भारत सरकार
विज्ञान और प्रौद्योगिकी मंत्रालय
जैव प्रौद्योगिकी विभाग
GOVERNMENT OF INDIA
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

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NO. PID-15011(11)/5/2020-PPB-DBT Dated 18.12.2020

OFFICE MEMORANDUM

Sub: Guidelines for the establishment of containment facilities: Biosafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility, 2020

In India, all activities related to Genetically Engineered organisms (GE organisms) or cells and hazardous microorganisms and products thereof are regulated as per the "**Manufacture, Use/Import/Export and Storage of Hazardous Microorganisms/ Genetically Engineered Organisms or Cells, Rules, 1989**" (Rules, 1989) notified by the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India under the **Environment (Protection) Act, 1986** (EPA 1986). "**The Regulations & Guidelines for Recombinant DNA Research and Biocontainment, 2017**" notified by the Department of Biotechnology vide OM No. **BT/BS/17/635/2015-PID, dated 01st April 2018** provide operational guidance on containment of biohazards and levels of biosafety that are required to be complied with by all the Institutional Biosafety Committees (IBSCs) and the host institutions involved in research, development and handling of the genetically engineered (GE) microorganisms and non-GE hazardous microorganisms.

2. The "**Guidelines for the Establishment of Containment Facilities: Biosafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility, 2020**" have been drafted in accordance with the national and international references, guidance and regulations.

3. The Department of Biotechnology hereby notifies the **"Guidelines for the Establishment of containment facilities: Biosafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility", 2020.**

4. The Guidelines can be accessed from www.dbtinida.nic.in and https://ibkp.dbtindia.gov.in/.

5. It is important to note that **specifications for BSL-2 facility are intended for Guidance purpose**, while those for **BSL-3 facility are essential components of compliance required for the Certification of the facility**.

6. With this notification, **Certification of BSL-3 Laboratories shall be mandatory for all organizations handling hazardous microorganisms for Research and Development purpose w.e.f 1st April, 2021**. Further, all new BSL-3 facilities will undergo Certification prior to commencement of facility operation. These guidelines shall be binding pan India for all public and private organizations involved in research, development and handling of the genetically engineered (GE) microorganisms and non-GE hazardous microorganisms.

7. The guidelines have approval of Review Committee of Genetic Manipulation (RCGM), the competent authority notified under Rules 1989 of Environment (Protection) Act, 1986.

(Dr. Nitin Kumar Jain)
Member Secretary, RCGM
Scientist F, DBT

GUIDELINES FOR THE ESTABLISHMENT OF CONTAINMENT FACILITIES: BIOSAFETY LEVEL 2 (BSL-2) & 3 (BSL-3) AND CERTIFICATION OF BSL-3 FACILITY

Department of Biotechnology
Ministry of Science and Technology
Government of India
2020

सत्यमेव जयते

डॉ. रेणु स्वरूप
DR. RENU SWARUP

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FOREWORD

Advances in Biotechnology research as well as existing, emerging and re-emerging micro-organisms have significant impact on public health. Preparedness towards a rapid response is therefore, critically important, with a strong network of public health laboratories as a key line of defence. Therefore, there is an urgent need to strengthen research laboratory biosafety practices and to forge more robust national laboratory networks. The laboratories should be devised such that they can support all three components of public health interventions, that is, diagnosis, surveillance and control. The Department of Biotechnology (DBT) recognizes biosafety and biosecurity as important issue and has been at the forefront to achieve these outcomes, as per the mandate under "Rules for the manufacture, use/import/export and storage of hazardous microorganisms/ genetically engineered organisms or cells, 1989" notified by the Ministry of Environment and Forests and Climate Change (MoEF&CC), Government of India under the Environment (Protection) Act, 1986.

In this regard, the Department of Biotechnology is pleased to release the "Guidelines for the Establishment of containment facilities: BioSafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility". The document describes the principle and components of containment and standards for the establishment of biosafety containment level 2 and 3 facilities and also the process of certification of BSL-3 facilities.

I would like to compliment Dr. Alka Sharma, Scientist G, DBT and Dr. Nitin Kumar Jain, Scientist F and Member Secretary, RCGM along with the Expert Committee Members; for their efforts in drafting the guidelines. I also acknowledge the contribution of Biosafety Support Unit, Regional Centre for Biotechnology, in preparation of this guidance document.

I am confident that these "Guidelines for the establishment of containment facilities:BioSafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility", would be beneficial for the Researchers in both, the setting up of the facility and getting it certified and in turn ensure that in this globalization era of modern biotechnology practices, the facilities in our country are the Global best in quality standard.

(Renu Swarup)

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PREFACE

In exercise of powers conferred through Environment (Protection) Act, 1986 and "Rules for the manufacture, use/import/export and storage of hazardous microorganisms/ genetically engineered organisms or cells, 1989", the Department of Biotechnology (DBT), Government of India had notified Regulations & Guidelines for Recombinant DNA Research and Biocontainment, 2017. As per these Guidelines, all existing BSL-3 and -4 facilities must be Certified by the DBT and all new facilities require Certification at the time of Commissioning operations. Thus, DBT embarked on instituting Guidelines for the Establishment of Biosafety levels 2, 3 and 4 facilities to deal with highly infectious and pathogenic organisms, in agreement with National Guidelines and at par with International standards.

Working with high risk pathogens requires diligence from the laboratory worker to maintain safe laboratory conditions. This includes extensive knowledge of both the pathogen and the procedures, proper training and rigorous adherence to the safety practices. We wish to emphasize that the guidance document recommends best practices for the safe conduct of work in a laboratory from a biosafety perspective. The document includes sections on the principle and practices of biosafety and risk assessment and important considerations for establishing the BSL-2 and BSL-3 facilities. With respect to the BSL-3 laboratory; technical standards for Engineering controls, essential tests to be conducted during the installation, validation procedures and Standard Operating Procedures, have been provided in depth.

In the Guidelines, we have harmonized recommendations with guidance issued and regulations promulgated by other interministerial committees. We are truly grateful to all the Experts for their proficiency and tireless efforts in shaping the Guidelines to this form. We acknowledge the critical inputs provided by Expert Committee constituted for this purpose, under the chairmanship of Prof. Rakesh Bhatnagar (Vice-Chancellor, Banaras Hindu University, Varanasi), esteemed members of the RCGM Committee and Experts from engineering background. We wish to thank them all for their dedication and hard work for without them, these Guidelines would not have been possible. We also acknowledge absolute commitment and dedication of Biosafety Support Unit, Regional Centre for Biotechnology, throughout the preparation of these guidelines.

We are glad to present the "Guidelines for the Establishment of containment facilities: BioSafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility". The purpose of this document is to provide guidance to Stakeholders including the architect and engineer contractor for planning, programming, designing, and constructing state-of-the-art facilities; that are vital for research and development; while ensuring compliance with biological safety, ultimately aimed towards public health. These guidlines applies to the establishment of BSL-2 facilities and establishment along with certification of BSL- 3 facilities nationwide.

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Dr. Nitin Kumar Jain
Member Secretary, RCGM
Department of Biotechnology
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Government of India

EXECUTIVE SUMMARY

In India, all activities related to Genetically Engineered organisms (GE organisms) or cells and hazardous microorganisms and products thereof are regulated as per the “Manufacture, Use/Import/Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, Rules, 1989” (Rules, 1989) notified by the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India under the Environment (Protection) Act, 1986 (EPA 1986). The Regulations & Guidelines for Recombinant DNA Research and Biocontainment, 2017 notified by the Department of Biotechnology provide operational guidance on containment of biohazards and levels of biosafety that are required to be complied with by all the IBSCs and the host institutions involved in research, development and handling of the genetically engineered (GE) microorganisms and non-GE hazardous microorganisms.

With increasing number of BSL 2 and BSL3 facilitates across the country, it was felt necessary to bring out a document on establishment of BSL 2 and BSL 3 containment facility and certification procedure. The “Guidelines for the Establishment of Containment Facilities: Biosafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility” notified here is an addition to the Regulations & Guidelines for Recombinant DNA Research and Biocontainment, 2017 with reference to the containment principle and procedures, containment levels, BSL-3 facility operation and certification.

The “Guidelines for the Establishment of Containment Facilities: Biosafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility” was drafted in accordance with the national and international reference, guidance and regulations substantiated by other inter-ministerial committees, recommendations from engineering experts, and detailed deliberations by the Expert Committee Chaired by Prof. Rakesh Bhatnagar, Vice Chancellor, Banaras Hindu University, Varanasi and RCGM experts.

The guidelines notified here provide the definition of containment, procedures and special practices, safety equipment and facility designs associated with various levels of containment in the Indian scenario. The establishment of the BSL-2 facility described in Chapter 2 addresses the requirements to be followed in the facility's design and related standards and practices for biosafety. Establishment of BSL-3 facility described in Chapter 3 addresses the additional design features and containment practices, recommendations for technical standards for the engineering controls for BSL-3 laboratory (Annexure I), essential tests during installation of BSL-3 facility (Annexure II), procedures for validation in BSL-3 facility (Annexure III), Standard Operating Procedures (SOPs) for specific considerations for working in the BSL-3 facility (Annexure IV), and mechanism of Certification for the operation of BSL-3 facility including the application forms, and checklist featuring set standards and procedures for BSL-3 operation for annual inspection (Annexure V).

These guidelines are applicable to diagnostics, research and development organizations, stakeholders as well as academic institutions.

This guidance document “Guidelines for the Establishment of Containment Facilities: Biosafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility” with subsequent additional information on technical standards for engineering controls, major tests to be conducted during installation, and methodology for validation of BSL-3 facility, after detailed deliberations from the experts was approved in the 184th and 191st meetings of the Review Committee on Genetic Manipulation (RCGM) held on 04th June 2020 and 15th October 2020, respectively.

It is important to mention that while the guidelines notified here provide specifications for BSL-2 facility, those for the BSL-3 facility are essential components of compliance required for the Certification of the BSL-3 facility.

CONTENTS

ABBREVIATIONS

ACH	Air Changes per Hour
ACR	Air Change Rates
BAS	Building Automation System
BMS	Building Management System
BC	Biological Containment
BIBO	Bag-In-Bag-Out
BSC	Biosafety Cabinet
BSL	Biosafety Level
CCTV	Closed-Circuit TeleVision
CDC	Centers for Disease Control and Prevention
CPCB	Central Pollution Control Board
DBT	Department Of Biotechnology
DDC	Direct Digital Control
DG	Diesel Generator
DLC	District Level Committee
DST	Department Of Science & Technology
ECBC	Energy Conservation Building Code
EPA	Environment (Protection) Act
ETP	Effluent Treatment Plant
GE	Genetically Engineered
GEAC	Genetic Engineering Appraisal Committee
HEPA	High Efficiency Particulate Air
HVAC	Heating, Ventilation and Air Conditioning
IATA	International Air Transport Association
IBSC	Institutional Biosafety Committee
ICAR	Indian Council Of Agricultural Research
ICMR	Indian Council Of Medical Research
ISO/IEC 17025	Accreditation based on standards entitled "general requirements for the competence of testing and calibration laboratories", published by the International Organization for Standardization
LAN	Local Area Network
MoEF&CC	Ministry Of Environment, Forest and Climate Change
NSF/ ANSI 49	American National Standard, which apply to Class II Biosafety Cabinets
PLC	Programmable Logic Controller
PPE	Personal Protective Equipment
RCGM	Review Committee On Genetic Manipulation
RDAC	Recombinant DNA Advisory Committee
rDNA	Recombinant DNA
RG	Risk Group
SBCC	State Biotechnology Coordination Committee
SOP	Standard Operating Procedures
SS-304	Stainless Steel - Grade 304 (Standard "18/8")
ULPA	Ultra Low Particulate Air
UPS	Uninterrupted Power Supply
WHO	World Health Organization

INTRODUCTION

Globally, biotechnology applications have seen a phenomenal advancement over the last few decades. Accordingly, biosafety and biosecurity practices pertaining to the safe handling and containment of infectious pathogens, emerging and re-emerging microorganisms along with hazardous biological materials are of utmost concern, as inadvertent or deliberate usage in addition to laboratory associated infections may have fatal consequences. This necessitates the institution of code of conduct, ethics and biosafety practices and adherence to globally harmonized regulations and guidelines, ultimately for ensuring the protection of public health and the environment.

In India, all activities related to Genetically Engineered organisms (GE organisms) or cells and hazardous microorganisms and products thereof are regulated as per the "*Manufacture, Use/Import/Export and Storage of Hazardous Microorganisms/ Genetically Engineered Organisms or Cells, Rules, 1989" (Rules, 1989)* notified by the Ministry of Environment, Forest and Climate Change (MoEF & CC), Government of India under the Environment (Protection) Act, 1986 (EPA 1986). The Six Competent Authorities and their mandate & functions as defined under *Rules, 1989* are:

Competent Authorities	Mandate & Functions
Recombinant DNA Advisory Committee (RDAC)	The RDAC functions in the Department of Biotechnology (DBT) and reviews developments in biotechnology at national and international levels and recommends suitable and appropriate safety regulations for India in recombinant research, use and applications from time to time.
Institutional Biosafety Committee (IBSC)	The IBSC is constituted by an occupier or any person including research institutions, handling hazardous microorganisms/genetically engineered organism(s) [at Research & Development (R&D) level]. The occupier or any person including research institutions prepares, with the assistance of the IBSC, an up-to-date on the site emergency plan according to the manuals/guidelines of the RCGM and makes available copies to the DLC/SBCC and RCGM/GEAC.
Review Committee on Genetic Manipulation(RCGM)	RCGM functions in the DBT to monitor the safety-related aspects in respect of on-going research projects and activities involving genetically engineered organisms/hazardous microorganisms. RCGM brings out Manuals of Guidelines specifying the procedure for regulatory process with respect to activities involving genetically engineered organisms in research use and applications including industry with a view to ensure environmental safety. All ongoing projects involving high risk category and controlled field experiments need to be reviewed to ensure that adequate precautions and containment conditions are followed as per the guidelines.

	The RCGM lays down procedures restricting or prohibiting production, sale, importation and use of such genetically engineered organism(s) or cells as are mentioned in the Schedule of Rules, 1989.
Genetic Engineering Appraisal Committee (GEAC)	The GEAC functions in the MoEF & CC and is responsible for approval of (i) activities involving large scale use of hazardous microorganisms and recombinants in research and industrial production from an environmental angle (ii) proposals relating to the release of genetically engineered organisms and products into the environment including experimental field trials. The committee or any person/s authorized by it shall have powers to take punitive actions under the Environment (Protection) Act, 1986.
State Biotechnology Coordination Committee (SBCC)	The SBCC periodically reviews the safety and control measures in the various installations/institutions handling genetically engineered organisms/hazardous microorganisms. The SBCC has powers to inspect, investigate and take punitive action in case of violations of statutory provisions through the Nodal Department and the State Pollution Control Board/Directorate of Health &/ Medical Services
District Level Committee (DLC)	The DLC monitors the safety regulations in installations/institutions engaged in the use of genetically modified organism(s)/ hazardous microorganism(s) and its/their applications in the environment. DLC/or any other person(s) authorized on this behalf shall visit the installation engaged in activity(ies) involving genetically engineered organisms, hazardous microorganisms, formulate information chart, find out hazards and risks associated with each of these installations and coordinate activities with a view to meet any emergency. The DLC shall also prepare an off-site emergency plan for field trials. The District Level Committee shall regularly submit its report to the SBCC/ GEAC.

The RDAC plays an advisory role, while IBSC, RCGM and GEAC are involved in regulatory and approval functions. SBCC and DLC are responsible for monitoring the activities related to GMOs at state and district levels, respectively.

The Review Committee on Genetic Manipulation, administered by the Department of Biotechnology, Ministry of Science and Technology, monitors the safety of on-going research projects or activities involving hazardous microorganisms, GE organisms, cells and products thereof. Within the purview of Rules, keeping in view the latest scientific developments, RCGM/GEAC issue guidelines from time to time on matters related to biosafety and biosecurity to be implemented pan India by all the institutions engaged in modern biotechnology involving hazardous microorganisms, GE organisms and cells and

products thereof. These guidelines shall be implemented through the Institutional Biosafety Committees (IBSCs).

In light of the above, the Department of Biotechnology is pleased to present "Guidelines for the establishment of containment facilities: Biosafety Level 2 (BSL-2) & 3 (BSL-3) and Certification of BSL-3 facility". The Guidance document covers the basic underlying principles and sstandard to be considered while establishing BSL-2 and BSL-3 laboratory including its testing, commissioning and validation.The Institutional Biosafety Committee (IBSC)s have been empowered for implementing and responding to institutional biosafety & biosecurity at the institution level and evaluating applications/ reports related to rDNA technology work involving the GE organisms and non-GE hazardous microorganisms in an organization. The IBSCs along with Laboratory In-charge are responsible for determining appropriate risk group classification and category of experiments and accordingly, proposing the required level of BSL facility. The responsibilities and functions of IBSCs have been detailed in the *"Handbook for Institutional Biosafety Committee (IBSC), 2020"*. Use of the appropriate BSL facility is binding pan India for all the public and private organizations involved in research, development and handling of GE organisms and non-GE hazardous microorganisms.

Chapter 1
PRINCIPLE AND COMPONENTS OF CONTAINMENT

Chapter 1: PRINCIPLE AND COMPONENTS OF CONTAINMENT

1.1. Principle

The principle is the protection of all identified elements from risk(s) posed by organisms (includes risk-inherent; GE and non-GE microorganisms, animal, plants, arthropods, aquatic animals, etc.) during their use in the laboratory. In practice, it should be achieved in the realization of the three interrelated steps:

- Identification of elements that should be protected: Containment measures should ensure the protection of the laboratory worker(s) (Primary elements) who have maximum possibility of exposure to the organism(s). In addition, the containment measure(s) should also prevent the escape of the organism(s) and, therefore, ensure the protection of persons outside the laboratory and the environment (Secondary elements).
- Identification of potential risk(s) associated with organism(s): It involves assessment of risk(s) associated with the organism(s) and their classification to appropriate risk groups based on the:
  - pathogenicity of the organism
  - modes of transmission and host range of the organism
  - availability of effective preventive treatments or curative medicines
  - capability to cause diseases to humans/animals/plants, and the
  - capability to cause epidemics.

  Based on the above information, the infective microorganisms are classified into four risk groups (Table 1), enabling the selection of the appropriate levels for the biosafety facilities. For an updated list of infective microorganisms under different risk groups refer "*Regulations and Guidelines on Biosafety of Recombinant DNA Research & Biocontainment, 2017*".

Table 1: Risk Group (RG) classification*

Risk Group (RG)	Description
RG 1 (no or low individual and community risk)	Microorganisms that are unlikely to cause human/ animal/plant disease.
RG 2 (moderate individual risk, low community risk)	Microorganisms that can cause disease in human /animal/ plant. The laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
RG 3 (high individual risk, low community risk)	A microorganism that usually causes serious or lethal human/ animal/ plant disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
RG 4 (high individual and community risk)	A microorganism that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

*Adopted from the Laboratory Biosafety Manual, Third edition, WHO, 2004.

- Determining the appropriate biosafety facility level for safe handling of organisms (includes risk-inherent GE and non-GE microorganisms, animals, plants, arthropods, aquatic animals, etc.).

1.2. Containment

Containment encompasses the safe methods (a combination of facilities, practices and procedures) for managing risk-inherent microorganisms, GE organisms or cells in the laboratory environment where they are being handled or maintained. Selection of appropriate containment strategies will ensure the safety of laboratory workers, outside people and the environment from hazardous microorganisms, GE organisms or cells by:

- Reducing the exposure, and
- Preventing their escape and establishment in a natural environment.

1.3. Factors in Containment

Depending on the nature of the work and organism involved, containment shall be different to ensure optimal protection to the workersand the environment. The levels of containment shall be determined based upon principle factors as described below:

1.3.1. Physical Containment

The physical containment of the microorganism under study, should be feasible to prevent or minimize its exposure to worker and environment. It is achieved through the use of three elements of containment, i.e., Procedures, Safety equipment(s) and Facility design(s).

The protection of personnel(s) and the immediate laboratory environment from exposure to organism (includes risk-inherent GE and non-GE microorganisms, animals, plants, arthropods, aquatic animals, etc.) is provided by 'Procedures' and the use of appropriate 'Safety equipment(s)' (Primary containment). The protection of the environment external to the laboratory from exposure to risk-inherent materials is provided by a combination of 'facility design' and operational practices (Secondary containment).

The elements are not in hierarchy and should be used with equal priorities in combination to ensure a successful containment.

1.3.1.1. Procedure

It is emphasized that good laboratory practices (GLP) is fundamental to laboratory safety and cannot be replaced by other means, which can only supplement it. The following must be followed by workers involved in research in handling of organism(s) in consideration of the:

- strict adherence to standard microbiological practices and techniques,
- selection of laboratory practices as required for ensuring safety,
- awareness of potential hazards, and
- providing/ arranging for appropriate training of personnel.

1.3.1.2. Safety Equipment

Any equipment that contributes to personnel protection either directly or indirectly from the hazardous biological material is considered for containment. It includes:

- Instruments like Biological Safety Cabinets (BSC), autoclave and a variety of enclosed containers (e.g. safety centrifuge cup). The BSC is one of the principal devices used to provide safety to the workers from hazardous microorganisms and infectious aerosols. Three types of BSCs (Class I, II, and III) are used in biosafety level facilities. The safety and functionality of each instrument must be monitored for effectiveness and calibrated annually. Equipment such as autoclaves and biological safety cabinets must be validated with appropriate methods before being placed taken into use. The results of the monitoring and calibration must be documented. Revalidation should take place at regular intervals, according to the manufacturer's instructions. If any equipment is found to be defective and the defect has not been corrected, the equipment must be clearly marked as defective and must not be used for any purpose until the defect has been corrected.
- Personal protective equipment (PPE) such as gloves, coats, gowns, shoe covers, boots, respirators, face shields and safety glasses, etc.

The Laboratory In-charge, after consultation with the biosafety officer and IBSC, should ensure that adequate equipment is provided and used properly. In selecting safe laboratory equipment, the general principles that should be considered include:

- Designed to limit or prevent contact between the operator and the infectious organisms.
- Constructed of materials that are impermeable to liquids, corrosion-resistant and meet structural strength requirements.
- Fabricated to be free of burrs and sharp edges.
- Designed, constructed and installed to facilitate simple operation and to provide ease of maintenance, accessibility for cleaning, and ease of decontamination, testing and validation.

These above mentioned points are general principles. Detailed performance and construction specifications are required to ensure that the equipment possesses necessary safety features, as detailed in Annexure I - III.

1.3.1.3. Facility Design

The design of the facility is important in providing a barrier to protect not only to persons working in the facility but also outside of the laboratory and those in the community from infectious organisms which may be accidentally released from the laboratory. Selection of the facility is to be determined based on risk group of microorganisms and category of experiments to be performed. These have been detailed in the "*Regulations and Guidelines for Recombinant DNA Research & Biocontainment, 2017*". Special consideration should be given to the following conditions:

- Creation of aerosols.
- Work with large volumes and/or high concentrations of microorganisms.
- Overcrowded, over equipped laboratories.
- Infestation with rodents or insects.

- Unauthorized entrance.

1.3.2. Biological Containment

Biological containment employs strategies that render an organism used for genetic engineering either incapable of survival or severely reduce its ability to survive or reproduce in the open environment. Such GE organisms would either remain viable only under the selective environmental conditions for which they were designed for or would carry self-contained mechanism(s) that could be induced when the need arises to eradicate such GE population. In addition to physical containment, such biological containment consequently ensures additional safety while working with GE organisms and provides more flexibility of handling organisms with higher risk(s).

It is always advisable to consider biological containment strategies especially if the final aim of the experiment is to release the organisms into the environment. In doing so, it is the responsibility of an investigator to first identify the possible risk(s) associated with the host, vector and modification(s) proposed and select appropriate strategies to reduce or limit:

- The risk(s) associated with the host organism.
- The infectivity of vector to specific hosts.
- The host-vector survival in the environment.

1.3.3. Laboratory Monitoring

Laboratory monitoring is a systematic, regular and preventive activity designed for corrective actions, if required. It is the responsibility of the Laboratory In-charge to ensure:

- Prevention of any unauthorized entry in the laboratory.
- Only allow entry of persons properly trained in laboratory safety.
- Personnel should be advised of special hazards and be required to know and follow standard practices and procedures. Such instructions should be prominently displayed near the entrance of the laboratory/facility.
- Persons at increased risk(s) of acquiring an infection or for whom infection may have unusually serious consequences (*e.g.* Immuno-compromised, Women during pregnancy, *etc.*) are informed of their risk(s) and should be restricted from entering the laboratory.
- To create a friendly environment where workers are following proper containment strategies and are fearless to report violations of the procedure(s), identify co-worker failings, express concerns and offer suggestions.
- All safety equipment are working properly and if not, maintenance of the equipment is done immediately. All civil structures are in good condition to ensure proper containment.
- A regular schedule for housekeeping is maintained.
- Prevention of diseases in the general or occupational environment.
- Documentation of daily laboratory activity for immediate consideration of emergency procedures in cases of breach in containment.

- Proper documentation of work involving both non-GE and GE organisms in thesame facility should be maintained to ensure that no unintentional cross-contamination of non-GE organisms occurs.
- Stringency in monitoring procedure(s) must be determined based on the biosafety level of the laboratory and should be determined by Laboratory In-Charge with consultation of scientific experts.

Further the procedures mentioned in the "*Regulations and Guidelines on Biosafety of Recombinant DNA Research & Biocontainment, 2017" should be strictly complied.*

1.3.4. Health and Medical Surveillance

The objectives of the health and medical surveillance of laboratory personnel are:

- Prevent individual from acquiring infection during the work.
- Early detection of laboratory-acquired infection.
- Assessing the efficacy of protective equipment and procedures.
- Ensure prophylactic vaccinations where needed and monitor booster regimens and assessment of sero-conversion, as applicable.

1.3.5. Decontamination and Disposal

Decontamination and disposal in laboratories are closely interrelated acts, since disinfection or sterilization constitute the first phase of disposal. All materials will ultimately be disposed of; however, in terms of daily use, only a portion of these will require actual removal from the laboratory for destruction. These will be referred as biological wastes that need specific treatment to render safe before discard. Steam autoclaving is the preferred method for all decontamination processes. Depending on the contaminated material for decontamination, it could be subjected to autoclaving and thereafter washing and reuse or recycling or autoclaving and disposal. The decontamination and disposal procedures mentioned in the "*Regulations and Guidelines on Biosafety of Recombinant DNA Research & Biocontainment , 2017" should be strictly complied.*

1.3.6. Emergency Procedures

Emergency contingency plans in consideration of every possible breach in biocontainment should be prepared for each laboratory as well as for the institution. These are best prepared by the laboratory In-charge in conjunction with his/her staff, biosafety officer and IBSC. This procedure offers the best prospect of success as it is the immediate staff that is most familiar with the hazards associated with the particular laboratory. Once the emergency plan is formulated, it should be pasted in a conspicuous place in the laboratory for immediate reference. Statutory rules and regulations for each of these will normally be laid down by the competent national or local authorities. Their assistance and guidance should be sought if necessary. The emergency procedures mentioned in the "*Regulations and Guidelines on Biosafety of Recombinant DNA Research & Biocontainment, 2017" should be strictly complied.*

1.3.7. Training

The hands-on proficiency training, mentorship, and didactic training are critical for establishing and evaluating the researcher's ability to work in a high-containment laboratory. Theoretical training helps laboratory workers develop an understanding of the underpinnings of biocontainment operations and the laboratory systems that support these operations. Hands-on practical training includes a comprehensive orientation to the specific facility in which the person will work to include a complete review and documented understanding of all the standard operating procedures; orientation to engineering aspects of the facility; overview of all safety procedures, including alarms and emergency operations; and an introduction to the care and use of a protective suit or glove box. A variety of individuals, from researchers to administrators and support staff to equipment service personnel, require some level of training before gaining access to high-containment laboratories; biosafety training programs have to be flexible to account for the research, model systems, facilities, job function, and the average cost of personnel training. The training module should encompass:

- Training would involve the use of entrances simply designed to demonstrate how one enters and exits the suite, general orientation on the use of air hoses, working within biological safety cabinets or glove boxes, storage and record-keeping of pathogens, clean-up, and decontamination following procedures or spills, solid and liquid waste management, use of autoclaves and other specialized equipment, communications with others inside and outside of the facility, and other general procedures.
- Open-ended training, working with live pathogens; the duration varies greatly depending upon the skills of the person and his or her ability to master all procedures necessary for independent work.
- Performance-based training standards developed from a set of core competencies that are critical for working in high containment laboratories.
- The final decision of when a person is allowed independent access is subjective and based on an assessment by the mentor and laboratory director; it is usually after the person has had extensive experience working in the facility. The time required to gain full independent access may also vary depending upon the kind of work the person will be undertaking.
- Information on variation in appropriate levels of protective equipment depending on the risks of the research conducted.
- Constant and continuous monitoring and training by experts to improve the laboratory skills and develop an awareness of current biosafety issues.
- Provisions to define biosafety training and protection standards.
- Provide realistic information about the hazards that exist in the high-containment facility to emergency responders and appropriate members of their community to help guide their response(s) in an emergency.

1.3.8. Certification

The biosafety laboratories need to be tested, commissioned and validated before they are made operational for research work. The design and operational parameters for

Containment Level 3 and 4 facilities have been enlisted in *"Regulations and Guidelines for Recombinant DNA Research and Biocontainment, 2017"*. The need for Certification of existing as well as new facilities was felt. Accordingly, an Expert Committee was constituted to draft standards for the establishment of BSL-3 and -4 facilities. The Certificate for BSL-3 facilities shall be issued for three years, with re-validation of essential parameters on annual basis.

The standards for establishing BSL-3 have been discussed in subsequent Chapters. The technical standards for engineering controls, essential tests to be conducted during installation and methodology for validation of BSL-3 laboratory have been detailed in Annexure I – III.

Chapter 2
ESTABLISHMENT OF BIOSAFETY LEVEL 2(BSL-2) FACILITY

Chapter 2: ESTABLISHMENT OF BIOSAFETY LEVEL 2(BSL-2) FACILITY

Biosafety Level 2 (BSL2) is applicable to clinical, diagnostic, teaching, research, or production facilities working with:

i. Isolation, cultivation and storage of risk group 2 microorganisms.
ii. Handling of environmental samples collected from environment that is unlikely to contain pathogens.
iii. Experiments on RG 2 microorganisms or isolates from environment mentioned above provided that the experiments will not increase environmental fitness and virulence of the microorganisms.
iv. Categories II genetic engineering experiments that pose low-level risk(s) to laboratory workers, community or the environment. Examples are:
    a. Experiments involving the use of infectious or defective RG 2 viruses in the presence of helper virus.
    b. Work with non-approved host/vector systems where the host or vector either does not cause disease in plants, humans or animals; and/ or is able to cause disease in plants, humans or animals but the introduced DNA is completely characterized and will not cause an increase in the virulence of the host or vector; experiments using replication defective viruses as host or vector.
    c. Experiments with approved host/vector systems, in which the gene inserted is: a pathogenic determinant; not fully characterized from microorganisms which are able to cause disease in humans, animals or plants; or an oncogene.
    d. Modification leading to persistent transient disruption of expression of gene(s) that are involved directly or indirectly in inducing pathogenicity, toxicity, survival, or fitness. Modification should be well characterized and the gene functions and effects are adequately understood to predict safety.
    e. Work involving fragments of Transmissible Spongiform Encephalopathy (TSEs) proteins or modified TSEs proteins that are not pathogenic and is not producing any harmful biological activity.
    f. Experiments in which DNA from RG 2 or 3 organisms are transferred into non-pathogenic prokaryotes or lower eukaryotes. However, handling of live cultures of RG 3 organism should be performed in BSL-3 laboratory.

BSL-2 differs from BSL-1 in that 1) Laboratory workers have advanced experience in handling of pathogenic agents and are supervised by scientists competent working with infectious agents and related procedures.; 2) restriction to the laboratory access when work is being conducted; and 3) procedures involving the generation of infectious aerosols or splashes are carried out in BSCs or other physical containment facilities. The following standards and special practices, safety equipment, and facility specifications should be followed by the BSL-2 facility, as stated below.

ELEMENTS	STANDARDS
2.1. Laboratory design and facilities	
Facility design	
	✓ An autoclave for decontamination of potentially hazardous laboratory wastes should be available in the same building as the laboratory. ✓ Biological safety cabinets for the handling of risk-inherent microorganisms of RG 2 should be used. ✓ Laboratory may be kept under constant CCTV surveillance. ✓ The biohazard warning symbol and sign (Fig. 1) must be displayed on the door(s) of the rooms where microorganisms of RG 2 are handled.  **Fig. 1**
Architectural	
Flooring and Base Materials	✓ Floor materials should be non-absorbent, skid-proof, resistant to wear, and also resistant to the adverse effects of acids, solvents, and detergents in normal use. ✓ Flooring should be monolithic or have a minimal number of joints. ✓ Floor surfaces should be easily cleanable and impervious to water. ✓ Joints in the flooring material should be kept to a minimum and sealed by hot welding. At wall junctions, the flooring should be coved to walls and sealed.
Walls	✓ Wall surfaces should be free from cracks, unsealed penetrations, and imperfect junctions with ceiling and floors. ✓ Materials should be washable with disinfectants.
Ceilings	✓ Ceilings such as washable lay-in acoustical tiles (with smooth surface) or any suitable suspended ceiling tiles should be provided for most laboratory spaces.
Windows	✓ Energy- efficient glass in windows should be recommended. ✓ Windows should be sealed and caulked.
Doors	✓ The international biohazard warning symbol must be displayed on the doors of the rooms where microorganisms of Risk Group 2 or higher risk groups are handled. ✓ Laboratory doors should be kept closed, Self-closing doors are recommended to aid in infection control. ✓ High-touch fittings with copper finish are recommended. ✓ Access restriction to laboratories for example, using swipe

	cards, fingerprint scanners or locks to control entry.
Ergonomics features	
Workbenches	✓ Workbenches should be made of non-absorbent materials, skid - proof, resistant to wear, and also resistant to the adverse effects of acids, solvents, and detergents in normal use. ✓ These should be monolithic or have a minimal number of joints between them which can sometimes harbour contaminating microorganisms.
Furniture	✓ Lab furniture should be sturdy and ergonomically effective. ✓ Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectants.
Storage Shelves	✓ The storage shelves should be wall-mounted or floor-mounted, with appropriate storage area.
Washing stations	
Sinks	✓ General laboratory sinks should be "all- in one' units to avoid the need for sealing around the sink. They should be of Stainless Steel and should drain directly to the Waste via a simple S-bend trap. ✓ Splash-proof sinks should be provided, especially at the washing areas of reusable items.
Hand- wash basins	✓ Hand wash basin should be provided along with Liquid Soap Dispenser/Hand Wash and Tissue paper towel (preferably Wall Mounted). ✓ It is recommended that the Taps should be lever- knee-or elbow or automatic sensor- operated. ✓ It is preferable to have Eyewash stations adjacent to hand-wash basins and roof mounted shower for quick wash/bath in case of accidental spill.
Service rooms and Support Spaces	
Storage	✓ Storage space must be adequate to hold supplies for immediate use and spare parts.
Washing	✓ Service rooms to accommodate autoclaves. ✓ Sinks for cleaning glassware. ✓ Preparation and sterilization of culture media.
Administration office	✓ Should have a provision of recording all reports of studies conducted on one site. ✓ The office should have a dedicated area for housing computer workstation, printers and storage space for records.
Staff Rooms	✓ Should have the provision of Protective clothing storage and other amenities.

Bio-hazardous waste temporary holding facility	✓ The universal biological hazard symbol should be posted on the storage area, door and waste containers. ✓ Large enough to contain all the hazardous material with spare capacity, the hazardous material should not be stored for more than 48 hours. ✓ Totally enclosed and secured from unauthorized access. ✓ Easy to clean and disinfect.
Safety instruments	
	✓ Autoclaves - to sterilize contaminated material. ✓ Biological safety cabinets to be used whenever: o Procedures with a high potential for creating hazardous aerosols. These may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers harbouring hazardous materials whose internal pressure may be different from the ambient pressure, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs. o High concentrations or large volumes of hazardous microorganisms are handled. Such materials may be centrifuged in the open laboratory if sealed heads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.
Personal Protective Equipment (PPE)	
	1. The use of laboratory coats, gowns or uniforms is required to prevent contamination of street clothing. It is preferable to use two layers of gloves while experimentation and outer layer should be disposed off immediately after working at BSL cabinet. 2. Goggles and face protection shield must be used when there is a potential for splashes of microorganisms or other hazardous materials. 3. Face mask and appropriate gloves should be worn as protection. 4. Appropriate gloves should be worn for all procedures and replaced/discarded frequently after handling infectious microorganisms. 5. All PPE should be removed so that the transfer of infectious materials to areas beyond is minimized. 6. Used disposable PPE should be disposed of with other contaminated waste and reusable PPE (i.e., goggles) should be appropriately decontaminated after use and before reuse. 7. Reusable protective clothing should be laundered through laboratory laundry facility only and it must not be taken home. The contaminated laundry should be placed in a biohazard bag

and autoclaved before laundry.

Drainage and waste Systems	
	✓ The internal drainage system should use the minimum of pipe work and remain water/airtight at all joints and connections. The entire drainage system should be made of acid-resistant materials. ✓ The internal drainage system should be connected to the main drainage system as far downstream as possible to ensure maximum dilution. ✓ The drainage system should allow easy access for inspection and maintenance.
Hot and Cold Water Systems	
	✓ Hot and cold water supplies to laboratories should be served by separate storage vessels and pipe work distribution systems. ✓ All pipe work, valves and flanges for water supply systems should be insulated and vapour sealed.
Waste management	
	✓ Decontamination and disposal mechanisms should be strictly adhered to the procedures mentioned in the *Regulations and Guidelines on Biosafety of Recombinant DNA Research & Biocontainment, 2017*. ✓ Separate identified places be marked for waste arrival, collection and decontamination. ✓ Autoclaves and sterilizers should be utilized for treatment of solid wastes.
Additional requirements	
Energy Conservation & Sustainability	✓ Heating, ventilation, cooling and lighting should be automatically controlled when not in use. ✓ Above facilities should be designed to meet the requirements of the Building Regulations norms.
Lighting	✓ Natural lighting should be used (optimally) where ever possible. Passive Solar Design should ensure that laboratory areas are located where they can benefit from natural daylight. ✓ Solar protection should be provided to minimise solar gain and control glare. This may include the use of sun shades, sunlight reduction glazing and window blinds.
Ventilation	✓ Ventilation systems for clean laboratories should maintain positive pressures at all times. ✓ Mechanical ventilation to internal rooms other than laboratories should provide minimum Air changes. It shall be necessary to maintain a comfortable condition; therefore, a low-velocity mechanical ventilation system should be used.

	✓ The supply air distribution system in the laboratory should not distort the unidirectional and stable airflow pattern required for fume cupboards and microbiological safety cabinets.
Extracts Systems (Lab Safety Airflow Systems)	✓ Extract fans should be located close to the point of discharge to maintain negative pressure. ✓ Staining areas should have bench extract systems that ensure air flows away from operators' faces. Low-level extract should be provided adjacent to equipment for use when solvents are changed or when specimens in formaldehyde are opened. ✓ External discharge arrangements for extract systems should be protected against back pressure from adverse wind effects. They should be located to avoid reintroduction of exhausted air into the building through air intakes and windows.
Control Systems	✓ All supply and extract systems should have local control systems in addition to the central main control. ✓ Supply and extract fans should be interlocked. This will ensure that the supply fan will not operate unless airflow is established with the extract system. ✓ Laboratory spaces should be comfort cooled without local humidity control. Large laboratory spaces should be zoned, with each zone equipped with a thermostat for individual control.
2.2. Procedures	
	✓ All contaminated liquid or solid materials should be decontaminated before disposal or reuse. As far as possible contaminated materials may be autoclaved / decontaminated within the lab, however if required to be autoclaved at a site away from the laboratory, it should be placed in durable leak-proof containers, which are closed before being removed from the laboratory. ✓ Containers used to collect, handle, process, store, or transport within a facility, potentially infectious materials must be durable, leak-proof and have a lid. The containers must be properly labelled with the contents and a biohazard symbol. ✓ Laboratory coats, gowns, or uniforms should be worn in the laboratory; laboratory clothing should not be worn in non-laboratory areas; contaminated clothing should be disinfected by appropriate means. ✓ Safety glasses, face shields and other protective devices should be worn to protect eyes and face from splashes and impacting objects. ✓ Only persons who have been advised of the potential hazards and meet any specific entry requirements should be allowed to enter the laboratory working areas.

- ✓ Add disinfectant to water baths to contain spread of infectious substances.
- ✓ Use sealed rotors, sealed buckets, or a guard bowl cover complete with gasket as well as safety centrifuge tubes (tube or bottle carrier with sealable cap or "O" ring cap) for potentially infectious samples/otherwise hazardous samples. Before use, tubes should be checked for cracks.
- ✓ All technical procedures should be performed to minimize the formation of aerosols and droplets. Whenever there is an increased risk(s) of aerosolization, work should be conducted in a biological safety cabinet.
- ✓ Always use secondary leak-proof containers when transporting samples, cultures, inoculated Petri dishes, and other containers of hazardous microorganisms. Packages containing viable microorganisms must be opened in a facility having an equivalent or higher level of physical containment unless the microorganism is biologically inactivated or incapable of reproduction.

2.3. Laboratory Monitoring

Monitoring

- ✓ Monitoring should ensure that:
  - Only highly trained personnel are entering in the facility.
  - Personnel working in the facility are not transporting the laboratory materials including hazardous organisms outside the laboratory environment either without permission or without proper transport strategy with prior approval from the competent authority.
  - Personnel working in the laboratory are well aware about the microorganism(s) to be handled and its associated risks.
  - Accidental spill or splashes are cleaned immediately, reported and recorded.

Building Management System

- ✓ No separate BMS is mandated, if the building has a BMS then the following points should be followed:
  - Engineering plant and equipment should be monitored and regulated by the BMS.
  - The BMS should also monitor, measure and record energy consumption for the facility.
  - Real-time centralized monitoring of temperature - sensitive operations: refrigerators, deep freezers.

2.4. Health and Medical Surveillance

- ✓ Pre-employment health surveillance is necessary. This screening should include the past medical history.

- ✓ Records of illness and absence should be kept by the Laboratory In-charge and it is the responsibility of the laboratory worker and his/her own medical officer to keep the in-charge informed of all absences due to illness.
- ✓ Women of child-bearing age should be made aware, in unequivocal terms, of the risk(s) to the unborn child of occupational exposures to hazardous microorganisms, such as Rubella, Cytomegalovirus *etc.*

2.5. Emergency Procedures

- ✓ All spills, accidents and overt or potential exposures to infectious materials should be reported immediately to the Laboratory In-charge and Bio safety Officer, and subsequently to IBSC.
- ✓ A written record should be prepared and maintained.
- ✓ Appropriate medical evaluation, surveillance and treatment should be provided.
- ✓ First Aid Kit should be available in the laboratory.

2.6. Fire Safety

- ✓ All necessary fire detection, protection and prevention should be provisioned in the laboratory. The principles of fire safety apply equally to new projects, alterations and upgrading of existing buildings.
- ✓ Consideration should be given to the fire safety strategy during the design stage. Operational aspects such as staff responsibilities, equipment provision, building and engineering layouts, should be made. Fire-Exit route should be displayed with location of the nearest exit.
- ✓ Installation of A, B, C type of fire extinguisher.
- ✓ Training of lab staff as new to operate.

Chapter 3
ESTABLISHMENT AND CERTIFICATION OF BIOSAFETY LEVEL 3 (BSL-3) FACILITY

Chapter 3: ESTABLISHMENT AND CERTIFICATION OF BIOSAFETY LEVEL 3 (BSL-3) FACILITY

Biosafety Level 3 (BSL-3) is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Keeping in view the principle and components of bio safety, as detailed in Chapter 1 and the requirements for BSL-2 as described in Chapter 2, additional safety and containment practices are required for working in BSL-3 laboratory. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. The considerations for establishment, technical standards for engineering controls, installation and validation of BSL-3 facility have been detailed in this Chapter and the following/subsequent Annexure.

Pre-design considerations for BSL-3 facility

An important consideration for the pre-design of BSL-3 facility is the site selection and determination of risk assessment of the site conditions based on objectives and scope of proposed work. BSL-3 laboratory should be ideally located in a stand alone building or it shall be separated from the regular buildings including administration, library, etc., that are frequently visited by employers or public. Access to the building is strictly restricted and only authorized persons can access through an anteroom/air lock facility, preferably controlled through electronic systems including surveillance systems with a provision for a 3-tiered security system built within the building. The construction site should meet the basic criteria of an uninterrupted supply of water and electricity. Geographical and climatic conditions, such as geological fault lines or extreme heat, cold or humidity may affect the laboratory design; therefore, an in-depth risk analysis based on geographical data of the selected site should be carried out in the area selected, for susceptibility to natural disasters like landslides and earthquakes. The proposed site should be away from High Tension Electric lines to avoid any related accidents. The risk of possible damage to the site from heavy rains and flooding should also be systematically evaluated. The proposed site should be subjected to soil testing to detect possible presence of 'borehole logs', type of soil, strength of soil to bear load of structure, compaction of soil, chemicals present in soil, moisture content, water table *etc*. Detailed analysis of soil properties at the site also needs to be undertaken in order to evaluate the suitability for construction and evaluation of site for seismic sensitivity.

Contemplating the above, the conceptual proposal for BSL-3 laboratory with detailed drawings and plans, justifying the objectives for the construction as per the requirements along with abiding with the national and international guidelines on bio safety and biosecurity, should be postulated. This involves preparing a detailed flowchart of the construction work and a schematic drawing to enable detailed planning. The drawings

must depict the layout of all laboratory areas to facilitate placement of essential on-site and stand-alone equipment (including autoclaves, biological liquid effluent decontamination plant/chemical kill tank, air handling units, exhaust filters). The plan should also indicate the placement of safety equipment, such as fire extinguishers, water sprinklers etc. within the facility. A detailed concept proposal with detailed drawings, plans as per requirements should be formulated. Qualified and experienced architects-engineers should be involved in design followed by experience construction agency/contractor. Strict monitoring should be done for proper facility construction and in time bound manner until the facility is validated and handed over to the institution.

The BSL-3 containment facility requires the strengthening of the operational and safety programmes over and above those for basic laboratories, that is, Bio safety Levels 1 and 2. The major additions and changes are in:

- Code of practice
- Laboratory design and facilities
- Special Laboratory equipment
- Health and medical surveillance

3.1. CODE OF PRACTICE

The code of practice for basic laboratories, that is, Bio safety Levels 1 and 2 applies except where modified as follows.

- In addition to the international biohazard warning symbol and sign, the laboratory access doors must identify the bio safety level and the name of the laboratory in-charge who controls access, and indicate any special conditions for entry into the area, e.g. Immunization.
- The laboratory in-charge must enforce the institutional policies that control access to the laboratory.
- Laboratory protective clothing must be of the type with solid-front or wrap-around gowns, scrub suits, cover-all, head covering and shoe covers or dedicated shoes. Front-buttoned standard laboratory coats are unsuitable, as are sleeves that do not fully cover the forearms. Laboratory protective clothing must not be worn outside the laboratory, and it must be decontaminated before it is laundered.
- Open manipulations of all potentially infectious material must be conducted within a biological safety cabinet.
- Respiratory protective equipment may be necessary for some laboratory procedures
- A laboratory-specific bio safety manual must be prepared and adopted. The bio safety manual must be readily available and accessible.
- The laboratory in-charge must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.

3.2. LABORATORY DESIGN AND FACILITIES

The laboratory design and facilities for basic laboratories, that is, Biosafety Levels 1 and 2 apply except where modified as follows:

- The laboratory must be separated from the main building or in an isolated area within the building. Additional separation may be achieved by placing the laboratory at the blind end of a corridor, or constructing a partition and door or access through an anteroom (e.g. a double-door entry or basic laboratory –Bio safety Level 2), describing a specific area designed to maintain the pressure differential between the laboratory and its adjacent space. The anteroom should have facilities for separating clean and dirty clothing.
- Anteroom doors may be self-closing and interlocking so that only one door is open at a time. A break-through panel may be provided for emergency exit use.
- Surfaces of walls, floors and ceilings should be water-resistant and easy to clean. Openings through these surfaces (*e.g.* for service pipes) should be sealed to facilitate the decontamination of the room(s).
- The laboratory room must be sealable for decontamination. The air-ducting systems must be leak-proof (sealed) and constructed to permit gaseous decontamination.
- Windows must be closed, sealed and break-resistant.
- A hand-washing station with hands-free controls should be provided near each exit door. Provision for a sensor-based or foot / elbow operated eye wash station should also be there.
- There must be a controlled ventilation system that maintains a directional airflow into the laboratory room. A very vital aspect of BSL-3 design and operation is the maintenance of negative pressure in the core facility where infectious pathogen(s) is/are to be handled. The supply and exhaust components of the ventilation system must be designed to maintain the laboratory all the time under a negative pressure as compared to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory. A dedicated non-re circulating, high-efficiency particulate air (HEPA) filtered, ventilation system should be provided. A heating, ventilation and air-conditioning (HVAC) control system shall be installed to prevent positive pressurization of the laboratory. A visual monitoring device with or without alarm(s) should be installed so that staff can at all times ensure that proper directional airflow into the laboratory room is maintained. Consideration should be given to the installation of audible or clearly visible alarms to notify personnel of HVAC system failure.
- All HEPA filters must be installed in a manner that permits decontamination and testing.
- Biological safety cabinets should be sited away from walking areas and out of crosscurrents from doors and ventilation systems.

- The exhaust air from Class II biological safety cabinets, which has passed through HEPA filters, must be discharged in such a way as to avoid interference with the air balance of the cabinet or the building exhaust system.
- An *in-situ*, double- door barrier autoclave for the decontamination of contaminated waste material should be available in the containment laboratory.
- Backflow-precaution devices must be fitted to the water supply. Vacuum lines should be protected with liquid disinfectant traps and HEPA filters, or their equivalent. Alternative vacuum pumps should also be properly protected with traps and filters.
- The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.
- The entry/exit should be through cloth change and additionally, through shower room while exit. The need and number of showers shall be as per the laboratory SOP and requirement.
- BSL-3 must have barrier steam Autoclave and fumigation chamber. Material supplies and equipment shall enter/exit through double door barrier Autoclave/ Fumigation chamber or Air Lock.
- There should be additional personnel change room contiguous with shower, depending on type of work in BSL-3 facility.
- Sewer and other vent lines must be protected by HEPA filters.
- Pressure gradient in the direction of more contamination with more negative pressure required.
- Air supply duct and exhaust duct within the contaminated BSL-3 lab should be air tight and preferably made of stainless steel. Duct material should be fumigation friendly. Duct must be tested for leakage by pressure decay test.
- All supply and exhaust air dampers should be airtight and tested before installation.
- Complete lab should be *in situ* tested for any leakage before commissioning by pressure decay test.
- All entry/exit gates should be air tight and gates must be tested for air leakage before installation and on annual basis thereafter by pressure decay test or soap bubble leak test.
- BSL-3 laboratory area must have at least 12-15 air changes per hour.

3.3. LABORATORY EQUIPMENT

The principles for the selection of laboratory equipment, including biological safety cabinets are the same as for the basic laboratory – Bio safety Level - 2. However, at Bio safety Level 3, manipulation of all potentially infectious materials must be conducted within a biological safety cabinet or other primary containment device. In addition:

- Equipment that may produce infectious aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory or outside.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
- Consideration should be given to equipment such as centrifuges, which will need additional containment accessories, for example, safety buckets or containment rotors.
- Equipment like centrifuges, cell-sorters for use with infected cells; may need additional local exhaust ventilation with HEPA filtration for efficient containment.

3.4 PROCEDURES

As described above, the BSL-3 laboratory has special engineering and design features. The technical standards for the engineering controls with respect to special safety practices, equipment, and facility requirements that apply to BSL-3 have been detailed at **Annexure I**. Essential tests during the installation and Validation procedure of a BSL-3 laboratory have been provided at Annexure II and III, respectively. The Standard Operating Procedures (SOPs) for specific considerations for working in the BSL-3 facility have been detailed at **Annexure IV**.

3.5 LABORATORY MONITORING AND INFORMATION NETWORK

Building Automation System is a dedicated, centralized Building Automation System (BAS) to monitor and maintain desired room conditions like pressure, temp and RH. The VFDs should be connected to the directly controlled Building Management System (BMS) system in a BSL-3 facility. Customized BMS along with controllers, Cloud based monitoring, sensors and control dampers, human machine interface unit, cabling interconnecting the units and integrator units for the Programmable Logic with remote display of alarm/ parameters, shall be designed, to remotely monitor and supervise all functions, equipped with appropriate alarms.

Biometric access control system: There should be provision for door interlock system with electro-magnetic locks and control panel. Access Control System having biometric finger print, key pad, LED screen, USB port for communication and data back-up should be available in the BSL-3 facility.

Connectivity: The Laboratory areas and support and service area shall be provided with Data and Voice points for communication. The system shall be complete with required conduit and wiring. The Data and Voice points shall be fully wired with CAT5/6 cable complete with output terminals. A suitable EPABX system shall be provided for required incoming and outgoing lines, as per the requirement. A system must be connected to a location that has personnel available for emergency response at all times work is being performed in a BSL-3 laboratory. There should be provision for Electronic transfer of

information to outside of containment, such that the practice of transferring documents from the BSL-3 to areas outside of containment can be eliminated

CCTV installations: CCTV System, complete with wall/ceiling mounted high resolution color cameras, multiplexer cum DVR, LED color monitor, associated power and control cabling along with required hardware and software, shall be provided. In addition to the laboratory area, the video surveillance shall monitor activity outside of the secured space including hallway entrance.

Security & Documentation: Due to the bio safety and bio security concerns over the usage of bio hazardous materials, access to the bio containment facility must be strictly limited to trained and authorized personnel. The same may be ensured through access card, biometric or PIN security access. Video surveillance cameras should be installed to provide live and recorded video activity inside the laboratory as well as outside of the secured space, in interior activity spaces, and covering materials of interest. The access control and video surveillance system devices should be coordinated to allow for recording and monitoring of entry and exit events. Due to the sensitivity of research, the physical security systems should be integrated to provide for real time monitoring and auditing of the video surveillance and electronic access control systems. All activities related to movement of laboratory personnel and material should be duly recorded and documented.

3.6. HEALTH AND MEDICAL SURVEILLANCE

The objectives of health and medical surveillance programmes for Bio safety Levels 2 also apply to containment laboratories, that is, Bio safety Level -3, except where modified as follows:

- Medical examination of all laboratory personnel who work in containment laboratories – Bio safety Level -3 is mandatory. This should include recording of a detailed medical history and an occupationally-targeted physical examination.
- After a satisfactory clinical assessment, the examinee may be provided with a medical contact card stating that he or she is employed in a facility with a containment laboratory – Bio safety Level- 3. This card should include a picture of the card holder, and always be carried by the holder. The name(s) of the contact persons to be entered will need to be agreed locally but might include the Laboratory In-charge, Medical Officer and/or Bio safety Officer. A template for the Medical Card is provided in "*Regulations and Guidelines on Biosafety of Recombinant DNA Research & Biocontainment, 2017*".

3.7. EMERGENCY PROCEDURES

Any unforeseen incident/breach in containment must be immediately reported to the regulatory authorities. The Laboratory In-charge and Biosafety Officer must be instantly notified. The IBSC must be informed about the emergency and response procedure. The IBSC must bring such incidents to the notice of RCGM/GEAC. All such instances shall

be duly recorded and reported. In addition to emergency procedures for BSL-2 laboratories, containment laboratories require additional considerations. These include:

Puncture wounds, cuts and abrasions

- The affected individual should remove protective clothing, wash the hands and any affected area(s), apply an appropriate skin disinfectant, and seek medical attention as necessary.
- The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

Ingestion of potentially infectious material

- Protective clothing should be removed and medical attention sought.
- Identification of the material ingested and circumstances of the incident should be reported, and appropriate and complete medical records should be kept.

Potentially infectious aerosol release (outside a biological safety cabinet)

- All persons should immediately vacate the affected area and any exposed persons should be referred for medical advice.
- The laboratory in-charge and the biosafety officer should be informed at once.
- Signs should be posted indicating that entry is prohibited.
- No one should enter the room for an appropriate amount of time (minimum 1 h), to allow aerosols to be carried away and heavier particles to settle.
- After which, decontamination should proceed, supervised by the laboratory in-charge and biosafety officer. Appropriate protective clothing and respiratory protection should be worn during the decontamination procedure. The facility may be allowed for re-entry only after it is cleared and approved.

Broken containers and spilled infectious substances

- Broken containers contaminated with infectious substances and spilled infectious substances should be covered with a cloth or paper towels.
- Disinfectant (as per laboratory SOP) should then be poured over these and left for the appropriate amount of time. The cloth or paper towels and the broken material can then be cleared away.
- The contaminated area should then be swabbed with disinfectant.
- All the materials assisting decontamination should be autoclaved or placed in an effective disinfectant.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. For example: Neoprene gloves, Nitrile exam style, Utility grade nitrile.

Breakage of tubes containing potentially infectious material in centrifuges not having sealable buckets

- If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (*e.g.* for 30 min) to allow settling.
- If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (*e.g.* for 30 min).
- In both instances, the biosafety officer should be informed.

- Appropriate gloves, should be worn for all subsequent decontamination operations.
- Forceps, or cotton held in the forceps, should be used to retrieve glass debris.
- All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a noncorrosive disinfectant known to be active against the organisms concerned.
- Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered.
- The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried.
- All materials used in the clean-up should be treated as infectious waste and appropriately decontaminated and discarded

Breakage of tubes inside sealable buckets (safety cups)

- All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved.
- Alternatively, the safety cup may be chemically disinfected.

The contact details of authorities to be contacted in case of emergency should be displayed on the facility. The following emergency equipment must be available:

- First-aid kit, including universal and special antidotes
- Appropriate fire extinguishers, fire blankets
- Others, as required:
- Full protective clothing
- Full-face respirators with appropriate chemical and particulate filter canisters
- Room disinfection apparatus, e.g. sprays and formaldehyde vaporizers
- Hazard area demarcation equipment and notices.

3.8. FIRE AND NATURAL DISASTERS

Fire and other services should be involved in the development of emergency preparedness plans for containment facilities.

Fire Detection and Alarm System: The BSL-3 Laboratory and support areas shall be provided with Fire detection and monitoring system with alarm facility along with provision for manual fire extinguishers. The Fire hazard must be notified through both local audible and visual alarms. Further, the fire alarm should be differentiated from other alarms (in the BMS), for easy identification and rapid response. The Fire Detection & Alarm System shall be complete with Smoke detectors, Heat detectors, Fire Alarm Panel, manual call points, response indicators, power and control wiring and cabling etc. complete in all respect. Fire Dampers provided in the supply and exhaust air systems shall be interlocked with the AHU blower motors such that in case of fire, the AHU fan motor should trip automatically. Volume Control Dampers, Fire dampers, air diverting vanes shall be provided in the supply and exhaust air ducting, as per the requirements and approved design.

Natural disaster: After a natural disaster, local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings. They should enter only when accompanied by a trained laboratory worker. Infectious materials should be collected in leak-proof boxes or strong disposable bags. Salvage or final disposal should be determined by biosafety staff on the basis of local ordinances.

3.9 STATUTORY APPROVALS

The approval from IBSC/RCGM/DBT needs to be sought. The required statutory approvals from authorities like Fire Authorities, Pollution Control Board, Electrical Inspectors, etc., if required and applicable, shall be obtained by the contractor. The parent institute shall only provide the required assistance in getting such clearance/s, as required.

3.10 TESTING, COMMISSIONING AND VALIDATION OF FACILITY

Testing: The testing of the on-site equipment should be initially performed by the construction contractor alone in the presence of a team involved. Functioning of all the critical parameters should be repeated and demonstrated to authorized person/team or project management consultant for the facility, in presence of the Laboratory In-charge and Biosafety Officer. Final testing and commissioning should take place in presence of committee / project team that may include a third party.

Commissioning: Commissioning procedure for the laboratory should be well designed and implemented to verify the safety aspects of facility operation. Commissioning ofthe facility shall include all critical elements such as airflow patterns and negative pressures within isolators, biosafety cabinets, temperature profiles in autoclaves, procedures for decontamination and sterilization, verification of light lux level, operation of HVAC systems, chilled water pumps capacity, air quantities at outlet diffusers / grilles, and air compressor, air curtains, steam boiler, clean room garment storage cabinet, floor traps, drains, dunk tanks, checking of ceiling panels, pass box, shower cabinets/air shower, water outlets, air leak in ducts as well as plenums, doors and view panels along with functioning of all the alarm systems. Additionally, ensure proper commissioning of all the basic requirements as per the approved layouts, including electrical connections (raw, essential and UPS), local area network (LAN) connections, servers, water connections, sewage connection, hardware fitting, alarms, telephones and intercoms, functioning of the BMS with all the desired parameters, fine setting of access control and all the inventories.

Validation: Validation of the BSL-3 facilityshould be conducted by ISO/IEC 17025/ Bureau of Indian Standards/ Quality Council of India certified agency, in liaison with the Laboratory In-charge and Biosafety Officer. The validation process aims to ensure biosafety and biosecurity concerns to the workers and the surrounding environment as well as adherence to biosafety regulations of the country.The procedure for Testing, Commissioning and Validation of the facility has been provided at **Annexure I**.

Prior to commencing of the laboratory for operations, following shall be observed:

- Staff training should be reinforced
- Mock drills along with validation of SOPs should be conducted
- A document describing the mandate and features of the laboratory would be a primary requirement

- Facility and Operation Manuals explaining biosafety aspects as well as maintenance of engineering systems should also be prepared.
- A Technical Manual should also be developed for the facility.

The validation should essentially incorporate the following:

- Clean room validation
- Air Balancing test
- Room negative Pressure Test
- Particle Count Test (at REST)
- Temperature & Relative Humidity test
- Light level test
- Spore strip test

ANNEXURE I

TECHNICAL STANDARDS FOR THE ENGINEERING CONTROLS FORBSL-3 LABORATORY

As discussed in the previous chapter, a BSL-3 laboratory must abide by the technical standards prescribed for the engineering controls. They have been listed below:

S. NO.	PARTICULARS
I	**FACILITY FEATURE**
1	Personnel Entry/Exit in lab through Clothing Change & Shower Rooms: Shower doors located between the clean and dirty change room should not to be opened simultaneously. These doors should be interlocked electronically and /or mechanically. Additionally visual and audible alarms are recommended. There should be manual override facility to interlocking in case of emergency. Facility should preferably have separate entry/ exit for Ladies and Gents wherever appropriate.
2	Materials, Supplies & Equipment should enter/ leave through Double Door Autoclave, fumigation chamber / Airlock with inter locked doors. Doors should be interlocked electronically and /or mechanically. Additionally, visual and audible alarms are recommended.
3	Work Conducted in Primary Containment Equipment: Work should be conducted in a Biosafety cabinet. The BSC may be selected based on protection assessment, work assessment, Chemical vapour generation assessment. In general, BSC Class II type B1 or B2 is recommended.
4	Hand Washing station: Should be placed near the laboratory exit. Provision for sensor-based or foot / elbow operated eye wash station should also be there.
5	Laboratory and animal room wastes from the containment area to be decontaminated or sterilized before disposal. A Double door barrier autoclave with interlocking of doors and other controls. Waste material is loaded in the autoclave from the facility and removed from outside the facility after autoclave selected cycle is completed.
6	Lab clothing: Lab clothing should be decontaminated by autoclaving before washing.
7	Animal cages: may be autoclaved or thoroughly decontaminated by cleaning with hot water at 82^{o}C.
8	Appropriate cautionary signs: Appropriate hazard sign should be pasted for BSL-3 on the main entrance door of the laboratory.
9	The facility should be located in a separate building or isolated zone within a building, as per “Box in Box” principle, *i.e.*, more containment area is surrounded by less containment area.

10	Pass through cabinets /dunk tanks for transfer of biological materials. Door interlocking with override facility. Dunk tank, as feasible, based on risk assessment.
11a	Steam Glassware sterilizer: Single door/ double door based on requirement.
11b	Ethylene Oxide barrier autoclave: should be used for materials which cannot be steam autoclaved.
12	Liquid Effluent (Bio-Waste) Treatment should be conducted at -50 Pa or more based on risk assessment and box in box principle.
13	Personnel change room is recommended for laboratory work.
14	Shower: the availability of shower within facility at the exit is recommended for a BSL-3 laboratory work but mandatory for high risk work. Drain water from such shower cannot & should not be disposed of in municipal waste without treatment. It must be ensured that such bath water flows down to designated drain for treatment and must not remain stagnant on floor. As per risk assessment permit for work on comparatively lower risk work, air shower may be considered as a substitute to water shower.
15	Lab contiguous with shower: These showers are in addition to shower at the exit of the facility and are required in animal houses and high risk areas. Drain water from such shower must be treated in effluent treatment system before disposing in municipal waste.
16	Work surfaces: bench tops impervious to water, resistant to acids, alkalis, organic solvents and moderate heat and should be monolithic.
17	Interior surfaces of walls, floors and ceilings: monolithic, resistant to liquids and chemicals, all penetrations to be sealed. All floor drains should have adequate water seal traps filled with chemical disinfectant (chemical would need frequent topping up). Epoxy resin is recommended for flooring. Epoxy, PU, low-lustre acrylic or latex enamel paint is recommended for walls. Wall and floor junction should be coved to walls and sealed.
18	All Windows should be fixed type and sealed windows should be made with unbreakable double glass.
19	Vacuum outlets (if provided) should be protected by HEPA filters & liquid disinfectant in traps. HEPA filters (with efficiency of 99.97% or better) must be validated annually or replaced annually.
20	Other liquid & gas services protected by backflow preventers, as per services required in lab.
21	Sewer and other vent lines protected by HEPA filters. HEPA filters must be validated annually.
22	Ventilation Facility: individual supply & exhaust air systems.
a	Air tight leak proof duct (Tested for leakages): Required
b	Single Pass / once through system (No Recirculation): Required

c	Directional Air Flow: Required
d	Pressure Gradient: Required
e	Supply/ exhaust fans interlocking: supply fan to start only after exhaust fan is switched on
f	Ventilation Containment Equipment: • Class III BSC: Required for high risk pathogens. Supply air through HEPA filters and exhaust through double HEPA filters in series in addition to pre-filters. • Class II BSC: HEPA supply and exhaust should be validated annually.
23	Direct Digital Control(DDC) and Building Automation System (BAS) are required
24	Leak tightness testing & validation of critical components of the biological containment system prior to final acceptance of the completed work is required, as per national guidelines. Duct work should be tested by soap bubble test or pressure decay method (as provided at **Annexure II**)
II	**ARCHITECTURAL CONSIDERATIONS**
25	Laboratory Layout
a	The designing of a biocontainment laboratory must consider laboratory personnel, material, pathogen flow routes; activities to be carried out; facilities including laboratory equipment required in containment laboratory. The engineering services required for the laboratory equipment and for working of the laboratory need to be identified so that provision can be kept for the services while designing the lab
b	BSL-3 laboratory design should be rodent and insects free.
c	Adequate means of outlets shall be provided from laboratories without breaching containment or leading to cross contamination. Air locks shall be provided at transitional points between the spaces of different biocontainment levels through which personnel and / or material must pass.
d	The entry/exit should be through cloth change and additionally, through shower room while exit. The need and number of showers shall be as per the laboratory SOP and requirement.
e	For equipment and other material which cannot be autoclaved, the facility should have Air-Lock with gaseous decontamination system and inter locking of doors so that only one door opens at a time. Once a dirty side door is opened, the clean side door should open only after decontamination cycle is completed. If formaldehyde is used for gaseous decontamination then it should be neutralised by ammonia. SOP must be developed by the Laboratory In-charge.

f	Facility must have an emergency exit in case of fire or other emergency and must have an override facility with interlocking of all exit doors. Facility specific SOP may be developed for exit from the facility, in case of emergency. Maintenance of emergency exit door may be done at regular intervals as developed for preventive maintenance. Emergency door should remain closed and its key should be kept in a glass box nearby. .
g	The emergency door must only be opened in the event of an emergency or at the time of mock drill. SOPs with frequency at least once in a year, should be developed by the Laboratory In-charge.
h	For large facilities, if wash rooms are required, then effluent from the wash room should also be decontaminated before disposing off in the municipal sewer system. Washroom drains must have a minimum 125mm trap and it should be ensured that trap is always filled with water for sealing and sewer line should have HEPA filter (99.97% or better efficiency) air vent.
26	Room Envelope and Interior Finish
a	The design should include construction materials and finishes that are compatible with respective research activities and decontamination methods. Floor should be made of epoxy material and seamless, impervious, abrasion resistant, non-slip when wet, cleanable. The floor should be able to withstand disinfectants and be washable with 82 °C hot-water containing detergents and the decontamination liquids under hose pressure. Walls of the labs must be constructed with non- porous materials with industrial grade epoxy paint.
b	Doors, frames, casework and bench tops should be non-absorptive; the use of organic materials should be avoided.
c	• Surfaces to be scratch, stain, moisture, chemical and heat resistant in accordance with laboratory function. • Surfaces to provide impact resistance in accordance with laboratory function. • Surfaces to be continuous and compatible with adjacent and overlapping materials (*i.e.* to maintain adhesion and a continuous perimeter); wall and floor welded seams are acceptable in BSL-3 laboratories.
d	Continuity of seal to be maintained between the floor and wall (a continuous cove floor finishes up the wall is recommended).
e	• Interior surfaces to minimize movement of gases and liquid through perimeter membrane. • Interior coatings should be gas and chemical resistant in accordance with laboratory function (should be able to withstand chemical disinfection, fumigation). • Interior coatings should be cleanable.

f	• Bench tops should not have open seams. • Bench tops should contain spillage of materials (*e.g.* with marine edges and drip stops). • Benches, doors, drawers, door handles, *etc* .should have rounded rims and corners.
g	• Backsplashes, if installed, should be tight placed along with wall and sealed at wall-bench junction.
h	Reagent shelving should be equipped with lip edges.
i	• Cabinet doors must be self-closing and lockable. • Drawers should be of one-piece construction and equipped with catches,
27	Heating Ventilation and Air-Conditioning (HVAC)
a	• For the purpose of isolation purpose, separate air handling systems should be installed in non-containment and containment zones. Further, AHUs should be installed in each isolation zone, wherever isolation is required within the containment area. • Each air-handling unit serving a containment area shall supply 100% fresh air. Wherever feasible and economical, heat recovery may be done from lab exhaust air.
b	• Direction of air flow should be from less contaminated area to more contaminated area. • Air flow within containment area should be from entrance to rear area. • All laboratory rooms must be provided with a visual monitoring device that indicates directional inward air-flow.
c	• There should be 6-8 air-changes per hour for offices, 10-12 air-changes for laboratory. • HVAC system should have provision for less air change during no occupancy to conserve energy.
d	• Differential pressure of minimum 15 Pascals should be maintained between separate functional spaces to ensure more negative pressure in those areas which are at higher risk of contamination. The BSL-3 facility is recommended to be maintained at differential negative pressure of 15, 30, 45 Pa. • All negative pressure in the containment area should be measured with reference to common single atmospheric set point.
e	• HVAC system must be capable of sensing and responding to unusual outside pressure conditions (like a storm) and maintaining negative pressure in the containment area during all weather conditions. • For the ease of maintenance, active components of HVAC system must be kept outside containment area with provision for sufficient space for maintenance of components. • The capacity of exhaust system must be approximately 15% more than the supply air system. Variable speed drive on the exhaust fan is recommended

	to facilitate room pressure control adjustments. • HVAC system must be controlled by electronic control system or BAS.
f	• Air duct for supply and exhaust should be air tight up to zero leakage dampers. Isolation valves should be provided in HEPA Filter housing to avoid any leakage of contaminated air and for provision of gaseous decontamination of laboratory, in case of breakdown of AHU. • Duct should be tested for any leakage before commissioning of laboratory by soap bubble test or pressure decay test at + 1000 Pascal pressure. Ducting should be of SS-304 material to avoid rusting of duct work.
g	• Structural stability to withstand 1.25 times maximum design pressure under supply and exhaust fan failure conditions (no wall distortion or damage should occur).
h	• The exhaust air from all the containment equipment and containment area shall be filtered through HEPA/ULPA filters before discharging outside. • Efficiency of HEPA/ULPA filters should be better than 99.97%. HEPA/ULPA filter housing shall have provision of Isolation valves in both upstream and downstream side for decontamination of HEPA/ULPA filters during maintenance. • HEPA filter housing should have pressure differential meter to know the status for any choking of filters.
i	• Pre-filters shall be installed in upstream side of HEPA filters to increase the life of HEPA filters in both supply air and exhaust air. • Exhaust pre filters can also be installed within the containment area where they can be changed easily. • Used pre-filters should be decontaminated before removal from the containment area.
j	• HEPA Filter housing should have *in-situ* leak testing facility to assure integrity and damage in installation of HEPA filter and leakage testing of HEPA filter sealing with its housing. • Supply and exhaust HEPA filters housings should be located as close as possible to the containment space to reduce the containment duct length. • HEPA filter housing should have provision for physical isolation using zero leakage bio seal dampers. Housing should also have provision of port for injecting chemical for decontamination of HEPA filters before their removal for replacement. • For existing facilities which do not have above provision, they should develop SOP for safe maintenance and HEPA filter replacement. Bag-In-Bag-Out (BIBO) HEPA filters may be preferred or HEPA filter housings may be kept under negative pressure.
k	• In areas with higher contamination, double HEPA filter in series are recommended. HEPA filters may be required to be installed in parallel depending upon the quantity of air to be filtered based on containment area.

	• HEPA filters may be designed for approximately 50% of its rated capacity so that there is sufficient margin for dust loading as capacity of filter reduces with the choking of HEPA filters.
l	• Exhaust should be discharged at a safe distance from the supply air, minimum distance between supply and exhaust should be 7m. • Exhaust air should be discharged in such a direction that it has minimum impact of storm/ wind direction. • Air from the containment space is to be discharged preferably from the roof and in vertically upward direction at a velocity greater than 3000 fpm (900 meter per minute)with cowl hood or at 135 degree inclination to prevent rain water entry.
m	• Outside air intake should be such that rain should not wet or clog the supply air filters. Air intake may be protected with 6 mm or 12 mm bird screen.
n	• As a general principle, design must ensure that failure of electrical, mechanical or power source will not shut down critical biocontainment system. Therefore sufficient redundancy should be kept based on risk analysis based on criticality and cost factor. • Redundant fan, pumps should be considered in supply and exhaust air ventilation systems. To prevent overheating of interior rooms, N+1 number of chillers should be considered (N being the number of best size chillers).
III	**MECHANICAL**
28	Air compressor, if required for pneumatic controls and for compressed air supply in the BSL-3, should be oil- and moisture- free, and installed outside the containment area. Sound level should be less than 60 DB and must be installed on vibration isolators. Air compressor must have moisture separator with automatic moisture drain valve.
29	Analysis may be done to have a centralised steam generator (oil fired boiler) or electric boiler, or stand-alone electric steam generators depending on requirement. Steam is required for autoclave, liquid waste decontamination and hot water generator for central heating/ personnel showers etc.
30	BSL-3 facility must have DG generator for 100% load requirement with AMF panel. Fuel storage capacity should be designed keeping in view local load shedding and continuous run of generator for long time due to electric supply break down. DG set should be housed in environment friendly enclosure and must meet CPCB norms. Noise level should be less than 60 DB. Standby generator is recommended. However, its cost and risk analysis may be done at the design stage.
IV	**SERVICES**
31	Service pipes shall be installed with slopping lines. Back flow preventers may be used to isolate branch water lines. For cleaning purpose and to avoid contamination, piping should be mounted at some gap from the wall.

32	Compressed air for instrument control and other requirements should be oil and moisture free, and must have small inline HEPA filters and back flow preventers.
33	Each floor drain should have minimum 125 mm deep trap, which should be directly connected to the liquid waste decontamination system. It must have cleanout plugs within the containment zone. Liquid waste pipe should be acid and chemical resistant, and leak proof. Drain pipe should be easily cleanable and preferably 150 mm in size.
34	A foot, elbow or automatic operated hand washing station should be provided near the exit of each functional space. Sink should be acid and chemical resistant, preferably of SS or epoxy coated resins.
V	**ELECTRICALS**
35	Separate power and lighting panels should be provided for containment and non-containment spaces. All main distribution panels should be located outside containment space for ease of maintenance.
36	All the inside and outside opening of the conduit, running from non-containment area to containment area and across different containment areas having differential negative pressure, should be sealed to prevent circulation of air.
37	Sealing should be done at accessible space for inspection and maintenance.
38	All lights should be energy efficient, as per Energy Conservation Building Code(ECBC).
39	Lighting should be in the range of 300 - 500 luxin the laboratory and 700-800 luxin cleaning cycle area.
40	All signage, emergency lights, BAS, communication network, exhaust system to maintain negative pressure and other critical equipment should be provided with on line UPS with minimum half an hour backup. There should be a standby UPS of 100% capacity of designed capacity UPS. The capacity of UPS shall be designed on the basis of emergency load of the facility.
41	A standby generator with AMF panel capable to bear 100% load of the facility should be available in case of power failure.
42	Electrical load should be divided equally on each phase.
43	There should be a centralized voltage regulator to supply +/- 10 % of rated electric supply. Voltage regulator shall be kept outside the facility.
44	Equipment which need servo voltage (+/- 2%) supply may be identified well in advance and central servo stabilizer may be kept outside the facility.
45	Separate cable with 100% capacity of load should be kept as stand-by and shall be used in case of any fault in the main cable to reduce down time.
46	Electrical load may be properly calculated during the design stage, by keeping in mind, any future addition of equipment(s).

47	Wiring for interlocking of double door entry, air locks, pass boxes, double door autoclaves should be well planned so that both the doors cannot be opened simultaneously.
48	The electrical system must have sufficient circuits and power to support decontamination need of the facility.
49	Earthing with copper earth plate 600 mm X 600 mm, 3 mm thick along with masonry enclosure and cover plate having 2.7 meter long watering pipe fitted with funnel for watering. Earthing should be separate for neutral and other for earthing as per Indian Electricity Rules. There should be separate similar earthing for electric supply and each generator. Voltage difference between neutral and earth should be less than 5V.
VI	**COMMUNICATION NETWORKS**
50	The laboratory should be equipped with communication network between containment area and outside support area.
51	Fax, LAN network should be provided for electronic transfer of information and data within the containment laboratory as well as from containment laboratory area to outside area. Electronic transfer of information and data should be encouraged, while avoiding paper transfer. However, in case paper has to be taken out from containment area to non-containment area, it must be de-contaminated through autoclave/ fumigation chamber/Air-lock.
52	It is recommended to install CCTV in containment area to monitor the laboratory working from outside the containment area.
VII	**CONTAINMENT PERIMETERS**
53	A double door barrier autoclave with bio seal should be located on containment barrier. Body of autoclave should be located outside of containment for ease of maintenance. Outside room (Clean side) of autoclave should be well ventilated.
54	Autoclave condensate drains should be directly connected to drain piping system and should be decontaminated along with liquid effluent in Effluent Treatment Plant (ETP).
55	Barrier autoclave and pass boxes should be equipped with interlocking door (electrically and /or mechanically) and visual or audible alarm to prevent opening of both doors simultaneously.
56	Autoclave should be PLC controlled and should record complete cycle real time data like temperature, steam pressure, type of cycle etc. A record for all autoclave cycles should be maintained.
57	Clean side door of barrier autoclave/ fumigation air lock should open only after completion of the decontamination cycle.
58	For materials that cannot be autoclaved (*e.g.* heat sensitive equipment, samples, films etc.), other proven technologies for material decontamination (*e.g.* chemical, gaseous) should be provided at the barrier. SOPs to be developed by the Laboratory In-charge.

59	Dunk tank and double door pass box with gaseous decontamination with interlocking of doors should be available at the barrier of the facility.
60	All penetrations should be sealed with non-shrinkable sealant and tested for leakage at the time of testing and commissioning.
61	Containment side of barrier autoclave, Necropsy room (Post mortem room) and effluent treatment room are highly contaminated areas. Hence, negative pressure of these areas should be accordingly kept at more negative value.
VIII	**DECONTAMINATION**
62	For personnel, change of laboratory clothing in change room and shower is recommended.
63	Air exit should occur only after HEPA filtration.
64	Liquid waste is decontaminated by steam liquid effluent treatment or by dosing chemical disinfectant.
65	Entry to effluent treatment system should be through cloth change room and shower room and staff must take shower before exit from the effluent treatment system. Effluent treatment system should be at negative pressure. The value of negative pressure may be set after doing risk analysis and keeping in view of negative pressure of its surrounding areas.
66	Solid wastes should be taken out of the facility through steam autoclave.
67	Equipment which cannot be autoclaved should be taken out after gaseous decontamination
IX	**TESTING, COMMISSIONING AND VALIDATION**
68	For Certification, the new facilities shall be tested, validated and commissioned. However, the existing facilities shall be validated, based on documentation submitted by ISO/IEC 17025/Bureau of Indian Standards/Quality Council of India certified organization**.** **Testing** Testing of equipment/ Critical components should be initially performed by the construction contractor in the presence of a team involved and test results are properly documented. The following equipment and systems must be tested during the construction: • Leak testing of supply and exhaust duct work (Duct of SS-304 recommended). • Factory testing of HEPA filters, HEPA filter Housings, Isolation valves, Air tight gates and other critical components. • Field testing of HEPA filters and HEPA filter housings after installation at site. • Leak testing of containment spaces (Recommended for and high-risk pathogens).

	• Differential pressures and/ or directional air flows between adjacent areas as per design parameters. • Field testing of Biological safety cabinets. Functioning of all critical parameters should be repeated and demonstrated to authorized person/ team or project management consultant for the facility, in presence of the Laboratory In-charge and Biosafety Officer. Final testing and commissioning should take place in presence of committee / project team that may include third party. All performance parameters and adjustments / replacements if any carried out during testing should be documented for future reference. Testing protocols are provided at **Annexure-II.**
69	**Commissioning**: Commissioning is the verification of physical construction with the design parameters/ predetermined performance criteria and it is one part of overall validation process. This requires verification and documentation of critical containment components, equipment start up, adjustments of parameters, control system calibration, balancing and performance testing. Commissioning procedure for the laboratory should be well designed and implemented to verify the safe aspects of facility operation. Commissioning of facility shall include all critical elements such as airflow patterns, negative pressures in different zones of the facility, biosafety cabinets, temperature profiles in autoclaves, procedures for decontamination and sterilization, verification of light lux level, operation of HVAC systems, measurement of chilled water pumps capacity, air quantities at outlet diffusers / grilles, air compressor capacity, air curtains, steam boiler, clean room garment storage cabinet, floor traps, drains, dunk tanks, checking of ceiling panels, pass box, shower cabinets/ air shower, water outlets, air leak in ducts as well as plenums, doors and view panels along with functioning of all the alarm systems. Additionally, commissioning of all the basic requirements as per the approved layouts, including electrical connections, emergency electric supply, UPS, local area network (LAN) connections, servers, water connections, sewage connection, hardware fitting, telephones and intercoms. Functioning of the BMS with all the desired parameters, fine setting of access control and all the inventories *etc.*
70	**Validation** Validation represents successful completion of commissioning and acceptance of operational protocols that meet the required design parameters, as per "*Regulations and Guidelines on Biosafety of Recombinant DNA Research & Biocontainment , 2017*". Performance based tests to be conducted at the time of validation are provided at **Annexure III**. It is also a verification of all approvals from statutory bodies like Fire safety, Municipal corporation, Pollution control board, Electrical Inspector, Natural climatic safety and Boiler Inspection

	Authority, as applicable. The validation process should also verify that following protocols have been developed in the facility: • Staff training has been reinforced. • SOPs for working in the facility have been developed. • Mock drill has been conducted. • A document describing the mandate and features of the laboratory has been developed. • Facility and Operation Manuals explaining biosafety aspects as well as maintenance of engineering systems has been prepared. • A Technical Manual should also be developed for the facility.
X	**CERTIFICATION**
71	For Certification, an inter-ministerial committee to be constituted. Committee shall include representatives/nominees of DBT, ICAR, DST, MOEF&CC, ICMR and Experts including Engineers. The engineer must have experience of installation, testing, commissioning and maintenance of BSL-3 facility. The committee shall examine the documentation submitted by the Laboratory In-charge. The committee may also consider visiting the facilities before certification. The committee to review the documents having validation done by an ISO/IEC 17025/Bureau of Indian Standards/Quality Council of India certified Third party organization. The Certificate shall be issued for three years. Documentation for Testing, Commissioning and Validation of the facility certifying compliance with due guidelines shall be preserved for future reference.
XI	**REVALIDATION**
72	Revalidation of certain containment components should be performed in normal routine without affecting the working of containment facility. Nature and frequency depends on no. of factors. For example, following components can be revalidated without affecting the working of facility. These need to be revalidated, on annual basis: • Revalidation of directional flow. • Revalidation of No. of air changes etc. • Detection of any visual leak in room perimeter. • Leakage through entry/ exit doors, Pass box, Air-Lock doors etc. • Re-calibration of sensitive controllers and gauges. • Monitoring of the efficacy of autoclaves (quarterly internally) • Monitoring the working of effluent decontamination system.

	• Monitoring resistance across HEPA filters through pressure differential meters installed across HEPA filters will indicate the necessity &frequency of replacing HEPA filters. • Biological safety cabinets need to be revalidated at least annually. Additionally after relocation of the cabinet, after electrical or mechanical maintenance and after HEPA filters are replaced. • Other containment equipment like IVC must be tested at least annually for HEPA filter integrity testing. • Integrity testing of supply and exhaust HEPA filter housing and scanning of HEPA filters must be done annually. HEPA filters must be decontaminated prior to testing. • Liquid effluent decontamination system and BAS must be tested annually. • Revalidation of critical components like integrity of room perimeter & duct work is necessary every time any major structural repair or modification in the structure or new installation of any critical equipment has been done. All the testing and revalidation must be done by qualified, competent, ISO/IEC 17025/Bureau of Indian Standards/Quality Council of India certified third party organization.

PROCESS FLOW FOR BSL-3 FACILITY CERTIFICATION

ANNEXURE II
ESSENTIAL TESTS DURING INSTALLATION OF BSL-3 LABORATORY

Leak testing of supply and exhaust duct work and Isolation Valves:

- Leak test should be conducted in all the duct portions that are potentially exposed to contamination i.e. from respective containment rooms to isolation valves.
- All welds and duct joints shall be fully exposed and accessible for inspection and repair until testing is completed and validated.
- Duct and plenums should be isolated by closing isolation valves, which shall be pressurized to 1000 Pa. All the joints need to be physically inspected for any leakage.
- If any leakage is found, it needs to be repaired and again pressurized to 1000 Pa. Leakage can be tested by soap bubble test or pressure decay method.

Acceptance:

- No leakage assessed by soap bubble test in duct is acceptable.
- Alternatively, duct work may be tested by pressure decay method.
- Pressure drop less than 0.1% duct volume /min is acceptable.

Factory testing of HEPA filter Housings, Isolation valves, Air tight gates and other critical components:

- HEPA filter Housing, Isolation valves, Air-tight gates and other critical components should be tested at the manufacturing unit by pressure decay method as described above, by an ISO/IEC 17025/Bureau of Indian Standards/Quality Council of India certified third party organization.

Acceptance:

- Pressure drop less than 0.1% duct volume /min is acceptable.

Factory testing of HEPA filters:

- HEPA filters should be tested at the manufacturing unit, by an ISO/IEC 17025/Bureau of Indian Standards/Quality Council of India certified third party organization.

Acceptance:

- HEPA filters having leakage less than 0.01% down to 0.3 micrometre particles are acceptable.

Field testing of HEPA filters and HEPA filter housings after installation at site:

- Integrity of HEPA filter and Filter housings should be tested in situ by Soap bubble test and Pressure decay method.

- HEPA Filters may be tested after installation as explained above.
- Any pinhole leakage found during scanning is repaired and scanned again.

Acceptance:

- No leakage in filter housing is acceptable.
- Alternatively, integrated filter housing may be tested by pressure decay method.
- Pressure drop less than 0.1% duct volume /min is acceptable.
- HEPA filter having leakage less than 0.01% down to 0.3 micrometer particles is acceptable.

Leak testing of containment spaces:

The purpose of testing of containment space is to determine and minimizing the leakage through walls, floors, ceilings, penetrations for service pipes, ducts, electrical conduits and other containment barriers of containment space. Testing is done by pressuring positive pressure by approximately 125 Pa and monitoring the air pressure during the test period. Testing is done using following method:

- Sealing the supply and exhaust openings, closing all doors and other openings in the containment perimeter.
- Installing inclined manometer/ pressure differential meter of minimum 0-1000 Pa scale and 10 Pa least count.
- Pressuring positive pressure to 125 Pa.
- Visual inspection of possible leakage spaces.
- Soap bubble testing.
- Repairing any leakage observed.
- Repeating the test with 250 Pa.
- Monitoring pressure decay test for 20 minutes.
- Record pressure differential after every minute.
- Release the pressure slowly after completion of monitoring period.

Acceptance:

- Pressure drops less than 125 Pa (Half of original 250 Pa) in 20 minutes, is acceptable.

Differential pressures and/ or directional air flows between adjacent areas as per design parameters:

- Differential pressure meter/ pressure manometers installed at predetermined spaces monitored and readings recorded while doors are operated as per SOP.
- Negative pressures of different pressure zones should not equalize during normal operating of doors.
- Directional air flow is tested with the visual inspection of smoke patterns while testing is conducted with the help of smoke generating pencil.

Acceptance:

- Unidirectional smoke pattern from area of low contamination to high contamination, is acceptable.

Field testing of Biological safety cabinets:

Standard, tests for BSC are classified into two groups:

- Critical performance tests that are required to confirm that the cabinet is functioning properly, include:
  - HEPA filter Installation integrity test.
  - Work zone integrity test.
  - Biosafety cabinet integrity test.
  - Down flow velocity test.
  - Face (Inflow) velocity test.
  - Air flow smoke pattern test
  - Supply and Exhaust Fan interlocking control test.
  - Alarm operational check
- Non-critical tests which relate to operator's safety, include:
  - Vibration
  - Sound level
  - Lighting
  - UV light

Acceptance:

- The results of biosafety cabinet installation, as per NSF/ ANSI 49 standards, are acceptable.

Testing of Air-lock:

- Air-lock doors should be tested for leakage by Soap bubble test and Pressure decay test as detailed above.
- Once dirty side door of air lock is opened, air lock gets contaminated; therefore, outer side door/ clean side door should only be opened after air lock has been decontaminated.
- Formalin fumigation is one of the methods recommended for gaseous decontamination.
- Testing of air lock should be done as per SOP adopted for the lab.

Acceptance:

- The working of air lock decontamination system as per SOP adopted, is acceptable.

Testing of steam autoclaves:

Steam autoclaves are tested depending upon the number of programs available in the Programmable Logic Controller (PLC), which shall be used in the facility. Effectiveness

of decontamination depends on loading factors, material being decontaminated that influence the temperature to which material is subjected and the contact time. Packaging, size of container and their placement in autoclave must allow steam penetration and must be arranged so that steam circulation in the autoclave is free. Some important decontamination programs are meant for/directed towards:

- Liquids
- Liquids with pre vacuum.
- Nonporous solid materials
- Nonporous solid materials with vacuum.
- Fabric material with pre and post vacuum.

It should be ensured that gauges, thermocouples are calibrated. Depending upon the temperature, the exposure period varies from material to material. For testing of effective sterilization, chemical indicators can be used. Biological indicators are recommended for the same.

Note: Chamber temperature should always be more than 121 or 134 °C as per the case. During testing if chamber temperature comes down from 121°C or from the set temperature, then time counter should be reset to zero.

Acceptance:

- Appropriate working of chemical/biological indicators, is acceptable.

Testing of Interlocking of airlock doors, pass box doors, entry- exit doors, autoclave doors *etc.*:

Interlocking of dirty side door and clean side door is tested as per SOP adopted for the lab. Both side doors should not be opened at the same time. Once the dirty side door is opened in air lock/ pass box/ autoclave, then the clean side door should open only after the chamber has been decontaminated as per the SOP adopted in the lab. In case of entry-exit doors, once the dirty side door is opened then the clean side door should open only after dirty side door has been closed and shower has been taken (it is also recommended that dirty side door should open after one air change of the shower room).

Acceptance:

- The working of interlocking of doors as per SOP adopted is acceptable.

ANNEXURE III
VALIDATION PROCEDURE FOR BSL-3 LABORATORY

Administrative validation to facilitate Operation and Maintenance to ensure safety of Occupants, Product and environment:

1. **Review background materials that affect maintenance operations:**
    - Obtain and review Commissioning Report.
    - Review architectural and mechanical drawings to ensure facility construction is as per design.
    - Review biosafety policies and procedures (SOPs) for the laboratory (facility) including training of laboratory staff including maintenance staff.
    - Review hazardous (infectious) waste decontamination procedures.
    - Assess laboratory accident response protocols.
    - Review integrated pest management program.
    - Review SOPs for document retention, maintenance and lab procedures.

2. **Inspect and Evaluate:**
    - Finishes, penetrations & caulking integrity for architectural elements such as doors, around the ceilings, lighting fixtures, electrical devices, *etc.* within containment to meet requirements for:
        - Clean-ability of all surfaces including furniture
        - Smoothness of all surfaces
        - Sealed seams and penetrations
        - Monolithic, slip resistant floors
        - Surface impermeability to liquids
        - Resistance of surfaces to chemical (organic solvents, acids, alkalis),disinfectants and moderate heat
        - Gas tightness for decontamination
        - Pest management requirements
        - Non-openable bio-seal windows.

3. **Inspect room layout, placement of equipment and equipment condition:**
    - Evaluate autoclave verification testing procedures, inspect log book
    - Evaluate access control and exit procedures
    - Evaluate availability of:
        a. Emergency equipment
        b. Emergency two way communication system
        c. System provided for electronic transfer of information to outside of containment area.
        d. Emergency lighting
        e. Availability and Working fire extinguishers
        f. Availability of chemical spill kit within containment

- Evaluate redundancy requirements for particular facility such as air handling units, exhaust fans, decontamination system components (*e.g.* pumps & HEPA filters)
- Assess location of BSL-3 labs in relation to BSL-2 support labs, offices and break rooms.
- Operational condition of doors.
- Presence of an anteroom with/without a shower.
- Storage provided for clean protective clothing and safety equipment
- Hands-free sink located near exit of laboratory
- Office location outside of containment.
- Inspect signage for proper posting
    a. Biohazard sign
    b. Agents used
    c. Names and telephone number for Laboratory In-Charge and Biosafety officer
- Special requirements such as required use of PPEs,
- Review list of all mechanical controls and their locations
- Review start up and shut down procedures in case of emergency

4. Evaluate maintenance frequency and review maintenance logs

- Autoclaves
- BSC filters
- Centrifuges
- Door/ equipment locks
- HVAC balancing
- HVAC belts
- HVAC Motors
- Lights
- Plumbing

Validation of Engineering Controls:

1. Validate that extra capacity is present on both supply and exhaust systems.
2. Ensure single pass air flow.
3. Measure directional air flow, pressure relationships, air changes and record data.
4. Directional air flow must be established from clean areas to contaminated areas. In the event that multiple containment zones exist within a laboratory sequentially more negative pressure differentials must be established so that the more contaminated spaces are maintained at a negative pressure with respect to less contaminated areas.
5. Pressure differentials across doorways must be measured using a device calibrated against a primary standard. Ideally, at least -15 Pa should be maintained from clean areas to more contaminated areas.

6. Develop HVAC system and electrical systems failure tests consistent with laboratory design parameters. Perform tests and record data. To verify correct operations these tests should include at a minimum:
    - Switching from Normal power to emergency power. UPS must be immediately on, there should be no lag while power failure. Generator must be functional in 1-2 minutes.
    - Switching from Emergency power to normal power.
    - Loss of supply fans (individual and in combination).
    - Loss of exhaust fans (individual and in combination).
    - Building automation system (BAS) maintains operational set points during all scenarios and return to normal operations.
    - Upon reboot BAS must retain operational set points.
    - If an uninterrupted power supply (UPS) is installed, verify operation of relays.
    - Provide UPS for BAS.
    - Assess if UPS is operational and has sufficient back-up time.
    - Ensure that laboratories are maintained at negative pressure with respect to less contaminated areas.
7. Assess HVAC equipment condition
    - Visually inspect
        - Belts
        - Belt guards
        - Wiring
        - Duct supports and connections
        - air dampers
        - Bearings
        - Ductwork system workmanship, joint type, damage, etc.
    - Ensure that motor operating temperatures are maintained within equipment specifications
    - Ensure that interlock between supply and exhaust fan is operational
    - Verify correct placement of biological safety cabinets with respect to supply and exhaust diffusers, doors and traffic patterns.
    - Use smoke at the face of the cabinet to ensure that the air curtain is not being disrupted by supply or exhaust diffusers placed in proximity of the cabinet(s) or opening and closing doors and traffic patterns.
8. Perform smoke tests to demonstrate directional airflow
    - Doors
    - Vents
    - Windows
    - Autoclave
    - Other vented areas.
9. Inspect and challenge door interlock systems and automatic door closers

- Door closers are required
    - Ensure that doors automatically close and latch
    - Interlocks required
    - Check operability
- Open and close doors in all possible sequences
- Ensure that delay set points are tight enough to preclude inadvertent over ride of interlock.

10. Test all alarms

- HVAC Failure Alarm
- Availability of air flow alarms showing if the room has gone positive under normal conditions or if door is open for greater than 20 seconds.
- Availability of a visual indication for personnel to be aware if the room is under positive or negative pressure prior to entering into the lab
- Review fire alarm annual documentation
- Review security alarm annual documentation

11. Discharge exhausts assessment:

- Inspect rooftop landscape for re-entrainment opportunities
- Minimum. 25 ft away from fresh air intake, 40 ft away from boiler stacks and 15 ft away from plumbing stacks
- Laboratory exhaust stacks, minimum 3m height above highest point on roof
- Check Exhaust stack locations and discharge velocities
- Exhaust velocity = 15-20 m/s or 3000-4000 fpm
- Aerosol-producing equipment exhaust through validated HEPA filtration devices
    - Ensure that continuous flow centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory.
- Ensure that discharge of local exhaust ventilation devices is removed from air intakes to prevent re-entrainment.

12. Verification of air change rates (ACR) in containment spaces

- ACR is determined during design based on sensible and latent heat loads contaminants and odors that require containment space usage
- Measure supply and exhaust air volumes using a device calibrated annually
- Calculate ACR; monitor trends
- In no case ACR should be less than 6/hour for lab office and 10/hour for laboratory and 12/ hour for animal facilities.

13. Review biological safety cabinet (BSC) validation data

- BSCs must be on an annual validation schedule
- Verify that BSCs are located away from doors and vents
- Verify that installation of BSC is correct for cabinet type.

- Inspect HEPA filter installations
- Review validation documentation for all exhaust HVAC HEPA installations
- Verify that HEPA filters are on portable air vacuum systems at point of use and at the barrier
- Visually inspect
    - Isolation valves for decontamination
    - Decontamination and challenge ports
    - Scanning access.

14. Validate Mechanical, Electrical, Plumbing Services

- Inspect for adequate illumination
- Verify that circuit breakers are outside of containment
- Backflow prevention for lab water system
- Sinks and drains properly marked
- Availability of emergency power for critical systems
- Availability of hands-free emergency eyewash
- Availability of emergency shower
- Caulking and sealing requirements for electrical devices such as conduits, boxes, lights, *etc.*
- Validate provision for dedicated vacuum pump, if present
- Inspect effluent decontamination system

15. Validate autoclave availability, operations and bioseal integrity

- Test interlocks
- Confirm cycle test load
- Visually inspect bioseal
- Smoke test for testing bioseal

- Validate maintenance of sterilization temperature of 121 degrees for 30 minutes. **Note:** Chamber temperature should always be more than 121 or 134°C, as per the case. During testing if chamber temperature comes down from 121°C or from set temperature, then time counter should reset to zero.

16. Additional environmental protection (*e.g.,* personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) is considered.

ANNEXURE IV
STANDARD OPERATING PROCEDURES (SOPs) FOR SPECIFIC CONSIDERATIONS FOR WORKING IN THE BSL-3 FACILITY

The Standard Operating Procedures (SOPs) must be developed by the Laboratory In-Charge and Biosafety Officer, along with IBSCs. A Biosafety Manual should also be prepared and adopted. The Biosafety and SOP manuals should be readily available to all users of the facility. There are several specific considerations for working in the BSL-3 facility, they have been broadly mentioned below:

Entry Procedures

Secured Entry

- ✓ Entry of new workers will be allowed only when accompanied with authorized personnel or once they passed the training.
- ✓ Controlled access entry required through fingerprint scan.
- ✓ Manual logging in log book required in the anteroom 1.
- ✓ Entry into Anteroom and Laboratory Space:
  - Location for manual logging in and shoe cover, head-cover, two layers of gloves: Anteroom 1
  - Location for Donning rest of PPE (Tyvek suit, N95, eye cover): Anteroom 2

Donning SOP

- ✓ Put on two pair of gloves, head-cover, shoe covers in Anteroom1
- ✓ Put on gown, N95 and eye cover in anteroom 2

*Make sure you are not wearing any sharp rings which will perforate the gloves.

Working within BSl-3 Laboratory

The use of *good laboratory practices and appropriate microbiological techniques*

- ✓ Being aware
- ✓ Proper pre-planning of procedures before entering
- ✓ Understanding the proper care and use of equipment within BSL-3
- ✓ Avoiding distractions as much as possible
- ✓ No work with infectious materials is conducted in open vessels on the open bench.

Use of Biosafety cabinets (BSC)

- ✓ Depending on the Class of cabinet, suitable HVAC system
- ✓ Gauges should be checked to ensure cabinet is operating within the validation parameters
- ✓ Use minimum equipment in the cabinet to allow for effective airflow.
- ✓ Keep grills clear and the sash at a proper level.
- ✓ Arm movement should be slow

- ✓ Waste container inside BSCs: small plastic pail is used for liquid waste and for solid waste a biohazard bag is used (such as paper and plastic wrappers of serological pipettes).
- ✓ Waste containers outside the BSCs: All waste inside the BSCs are collected in a covered waste container lined with double biohazard bags on the floor adjacent to the BSCs.

SOP: Small Spill Response in BSCs

- ✓ Work should be halted as soon as possible after a spill occurs.
- ✓ Clean the spill as soon as safely possible in following way.
- ✓ Cover the spill with disinfectant adopted by lab and cover with paper towel to cover the entire spill.
- ✓ Leave for appropriate time and then pick up the paper towel and place in the biohazard bag in the BSC.
- ✓ Resume work.

SOP: Movement of culture from

- ✓ No culture should be moved around in the BSL-3 laboratory without containing them in secondary container.

SOP: Small Spill Response outside BSCs

- ✓ Leave the room immediately, lock the door, post a warning sign and inform your Laboratory In-Charge.
- ✓ Stop all work and inform all the workers in the BSL-3 and Leave the BSL-3 after proper doffing and inform others working in the lab or planning to work in the lab.
- ✓ All the workers must leave the BSL-3.
- ✓ If clothing is contaminated, remove and turn the exposed side of fabric in on itself and place in biohazard container.
- ✓ Wait at least 30 minutes before re-entering the lab to allow dissipation of aerosol created by the spill.
- ✓ During this time, review clean-up procedures, assemble material and contact biosafety officer.
- ✓ Don fresh PPE
- ✓ Cover spilled material with an absorbent paper towel/tissue. Once the absorbent material is in place over the spill, wet the material with Lysol or formalin. Pour more around the spill. Use more concentrated disinfectant if the volume of material will significantly dilute the disinfectant.
- ✓ Allow 15 minutes contact time.
- ✓ Use forceps to place sharp objects into a sharps container. Using an autoclavable dustpan and squeegee, tongs, etc., transfer all contaminated materials (paper towels, gloves, lab ware, *etc*.) to plastic bags and treat as waste.
- ✓ Wipe surrounding surfaces with disinfectant to cover all splash areas. Wipe flat surfaces to remove any material that may have splashed out and settled on those surfaces.

- ✓ Place all contaminated materials into a biohazard bag for autoclaving.
- ✓ Complete an Accident Report form and also mention if one was exposed.

SOP: Glove puncture with contaminated instrument or pipette tip

- ✓ Two layers of gloves to be used while experimentation and outer layer should be disposed off immediately after working at BSL cabinet.
- ✓ Decontaminate the top gloves with 70% ethanol and take it off inside the BSC.
- ✓ Spray more 70% ethanol on the inside gloves and takeout your hands out of the BSC and check for is integrity.
- ✓ If the inside gloves are not pierced, then put on the second pair of gloves and resume work.
- ✓ If the inside gloves are damaged, then leave the BSL-3 and don off according to SOP and wash your hands with soap and water. And check of any injury, if no injury, report the damaged gloves and resume work after proper donning.
- ✓ If skin injury is noticed, then do not squeeze the wound to induce bleeding.
- ✓ Avoid use of abrasive chemical soaps or disinfectant washes as they can decrease skin integrity.
- ✓ Contact the laboratory director and biosafety office and see the medical doctor immediately.
- ✓ Complete an Accident Report form and also mention if one was exposed.

Centrifuges and microfuge: Proper use

- ✓ Opening centrifuge rotor heads and caps must also be done inside a BSC.
- ✓ Rotors should be taken out from BSC in a closed container.

Sharps handling and disposal

- ✓ Use of hypodermic needles and Pasteur pipettes is restricted in the BSL-3 lab.
- ✓ If needles are to be used, "safe" or protected needle devices are recommended.
- ✓ Extreme care should be used to avoid auto-inoculation and aerosol generation. Contaminated sharps must be promptly placed in a puncture-resistant sharps container and decontaminated before disposal.
- ✓ Broken glassware must not be handled directly by hand.
- ✓ Plastic ware should be substituted for glassware whenever possible.

Autoclaving/Decontamination Procedures

- ✓ Decontamination should be done by agent adopted as per lab SOP. For example: vesphene, lysol.
- ✓ All solid waste should be placed into a biohazard disposal bag and then autoclaved.
- ✓ All contaminated liquids to be autoclaved after decontamination, preferably, placed in closed, labelled, and leak-proof containers that have been surface decontaminated prior to removal from the containment zone
- ✓ Material to be removed from the facility shall be properly decontaminated by autoclaving or by chemical disinfection. Reuse should be avoided. However, if

essentially needed, reusable containers shall be decontaminated by immersion in an appropriate disinfectant for recommended time (care should be taken to fill completely). The items will be then drained and autoclaved out of the facility.

Facility upkeep and cleaning

- ✓ Researchers will perform all daily housekeeping routines within the BSL-3 lab, including trash removal. All the cleaning and decontamination procedures shall be performed only by individuals authorized to work in the BSL-3 facility.
- ✓ Large equipment, such as incubators and centrifuges, will have inner and outer surfaces damp-wiped with disinfectant on a routine basis.
- ✓ Water baths: shall be rinsed periodically with a suitable chemical decontaminant. It is recommended to add copper sulphate to the water.
- ✓ Lab notebooks shall not be brought into the containment section of the lab. If essential, any notes/papers to be taken outside should be decontaminated by treating with a suitable agent like vesphene and then, taken out through autoclave/ fumigation chamber/Air-lock.

Exit procedure

- ✓ Secure infectious materials
- ✓ Waste disposal/decontamination
- ✓ Disinfect work surfaces
- ✓ Doffing and its proper disposal: Disposable PPE should be placed into a biohazard container
- ✓ Hand washing
- ✓ Exit

Doffing SOP

- ✓ Decontaminate your gloves and shoe covers with as per laboratory SOP like with vesphene/Lysol/ 1% Sodium Hypochlorite solution in Anteroom 2.
- ✓ Take off bodysuit so that no outside portion of gown touches the inside clothing and remove gown and place it in biohazard bag.
- ✓ Perform Remove first pair of gloves and place it in biohazard bag.
- ✓ Remove eye protection and spray with disinfectant and place it in the storage cabinet.
- ✓ Remove N95 mask and discard.
- ✓ Before entering the Anteroom1 and remove shoe covers and last pair of gloves and discard in biohazard bag.
- ✓ Wash and sanitize your hands. Water/air shower as postulated in the Laboratory SOP.
- ✓ Record your exit time in the log book in the Anteroom 1 and exit.

Transport of infectious material

- ✓ Infectious materials within country must be packaged to withstand breakage and leakage of contents and be labelled, as specified in the regulations listed

International Air Transport Association's (IATA) Dangerous Goods Regulations and Biosafety unit of DBT.

- ✓ All shipment should be recorded. Import/export of select agents and toxins must be registered with the RCGM.

Incident Response Procedures/ Emergency Response Measures/ Post-exposure practice and procedures

- ✓ Spills and accidents that result in overt or potential exposure to infectious material should be immediately reported to the laboratory In-Charge and Biosafety Officer.
- ✓ Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.

Contact Information/Responsible official/Biosafety Officer

- ✓ Check that phone is operational and emergency numbers are posted.
- ✓ Do not work in the facility unless a means to summon help in an emergency situation is available.
- ✓ Any problems should be reported to the lab director.

Safety SOP

- ✓ Identification of responsible official and biosafety officer for BSL-3 facility.
- ✓ Certification of all personnel working within containment and the process followed to certify them.
- ✓ Use, storage and disposal of Personal Protective Equipment.
- ✓ Documented limited personnel access to facility.
- ✓ All new research personnel must get the appropriate training by IBSC designated training cell, before beginning to use the facility
- ✓ Procedures to enter facility for maintenance.
- ✓ Hand washing procedures are in place.
- ✓ Ensure use of mechanical pipetting devices. No mouth pipetting.
- ✓ Use of sharps prohibited unless absolutely required and then use should be managed by protocol.
- ✓ Procedures in place to minimize production of aerosols.
- ✓ Decontamination procedures are in place.
- ✓ Training program is in place and documentation available for training and refresher courses of all personnel including maintenance staff working in the BSL-3 facility.
- ✓ A biosafety manual specific to the laboratory must be prepared and adopted by the facility.

The following records have to be maintained

- ✓ Training and refresher training to be documented; records to be kept on file.

- ✓ Inventory of stocks of pathogens, toxins, and other regulated infectious material in long-term storage to be maintained, including location and risk group.
- ✓ Provision for detection of a missing sample in a timely manner.
- ✓ Map and physical specifications of BSL-3.
- ✓ Records of regular inspections of the containment zone and corrective actions to be kept on file.
- ✓ Records of regular inspections of the containment zone and corrective actions to be kept on file.
- ✓ A record of all individuals entering and exiting the containment zone to be maintained and kept on file.
- ✓ Records of routine decontamination and it's verification
- ✓ Records of incidents and it's response

Validation

- ✓ Facilities must be revalidated annually.
- ✓ BSCs should be preferably revalidated 6 monthly.

ANNEXURE V

CERTIFICATION OF BSL-3 FACILITY

A. Application for certification

This application is for the certification of a facility to the specified containment level-3. Applicant is required to submit the information to RCGM in the prescribed format to obtain necessary certificate. Submission of incorrect or incomplete information to RCGM may delay or may disqualify to grant the certification and it may attract penal actions as per those mentioned in Environment (Protection) Act, 1986. Additional information may be required and will be notified on case by case basis. The certificate will be valid for a period of 3 years and to be renewed after re-validation. The certificate holder should ensure to comply with the conditions of the certification.

1. Basic Information

Organization details

Name of organization:

Address:

Contact:

IBSC registration details:

Applicant details

Name of the Applicant:

Designation:

Address:

Telephone No.:

Fax No.:

E-mail:

Application type

New ☐	Renew ☐

If applying for renewal of certificate, please indicate the RCGM certification number:_____

2. Facility Inspection

The facility may be inspected by the inter-ministerial committee constituted. Committee shall include representatives/nominees of DBT, ICAR, DST, MOEF&CC, ICMR and Experts including Engineers. The engineer with experience of installation, testing, commissioning and maintenance of BSL-3 facility. The committee shall examine the documentation submitted by the Laboratory In-charge. The committee to review the documents having validation done by an ISO/IEC 17025/Bureau of Indian Standards/Quality Council of India certified Third party organization.

Appropriate inspection checklist for evaluation of facility design and operational practices within the facility should be filled at the time of inspection. Laboratory In-charge must be present at the time of inspection. The filled checklist duly signed by committee must be submitted to RCGM along with this form for further evaluation and issue of Certificate.

Inspection Report

i.	Is inspection checklist duly filled post inspection?	Yes ☐	No ☐
ii.	Inspection Report & Checklist attached? (Note: Only a single checklist should be submitted even if the facility is inspected by more than one person.)	Yes ☐	No ☐
iii.	Does the facility meet all requirements contained in this guideline?	Yes ☐	No ☐
If NO, please provide details of:			
a.	Which requirements in the relevant guidelines are not met; and		
b.	What strategies you suggest to manage any risks that may arise or reasons why it is considered that the requirement or condition is not necessary to achieve containment.		

a. -------Enclose separate sheet, if required-------
c. Please provide any other information that may assist the RCGM in making a decision about this application. d. -------Enclose separate sheet, if required------- e.

3. Declaration of the organization seeking certification

This declaration must be completed and signed by the utmost authority of the organization, or a person with the authority to sign on behalf of the organization.

I DECLARE THAT:

- I am duly authorized to sign this declaration;
- I have extended full cooperation to the inspector(s) during their visit
- The information supplied on this form and any other attachment is true and correct; and
- I am aware that the making of a false or misleading statement may be punishable by imprisonment or a fine under the Environment (Protection) Act, (1986).

Date

Place **Name of authority with official seal**

Declaration of the Committee upon INSPECTION

I DECLARE THAT:

- I have personally inspected the facility on …….
- I have recorded the observation in this form during the visit.
- My decision was not influenced and full support was extended to me during inspection.
- I attest that the information contained herein is accurate and complete to the best of my knowledge and belief.

Date

Place

B. Application Checklist for BSL-3 Facility (Documentation to be submitted by the Laboratory In-charge for examination by the committee/ for inspection by the committee of the facility)

B.1 Checklist for evaluation of Facility design

	Requirements of Facilities	Yes	No	Remarks
1	The facility must be a fully enclosable space, bounded by walls, doors, windows, floors and ceilings, which permit operation of the facility under negative pressure.			
2	The facility must be constructed to enable gaseous decontamination of the whole facility.			
3	All facility penetrations must be fitted with seals to minimize air leakage.			
4	All windows in the facility must be closed and sealed.			
5	The facility boundaries (walls, windows, doors, floors, ceilings etc.) must be constructed to prevent the incursion of pests.			
6	Where present, liquid drainage exits must be protected against entry and exit of invertebrate or other animals by the use of screens, liquid traps or an equivalent effective method. Where a screen is used, the apertures of the screen must be small enough to prevent entry or exit of invertebrates or other animals.			
7	The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Additional separation may be achieved by using a laboratory at the blind end of a corridor, a partition and door, a double-door system where entry to the laboratory must be through an ante-room or airlock.			
8	Airlock doors must be self-closing and fitted with seals at the top, bottom and both sides of the door. Airlock doors must contain a viewing panel unless the airlock functions as a shower airlock.			
9	Where the facility shares an airlock with an ABSL3 animal or invertebrate facility, or if animals or invertebrates are handled within the facility, any openings in the walls or ceiling, such as ventilation inlets and outlets must be screened. The screens must be fixed and sealed against their mounting. The apertures of the screen must be small enough to prevent entry or exit of invertebrates or other animals.			

10	Provision must be made for viewing of work areas from outside the facility.			
11	Walls, ceiling, and floors are smooth, easily cleanable, impermeable to liquids, and resistant to the chemicals and disinfectants.			
12	Adequate illumination is ensured for carrying out all activities.			
13	Laboratory furniture is sturdy and open spaces between and under benches, cabinets, and equipment is accessible for cleaning.			
14	Bench tops is impervious to water and resistant to disinfectants, acids, alkalis, organic solvents, and moderate heat.			
15	Biological safety cabinets for handling of infectious microorganisms of risk group 3 are available.			
16	Piped gas supplies to the facility must have reverse flow prevention on outlets located within the BSC.			
17	There must be a ventilation system that establishes a negative pressure into the laboratory so that there is a directional air flow from the corridor or the basic laboratory to the working area of the containment laboratory. Personnel must verify that proper direction air flow (into the laboratory) is achieved.			
18	The work area must be maintained at an air pressure of at least 50 Pa below the pressure of adjacent areas outside the facility when both doors of the airlock are closed. When either door of the airlock is open, the work area pressure must remain at least 25 Pa below that of adjacent areas outside of the PC3 containment barrier.			
19	The work area must be equipped to measure and display the pressure difference between the facility and the areas adjacent to the facility. The display must be located so that it can be read immediately before entering the facility.			
20	The facility must be equipped with an alarm that will alert relevant persons both inside and outside the facility and be immediately activated when the pressure in the facility is more than 25 Pa above the set point.			
21	Provisions for autosensing alarm for fire and other emergencies that evacuation.			
22	Backup power source in event of power failure.			
23	The facility must have an emergency stop button for the ventilation system, which is easily accessible in case of an emergency. The emergency stop button must operate			

	independently of the main ventilation control and main facility pressure control system such that emergency isolation of the ventilation can be implemented in the event of central control malfunction.			
24	Supply or replacement air to the facility are HEPA filtered.			
25	The exhaust filter must be a HEPA filter and must be tested by qualified person. The exhaust HEPA filter must be mounted in a gas-tight housing, with sealed access doors and the ductwork between the facility and the HEPA filter housing must also be gas-tight. The design and location of the filter housing must allow for access to and integrity testing of the HEPA filter.			
26	Access to the laboratory area should be designed to prevent entrance of free-living arthropods and other vermin.			
27	Wash-basins are provided in each laboratory or any other means of decontamination of hands provided.			
28	The following water supplied to the facility must be protected against backflow by registered testable devices that have a high hazard rating for protection against both back-pressure and back-siphon age. Laboratories sink outlets. Outlets within a BSC or other aerosol containment equipment. Direct connections to an autoclave.			
29	Designated storage or hanging provisions for personal protective equipment available in facility.			
30	Eyewash equipment is provided.			
31	The international biohazard warning symbol and sign are displayed on the doors of the rooms where microorganisms of Risk Group 3 or higher risk groups are handled.			
32	Shower facility must be available in the facility before exit.			
33	Class II biological safety cabinets are placed in proper place.			
34	Incinerators, if used, must have dual combustion chambers.			
35	An autoclave, preferably of double ended type with interlocked doors with the inner door opening to the facility and outer door opening externally to the facility is available.			
36	Refrigerators, freezers, incubators, etc. that contain bio hazardous materials for storage must be labelled with a biohazard symbol.			
37	Proper wastewater treatment facility available, working properly.			
	Additional requirements for IBSL-3			
	1. The arthropod facility should be provided with an			

access room. The access room should be fitted with insect-control units for example an electric insect-control device or an ultra-violet insect zapper. 2. Access room doors should be sealed to be arthropod-proof. 3. If risk assessment requires additional mitigation measures for arthropod containment, an anteroom may be provided with a sink and vacuum system to enable personnel to remove any arthropods, eggs or larvae from their person before leaving the facility.			

B.2 Checklist for evaluation of Facility operation

Operation checklist	Yes	No	Remarks
1. Measures are available to restrict access to lab. 2. Periodic inter inspection and audit of the facility is available. 3. Record keeping of biosafety policies and procedures (SOPs) for the laboratory (facility) including training of laboratory staff including maintenance staff. 4. Record keeping of SOPs for document retention, maintenance and lab procedures. 5. Inspect signage for proper posting • Biohazard sign • Agents used • Names and telephone number of Laboratory In-Charge and Biosafety officer 6. Eating and drinking was not observed and no food/drinks stored in work areas. 7. Personal protective equipment is clean, available and used appropriately; not worn outside of lab. 8. Biosafety cabinets (BSCs) are field tested and certified annually. Date of last certification: _____ 9. Autoclaves are maintained, calibrated and tested. Date of last calibration: _____ 10. Aerosol generating activities (sonication, vortexing, homogenizing) are performed inside BSC for risk group 2 microorganisms. 11. Centrifuge safety cups or sealed rotors are used to centrifuge RG 2 microorganisms. 12. Personnel employ safe handling of sharps. 13. Work areas are decontaminated regularly after work and after known contamination. 14. Personnel know how to clean up a spill. 15. All bio hazardous waste containers are closed or covered when			

not actively adding waste. 16. Autoclave bags with biohazard symbol are available and used for decontamination. 17. Lab personnel are up to date on required safety training and lab specific training. 18. Personnel know symptoms associated with organisms used in the lab. 19. Personnel know how to handle exposures and to report accidents immediately. 20. Review start up and shut down procedures in case of emergency 21. Evaluate maintenance frequency and review maintenance logs for Autoclaves, BSC filters, Centrifuges, Door/ equipment locks HVAC balancing, HVAC belts, HVAC Motors, Lights, Plumbing 22. Appropriate laboratory operation manual is accessible to personnel. 23. Record of HVAC system and electrical systems failure tests (as per Annexure-III):. 24. Record of smoke tests to demonstrate directional airflow. • Doors • Vents • Windows • Autoclave • Other vented areas 25. Test for all alarms (as per Annexure-III): 26. Record of work is duly registered in the register available. 27. Personnel know the process of registering and reporting in case of accidents 28. Verification of air change rates (ACR) in containment spaces. In no case ACR should be less than 6/hour for lab office and 10/hour for laboratory and 12/ hour for animal facilities 29. Record of biological safety cabinet (BSC) validation data (as per Annexure-III):. 30. A training program is available for fresh candidate. 31. Record of medical examination of all laboratory personnel including past medical history is available. 32. Baseline serum sample are stored for future reference. 33. Immuno compromised personnel are not employed. 34. Medical contact card is available for all personnel. 35. Laboratory monitoring plan is available and working including periodic surveillance. 36. Inspect Mechanical, Electrical, Plumbing Services (as per Annexure-III).			

37. Proper waste management plan available and is adopted. 38. Validate autoclave availability, operations and bio seal integrity (as per Annexure-III). 39. All instructions related to waste management are posted inside and outside of laboratory and must be visible clearly. 40. Inspection of personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination.			

GLOSSARY

Biohazardous waste	Any waste containing infectious materials or potentially infectious substances.
Biosafety	The maintenance of safe conditions in biological research to prevent harm to workers, non-laboratory organisms and the environment.
Biosafety cabinet (BSC)	An enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogens requiring a defined biosafety level.
Biosafety level (BSL)	A safety or Pathogen/Protection level, is a set of biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility.
Competent authority	An authority responsible for the implementation and application of health measures.
Containment	Safe methods (Combination of facilities, practices and procedures) for managing hazardous microorganisms, genetically engineered organisms or cells in the laboratory environment where they are being handled or maintained.
Contamination	The unintentional presence of an infectious organism on a human or animal body surface, instruments, product, parcels *etc* that may raise issues related to public health.
Disease	An illness due to a specific infectious organism or its toxic products that arises through transmission of that organism or its products from an infected person, animal or reservoir to a susceptible host, either directly or indirectly through an intermediate plant or animal host, vector or the inanimate environment
Decontamination	A procedure whereby health measures are taken to eliminate an infectious organism or toxic chemical agents.
Disinfection	A process that eliminates all pathogenic microorganisms, with the exception of bacterial spores, from inanimate objects, for the purpose of minimizing risk of infection.
Hazardous microorganisms	These are risk inherent microorganisms that may cause harm or likely to cause harm to public health and environment.
Health hazard	A factor or exposure that may adversely affect the health of a human population.
Health measure	Procedures applied to prevent the spread of disease or contamination; a health measure does not include law enforcement or security measures.
Infective microorganism	Infective microorganisms are those that could get access and colonize on human, animal or plant. It may or may not cause disease.
Infection	The entry and development or multiplication of an infectious organism in the body of humans and animals that may constitute a public health risk.
Pathogen	Organism that infect and could cause disease. Pathogens exhibit

	different degree of virulence trait (the ability to cause host cell damage) and so vary in pathogenicity (ability to cause disease).
Personal Protective Equipment	Specialized clothing and equipment designed to create a barrier against health and safety hazards; examples include eye protection (e.g. goggles or face shields), gloves, surgical masks and particulate respirators.
Public health	The science and art of preventing disease, prolonging life and promoting health through organized efforts of society. It is a combination of sciences, skills, and beliefs that is directed to the maintenance and improvement of the health of all people through collective or social actions. The goals are to reduce the amount of disease, premature death and disease produced discomfort and disability in the population.
Risk	A situation in which there is a probability that the use of, or exposure to an organism or contaminated product will cause adverse health consequences or death.
Risk assessment	The qualitative or quantitative estimation of the likelihood of adverse effects that may result from exposure to specified health hazards.
Risk Groups	Classifications that describe the relative hazard posed by infectious agents or toxins in the laboratory.

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