	2
Slow procurement processes within the public service	2
The need for corrective maintenance in airlocks	1
The need for corrective maintenance in pass-through	1
The need for corrective maintenance in the access control system	1
Lack of an automation system	1
Absence of a contract with a specialized company to provide maintenance services for HCBL	1
High turnover rates among maintenance staff	1
Challenges with supplies such as water, compressed air, and steam	1
The equipment has been in operation for an extended period	1
Difficulty in sourcing replacement parts for BSCs	1
Challenges in validating HEPA filters	1
Challenges in validating EDS	1
Lack of a device to decontaminate BSCs	1

Table A55 – List of the primary biorisk management challenges faced by the HCBLs participating in the survey.

Challenge faced	Frequency of similar manifestations
Lack of human resources	6
Lack of financial resources	6
Lack of capacity-building programs	5
The need to improve the management processes	2
The need to enhance the bidding processes	2
The need to enhance the biosafety culture among HCBL users	2
Limited support from decision-makers	1
The need to adequate the occupational health program	1
Inadequate availability of service providers capable of meeting HCBL demands	1
High users' turnover	1
The old age of the building	1
BOs do not exclusively allocate their time to biorisk management responsibilities	1

APPENDIX B – Questionnaire (Template)

Questionnaire about High-Containment Biological Laboratories

1. Name of the person responsible for filling in:
2. Email from the person responsible for filling in:
3. Position of the person responsible for filling in:
4. Function of the person responsible for filling in:

LABORATORY IDENTIFICATION

5. Lab Name/ Acronym:
6. Lab linkage
 - ☐ Federal Public
 - ☐ State Public
 - ☐ Municipal Public
 - ☐ Private
 - Other:
7. In the case of public lab, please inform the governmental body to which is linked:
8. City and State where the lab is located:

LAB SCOPE AND OPERATION

9. What's the lab purpose? *(Mark all applied)*
 - ☐ Service provider
 - ☐ Research
 - ☐ Teaching
 - ☐ Supply producer
 - ☐ Other:
10. Main pathogens handled in the BSL-3:
11. Does the lab handle airborne pathogens?
 - ☐ Yes
 - ☐ No
 - ☐ I don't know
12. Does the lab belong to any national network?
 - ☐ Yes
 - ☐ No
 - ☐ I don't know
13. If so, please inform which network (s):
14. Does the lab belong to any international network?
 - ☐ Yes
 - ☐ No
 - ☐ I don't know
15. If so, please inform which network (s):
16. What's the lab coverage (consider the widest)?
 - ☐ Municipal
 - ☐ State
 - ☐ National
 - ☐ International
17. Please inform the main lab outputs. E.g.: annual average number of samples received, volume of supplies produced, assays carried out, research projects completed etc.:

DESIGN AND CONSTRUCTION

18. When was the lab designed?

19. How much was the design?
20. Was carried out a risk assessment for the design?
 - ☐ Yes
 - ☐ No
 - ☐ I don't know
21. If so, what professionals were involved in the risk assessment?
22. Are the currently activities the same of those foreseen in the design?
 - ☐ Yes, totally
 - ☐ Yes, most of them are
 - ☐ Most activities are not
 - ☐ No, the currently activities are totally different from those foreseen in the design
23. Which was the financial source for the design?
24. Which was the company in charge of the design?
25. When was the lab built (year of beginning and conclusion)?
26. Mark the most appropriate response to the kind of construction:
 - ☐ New lab
 - ☐ Expansion
 - ☐ Remodeling
 - ☐ Other:
27. How much was the construction?
28. Does the value reported include other facilities besides the BSL-3 lab?
 - ☐ Yes
 - ☐ No
29. If so, please inform which facilities and respective surfaces:
30. Which was the financial source for the construction?
31. Which was the company in charge of the construction?
32. Please describe in few words how the construction was inspected.
33. Was the construction process inspected and/or managed by an independent company?
 - ☐ Yes, throughout the construction time
 - ☐ Yes, during part of the construction time
 - ☐ No
 - ☐ I don't know
34. Was the lab commissioned?
 - ☐ Yes
 - ☐ No
 - ☐ I don't know
35. If so, please mark the equipment/systems included in the commissioning: *(Mark all applied)*
 - ☐ HVAC
 - ☐ Electrical systems
 - ☐ Autoclaves
 - ☐ Fumigation chamber
 - ☐ Biosafety Cabinets
 - ☐ Pass-Through/dunk tank
 - ☐ Waste treatment system
 - ☐ Alarms
 - ☐ Automation
 - ☐ Access control
 - ☐ Sealing
 - ☐ Other:

36. Was the lab certified?
☐ Yes
☐ No
☐ I don't know
37. If so, which body was in charge of the certification?
38. Is certification renewed periodically?
☐ Yes
☐ No
☐ Not applicable (lab has not been certified)
☐ I don't know
39. How often is the certification renewing?
☐ Biannual
☐ Annual
☐ Every 2 years
☐ More than 2 years
☐ I don't know
☐ Not applicable (lab has not been certified)
☐ Other:
40. When it was conducted the last certification renewing?
41. In the case the laboratory has not been certified, who assigned its biosafety level?
42. Does the lab carry out periodically certifications/qualifications of the equipment/systems?
☐ Yes
☐ No
☐ I don't know
43. If so, which equipment/systems are included in the periodic certification/qualification? *(Mark all applied)*
☐ HVAC
☐ Electrical systems
☐ Autoclaves
☐ Fumigation chamber
☐ BSCs
☐ Pass-Through
☐ Waste treatment system
☐ Alarms
☐ Automation
☐ Access control
☐ Sealing
☐ Other:
44. How do you rate the quality of the road access to the lab?
- | | 1 | 2 | 3 | 4 | 5 | |
|----------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------|
| Terrible | ☐ | ☐ | ☐ | ☐ | ☐ | Excellent |
45. In your opinion, does the design have relevant shortcomings?
☐ Yes
☐ No
☐ I don't know
46. If so, please describe the main shortcomings:

FACILITY FEATURES

47. What's the BSL-3 footage?

48. What's the BSL-3 lab workspace footage?
49. Number of floors (including non-biocontained floors):
50. Number of biocontained floors:
51. Is the BSL-3 lab conjugate to BSL-2 and or BSL-1 labs?
- ☐ Yes BSL-2
 - ☐ Yes BSL-1
 - ☐ Yes BSL-2 and BSL-1
 - ☐ No
52. Are the walls and ceiling demonstrably sealed?
- ☐ Yes
 - ☐ No
 - ☐ Partially
 - ☐ I don't know
53. Are there windows in the BSL-3 facing the outside area?
- ☐ Yes
 - ☐ No
54. If so, please mark the kind of windows:
- ☐ Single glass
 - ☐ Single armored glass
 - ☐ Double glass
 - ☐ Double armored glass
 - ☐ Other:
55. Are these windows demonstrably sealed?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
56. Are there windows in the BSL-3 facing the inner area?
- ☐ Yes
 - ☐ No
57. If so, please mark the kind of windows:
- ☐ Single glass
 - ☐ Single armored glass
 - ☐ Double glass
 - ☐ Double armored glass
 - ☐ Other:
58. Are these windows demonstrably sealed?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
59. Are there curtains or shutters inside the lab?
- ☐ Yes
 - ☐ No
60. Which is the kind of floor coatings installed in the BSL-3 lab? (e.g., epoxy, vinyl, ceramics, etc)
61. Which is the kind of materials used for workbench and furniture? (e.g., stainless steel, corian, MDF, granite, ceramics, etc.)
62. Are there currently cracks on the inner walls?
- ☐ Yes
 - ☐ No
63. Are there currently cracks on the floor?
- ☐ Yes
 - ☐ No

WATER AND GASES SUPPLY

64. What's the source of potable water for the BSL-3 lab?

- () Public supply
 () Artesian well
 () I don't know
 () Other:

65. How do you rate how often the water supply is cut off?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

66. How do you rate how often the activities in the BSL-3 lab are harmed due to a lack of water?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

67. Does the lab have any mechanism to save water?

- () Yes
 () No
 () I don't know

68. If so, please describe the mechanism:

69. Are there floor drains inside the lab?

- () Yes
 () No
 () I don't know

70. Are there backflow prevention devices in the water and gas pipes?

- () Yes
 () No
 () I don't know

71. How do you rate how often leaks are observed in the water piping?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

72. Are the pipes and conduits visible and easily cleanable?

- () Yes, all of them
 () Most of them are
 () Most of them are not
 () No

73. Is there compressed air supply?

- () Yes
 () No
 () I don't know

74. If so, are there backflow prevention devices in compressed air pipes?

- () Yes
() No
() I don't know

75. How do you rate how often the compressed air supply is cut off?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

76. Are there gas cylinders located inside the lab?

- () Yes
() No
() I don't know

77. If so, what kind of gases are storage in these cylinders?

ACCESS

78. Please describe how is the access to the BSL-3 lab:

79. Is the access to the BSL-3 lab through a mechanism that includes interlocking doors?

- () Yes
() No
() I don't know

80. Which are the materials used to manufacture the doors installed inside the BSL-3 lab (e.g., stainless steel, wood, aluminum, etc.)

81. Does the lab keep records of each access to the BSL-3 area?

- () Yes, the lab records any access electronically
() Yes, the lab records any access manually
() Yes, the lab records accesses electronically, but only for visits
() Yes, the lab records accesses manually, but only for visits
() No, the lab does not record accesses
() Other response:

82. Is there changing room dedicated exclusively to the BSL-3 access?

- () Yes
() No

83. If so, are there exclusive changing rooms for men and women?

- () Yes
() No
() Not applicable

84. Are the changing rooms clearly divided into "clean" and "dirty" areas?

- () Yes
() No
() Not applicable

85. Are the changing rooms provided with a shower?

- () Yes
() No
() Not applicable

86. If so, is bathing mandatory?
- ☐ No
 - ☐ Yes, just to enter
 - ☐ Yes, just to exit
 - ☐ Yes, both to enter and exit
 - ☐ I don't know
 - ☐ Not applicable
87. Is bathing controlled by an automated system?
- ☐ Yes
 - ☐ No
 - ☐ Not applicable
88. Please inform the total number of showers present in the changing rooms:
89. Is bath water drained to a waste treatment system?
- ☐ Yes
 - ☐ No
 - ☐ Not applicable
 - ☐ I don't know
90. Is the air of bath box filtered (to decontaminate aerosols generated during bathing)?
- ☐ Yes
 - ☐ No
 - ☐ Not applicable
 - ☐ I don't know
91. How do you rate the footage from the changing rooms, considering the current staff?
- ☐ Insufficient
 - ☐ Suitable
 - ☐ Oversized
 - ☐ I don't know
 - ☐ Not applicable
92. Is there a SOP describing the training procedures for visitors' entrance?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
93. If so, please rate how often the procedure is fulfilled:

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

HVAC SYSTEM

94. Is the lab air pressure negative in comparison to the outside environment?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
95. Are there differential air pressures between the BSL-3 rooms?
- ☐ Yes
 - ☐ No
 - ☐ I don't know

96. Please mark the most appropriate answer related to the air pressure monitoring:

- () The air pressure is not monitored
- () The air pressure is manually monitored
- () The air pressure is automatically monitored
- () The air pressure is manually and automatically monitored
- () I don't know
- () Other answer:

97. How do you rate how often the lab air pressure does not fulfil the established parameters?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

98. Are there filters installed in the air supply?

- () Yes
- () No
- () I don't know

99. If so, please describe the kind of filters installed:

100. Are there filters installed in the air exhaustion?

- () Yes
- () No
- () I don't know

101. If so, please describe the kind of filters installed:

102. Is the air exhaust filtration system redundant?

- () Yes
- () No
- () I don't know

103. How do you decontaminate the filters when replacing them?

104. Does the lab periodically monitor the efficiency of the filters installed in the air exhaust system?

- () Yes
- () No
- () I don't know
- () Not applicable

105. Are the exhaust air filter boxes installed in a biocontained room?

- () Yes
- () No
- () I don't know
- () Not applicable

106. Is the air supply system redundant?

- () Yes
- () No
- () I don't know

107. Is the air exhaust system redundant?

- () Yes
- () No
- () I don't know

108. Does the HVAC system have sealing dampers?

- () Yes
- () No
- () I don't know

109. How many air changes per hour is provided in the BSL-3 rooms?

110. Does the HVAC system permit air recirculation? If so, which is the percentage of the recirculated air?

111. How do you rate the security level of the place where the Air Handling Units are installed?

	1	2	3	4	5	
Totally insecure	☐	☐	☐	☐	☐	Totally secure

112. Was the HVAC system nationally manufactured?

- () Yes
 () No
 () Partially
 () I don't know

113. Please mark the most appropriate response related to the air temperature monitoring:

- () The air temperature is not monitored
 () The air temperature is manually monitored
 () The air temperature is automatically monitored
 () The air temperature is manually and automatically monitored
 () I don't know
 () Other answer:

114. How do you rate how often the lab air temperature does not fulfil the established parameters?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

115. How do you rate the quality of the external services provided to the HVAC system maintenance/certification?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

ELECTRICAL SYSTEMS

116. Does the BSL-3 have dedicated power transformers?

- () Yes
 () No
 () I don't know

117. If so, how many power transformers are installed?

118. What's the transformers' capacity (kVA)?

119. What's the percentage of the lab demand met by the current power supply capacity on average?

- () Less than 100%
 () Between 100% and 150%
 () Between 150% and 200%
 () More than 200%
 () I don't know

120. What's the percentage of the lab demand met by the current power supply capacity during the peak of consumption?

- () Less than 100%
- () Between 100% and 150%
- () Between 150% and 200%
- () More than 200%
- () I don't know

121. How do you rate the security level of the place where the power transformers are installed?

	1	2	3	4	5	
Totally insecure	☐	☐	☐	☐	☐	Totally secure

122. Is the BSL-3 level covered by an UPS system?

- () Yes
- () No
- () I don't know

123. If so, how long is its autonomy?

124. Which is the equipment/systems covered by the UPS?

- () Automation
- () HVAC
- () Waste treatment system
- () Access control
- () Fumigation chamber
- () Sealed doors
- () Pass-Through/dunk tank
- () Firefighting systems
- () Biosafety Cabinets
- () Incubators
- () Ultra freezers
- () Freezers and refrigerators
- () Not applicable
- () I don't know
- () Other:

125. Is the BSL-3 covered by power generators?

126. If so, how many power generators are installed?

127. What's the power generators' capacity (kVA)?

128. Are power generators tested periodically by a specialized technical team?

- () Yes
- () No
- () I don't know

129. If so, what is the frequency of the test?

- () More than once per month
- () Monthly
- () Bimonthly
- () Quarterly
- () Biannually
- () Annually
- () Less than once a year
- () Other:

130. When it was conducted the last test on the power generators?

131. Which is the equipment/systems covered by the power generator?

- ☐ Automation
- ☐ HVAC
- ☐ Waste treatment system
- ☐ Access control
- ☐ Fumigation chamber
- ☐ Sealed doors
- ☐ Pass-Through/dunk tank
- ☐ Firefighting systems
- ☐ Biosafety Cabinets
- ☐ Incubators
- ☐ Ultra freezers
- ☐ Freezers and refrigerators
- ☐ Not applicable
- ☐ I don't know
- ☐ Other:

132. Is there currently a preventive maintenance service for the power generators provided by a specialized technical team?

- ☐ Yes
- ☐ No
- ☐ I don't know

133. How do you rate the security level of the place where the power generators are installed?

1	2	3	4	5
Totally insecure	☐	☐	☐	☐
Totally secure				

134. How do you rate how often the power electricity is cut off, determining the need to put into operation the UPS and power generators?

1	2	3	4	5
Never	☐	☐	☐	☐
Always				

135. How do you rate how often there is any impact on the activities carried out by the lab when there is a power electricity cut off? (*OBS: If the UPS and power generators always provide the power without any interruption, the answer should be 1 – never*)

1	2	3	4	5
Never	☐	☐	☐	☐
Always				

136. Does the lab have any program or mechanism to save electricity?

- ☐ Yes
- ☐ No
- ☐ I don't know

137. If so, please describe that program or mechanism.

FUMIGATION CHAMBER/AIR LOCKS

138. How is the entrance and exit procedures for large volumes in the BSL-3 lab?

139. Does the lab have Fumigation Chamber/Air Locks?

() Yes.

() No.

140. If so, how many?

141. Do the Fumigation Chamber/Air Locks have an interlocking door system?

() Yes

() No

() I don't know

() Not applicable

142. Are the Fumigation Chamber/Air Locks sealed?

() Yes

() No

() I don't know

() Not applicable

143. What is the method used for decontaminating the Fumigation Chamber/Air Locks?

144. Has the decontaminating method been validated?

() Yes

() No

() I don't know

() Not applicable

145. Do the Fumigation Chamber/Air Locks have their own system for inflating and exhausting the air?

() Yes

() No

() I don't know

() Not applicable

146. Are there cracks inside the Fumigation Chamber/Air Locks?

() Yes

() No

() I don't know

() Not applicable

147. Are the Fumigation Chamber/Air Locks nationally manufactured?

() Yes

() No

() Part of them are

() I don't know

() Not applicable

148. How do you rate how often are observed operational failures in the Fumigation Chamber/Air Locks?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

149. How do you rate the quality of the external services provided to the Fumigation Chamber/Air Locks maintenance?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

SAS/PASS-THROUGH/DUNK TUNKS

150. Which equipment/systems does the lab have to enter/exit samples and other small volumes materials?
151. Do those equipment/systems have an interlocking door system?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
152. What is the method used for decontaminating the samples and other small volumes materials?
153. Has the decontaminating method been validated?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable

WHOLE LAB DECONTAMINATION

154. Is there a set periodicity for the decontamination of the laboratory as a whole?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
155. If so, what is the frequency?
- ☐ More than once per semester
 - ☐ Biannually
 - ☐ Annually
 - ☐ Every two years
 - ☐ I don't know
 - ☐ Not applicable
 - ☐ Other:
156. What is the method used for decontaminating the lab as a whole?
157. Has the decontaminating method been validated?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable

EFFLUENT DECONTAMINATION SYSTEM

158. What is the method used for decontaminating the effluent generated in the BSL-3 lab?
- ☐ Chemical
 - ☐ Thermal
 - ☐ Thermo-Chemical
 - ☐ Other:
159. Has that method been validated?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
160. Please enter the name of the EDS manufacturer?
161. Is the effluent decontamination system installed in a biocontained area?
- ☐ Yes
 - ☐ No
 - ☐ Not applicable

162. How do you rate how often leaks are observed in the effluent decontamination system?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

163. Is there redundancy of the effluent decontamination system?

- () Yes
() No
() Not applicable

164. What is the maximum capacity of the effluent decontamination system?

165. How do you rate how often that capacity meets the lab demand?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

166. Are carried out periodically qualification tests for the effluent decontamination system?

- () Yes
() No
() I don't know
() Not applicable

167. If so, what is the frequency of the test?

- () Biannually
() Annually
() Every two years
() I don't know
() Not applicable
() Other:

168. When it was conducted the last qualification test?

169. Does the lab keep records of each effluent decontamination cycle?

- () Yes
() No
() I don't know
() Not applicable

170. How do you rate how often are observed operational failures in the effluent decontamination system?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

171. How do you rate the quality of the external services provided to the effluent decontamination system maintenance/qualification?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

AUTOCLAVES

172. How many autoclaves are there in the BSL-3 labs and what is their respective capacity?
173. Does the number and capacity of autoclaves meet the lab demands?
- ☐ Yes, always
 - ☐ Yes, usually
 - ☐ No, usually
 - ☐ No, never
174. What are the kinds of autoclaves present in the BSL-3 lab?
- ☐ Vertical
 - ☐ Horizontal, simple door
 - ☐ Horizontal, double door
 - ☐ Horizontal, double door, border
 - ☐ Chamberlain
 - ☐ Benchtop
 - ☐ Other:
175. If there are border autoclaves, do their doors have an interlocking system?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
176. If there are border autoclaves, do they have bioseal?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
177. Please enter the name of the manufacturers of the autoclaves present in the BSL-3 lab:
178. Have the autoclaves cycles been validated for the different types of loads?
- ☐ Yes, for all kinds of loads
 - ☐ Yes, but not for all kinds of loads
 - ☐ No
 - ☐ I don't know
179. Is the output of deaerators and condensates from border autoclaves taken to the effluent decontamination system?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
180. Does the lab keep records of each autoclaving cycle?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
181. How do you rate how often are observed operational failures in the autoclaves?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

182. How do you rate the quality of the external services provided to the autoclaves maintenance?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

183. In addition to autoclaves, does the BSL-3 lab have other equipment/systems for decontamination of solid waste?

- () Yes
() No
() I don't know

184. If so, please enter the kind of equipment/systems:

BIOSAFETY CABINETS (BSCs)

185. How many BSCs are currently installed on BSL-3 lab?

186. Among the installed BSCs, how many are currently out of use (in maintenance, with expired certification or for any other reason)?

187. What are the BSCs classes?

188. Please enter the name of the manufacturers of the BSCs present in the BSL-3 lab:

189. Does the current location of BSCs meet the requirements to avoid external interferences, like from the HVAC system, doors openings, etc.?

- () Yes, always
() Yes, usually
() No, usually
() No, never
() I don't know

190. Are carried out periodically certification tests for BSCs?

- () Yes
() No
() I don't know

191. If so, what is the frequency of the tests?

- () Biannually
() Annually
() Every two years
() I don't know
() Not applicable
() Other:

192. When it was conducted the last certification test?

193. Among the currently installed BSCs, how many are with the certification expired?

194. Is there a SOP to training users for proper use of BSCs?

- () Yes
() No
() I don't know

195. If so, do the technicians follow the recommended protocols?

- () Yes, always
() Yes, usually
() No, usually
() No, never
() I don't know

196. Does the lab use to use Bunsen burner inside the BSCs?
 () Yes
 () No
 () I don't know
197. Please describe the cleaning procedures after using a BSC.
198. Please describe the method applied to the UV bulb efficacy control.
199. Are samples and other potentially infectious materials handled exclusively in BSCs?
 () Yes
 () No
 () I don't know
200. How do you rate how often are observed operational failures in the BSCs?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

201. How do you rate the quality of the external services provided to the BSC maintenance/certification?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

FREEZERS AND REFRIGERATORS

202. How many ultra-freezers are currently installed on the BSL-3 lab?
203. Please enter the name of the manufacturers of the ultra-freezers present in the BSL-3 lab and their respective capacities:
204. Among the installed ultra-freezers, how many are currently out of use (in maintenance or for any other reason)?
205. Does the current number of refrigerators, freezers and ultra-freezers meet the needs of the BSL-3 lab?
 () Yes
 () No
 () I don't know
206. Are the refrigerators, freezers, and ultra-freezers models suitable for laboratory use or are they household equipment?
 () All equipment are suitable for laboratory use
 () Most equipment are suitable for laboratory use
 () Most are household equipment
 () All of them are household equipment
207. Please mark the most appropriate response related to the equipment temperature monitoring:
 () The equipment temperature is not monitored
 () The equipment temperature is manually monitored
 () The equipment temperature is automatically monitored
 () I don't know
208. Does the BSL-3 lab have a dedicated room to refrigerators, freezers, and ultra-freezers?
 () Yes
 () No
 () I don't know

209. How do you rate the quality of the external services provided to the ultra-freezers?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

CENTRIFUGES

210. Are there centrifuges installed in the BSL-3 lab?

- () Yes
- () No
- () I don't know

211. If so, are the rotors or cups sealed?

- () Yes
- () No
- () I don't know
- () Not applicable

212. Is there a SOP for periodically decontamination of centrifuges?

- () Yes
- () No
- () I don't know
- () Not applicable

213. If so, is the recommended decontamination protocol followed?

- () Yes, always
- () Yes, usually
- () No, usually
- () No, never
- () Not applicable

OTHER EQUIPMENT

214. In addition to the equipment mentioned in the previous sections, does the BSL-3 lab have other relevant equipment (e.g., ventilated racks, sequencers, tissue digesters, etc.)? If so, please enter which equipment:

LABORATORY ANIMALS

215. Does the lab handle animals in high-containment areas?

- () Yes
- () No

216. If so, please inform the suppliers of the animals.

217. Which species are handled?

218. Are different species handled in the same room at the same time?

- () Yes
- () No
- () I don't know

219. Are the rooms used for handling animals used exclusively for that purpose?

- () Yes, all of them
- () Yes, part of them
- () No

220. Are the rooms the primary barrier? Otherwise, please inform which equipment (such as ventilated racks and isolators) are used as primary barriers:

221. If equipment such as ventilated racks and isolators are used as primary barriers, please inform their number, capacity, and manufacturers.

222. If equipment such as ventilated racks and isolators are used, are they certified periodically?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
223. If so, how often is the certification?
- ☐ Biannual
 - ☐ Annual
 - ☐ Every 2 years
 - ☐ I don't know
 - ☐ Not applicable (lab has not been certified)
 - ☐ Other:
224. When it was conducted the last qualification test?
225. If the room is the primary barrier, is there an anteroom (or changing room) with interlocking doors to access the animals room?
- ☐ Yes
 - ☐ No
 - ☐ Not applicable
226. If animal necropsies are performed, where the procedure is carried out (BSCs, necropsy room, bench, etc.)?
227. Have the technicians who handle the animals received formal training on the concepts of animal welfare?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
228. Is specific risk assessment carry out for the use of laboratory animals?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
229. Does the lab have an Institutional Animal Care and Use Committee (IACUC) officially constituted?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
230. If so, is the IACUC up to date with the national regulations, including its register under the National Council?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
231. Do the conditions of space, temperature, humidity, light cycles, acoustics, watering, and feeding meet animal welfare requirements for the handled species?
- ☐ Yes
 - ☐ No
 - ☐ Partially
 - ☐ I don't know
232. Does the room where the animals are kept have a CCTV system enabling continuous monitoring?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
233. What is the method used for the decontamination of animal carcasses?

234. Has the method reported in the previous response been validated?

- ☐ Yes
- ☐ No
- ☐ I don't know
- ☐ Not applicable

235. How often are there incidents or accidents involving the handling of live animals?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

236. In the case of manipulation of arthropods, what are the procedures to avoid their release?

237. In the case of manipulation of arthropods, how do rate the risk of releasing animals?

- ☐ Insignificant
- ☐ Small
- ☐ Medium
- ☐ High

238. In the case of arthropod manipulation, did the constructive aspects follow specific requirements for the species handled?

- ☐ Yes
- ☐ No
- ☐ Partially
- ☐ I don't know

UNIFORMS AND PPE

239. Are uniforms available for exclusive use in the BSL-3 lab?

- ☐ Yes
- ☐ No
- ☐ I don't know

240. Is the uniform available suitable (quantity and quality) for all BSL-3 users?

- ☐ Yes (both quantity and quality)
- ☐ Yes (just quantity)
- ☐ Yes (just quality)
- ☐ No

241. What is the method used to decontaminate the uniforms?

242. Are the kinds of PPE provided suitable to all activities carried out in the BSL-3 lab?

- ☐ Yes, always
- ☐ Yes, usually
- ☐ No, usually
- ☐ No, never

243. Regarding the number of available PPE:

- ☐ Is always sufficient
- ☐ Is usually sufficient
- ☐ Is usually insufficient
- ☐ Is always insufficient

244. Does the lab use to reuse disposable PPE?

- () Yes, always
- () Yes, usually
- () No, usually
- () No, never

245. Is there a SOP containing guidance on the proper use of PPE?

- () Yes
- () No
- () I don't know

246. If so, how do you rate how often the procedures are followed by BSL-3 users?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

HUMAN RESOURCES AND TRAINING

247. How many people currently perform activities in the BSL-3 lab?

248. What is the minimum educational level required to the BSL-3 users?

249. Is there a SOP containing the capacity building pathway for BSL-3 users, emphasizing the biosafety and biosecurity issues?

- () Yes
- () No
- () I don't know

250. If so, how do you rate how often the procedures are followed?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

251. What is the approximate workload of the biosafety and biosecurity capacity building training (considering theory plus practice time)?

252. How do you rate the level of awareness of technicians in relation to biosafety procedures?

	1	2	3	4	5	
Very low	☐	☐	☐	☐	☐	Very high

253. How many cleaning professionals are currently allowed to access the BSL-3 lab?

254. Have the cleaning staff received training on biosafety issues?

- () Yes
- () No
- () I don't know
- () Not applicable

255. If so, how many hours of training (considering theory plus practice time), on average?

256. How many IT professionals are currently allowed to access the BSL-3 lab?

257. Have the IT staff received training on biosafety issues?

- () Yes
- () No
- () I don't know
- () Not applicable

258. If so, how many hours of training (considering theory plus practice time), on average?
259. Does the laboratory have an engineer with biosafety expertise on its workforce?
- () Yes
() No
() I don't know
260. Does the laboratory have a permanent maintenance team with biosafety expertise on its workforce?
- () Yes
() No
() I don't know
261. How many maintenance professionals are currently allowed to access the BSL-3 lab?
262. Have the maintenance team received training on biosafety issues?
- () Yes
() No
() I don't know
() Not applicable
263. If so, how many hours of training (considering theory plus practice time), on average?
264. Does the lab have a biosecurity officer?
- () Yes
() No
265. If so, does his/her position allow independence of those responsible for implementing the program of work?
- () Yes
() No
() I don't know
() Not applicable
266. Does the biosecurity officer dedicate his/her time exclusively to this activity?
- () Yes
() No
() I don't know
() Not applicable
267. What is the biosecurity officer academic background?
268. Has the biosecurity officer received training on biosafety issues before starting his/her activities related to this position?
- () Yes
() No
() I don't know
() Not applicable
269. If so, how many hours of training (considering theory plus practice time)?
270. How many hours of refresher training on biosafety issues does the biosafety officer receive in an annual basis (on average)?

WORKERS' HEALTH CARE

271. Does the lab include in its risk assessment pathway issues related to the BSL-3 users' health?
- () Yes
() No
() I don't know
272. Does the lab provide periodic medical exams for its employees?
- () Yes
() No
() I don't know

273. If so, do the medical exam include specific tests related to the activities carried out in the BSL-3 lab?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
274. Does the lab have a first aid station?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
275. If so, how many professionals work in the first aid station and what are their respective education background?
276. Are the BSL-3 users allowed to drink water in the BSL-3 lab?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
277. If so, what procedures are adopted to prevent contamination of employees during water intake?
278. Is food intake allowed in the BSL-3 lab?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
279. If so, what procedures are adopted to prevent contamination of employees during food intake?
280. Are the BSL-3 users allowed to take their cell phones in the BSL-3 lab?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
281. How many hours per day, on average, do users stay within the BSL-3 lab?
282. Has the lab defined a maximum period of stay for users within the BSL-3 lab?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
283. If so, what is the given period?
284. Is any survey carried out to assess the well-being of the professional working in the BSL-3 lab?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
285. If so, what is the frequency of the survey?
286. Was the lab designed considering accessibility requirements?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
287. Which communication tools are present in BSL-3 lab?
- ☐ Telephone
 - ☐ Intercom
 - ☐ Internet
 - ☐ Radio
 - ☐ None
 - ☐ I don't know
 - ☐ Other:

288. If internet service is provided in BSL-3 lab, how do you rate the quality of this service?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

289. Does the lab have emergency shower within the BSL-3 lab?

- () Yes
() No
() I don't know

290. Does the lab have emergency eye washing within the BSL-3 lab?

- () Yes
() No
() I don't know

BIOSECURITY

291. How do you rate the frequency of biosafety-related incidents in your institution (considering not only the BSL-3 lab)?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

292. How do you rate the risk of an external-sourced criminal event occurring in the BSL-3 lab?

	1	2	3	4	5	
Very low	☐	☐	☐	☐	☐	Very high

293. Has there ever been a report of theft or steal of materials from the BSL-3 lab?

- () Yes
() No
() I don't know

294. How do you rate the quality of street lighting that gives access to the lab?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

295. Has the lab ever been sabotaged?

- () Yes
() No
() I don't know

296. How do you rate the risk of sabotage in your institution?

	1	2	3	4	5	
Very low	☐	☐	☐	☐	☐	Very high

297. Does the lab have a guard service?

- () Yes
() No
() I don't know

298. If so, how many hours per day is the service provided?

299. Is the guard service provided on weekends and holidays?
- ☐ Yes
 - ☐ No
 - ☐ Partially
 - ☐ I don't know
300. Are there additional security barriers surrounding the BSL-3 facility? *(Mark all applied)*
- ☐ Regular fence
 - ☐ Wrought iron fence
 - ☐ Electric fence
 - ☐ CCTV
 - ☐ Presence sensor/Motion detector
 - ☐ There are no additional barriers
 - ☐ Other:
301. Is there any cooperation agreement established with security authorities such as military service, federal police, local police, FBI, etc.?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
302. Do the rooms where high-risk biological agents are stored have their own access control?
- ☐ Yes, all of them
 - ☐ Yes, some of them
 - ☐ No
 - ☐ I don't know
303. Is the equipment where high-risk biological agents are stored locked?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
304. Is there inventory and records of all transportation of high-risk biological agents?
- ☐ Yes
 - ☐ No
 - ☐ Partially
 - ☐ I don't know
305. Is there a protocol to evaluate the adequacy of receiving external visits?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
306. Is there a protocol for evaluating criminal records and behavioral profile before hiring workers who will access the BSL-3 lab?
- ☐ Yes
 - ☐ No
 - ☐ Partially
 - ☐ I don't know
307. Is there a SOP to transporting high-risk biological agents?
- ☐ Yes
 - ☐ No
 - ☐ I don't know

308. If so, how do you rate how often the procedures are followed by BSL-3 users?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

309. How do you rate how often high-risk biological agents are removed from the BSL-3 to be transported to another lab?

	1	2	3	4	5	
Never	☐	☐	☐	☐	☐	Always

310. Does the lab have workers certified in the IATA standards for biological assets transportation?

- () Yes
() No
() I don't know

311. If so, is the certificate up to date?

- () Yes
() No
() I don't know
() Not applicable

312. How do you rate the security level of the data generated/stored in the BSL-3 lab?

	1	2	3	4	5	
Very low	☐	☐	☐	☐	☐	Very high

313. Have already been reported incidents involving the security of the data generated/stored in the BSL-3 lab?

- () Yes
() No
() I don't know

GMOs

314. Does the lab work with genetic engineering, like DNA recombination methods?

- () Yes
() No
() I don't know

315. If so, does the lab carry out specific risk assessment for this purpose?

- () Yes
() No
() I don't know

316. Does the lab have a Quality Certificate for Biosafety?

- () Yes
() No
() I don't know
() Not applicable

RISK ASSESSMENT

317. Is there a SOP defining how to do risk assessment for the activities carried out in the BSL-3 lab?
 () Yes
 () No
 () I don't know
318. Has the risk assessment been done for all activities currently carried out in the BSL-3 lab?
 () Yes
 () No
 () I don't know
319. If so, is the risk assessment periodically reviewed?
 () Yes
 () No
 () I don't know
320. When it was conducted the last revision?
321. What is the position of the professional in charge of the risk assessment elaboration?
322. What is the function of the professional in charge of the risk assessment elaboration?
323. Has the person in charge of the risk assessment elaboration received specific training for this purpose?
 () Yes
 () No
 () I don't know
 () Not applicable
324. If so, which organization offered the training?
325. How do you rate the quality of the risk assessment carried out?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

AUTOMATION

326. Does the lab have an automation system for monitoring and/or controlling equipment and systems used in the BSL-3 lab?
 () Yes
 () No
 () I don't know
327. If so, which equipment/systems are automatized monitored/controlled? *(Mark all applied)*
 () HVAC
 () Electrical systems
 () Access control
 () Waste treatment system
 () Fumigation chamber/Air locks
 () Autoclaves
 () Other:
328. How do you rate the quality of the automation software?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

329. Does the automation system have audible or visual alerts in case of deviation from critical parameters?
() Yes
() No
() I don't know
() Not applicable
330. How many employees are currently able to operate the automation system? Which is their academic background?
331. Are the data generated by the automation system being backed up?
() Yes
() No
() I don't know
() Not applicable
332. If so, what is the frequency of the backup?
333. Does the lab have a software for recording data related to preventive maintenance plans?
() Yes
() No
() I don't know

OTHER MANAGEMENT AND ADMINISTRATIVE/FINANCIAL ISSUES

334. Does the institution carry out annual budget planning?
() Yes
() No
() I don't know
335. If so, what is the institution's annual budget for the current year (just running costs without considering investments)?
336. What is the institution's annual budget for the current year for investments?
337. What was the total amount of running costs in the previous year (without considering investment expenses) related to the BSL-3 lab?
338. What was the approximated cost for maintenance expenses in the previous year?
339. What was the approximated cost for supplies purchasing in the previous year?
340. What was the total amount of investment expenditures in the previous year, considering the laboratory as a whole?
341. What was the total amount of investment expenditures in the previous year, considering the BSL-3 lab?
342. What are the sources of funds to cover operating costs?
343. What are the sources of funds to cover investment expenses?
344. Has the operation of the BSL-3 lab been suspended in the last 12 months due to lack of funds for the acquisition of supplies?
() Yes
() No
() I don't know
345. If so, for how long?
346. Has the operation of the BSL-3 lab been suspended in the last 12 months due to lack of funds for equipment and/or systems maintenance?
() Yes
() No
() I don't know
347. If so, for how long?

348. How do you rate the financial condition of the laboratory, specifically considering the BSL-3 management?

	1	2	3	4	5	
Resources are always insufficient	☐	☐	☐	☐	☐	Resources are always sufficient

349. Have the activities of the BSL-3 lab been suspended at some point due to lack of demands?

- () Yes
() No
() I don't know

350. If so, how often the activities are suspended for this reason (lack of demand)?

	1	2	3	4	5	
Very rarely	☐	☐	☐	☐	☐	Very often

351. Does the institution currently have partnerships with others to use the BSL-3 lab?

- () Yes
() No
() I don't know

352. How do you predict the volume of laboratory activities for the coming years in comparison to the current demand?

- () Similar
() Greater
() Lower
() No demands are expected for the coming years

353. Are there demands currently unmet due to lack of a higher-level biocontainment structure?

- () Yes
() No
() I don't know

354. If so, please describe these demands:

355. Does the lab have an internal biosafety committee (or similar)?

- () Yes
() No
() I don't know

356. Does the lab have its own Biosafety Manual?

- () Yes
() No
() I don't know

357. Does the lab have its own documental structure for managing biorisk?

- () Yes
() No
() I don't know

358. If so, how do you rate the level of awareness that BSL-3 users have of this documentation?

	1	2	3	4	5	
Terrible	☐	☐	☐	☐	☐	Excellent

359. Does the lab have a documented contingency plan for emergencies?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
360. Is there a SOP to recording accidents?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
361. How many accidents are recorded, on average, per year, considering the whole lab (not just the BSL-3 lab)?
362. Did any of these accidents lead to morbidity or mortality due to laboratory acquired infection? If so, please briefly describe:
363. How many accidents are recorded, on average, per year, considering just the BSL-3 lab?
364. Did any of these accidents lead to morbidity or mortality due to laboratory acquired infection? If so, please briefly describe:
365. Does the lab carry out periodic internal audits addressing biosafety aspects?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
366. If, how often the internal audits are carried out?
- ☐ Biannual
 - ☐ Annual
 - ☐ Every 2 years
 - ☐ I don't know
 - ☐ Not applicable
 - ☐ Other:
367. When it was conducted the last internal audit?
368. Has the laboratory already received external audit addressing biosafety aspects?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
369. If so, when it was conducted the last external audit?
370. Which body/company/institution carried out the external audit?
371. Are the methods applied in the lab supported by legislation or reference guidelines/standards?
- ☐ Yes, always
 - ☐ Yes, usually
 - ☐ No, usually
 - ☐ No, never
 - ☐ I don't know
 - ☐ Not applicable
372. Are the methods applied in the lab accredited by an accreditation body?
- ☐ Yes, always
 - ☐ Yes, usually
 - ☐ No, usually
 - ☐ No, never
 - ☐ I don't know
 - ☐ Not applicable
373. In your opinion, are there gaps in the regulation/legislation of the activities carried out by the BSL-3 labs?
- ☐ Yes
 - ☐ No
 - ☐ I don't know

374. If so, please briefly mention what these shortcomings would be:
375. Does the lab have a waste management plan?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
376. Does the lab have a contract with specialized company for solid waste disposal?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
377. Is the lab up to date with all its legal obligations with the fire department?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
378. Which is the fire detection system inside the BSL-3 lab?
- ☐ None
 - ☐ Smoke detector
 - ☐ Heat detector
 - ☐ I don't know
 - ☐ Other:
379. Does the lab have a fire brigade?
- ☐ Yes
 - ☐ No
 - ☐ I don't know
380. If so, are the BSL-3 users brigade members?
- ☐ Yes, all of them
 - ☐ Most of them are
 - ☐ Most of them are not
 - ☐ No
 - ☐ I don't know
 - ☐ Not applicable
381. What are the main maintenance issues faced in the BSL-3 lab?
382. What are the main challenges in managing the BSL-3 lab?

APPENDIX C – Free Consent Agreement (Template)

FREE CONSENT AGREEMENT

You are being invited to participate in a research project entitled "*High containment laboratories: situational diagnosis, comparative critical analysis and future perspectives considering the international recommendations on biosafety and biosecurity*", developed by student *André de Oliveira Mendonça*, enrolled in the doctoral program in Applied Biochemistry of the Federal University of Viçosa, Brazil (student identification number: 104711), under the advisor of Professor *Cláudio Mafra*.

I declare, through this term, that I agree to fill out a questionnaire regarding the biosafety and biosecurity aspects of the laboratory where I work as part of the field activities related to the afore mentioned project.

I state that I have agreed to participate of my own free will, without suffering any embarrassment and without receiving any financial support or having any burden, with the sole purpose of collaborating for the success of the research. I am aware that I will be able to withdraw this consent at any time before the conclusion of this project.

I was informed about the strictly academic objective of the study, which, in general terms, consists of a diagnosis of the situation related to the structure and operation of high-level containment facilities installed in Brazil, and compare it with the status observed in other countries, especially in the United States of America. From this comparison, the stud aims to purpose structuring actions to improve the governance and oversight of the Brazilian laboratories, considering a cooperative approach between different governmental bodies.

My collaboration will be done on a voluntary basis, through filling out the questionnaire, being aware that only the student and its advisor will have access to the information provided and **that they have committed to maintain full confidentiality about it.**

I was informed that the presentation of the information obtained in the study will be made in a compiled manner, without individualized identification of the laboratories participating in the study, in order to prevent any damage to the image of these institutions. The information collected will be held by the researcher in a hard disk for a period of 10 years and after that will be deleted.

Participants will receive free, in electronic format, the scientific publications generated from the collection of the data.

The completion of the questionnaire does not bring any cost to the participant, however, if there is any damage resulting from the research to the participant, we undertake to guarantee the indemnification.

Estimated time to complete the questionnaire: 2 hours.

I was informed, also, that I may consult those responsible for this study at any time, including to rectify or supplement information, through the e-mails andre.d.mendonca@ufv.br and mafra@ufv.br, telephone (+1 832 5959) and at the address: 1650, Arrington Road, ap 1137, College Station, Texas. ZIP Code: 77845.

Doubts about the ethical nature of the research, as well as complaints may be sent to the Research Ethics Committee of the Federal University of Viçosa (CEP/UFV), through the following channels: email cep@ufv.br; phone (+55 31 3612 2316); or mail address: Universidade Federal de Viçosa / Comitê de Ética em Pesquisa com Seres Humanos; Edifício Arthur Bernardes, subsolo Av. PH Rolfs, s/n – Campus Universitário Viçosa/MG, Brasil; CEP: 36570-900.

I was informed about the availability of Word and PDF versions of the questionnaire, allowing the gradual completion of the questionnaire.

I am also aware of the risk related to not knowing all the answers. In this case, the risk will be mitigated as follows: no answer is mandatory.

The main benefits obtained from completing the questionnaire are:

- a) For the participant: reassessment of the biosafety status of the laboratory.
- b) For society: diagnosis of the situation of high containment laboratories installed in Brazil, allowing the proposition of policies to improve the governance and oversight of these laboratories.

I attest to receipt of a signed copy of this Free Consent Agreement.

City, State

Date

Participant's signature: _____

Student's signature: _____

Advisor's signature: _____