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

सूचना का अधिकार

MINISTRY OF SCIENCE & TECHNOLOGY

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No.BT/BS/17/635/2015-PID

Dated: 1st April, 2018

OFFICE MEMORANDUM

Sub.: Regulations and Guidelines for Recombinant DNA Research and Biocontainment, 2017

The tools of recombinant DNA research have changed dramatically in the last few years with new methods of gene-editing and these offer enormous opportunities for understanding of science and enabling new discoveries; yet proper containment guidelines are absolutely essential if this is to be done in a responsible manner. Therefore, Department of Biotechnology has undertaken consolidation and updating of earlier guidelines namely "Recombinant DNA safety guidelines, 1990"; "Revised Guidelines for Safety in Biotechnology, 1994" and Part of "Revised guidelines for research in transgenic plants, 1998" in the areas of laboratory biosafety through appropriate containment at R&D level. Through intense national and international consultations, inter-ministerial / departmental consultation and inputs from concerned stakeholders, the new guideline titled **"Regulations and Guidelines for Recombinant DNA Research and Biocontainment, 2017"** is now made available.

2. In accordance with the Allocation of Business Rules 1961 of Government of India, as notified vide Notification No. CD-172/86 dated 27.02.1986 and Notification No. CD-87/87 dated 31.01.1987 and the power conferred through the Sections 6, 8, & 25 of the Environment (Protection) Act, 1986 (EPA, 1986) read along with the Central Government Gazette Notification No. GSR 1037(E), dated 05.12.1989 issued by the Ministry of Environment & Forests, New Delhi and based on the recommendations of Review Committee on Genetic Manipulation (RCGM) in its meeting held on 14.11.2017, the Department of Biotechnology hereby notify "Regulations and Guidelines for Recombinant DNA Research and Biocontainment, 2017" for regulating recombinant DNA research through Institutional Biosafety Committees (IBSCs) and mechanisms that regulate them. The guidelines include a whole range from the research, contained/ laboratory use; import/export; storage and handling; manufacturing; disposal and emergency procedure and facility certification. The guidelines supersede and replace the three guidelines mentioned above.

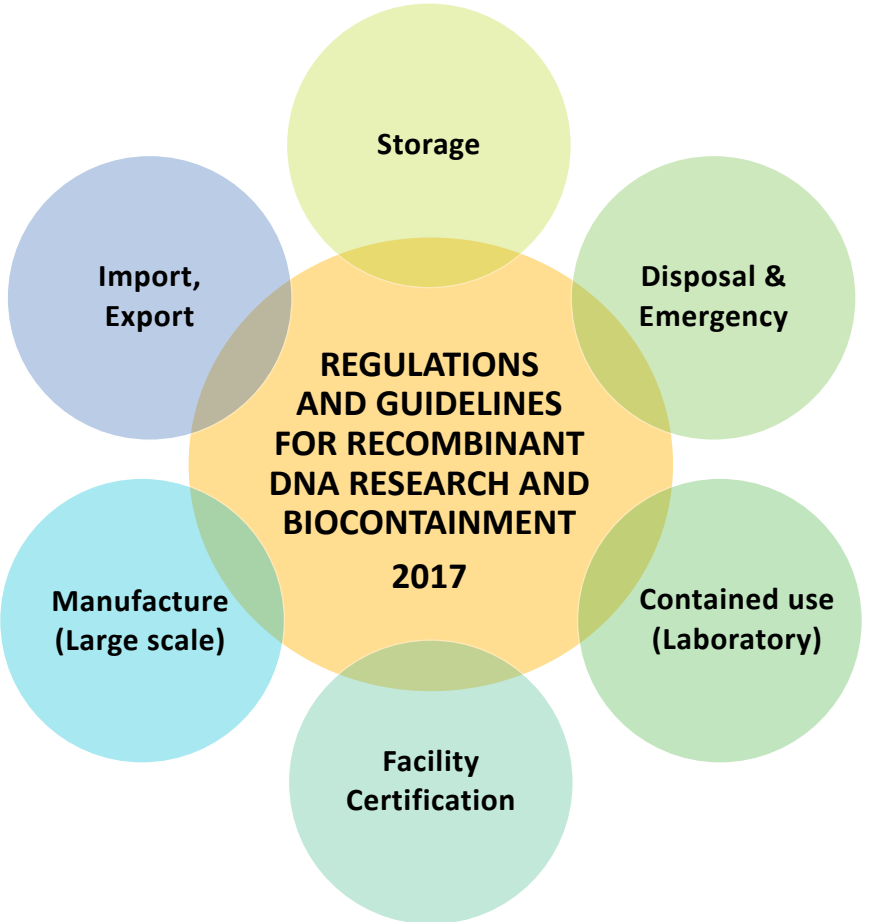
3. As per the provision Rules 1989 of EPA, 1986, all IBSCs and host institutions involved in research, development and handling of Genetically Engineered (GE) organisms and non-GE hazardous microorganisms and products thereof are required to comply with these guidelines with immediate effect. Non-compliance shall attract the provisions of Sections 15, 16 & 17 of EPA, 1986.

4. The "Regulations and Guidelines for Recombinant DNA Research and Biocontainment, 2017" are notified at www.dbtindia.nic.in

(S.R. Rao)
Member Secretary, RCGM &
Scientists-H, DBT

To

1) All Ministries/ Departments of Government of India
2) All Institutional Biosafety Committees
3) Communication Cell, DBT



Department of Biotechnology
Ministry of Science and Technology
Government of India

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PREFACE

Under the Rules 1989 of Environment (Protection) Act 1986, laboratory biosafety through appropriate containment has been identified as the fundamental part of any biological research. In this direction, DBT had earlier published three guidelines namely "Recombinant DNA safety guidelines, 1990" "Revised Guidelines for Safety in Biotechnology, 1994" and "Revised guidelines for research in transgenic plants, 1998".

During the last two decades, rapid advancement in biology and biotechnology research globally and in India, both in public and private sector institutions, necessitated that the above guidelines are reviewed, updated and harmonised with global best practices and guidelines. Further, research on emerging and re-emerging infections and potential risk associated in handling the pathogenic organisms required to put in place stringent yet practical regulations and guidelines for ensuring biosafety measures for protection of public health and environment.

RCGM, the apex body working under Rules 1989 has the mandate to monitor the safety of on-going research projects or activities involving hazardous microorganisms, GE organisms and cells and products thereof. RCGM therefore, has made an extensive effort to update and bring out a consolidated guideline at par with International best practices to prescribe the containment measures for storage, growth, research, manufacture, exchange, import and export of GE and non-GE organisms (microorganisms, animals, plants, arthropods, aquatic organisms) and products of such organisms.

As an outcome of efforts involving several deliberations, national and international expert consultations and stakeholder engagements, RCGM is pleased to present "Regulations and Guidelines on Biosafety of Recombinant, DNA Research and Biocontainment, 2017". The Guidelines cover the regulations on biosafety of rDNA research and handling of hazardous microorganisms and GE organisms or cells in India. It has described stringent and robust facility structures for handling of microorganisms, animals, plants, insects and aquatic organisms and has provided clear instruction on disposal and decontamination of laboratory wastes, emergency procedures etc. The guidelines include a list of risk group agents and determined appropriate containment level for their handling in India. Within the purview of Rules, Institutional Biosafety Committees have been empowered to take adequate precautionary measures for research conducted on risk group 1 and 2 organisms. The approval from RCGM is only required for experiments involving risk group 3 and 4 organisms.

In addition, a separate laboratory certification system for handling of risk group 3 and 4 organisms has been developed. Following implementation of these guidelines, it shall be mandatory for all existing high containment facilities at biosafety levels 3 and 4 to acquire this accreditation for working with risk group 3 and 4 organisms. Adoption of these guidelines shall be binding pan India for all public and private organizations involved in research, development and handling of GE organisms (organism includes microorganisms, animals, plants, arthropods, aquatic animals, etc.) and non-GE hazardous microorganisms (microorganism includes parasites, protozoa, algae, fungi, bacteria, virus, prions, etc.) and products produced through exploration of such organisms.

I am confident that this document will provide the much needed clarity to all stakeholders on biosafety and biosecurity requirements for manufacture, use, import, export and exchange of hazardous microorganism, GE organisms and cells.

I extend my sincere acknowledgements to all expert members, contributions of stakeholders from industry, academia and civil societies for preparing for their inputs in preparation of this document on biosafety in recombinant DNA research and containment yet addressing biosecurity issues.

S.R. Rao
Senior Advisor & Member Secretary, RCGM
Department of Biotechnology
Ministry of Science & Technology
Government of India

सत्यमेव जयते

के. विजयराघवन
K. VijayRaghavan

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MESSAGE

I am delighted to release new Regulations and Guidelines for Recombinant DNA Research and Biocontainment from the Department of Biotechnology, Government of India. The guidelines issued in 1990s have been consolidated and updated. The new guidelines include a whole range from the research, contained/laboratory use; import/export; storage and handling; manufacturing; disposal and emergency procedure and facility certification.

These guidelines have been prepared through intense consultations, nationally as well as internationally, and meet highest global standards. These guidelines will be very valuable for a country such as India where cutting edge biotechnology is done with a huge growth in our biotech at R&D level, applications and industry.

The tools of recombinant DNA research have changed dramatically in the last few years with new methods of gene-editing and these offer enormous opportunities for humanity and for understanding of science and enabling new discoveries; yet proper containment guidelines are absolutely essential if this is to be done in a responsible manner. The new guidelines will facilitate such responsible use through institutional biosafety committees and mechanisms that regulate them.

I look forward to getting feedback from the community on these processes and see how their implementation is most effectively done.

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CONTENTS

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ABBREVIATIONS

ABSL	-	Animal Biosafety Level
AqBSL	-	Aquatic Organism Biosafety Level
BC	-	Biological Containment
BSC	-	Biosafety Cabinet
BSL	-	Biosafety Level
CDC	-	Centers for Disease Control and Prevention
DBT	-	Department of Biotechnology
DLC	-	District Level Committee
EPA	-	Environment (Protection) Act
GE	-	Genetically Engineered
GEAC	-	Genetic Engineering Appraisal Committee
GLSP	-	Good Large Scale Practices
GOI	-	Government of India
HEPA	-	High Efficiency Particulate Air
IBSC	-	Institutional Biosafety Committee
IBSL	-	Insect/Arthropod Biosafety Level
MoEF&CC	-	Ministry of Environment, Forest and Climate Change
NBPGR	-	National Bureau of Plant Genetic Resources
NIH	-	National Institute of Health, USA
PBSL	-	Plant Biosafety Level
PI	-	Principal Investigator (R&D/Industry/Others)
PPE	-	Personal Protective Equipment
RCGM	-	Review Committee on Genetic Manipulation
RDAC	-	Recombinant DNA Advisory Committee
rDNA	-	recombinant DNA
RG	-	Risk Group
SBCC	-	State Biotechnology Co-ordination Committee

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INTRODUCTION

As mandated in the Rules, 1989 of Environment (Protection) Act, 1986, Review Committee on Genetic Manipulation (RCGM) administered by the Department of Biotechnology, Ministry of Science and Technology has updated "Recombinant DNA safety guidelines, 1990"; "Revised Guidelines for Safety in Biotechnology, 1994" and "Revised guidelines for research in transgenic plants, 1998" and prepared "Regulations and Guidelines on Biosafety of Recombinant DNA Research and Biocontainment, 2017". These guidelines are based on current scientific information, best practices and from the experience gained while implementing the biosafety frameworks within the country. A series of consultation with researchers, experts, academicians, concerned Ministries/departments and other stakeholders was carried out during preparing this guideline. This document specifies the practices for handling (Manufacture, Use, Import, Export, Exchange and Storage) of hazardous biological material, recombinant nucleic acid molecules and cells, organisms and viruses containing such molecules to ensure an optimal protection of public health and of the environment. The document provides clarity on biosafety requirements and recommendations for laboratory facilities such as facility design, biosafety equipment, personal protective equipment, good laboratory practices and techniques, waste management, etc.

OBJECTIVES

i. Outline the general principles of containment and establish a minimum standard for laboratories that must be adopted pan India for all handling of genetically engineered (GE) organisms (organism includes microorganisms, animals, plants, arthropods, aquatic animals, etc.) and non-genetically engineered (non-GE) hazardous microorganisms (microorganism includes parasites, protozoa, algae, fungi, bacteria, virus, prions, etc.).

ii. Identify the levels of risk(s) associated with GE organisms and non-GE hazardous microorganisms and classification of those organisms into their respective risk groups to select appropriate containment facilities. It also covers certification of containment facilities.

iii. Prescribe criteria for Manufacture, Use, Import, Export, Exchange and Storage of any hazardous microorganisms, GE organisms or cells and product(s) produce through exploration of such organisms.

iv. Ensure that national authorities, institutions and all other stakeholders involved in research & development are well informed or have access to information on safety thereby facilitating the safe use and handling of hazardous microorganisms, GE organisms or cells and product(s) produce through exploration of such organisms.

v. Emphasize the need and responsibility of all national authorities institutions and all other stakeholders involved in research to ensure that the public is well informed about the containment strategies followed in India.

SCOPE

This document covers regulatory scope on rDNA research and handling of hazardous microorganisms and GE organisms or cells in India.

Adoption of these guidelines shall be binding pan India for all public and private organizations involved in research, development and handling of GE organisms (organism includes microorganisms, animals, plants, arthropods, aquatic animals, etc.) and non-GE hazardous microorganisms (microorganism includes parasites, protozoa, algae, fungi, bacteria, virus, prions, etc.) and products produced through exploration of such organisms**.**

Note: These guidelines do not override any other existing regulations or guidelines, unless specified here.

CHAPTER 1

REGULATIONS AND COMPETENT AUTHORITIES

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CHAPTER 1
REGULATIONS AND COMPETENT AUTHORITIES

1.1. SCOPE OF REGULATIONS

These regulations are to implement the provisions of Rules 1989 of Environment (Protection) Act, 1986 for the manufacture, use, import, export and storage of hazardous microorganisms, GE organisms or cells and products thereof which applies to the whole of India in the following specific cases:

i. Sale, offers for sale, storage for the purpose of sale, offers and any kind of handling over with or without a consideration;

ii. Exportation and importation;

iii. Production, manufacturing, processing, storage, import, drawing off, packaging and repacking of the GE Products;

iv. Production, manufacture etc. of drugs and pharmaceuticals, food and food components, distilleries and tanneries, etc. which make use of hazardous micro-organisms or GE organisms one way or the other.

1.2. DEFINITION APPLICABLE AS PER RULES, 1989

Definitions applicable to this guideline as per Rules, 1989 unless the context requires:

i. "Biotechnology" means the application of scientific and engineering principles to the processing of materials by biological agents to produce goods and services;

ii. "Cell hybridisation" means the formation of live cells with new combinations of genetic material through the fusion of two or more cells by means of methods which do not occur naturally;

iii. "Gene Technology" means the application of the gene technique called genetic engineering, include self-cloning and deletion as well as cell hybridisation;

iv. "Genetic engineering" means the technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside the organism or the cell is inserted into said cell or organism. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self-cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material;

v. "Microorganisms" shall include all the bacteria, viruses, fungi, mycoplasma, cells lines, algae, protozoans and nematodes indicated in the schedule and those that have not been presently known to exist in the country or not have been discovered so far.

1.3. COMPETENT AUTHORITIES

The Rules 1989 are broad in scope and covers area of research as well as large scale handling of hazardous microorganisms, GE organisms or cells and products thereof. In order to implement the Rules in the entire country, six competent authorities and their roles have been notified (Table 1) for:

i. Regulation and control of contained research activities with hazardous microorganisms, and GE organisms.

ii. Regulation and control of large scale use of GE organisms in production activity.

iii. Import, export and transfer of hazardous microorganisms, GE organisms and products thereof.

iv. Release of GE organisms and products thereof in environmental applications under statutory provisions.

Table 1: Competent Authorities under Rules, 1989

Competent Authorities	Role
Recombinant DNA Advisory Committee (RDAC)	Advisory
Institutional Biosafety Committee (IBSC)	Regulatory/ Approval
Review Committee on Genetic Manipulation (RCGM)	
Genetic Engineering Appraisal Committee (GEAC)	
State Biotechnology Coordination Committee (SBCC)	Monitoring
District Level Committee (DLC)	

1.3.1. RECOMBINANT DNA ADVISORY COMMITTEE (RDAC)

This committee functions in the Department of Biotechnology with a role to:

i. Review developments in Biotechnology at national and international levels.

ii. Shall recommend suitable and appropriate safety regulations for India in recombinant research, use and applications from time to time.

iii. Evolve long term policy for research and development in Recombinant DNA research.

1.3.2. REVIEW COMMITTEE ON GENETIC MANIPULATION (RCGM)

This committee functions from the Department of Biotechnology to monitor the safety related aspect in respect of on-going research projects or activities involving hazardous microorganisms, GE organisms and cells and products thereof. The RCGM includes representatives of (a) Department of Biotechnology (b) Indian Council of Medical Research (c) Indian Council of Agricultural Research

(d) Council of Scientific and Industrial Research (e) other experts in their individual capacity. RCGM may appoint sub groups to assist RCGM on matters related to risk(s) assessment, in reviewing of existing and preparing new guidelines.

RCGM is mandated to bring out manuals of guidelines specifying procedure for regulatory process with respect to activities involving GE organisms in research use as well as industrial and environmental applications with a view to ensure human health and environmental safety. All ongoing research projects involving hazardous microorganisms, GE organisms or cells and products thereof shall be reviewed to ensure that adequate precautions and containment conditions are being met.

RCGM lays down procedures restricting or prohibiting production, sale, importation and use of such hazardous microorganisms (Annexure 1), GE organisms or cells.

1.3.3. INSTITUTIONAL BIOSAFETY COMMITTEE (IBSC)

This committee is constituted by all institutions handling hazardous microorganisms and/or GE organisms. The committee is the nodal point for implementation of the biosafety guidelines and for the interactions within the institution. The committee comprises of the Head of the Institution, Scientists engaged in the recombinant DNA work, a medical doctor and a nominee of the Department of Biotechnology. Institutions handling risk-inherent microorganisms or GE organisms shall prepare, with the assistance of the Institutional Biosafety Committee (IBSC), on-site emergency plan and update from time to time according to the manuals/guidelines of the RCGM and make available as required copies to the District Level Committee/State Biotechnology Co-ordination Committee and the Genetic Engineering Appraisal Committee.

The constitution, composition, role and functions, information for compliance requirements, processes to be followed while dealing with hazardous microorganisms, GE organisms or cells and product thereof in line with Rules 1989 is described in "Guidelines and Handbook for Institutional Biosafety Committee". Adherence to the guideline shall be binding for all IBSCs.

1.3.4. GENETIC ENGINEERING APPRAISAL COMMITTEE (GEAC)

Genetic Engineering Appraisal Committee (GEAC) [formerly known as Genetic Engineering Approval Committee (GEAC); name changed through Gazette notification, G.S.R No. 613 dated 16th July 2010], has been established under the Ministry of Environment, Forest and Climate Change (MoEF&CC). The major functions of GEAC as prescribed in the Rules 1989 are:

i. To appraise activities involving large scale use of hazardous microorganisms GE organisms or cells in research and industrial production from the environmental angle.

ii. To appraise proposals relating to release of GE organisms and products into the environment including experimental field trials.

iii. The committee or any persons authorized by it has powers to take punitive action under the Environment (Protection) Act, 1986.

Submission of applications to GEAC, information on current composition, meeting deliberations etc. is available at http://geacindia.gov.in/index.aspx.

1.3.5. STATE BIOTECHNOLOGY CO-ORDINATION COMMITTEE (SBCC)

The State Biotechnology Co-ordination Committee (SBCC) is a monitoring committee at State level. It shall have powers:

i. To inspect, investigate and to take punitive action in case of violations of statutory provisions through the State Pollution Control Board (SPCB) or the Directorate of Health etc.

ii. To review periodically the safety and control measures established at various institutions handling GE organisms.

iii. To act as nodal agency at the State level to assess the damage, if any, due to release of GE organisms and to take on site control measures.

1.3.6. DISTRICT LEVEL COMMITTEE (DLC)

There shall be a District Level Biotechnology Committee (DLC) in the districts wherever necessary under the District Collectors to monitor the safety regulations in installations engaged in the use of genetically modified organisms/ hazardous microorganisms and its applications in the environment.

The District Level Committee/or any other person/s authorized in this behalf shall visit the installation engaged in activity involving hazardous microorganisms, GE organisms or cells, and , formulate information chart, find out hazards and risk(s) associated with each of these installations and coordinate activities with a view to meeting any emergency. They shall also prepare an off-site emergency plan. The District Level Committee shall regularly submit its report to the SBCC/ GEAC.

CHAPTER 2

PRINCIPLES AND COMPONENTS OF CONTAINMENT

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CHAPTER 2
PRINCIPLES AND COMPONENTS OF CONTAINMENT

2.1. CONTAINMENT

Containment encompasses safe methods (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 strategy will ensure safety to laboratory workers, outside people and the environment from hazardous microorganisms, GE organisms or cells by:

i. Reducing the exposure, and

ii. Preventing their escape and establishment in a natural environment.

2.2. 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 laboratory. In practice, it should be achieved in realization of three interrelated steps:

i. **Identification of elements that should be protected**:

   Containment measures should ensure protection of laboratory worker(s) (Primary elements) who have maximum possibility of exposure to the organisms. In addition, the containment measure should also prevent the escape of organisms and so ensure protection of persons outside the laboratory and the environment (Secondary elements).

ii. **Identification of potential risk(s) associated with organism(s)**:

   It involves assessment of risk(s) associated with the organisms and their classification to appropriate risk groups based on:

   a. Pathogenicity of the organism towards humans/animals/plants.

   b. Modes of transmission and host range of the organism.

   c. Availability of effective preventive treatments or curative medicines.

   d. Capability to cause epidemics.

Based on the above information, infective microorganisms can be classified into four risk groups (Table 1) that allow selection of appropriate biosafety level facilities. In this document, an updated list of infective microorganisms under different risk groups has been provided in Annexure 1.

Table 1: Risk Group (RG) classification

Risk Group	Description
RG 1	A microorganism that is unlikely to cause human/ animal/plant disease.
RG 2	A microorganism that can cause disease in human /animal/ plant but the laboratory exposures may or may not cause serious infection to individual and risk(s) of spread of infection is limited.
RG 3	A microorganism that usually causes serious/lethal human/ animal/ plant disease but does not ordinarily spread from one infected individual to another.
RG 4	A microorganism that usually causes serious/lethal human/ animal/ plant disease and that can be readily transmitted from one individual to another, directly or indirectly.

iii. **Determining appropriate biosafety level facility for safe handling of organisms** (includes risk-inherent; GE and non-GE microorganisms, animals, plants, arthropods, aquatic animals, etc.) (Table 2).

Table 2. Different biosafety level facilities

Organism	Facility designation	Safety Levels
Microorganisms	Biosafety Level (BSL)	BSL-1 to BSL-4
Animal	Animal Biosafety Level (ABSL)	ABSL-1 to ABSL-4
Plant	Plant Biosafety Level (PBSL)	PBSL-1 to PBSL-4
Arthropods/Insects	Insect Biosafety Level (IBSL)	IBSL-1 to IBSL-4
Aquatic organisms	Aquatic Organism Biosafety Level (AqBSL)	AqBSL-1 to AqBSL-3

- ✓ Those agents not listed in Risk Group (RG) 2, 3 and 4 are not implicitly classified as RG 1. For such agents, a risk(s) assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed and then placed in an appropriate Risk Group.
- ✓ Genetic engineering can alter/change the overall risk(s) of an organism depending on the genetic modification. Hence, irrespective of cases, re-evaluation of risk(s) associated with the GE organism will be required to assign requisite containment levels. The risk assessment approaches for GE organisms is presented in Annexure 2.
- ✓ Appropriate containment level for handling of microorganisms, animals, plants, arthropods, aquatic organisms (both GE and non-GE), is described in this document.

2.3. FACTORS IN CONTAINMENT

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

2.3.1 PHYSICAL CONTAINMENT

The strategy is to physically confine the organism under study that can be feasibly adopted to prevent or minimize its exposure to worker and environment ensuring the risk(s) can be prevented or mitigated. 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 organisms (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.

Appropriate combination of these elements lays the foundation for selection of containment facilities for working with organisms/cells pertaining to different risk groups.

2.3.1.1 Procedure

These must be followed by workers involved in research and handling of organism in consideration of:

i. Strict adherence to standard microbiological practices and techniques.

ii. Awareness of potential hazards.

iii. Providing/arranging for appropriate training of personnel.

iv. Selection of safety practices in addition to standard laboratory practices if required. It is emphasized that good laboratory practice is fundamental to laboratory safety and cannot be replaced by any other means, which can only supplement it.

Note: Handling of GE and non-GE organism(s) is the same laboratory will require extra precautionary measures so as to prevent unintentional cross-contamination of non-GE organisms. Means of preventing cross-contamination of other work by GE organisms could include physical separation of the work or separation of the work at different times and ensure proper decontamination prior to commencing work with non-GE organisms.

2.3.1.2 Safety Equipments

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

i. Instruments like biological safety cabinets, autoclave and a variety of enclosed containers (e.g. safety centrifuge cup). The biological safety cabinet (BSC) is one of the principal devices used to provide workers safety from hazardous microorganisms and infectious aerosols. Three types of BSCs (Class I, II, and III) are used in biosafety level facilities. Safety and functionality of each instrument must be monitored monthly for effectiveness and calibrated annually before commencing operations. Equipment such as autoclaves and biological safety cabinets must be validated with appropriate methods (usually by a certified examiner) before being taken into use. The results of the monitoring and calibration must be documented. Recertification 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 to show that it is defective and must not be used for any purpose until the defect has been corrected.

ii. Personal protective equipment (PPE) such as gloves, coats, gowns, shoe covers, boots, respirators, face shields and safety glasses, etc.

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

i. Designed to limit or prevent contact between the operator and the infectious organisms.

ii. Constructed of materials that are impermeable to liquids, corrosion-resistant and meet structural strength requirements.

iii. Fabricated to be free of burrs and sharp edges.

iv. Designed, constructed and installed to facilitate simple operation and to provide ease of maintenance, accessibility for cleaning, and ease of decontamination and certification testing.

These are general principles. Detailed performance and construction specifications may be required to ensure that the equipment possess necessary safety features.

2.3.1.3 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 facility is to be determined based on risk(s) associated with the organism. Details of facility design for hazardous microorganisms, GE organisms and cells are mentioned in this guideline. Although, special attention should be paid to conditions like:

i. Creation of aerosols.

ii. Work with large volumes and/or high concentration of microorganisms.

iii. Overcrowded, over equipped laboratories.

iv. Infestation with rodents or insects.

v. Unauthorized entrance.

2.3.2 BIOLOGICAL CONTAINMENT

Biological containment employs strategies that render an organism used for genetic engineering either incapable of survival in the open 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 need arises to eradicate such GE population. In addition to physical containment, such biological containment hence 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:

i. The risk(s) associated with host organism.

ii. The infectivity of vector to specific hosts.

iii. The host-vector survival in the environment.

Note: Biological containment must not be considered a standalone containment strategy.

2.3.3 LABORATORY MONITORING

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

i. Prevention of any unauthorized entry in the laboratory. Entry and exit procedures must cover:

   a. Authorization for laboratory staff, visitors, maintenance, visiting scholars.

   b. Entry/exit log or other method to monitor authorized entry.

   c. Required personal protective equipment.

   d. Escort requirements.

   e. Removal of interfering objects and viable cultures.

A manual of laboratory monitoring should be prepared and kept in the facility for information and ready reference to the workers.

f. Disinfection procedure prior to entry and maintenance.

g. Disinfection procedure for exiting.

ii. Allow entry of person having proper training in laboratory safety and immunization, if required (For handling RG 3 and above organisms). Personnel training in biosafety is the key for prevention of laboratory-acquired infections, incidents and accidents ensuring success of any containment strategy. Based on the organism to be handled and the nature of work, training program to be developed and laboratory in charge must play the key role in training of laboratory staff. A training program must include information on safe handling of organisms of different risk groups that are commonly encountered by all laboratory personnel, involving any possible exposure scenarios and decontamination and emergency plan strategies. Training should involve class room work as well as significant one-on-one mentoring in the lab before an individual is allowed to work alone. It may include:

a. Inhalation risk(s) (i.e. aerosol production), such as using loops, streaking agar plates, pipetting, making smears, opening cultures, taking blood/serum samples, centrifugation etc.

b. Ingestion risks, such as handling specimens, smears and cultures.

c. Risk(s) of percutaneous exposures, through the use of syringe and needle techniques.

d. Animal handling that may result in bites and scratches.

e. Handling of blood and other potentially hazardous pathological materials.

f. Decontamination and disposal of infectious material.

g. Emergency procedures in case of unwanted breach in containment.

iii. Personnel should be advised of special hazards and required to read and follow standard practices and procedures.

iv. Persons at increased risk(s) of acquiring infection or for whom infection may have unusually serious consequences (e.g. Immunocompromised, Women during pregnancy, etc.) are informed of their risk(s) and should be restricted from entering the laboratory. Panel of expert scientists of the institute/organization/universities need to review and decide on case by case basis.

v. To create an open environment where workers are following proper containment strategies and are fearless to report violations of procedure, identify coworker failings, express concerns and offer suggestions.

vi. All safety equipments are working properly and if not, maintenance of the equipment is made immediately. All civil structures are in good condition to ensure proper containment.

vii. A regular schedule for housekeeping is maintained.

viii. Prevention of diseases in the general or occupational environment.

ix. Documentation of daily laboratory activity for immediate consideration of emergency procedures in cases of breach in containment.

x. Proper documentation of work involving both non-GE and GE organisms in a same 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 biosafety level of the laboratory and should be determined by laboratory supervisors with consultation of scientific experts.

2.3.4 HEALTH AND MEDICAL SURVEILLANCE

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

i. Prevent individual from acquiring infection during the work

ii. Provide for early detection of laboratory-acquired infection.

iii. Provide for assessing the efficacy of protective equipment and procedures.

iv. Provide for prophylactic vaccinations where needed and monitor booster regimens and assessment of sero conversion, in applicable cases.

It is the responsibility of the employing authority and/or the facility in-charge to ensure that health and medical surveillance of laboratory personnel is carried out.

2.3.5 DECONTAMINATION AND DISPOSAL

Decontamination and disposal in laboratories are closely interrelated acts, since disinfection or sterilization constitute the first phase of disposal. All materials and equipment will ultimately be disposed off; however, in terms of daily use, only a portion of these will require actual removal from the laboratory or destruction. These will be referred as biological wastes that need specific treatment to render safe before discard. These include:

Steam autoclaving is the preferred method for all decontamination processes. Materials for decontamination and disposal should be placed in containers, e.g. autoclavable plastic bags that are colour-coded according to whether the contents are to be autoclaved and/or incinerated. Alternative methods may be adopted only if they remove and/or kill risk-inherent organisms.

The principal questions to be answered prior to disposal of any objects or materials from laboratories dealing with risk-inherent organisms or tissues are:

i. Have the objects or materials been effectively disinfected or sterilised by an approved procedure? If not, how the materials will be stored before effective disinfection and/or sterilization?

ii. Will the materials be disinfected or sterilised on site or transferred to other laboratory/ area and how it will be transferred?

iii. How the disinfected or sterilised material will be disposed off?

iv. Does disposal of the disinfected or sterilized objects or materials involve any additional potential hazard, biological or otherwise, to those carrying out the immediate procedure or those who might come into contact with the objects or materials outside the laboratory complex?

As part of disposal mechanism:

i. All equipment must be decontaminated before being repaired, maintained, or removed from the laboratory.

ii. Separate approach of storage and disinfection procedure should be adopted and the same should be well informed to the personnel working in the facility.

iii. If on site disinfection/ sterilization is not possible, transport of materials should be carried out by trained staff under appropriate storage container that must be leak proof and tightly sealed.

iv. Designated waste disposal area should be available within each facility.

v. Wastewater released from laboratories should not be allowed to mix to general public sewage system. Provision should be made to collect such effluents coming from all laboratories and should be treated for proper decontamination before disposal. Microbiological testing may be performed periodically and record should be maintained.

vi. All employees who handle biological waste shall be trained regarding the proper segregation, handling, packaging, labelling, storage, and treatment of biological waste. Refresher training is required annually. A training manual must be developed and available in the facility.

vii. For facilities that work with RG 3 and above organism and/or perform Category III and above genetic engineering work, it will be mandatory to maintain written records on decontamination and disposal. It should include: Date of treatment, method/conditions of treatment, name of the persons performing the treatment, a written procedure for the operation and testing of any equipment used and a written procedure for the preparation of any chemicals used in treatment.

viii. Appropriate disposal mechanisms for chemical or radiological wastes should be developed in discussion with biosafety officer and appropriate local and national competent authorities in conjunction with current mechanism.

Unless mentioned in the operational practices in specific containment facility, instructions on disposal of biological materials (classified as Solids, Liquids, Sharps, and Pathological) should be adhered to those mentioned in Table 3.

Selection of appropriate decontamination and disinfection strategies for biomedical waste treatment and disposal facilities should be in accordance to those mentioned in the "Revised Guidelines for Common Bio-medical Waste Treatment and Disposal Facilities" (2016) developed by Central Pollution Control Board (CPCB).

Table 3: Instruction on disposal of laboratory wastes

Laboratory waste type	Disposal Container (s)	Disposal requirements
Solid wastes E.g. All used gloves, paper towels, gauze, wipes, absorbents, disposable Petri dishes, culture vials, plastic wares, plants or any parts/ tissues, seeds, soil/soil substitute (perlite, vermiculite, peat mass, etc.)	i. The primary container should be leak and puncture proof and must have lid. ii. A label of "biological waste" should be visible on container. iii. It should be lined with a biohazard bag of mandated colour that is leak proof and able to withstand autoclave conditions.	i. Keep container and lid clean at all times. ii. Maintain access to the container - do not put materials on lid. iii. Lid must be in place when waste is not being added to container. iv. No liquid should be discarded along with solid waste. v. The biohazard bag should be 3/4th fill maximum. vi. Do not overfill. vii. To transport container(s) outside the facility for decontamination, ensure that the biohazard bag is sealed and the lid is tightly closed. A trolley for transport is preferred. Do not toss. viii. All solid hazardous waste must be autoclaved. ix. Prior to autoclaving, crisscross the bag's biohazard symbol and/or markings with heat sensitive autoclave tape. x. Ensure the autoclave is set for the appropriate time– selection of time and pressure should ensure proper decontamination. For e.g. Organism containing TSE must be autoclaved at a higher setting.

		xi. Once the waste is autoclaved, mark the autoclaved bag with an "Autoclaved/ Decontaminated" sticker. xii. Place the autoclaved bag into an opaque bag of mandated colour and seal it. xiii. Store the bag in a place that could be collected for disposal by cleaning personnel. xiv. As an alternative to autoclaving, other waste disposal methods may be employed as prescribed by the local competent authorities (Pollution Control Board).
Liquid Waste E.g. Any media, liquids coming from Petri dishes, culture vials, lab equipment, recombinant Nucleic Acids (rNA) in all forms (natural and synthetic e.g., DNA, RNA, shRNA, etc.).	i. The container should be leak and puncture proof and must have lid. ii. A label of "biological waste" should be visible on container.	i. Liquid waste must be separated from solid waste. ii. Liquid waste must be decontaminated on site with an appropriate disinfectant/bleach with appropriate period of exposure. iii. Flush the disinfected material down the sink, allowing the cold water to run for a period of time (at least 5 minutes). iv. Do not flush non-aqueous solutions, such as liquefied agarose or unfiltered broths, down the drain as they will clog the drain pipes. Note: Liquid waste generated from higher containment laboratory (BSL-2 and above) should be autoclaved.

Sharps E.g. All needles, syringes, scalpels, razor blades, pipette tips, Pasteur pipettes, glass ware, capillary tubes, slides and cover slips, contaminated broken glassware.	i. The container must be rigid, leak proof, puncture proof and have lid. ii. Keep baffle in place. iii. Line with a biohazard bag of mandated colour. iv. Label with "BIOHAZARD SHARPS" sticker.	i. All sharps must be placed in appropriate sharps container. ii. Once the container is ¾ full, close the top of the container. iii. Sharps contaminated with biological materials must be autoclaved before disposal.
Pathological E.g. Animal carcasses suspected to be or potentially infected; tissues, organs and any body parts; bedding from animal cages, etc.	Must be leak-proof and puncture-proof. Lid must be in place when waste is not being added to container. Line with a red or orange biohazard bag. Label with "BIOHAZARD" and "PATHOLOGICAL WASTE" stickers.	Same as 'solid waste'. Incineration of carcasses.

2.3.6 EMERGENCY PROCEDURES

Emergency contingency plans in consideration of every possible breach in biocontainment should be prepared for each individual laboratory as well as for the institutions. These are best prepared by the individual laboratory supervisor in conjunction with his staff and the biosafety officer. This procedure offers the best prospect of success as it is the immediate staffs that are most familiar with the hazards associated with a particular laboratory.

Once the emergency plan is formulated, it should be pasted in 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.

Emergency plan should provide for:

i. Breakage and spillage.
ii. Accidental injection, cuts and abrasions.
iii. Accidental ingestion of potentially hazardous material.
iv. A potentially hazardous aerosol release (other than in a safety cabinet).
v. Breakage of tubes in centrifuges not having safety cups.
vi. Chemical, fire, electrical and radiation.
vii. Flood and natural disaster.
viii. Vandalism.
ix. Miscellaneous emergencies including falls due to wet floor, ill health, seizures etc.

In addition, emergency plan should also provide:

i. Emergency contact numbers, and contact details of other relevant emergency services available.
ii. Details of emergency equipment and its location.

Note: Apart from these, emergency procedures for containment in case of biological disasters should be according to National Disaster Management Guidelines — Management of Biological Disasters, 2008.

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CHAPTER 3

OPERATIONAL GUIDES ON CONTAINMENT

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CHAPTER 3
OPERATIONAL GUIDES ON CONTAINMENT

3.1. MICROBIOLOGICAL BIOSAFETY LEVEL (BSL) FACILITIES

3.1.1 PURPOSE

BSL facilities are the fundamental laboratory structures for containment purposes. Such facilities will be suitable for:

i. Isolation, cultivation and storage and experiments on hazardous microorganisms.

ii. Genetic engineering of organisms and their safe handling.

iii. Handling of toxins, tissues, etc.

3.1.2 TYPES OF MICROBIOLOGICAL BIOSAFETY LEVEL FACILITIES

3.1.2.1 Biosafety Level 1 (BSL-1):

BSL-1 will be applicable for:

i. Isolation, cultivation and storage of Risk Group (RG) 1 microorganisms those are abundant in natural environment.

ii. Experiments on RG 1 microorganisms provided that the experiments will not increase environmental fitness and virulence of the microorganisms.

iii. **Category I genetic engineering experiments on microorganism:**

This category includes experiments which generally do not pose significant risk(s) to laboratory workers, community or the environment and the modifications have no effect on safety concerns. Examples are:

a. Insertions of gene into RG 1 microorganism from any source, deletions, or rearrangements that have no adverse health, phenotypic or genotypic consequence. Modification should be well characterized and that the gene functions and effects are adequately understood to predict safety.

b. Experiments involving approved host-vector systems (refer to Annexure 3) provided that the donor DNA is originated from RG 1 microorganism, not derived from pathogens. The DNA to be introduced should be characterized fully and will not increase host or vector virulence.

c. Experiments involving the fusion of mammalian cells which generate a non-viable organism, for example, the construction of hybridomas to generate monoclonal antibodies.

d. Any experiments involving microorganism belonging to RG 1. For e.g. self-cloning, fusion of protoplasts between non-pathogenic RG 1 organism.

Before commencement of Category I GE experiments, the investigator should intimate the IBSC about the objective and experimental design of the study along with organisms involved. IBSC should review the same as and when convened for record or action if any.

It is desirable to designate a separate area in the facility with proper labelling for Category I GE experiments to avoid any chances of contamination.

3.1.2.2. Biosafety Level 2 (BSL-2):

BSL-2 will be applicable for:

i. Isolation, cultivation and storage of RG 2 microorganisms.

ii. Handling of environmental samples collected from environment that is unlikely to contain pathogens. Isolation of microorganisms from those samples and subsequent experiments.

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. **Category II genetic engineering experiments on microorganism:**

These experiments may 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.

All category II GE experiments require prior authorization from IBSC before the commencement of the experiments through submission of information in the prescribed proforma.

It is desirable to designate a separate area in the facility with proper labelling for Category II GE experiments to avoid any chances of contamination.

3.1.2.3 Biosafety Level 3 (BSL-3):

BSL-3 will be applicable for:

i. Isolation, cultivation and storage of RG 3 microorganisms.

ii. Handling of environmental samples collected from environment that is likely to contain pathogens of potential disease consequences. Isolation of microorganisms from those samples and subsequent experiments.

iii. Experiments on RG 3 microorganisms or isolates from environment mentioned above provided that the experiments will not increase environmental fitness and virulence of the microorganisms.

iv. **Category III and above genetic engineering experiments on microorganism:**

These kinds of experiments pose moderate to high risk(s) to laboratory workers, community or the environment. Examples are:

a. Experiments on RG 2 and RG 3 microorganisms where insertion of gene directly involved in production of toxin or allergen or antimicrobial compounds.

b. Insertions of gene into RG 3 microorganisms from any source, deletions, or rearrangements that affect the expression of genes, whose functions or effects are not sufficiently understood to determine with reasonable certainty if the engineered organism poses greater risk(s) than the parental organism.

c. Insertions of nucleic acid from any source, deletions, or rearrangements that have known or predictable phenotypic or genotypic consequence in the accessible environment that are likely to result in additional adverse effects on human and/or animal health or on managed or natural ecosystems, e.g., those which result in the production of certain toxins.

d. Research involving the introduction of nucleic acids (recombinant or synthetic) into RG 3 organisms or organisms listed in SCOMET items (http://dgft.gov.in).

e. Genetic engineering of organisms isolated from environment where there are reported cases of disease prevalence and possibility of presence of infectious microorganisms.

All category III and above GE experiments require prior authorization from IBSC and subsequent approval from RCGM before commencement of the experiments through submission of information in the prescribed proforma.

3.1.2.4. Biosafety Level 4 (BSL-4):

BSL-4 laboratory is the maximum containment laboratory. Strict training, strictly restricted access and supervision are required and the work must be done under stringent safety conditions and positive pressure personnel suits. BSL-4 will be suitable for:

i. Isolation, cultivation and storage of RG 4 microorganisms.

ii. Handling of samples collected from environment/patients that are likely infected with RG 4 organisms with serious/fatal health effects.

iii. Experiments on RG 4 microorganisms or isolates from environment/patients mentioned above to find remedial measures.

iv. Category III and above genetic engineering experiments on microorganisms involving introduction of nucleic acids (recombinant or synthetic) into RG 4 microorganisms or exotic agents.

Note:

i. BSL facilities are not meant for:

   a. Permanently housing/keeping/rearing of any animals, arthropods or aquatic organisms for longer than the time required to complete laboratory procedures on them.

   b. The growing of any plants, except those in tissue culture bottles or fully contained in a plant growth chamber.

ii. Genetic engineering experiments not covered under any of the above four categories will require case by case evaluation for selection of appropriate containment strategies. Prior

approval and/or permission from the IBSC and/or the RCGM shall be required to initiate such experiment. Few examples are:

a. Clubbing of experiments pertaining to different categories.
b. Any experiments involving primates, dogs, large animals, and human participants within the laboratory.
c. Experiments involving the use of infectious or defective RG 3 and above viruses in the presence of helper virus.
d. Experiments using DNA which encodes a vertebrate toxin having an LD_{50} of less than 100 µg/kg.
e. Experiments with genes that alter the growth status of cells such as oncogenes, cytokines and growth factors.
f. Experiments aimed at controlling natural populations.

iii. All existing BSL-3 and 4 facilities must be certified by RCGM. A format for certification is available in this guideline.

iv. The new BSL-3 and 4 facilities shall require certification at the time of commissioning operations as per the format.

3.1.3. OPERATIONAL GUIDE FOR BSL-1 FACILITY

A) Facility design

i. Facility should be a fully enclosed space bounded by walls, doors, windows, floors and ceilings.

ii. Ample space must be provided for the safe conduct of laboratory procedures.

iii. Walls, ceiling, and floors should be smooth, easily cleanable, impermeable to liquids, and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be slip resistant. Exposed pipes and ducting should stand clear of walls. Horizontal runs should be avoided to prevent dust collection.

iv. Adequate illumination should be ensured for carrying out all activities. Undesirable reflection is to be avoided.

v. Bench tops should be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat.

vi. Laboratory furniture should be sturdy and open spaces between and under benches, cabinets and equipment should be accessible for cleaning.

vii. Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on bench tops and in the aisles. Additional long-term storage space, conveniently located outside and working areas, should also be provided.

viii. Wash-basins, with running water, should be provided in each laboratory room, preferably near the exit.

ix. Doors should have appropriate fire ratings, be self-closing, and have vision panels.

x. There are no specific ventilation requirements. In planning new facilities, consideration should be given for providing a mechanical ventilation system that provides an inward air flow and exhaust without recirculation. If there is no mechanical ventilation, windows should be openable, preferably having fly proof screens. Skylights should be avoided.

xi. Drainage exits should be fitted with barriers to prevent entry of arthropods and rodents.

xii. Space and facilities should be provided for the safe handling and storage of solvents, radioactive materials and compressed gases.

xiii. Safety systems should cover fire, electrical emergencies, emergency shower and eyewash facilities.

xiv. First-aid areas or rooms suitably equipped and readily accessible should be available.

xv. A good-quality and dependable water supply is essential. There should be no cross-connections between sources for laboratory purposes and the drinking water supply. The public water system must be protected by a back-flow preventer.

xvi. A reliable electricity supply with adequate capacity should be available. There should be emergency lighting to permit safe exit. A standby generator with automatic cut-off is desirable for the support of essential equipment-incubators, freezers, etc.

xvii. There should be an insect and rodent control measures.

xviii. Facilities for storing outer garments and personal items and for eating and drinking should be provided outside the working areas.

xix. "No Smoking" "No Eating" and "No Drinking" signs should be displayed clearly inside and outside the laboratory.

xx. Access to the laboratory area should be designed to prevent entrance of free-living arthropods and other vermin.

B) Safety Equipments

i. Pipetting aids-to replace mouth pipetting.

ii. Screw-cap tubes and bottles - to provide positive specimen containment.

iii. Disposable Pasteur pipettes, whenever available, to avoid glass.

iv. Sterile plastic disposable transfer loops and spreader etc. to avoid incineration of regular loops, glass spreader etc.

C) Personal Protective Equipment

Working in BSL-1 laboratory do not require any Personal Protective Equipment (PPE), although care should be made to avoid spillage of biological material on street clothing for which use of apron is recommended.

D) Procedures

i. Mouth pipetting should be prohibited.

ii. Eating, drinking, storing food, and applying cosmetics should not be permitted in the laboratory work area.

iii. Avoid touching various body parts while handling the microorganisms.

iv. Wash hand after entering, post work and before leaving the laboratory with sanitizing agents.

v. The laboratory should be kept neat, clean and free of materials not pertinent to the work.

vi. Work surfaces should be decontaminated at least once a day and after any spill of potentially dangerous material.

vii. Members of the staff should wash their hands after working before leaving the laboratory.

viii. All technical procedures should be performed in a way that minimizes the creation of aerosols.

ix. Laboratory doors would be kept closed when work is in progress.

x. Children are not permitted in laboratory working areas.

E) Laboratory monitoring

i. There should be no unauthorized entry in the laboratory.

ii. Only the trained personnel to enter the laboratory.

iii. Entry and exit should be limited when work is in progress.

iv. Immediately after work, the workplace and the used instruments should be cleaned with a disinfectant and the materials used in work should be placed back to its position.

v. No viable cultures are left unattended and either stored or incubated as per need.

vi. Record of work should be duly registered in the register available.

F) Waste management

There is no specific requirement on waste management in BSL-1 facility. However, waste disposal procedure must meet the pollution control requirements. Any effluents from laboratories should be pre-treated and microbiological testing of treated effluents along with record should be available.

G) Health and Medical Surveillance

These microorganisms are unlikely to cause human or animal diseases of veterinary importance. Ideally, however, staff members should be subjected to a pre-employment health surveillance procedure regarding medical history. Prompt reporting of illness or laboratory accident is desirable and all staff members should be made aware of the importance of maintaining good laboratory safety practice.

H) Emergency procedures

All spills, accidents and overt or potential exposures to infectious materials should be reported immediately to the laboratory supervisor. A written record should be prepared and maintained. Appropriate medical evaluation, surveillance and treatment should be provided.

3.1.4. OPERATIONAL GUIDE FOR BSL-2 FACILITY

The operational program for BSL-1 laboratory will also apply to Biosafety Level 2 laboratory, with additional modifications as follows:

A) Facility design

i. An autoclave for decontamination of potentially hazardous laboratory wastes should be available in the same building as the laboratory.

ii. Biological safety cabinets for handling of risk-inherent microorganisms of RG 2 should be used.

iii. Laboratory may be kept under constant CCTV surveillance.

iv. 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

B) Safety instruments

i. Autoclaves - to sterilize contaminated material.

ii. Biological safety cabinets to be used whenever:

   a. 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.

   b. 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.

C) Personal Protective Equipment

i. The use of laboratory coats, gowns or uniforms is required to prevent contamination of street clothing.

ii. Goggles and face protection must be used when there is a potential for splashes of microorganisms or other hazardous materials.

iii. Face mask and appropriate gloves may be worn as protection while handling animals.

iv. Appropriate gloves should be worn for all procedures that may involve accidental direct contact with blood, infectious materials, or infected animals. Gloves should be removed aseptically and autoclaved with other laboratory wastes before disposal. When disposable gloves are not available, re-usable gloves should be used. Upon removal they should be cleaned and disinfected before re-use.

v. All PPE should be removed so that the transfer of infectious materials to areas beyond where they or animals are being handled is minimized.

vi. Used disposable PPE should be disposed off with other contaminated waste and reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse.

vii. Reusable protective clothing should be laundered through laboratory laundry facility only and it must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before being placed with other items to go to laundry.

D) Procedures

i. All contaminated liquid or solid materials should be decontaminated before disposal or reuse; contaminated materials that are to be autoclaved or incinerated at a site away from the laboratory should be placed in durable leak-proof containers, which are closed before being removed from the laboratory.

ii. 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.

iii. 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.

iv. Safety glasses, face shields and other protective devices should be worn to protect eyes and face from splashes and impacting objects.

v. Only persons who have been advised of the potential hazards and meet any specific entry requirements (e.g. immunization) should be allowed to enter the laboratory working areas.

vi. Hypodermic needles and syringes should not be used as a substitute for automatic pipetting devices in the manipulation of infectious fluids. Cannulas should be used instead of sharp needles wherever possible.

vii. Never wear contact lenses when working with infectious microorganisms.

viii. Add disinfectant to water baths to contain spread of infectious substances.

ix. 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.

x. 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.

xi. 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.

E) Laboratory monitoring

Monitoring should ensure that:

i. Only highly trained personnel are entering in the facility.

ii. Person working in the facility are not transporting the laboratory materials including hazardous organism outside the laboratory environment either without permission or without proper transport strategy with prior approval from competent authority.

iii. Person working in the laboratory are well aware about the microorganism(s) to be handled and its associated risks.

iv. Accidental spill or splashes are cleaned immediately, reported and recorded.

F) Waste management

Decontamination and disposal mechanism should be in strict adherence to those mentioned in "DECONTAMINATION AND DISPOSAL".

i. Autoclaves and sterilizers for treatment of solid wastes need specially designed accommodation and services.

ii. Incinerators may need to be of special design and equipped with after burners and smoke-consuming devices.

G) Health and Medical Surveillance

i. Pre-employment health surveillance is necessary. This screening should include the past medical history. A clinical examination and the collection of a baseline serum sample would be advantageous and, in some cases, may be necessary.

ii. Records of illness and absence should be kept by the facility in-charge and it is the responsibility of the laboratory worker and his own medical officer to keep the facility in-charge informed of all absences due to illness.

iii. 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. The precise steps taken to protect the foetus will vary, depending on the microorganisms to which exposure may occur.

H) Emergency Procedures

Same as BSL-1

3.1.5. OPERATIONAL GUIDE FOR BSL-3 FACILITY

This level of containment requires strengthening of BSL-2 laboratory operational and safety programmes as well as the provision of added structural safeguards and the mandatory use of biological safety cabinets. Therefore, the facility in-charge must first comply with the BSL-2 guidelines and additionally have those specific for BSL-3 facility. The major changes are in: Procedures, Facility design and Health and medical surveillance. Laboratories in this category should be registered or listed with the appropriate national authority(ies).

A) Facility design

i. The laboratory should 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 should be through an ante-room or airlock. 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. Physical mechanisms (e.g., interlocking or alarm system) must be in place to ensure that only one door can be opened at any time.

ii. The surfaces of walls, floors, and ceilings should be water resistant and easy to clean. Openings in these surfaces should be sealed to facilitate decontaminating the area.

iii. A foot or elbow-operated wash-hand basin should be provided near each laboratory exit door.

iv. Windows in the laboratory should be closed and sealed.

v. Access doors to the laboratory should be self-closing and lockable.

vi. An autoclave for decontamination of laboratory wastes should be available within the laboratory. If infectious wastes have to be removed to another area in the same building for disinfection, they should hold and transported in a covered, leak-proof container.

vii. There should be a ventilation system that establishes a negative pressure into the laboratory. Personnel must verify that proper direction air flow (into the laboratory) is achieved.

viii. 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 BSL-3 containment barrier.

ix. 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.

x. 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.

xi. 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.

xii. Supply or replacement air to the facility must have HEPA filtered.

xiii. The exhaust air from the facility must pass through 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.

xiv. The building exhaust system can be used for this purpose if the exhaust air is not recirculated to other areas of the building. Air within the laboratory can be recirculated.

xv. In laboratories that have supply air systems, the supply air and exhaust air systems are interlocked to ensure inward air flow at all times.

xvi. The HEPA-filtered exhaust air from Class I and Class II biological safety cabinets should be discharged directly to the outside or through the building exhaust system.

xvii. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through a building exhaust air system, it should be connected to this system in such a way as to avoid any interference with the air balance of the cabinet or building exhaust systems.

xviii. Designated areas or hanging areas for PPE must be available within each work area.

xix. The facility must be constructed to enable gaseous decontamination of the whole facility.

xx. Where the facility shares an airlock with a contained animal or invertebrate facility, or if animals or invertebrates are handled within the facility, any openings in the wall or ceiling, such as ventilation inlets and outlets must be screened. The screens must be fixed and sealed against their mounting. The aperture of the screen must be small enough to prevent entry or exit of invertebrates or other animals.

xxi. 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.

xxii. The following water supplies 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-siphonage:

   a. Laboratory sink outlets
   b. Outlets within a BSC or other aerosol containment equipment
   c. Direct connections to an autoclave.

xxiii. Backflow prevention must isolate the facility to the exclusion of all other areas.

xxiv. The work area in the facility must contain eyewash equipment (either plumber eyewash equipment or single use packs of sterile use irrigation fluids).

xxv. Piped gas supplies to the facility must have reverse flow prevention on outlets located within the BSC.

xxvi. Shower facility must be available in the facility before exit.

xxvii. Appropriate areas should be available for donning and doffing of the laboratory clothing.

xxviii. A constant CCTV surveillance should be in place.

B) Safety equipment

i. The principles for the selection of instrument, including biological safety cabinets, are the same as per the BSL 2 laboratory except that all activities involving infectious materials are to be conducted in biological safety cabinets (Class II), with other physical containment devices.

ii. Refrigerators, freezers, incubators, etc. that contain biohazardous materials for storage must be labelled with a biohazard symbol.

iii. Equipment that may produce biohazardous aerosols has engineered containment to prevent exposures to people or the environment. For example, additional containment accessories like safety buckets or containment rotors should be used to operate centrifuge. Additional local exhaust ventilation with HEPA filtration may be required while using containment equipments. Devices are to be tested at least every twelve months.

iv. An autoclave that is suitable for the load size and type of material to be decontaminated. The autoclave must not be located in the airlock. The autoclave should preferably be of double ended type with interlocked doors with the inner door opening to the facility and outer door opening externally to the facility.

v. Incinerators, if used, must have dual combustion chambers. The temperature in the primary chamber should be at least 800°C and that in the secondary chamber at least 1000°C.

C) Personal Protective Equipment

i. Protective solid-front laboratory clothing shall be worn by workers when in the laboratory and shall NOT be worn outside the laboratory.

ii. An eye protection policy should be in place. Eye protection devices must be worn when the chance of eye contamination exists.

iii. Respiratory protective equipment should be used if the microorganism has higher possibility of spread through aerosolization.

iv. Appropriate gloves must be worn.

v. Cleaning and re-use conditions (if permitted) should be clearly defined.

D) Procedures

i. All personnel must demonstrate proficiency in the practices and procedures specific to their responsibilities before being authorized to work in the BSL-3 containment area.

ii. The two-person rule should apply, whereby no individual works alone within the laboratory.

iii. A hazard warning sign should be displayed on laboratory doors, identifying the microorganism, the name of the laboratory supervisor and other responsible person(s) and indicating any special conditions of entry into the area (immunizations, etc.) (Fig. 2).

iv. Biohazardous materials should be stored in labelled secondary containers.

v. Any equipment must be decontaminated before in-place (or removal for) repair or maintenance.

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Hazard Identity:

Responsible Investigator: _____

IN CASE OF EMERGENCY

Mobile: _____

Daytime Phone: _____

Home Phone: _____

Fig. 2

vi. Where equipment could create a biohazardous aerosol, a containment procedure should be in place with consultation of experts.

vii. Shutdown and clearance procedures for periods of major maintenance, repair, equipment replacement, etc. should be discussed and adopted.

viii. Laboratory clothing that protects street clothing (i.e. solid front or wrap-around gowns, scrub suits, coveralls, etc.) must be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing must not be worn outside the laboratory and must be decontaminated before being laundered.

E) Laboratory Monitoring

Same as BSL-2.

F) Waste Management

i. All instructions related to waste management should be posted inside and outside of laboratory and must be visible clearly.

ii. All biohazardous waste must be decontaminated in the facility or building (preferably within facility). Autoclave should be maintained, calibrated and tested. Autoclave use records must be maintained.

iii. Procedures for disposal of biohazardous waste that also include chemical or radioactive waste are developed in consultation with the Biosafety Officer.

iv. The statutory rules and regulations laid down by the National Competent Authority for design and operation of facility handling radioactive material should be followed.

v. The statutory rules and regulations for disposal of radioactive waste laid down by the National Competent Authority should be followed.

G) Health and medical surveillance

In addition to provisions mentioned in BSL-2, following should be considered:

i. Medical examination of all laboratory personnel working in the containment laboratory is mandatory. This examination should include a detailed past medical history and clinical examination.

ii. A baseline serum sample should be obtained and stored for future reference.

iii. Employees either immunocompromised or being treated with immunosuppressive drugs should not be employed in containment laboratories.

Following a satisfactory clinical assessment report, the examinee should be provided with the medical contact card (Fig. 3)

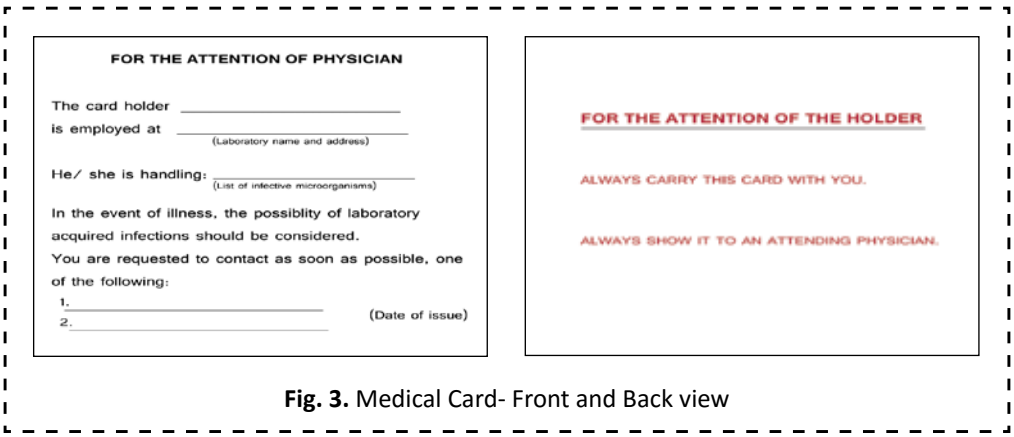

Fig. 3. Medical Card- Front and Back view

NOTE: The contact person's name to be entered on the front of the card. It might include the names of the facility in-charge, the medical officer, or the biosafety officer. It is suggested that this card should be wallet sized and it should always be carried by the holder.

H) Emergency Procedures

i. All who work in the vicinity must understand the importance of emergency plan in BSL-3 level.

ii. An easily accessible pill kit for containment of infectious organisms must be maintained.

iii. Biohazard spills must be decontaminated, contained and cleaned up by properly trained and equipped staff.

iv. Spills/accidents that result in potential exposure must be immediately reported to the facility in-charge and the biosafety officer.

v. A written report of any spills, exposure, failures of containment, mechanical breakdown, and maintenance problems should be submitted to the Biosafety Officer within five workdays.

vi. Each worker must be knowledgeable of the steps to take if a (probable) exposure occurs. A post-exposure management plan should be in place.

vii. Laboratory shutdown and clearance procedure must be followed before initiation of further work in case of high level of exposure.

viii. Any breach in containment should be immediately reported to facility in-charge, IBSC and RCGM.

3.1.6 OPERATIONAL GUIDE FOR BSL-4 FACILITY

Biosafety Level 4 (BSL-4) laboratory is for work with RG 4 microorganisms that have serious health and environmental consequences upon escape. Hence, such laboratory must be constructed under expert supervisions and with intensive consultations with institutions and agencies that had prior experience of operating a similar facility.

Construction and operationalization of such laboratory must require prior approval from competent national authorities and should be under strict observations of appropriate national authorities in addition to the control of implementing institute.

In addition to features proposed for BSL-3 laboratory, following factors should be considered strictly for construction and operation of BSL-4 laboratory:

A) Facility design

i. **Controlled access**

   a. Facility must be located in a separate building or in a clearly delineated zone within a secure building.

   b. Isolation of facility must be ensured by deploying strict security systems.

   c. Entry and exit of personnel and supplies must be through airlock systems.

d. On entering, personnel must put on a complete change of clothing; before leaving, they must shower before putting on their street clothing.

ii. **An efficient primary containment system** must be in place, consisting of one or a combination of the following:

Cabinet room

a. The cabinet room housing Class III biological safety cabinet must be separated from outside environment by two air lock entry doors. Handling of Risk Group 4 microorganisms must be performed in this room and nothing must be taken out of the room. All required instruments for study must be placed inside the cabinet room.

b. Cabinet room must be attached with outer and inner changing rooms fitted with shower.

c. An arrangement of interlocked autoclave or fumigation chamber must be kept to transfer materials inside the cabinet room.

Suit laboratory

a. A protective suit laboratory is designed and maintained to provide personnel protection equivalent to that provided by Class III biological safety cabinets and must be attached with changing and decontamination rooms.

b. It must be attached with outer and inner changing rooms fitted with shower.

c. Entry into the suit laboratory is through an airlock fitted with airtight doors.

d. Exit through a chemical shower facility to decontaminate the surface of the positive pressure suit. In the event of an emergency exit or failure of the chemical shower system, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

e. An appropriate warning system for personnel working in the suit laboratory must be provided for use in the event of mechanical system or air failure.

f. Decontamination room, fitted with decontamination shower system must also be attached with suit laboratory.

iii. **Controlled air system**

a. Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. All HEPA filters must be tested for efficiency and certified annually from authorized service providers. Decontamination of filters must be performed by experienced certified person.

b. Design of ventilating systems for cabinet room and suit laboratory must be different as explained below:

Cabinet room

a. A dedicated non-recirculating ventilating system for the cabinet laboratory is required.

b. The supply air to the Class III biological safety cabinet(s) may be drawn from within the room through a HEPA filter mounted on the cabinet or supplied directly through the supply air system.

c. Exhaust air from the Class III biological safety cabinet must pass through two HEPA filters prior to release outdoors. The cabinet must be operated at negative pressure to the surrounding laboratory at all times.

Suit laboratory

a. Personnel must wear a positive pressure supplied air protective suit.

b. Dedicated room air supply and exhaust systems are required.

c. The supply and exhaust components of the ventilating system are balanced to provide directional air flow within the suit area from the area of least hazard to the area(s) of greatest potential hazard.

d. Redundant exhaust fans are required to ensure that the facility remains under negative pressure at all times.

e. The differential pressures within the suit laboratory and between the suit laboratory and adjacent areas must be monitored.

f. Air flow in the supply and exhaust components of the ventilating system must be monitored and an appropriate system of controls must be used to prevent pressurization of the suit laboratory. HEPA-filtered supply air must be provided to the suit area, decontamination shower and decontamination airlocks or chambers.

g. Exhaust air from the suit laboratory must be passed through a series of two HEPA filters prior to release outdoors.

h. Alternatively, after double HEPA filtration, exhaust air may be recirculated but only within the suit laboratory. Under no circumstances shall the exhaust air from the Biosafety Level 4 suit laboratory be recirculated to other areas.

iv. **Emergency lighting and communication systems** inside and outside of the facility must be provided.

B) Safety equipments

i. All operations must be conducted within Class III biological safety cabinet.

ii. Separation of works may be achieved by Flexible-film isolators to similar standards.

iii. Interlocked autoclave or fumigation chamber must be available for decontamination of hazardous biomaterials.

C) Personal Protective Equipments

i. One-piece positive pressure suit should be ventilated by a life support system. The life support system is provided with alarms and emergency break-up breathing air tanks.

D) Laboratory practices

i. Access is strictly limited.

ii. Before entering the cabinet/suit room, worker must pass through outer and inner changing rooms fitted with shower.

iii. Supplies and materials that are not brought into the cabinet room through the changing area must be introduced through a double-door autoclave or fumigation chamber. Once the outer door is securely closed, staff inside the laboratory can open the inner door to retrieve the materials. The doors of the autoclave or fumigation chamber are interlocked in such a way that the outer door cannot open unless the autoclave has been operated through a sterilization cycle or the fumigation chamber has been decontaminated.

iv. Personnel who enter the suit area are required to wear a one-piece, positively pressurized, HEPA filter-supplied air suit. Air to the suit must be provided by a system that has a 100% redundant capability with an independent source of air, for use in the event of an emergency.

v. Before leaving the laboratory, personnel must pass through chemical decontamination shower to decontaminate the surface of the positive pressure suit.

E) Laboratory monitoring

Same as BSL-3

F) Waste Management

All hazardous biomaterials must be strictly decontaminated within facility within 24 hour. Transport is not allowed. All materials including liquid waste must be autoclaved before disposal.

i. *Sterilization of waste and materials*. A double-door pass through autoclave is provided.

ii. *Decontamination of effluents*. All effluents from the maximum containment laboratory are to be rendered safe, including the shower water.

G) Health and medical surveillance

Same as BSL-3

H) Emergency procedures

An effective emergency programme must be devised on case by case basis with proper amendments of BSL-3 emergency procedures wherever applicable. In the preparation of this programme active cooperation with national and local health authorities must be established. Other emergency services, e.g., fire, police, receiving hospitals, must likewise be involved.

3.2. CONTAINMENT FOR LARGE SCALE OPERATIONS OF GENETICALLY ENGINEERED (GE) MICROORGANISMS

i. In the guidelines, experiments beyond 100 litre capacity for research as well as industrial purposes are considered as large scale experimentation/operations, which are generally used for production of bioethanol, enzymes, biochemicals and proteins for non-therapeutic applications, etc.

ii. For large scale or production, two physical containment levels are established:

   a. **BSL-1 Large scale facility:** It is recommended for large-scale research or production of viable microorganisms containing recombinant or synthetic nucleic acid molecules that require BSL-1 containment at the laboratory scale.

   b. **BSL-2 Large Scale facility:** It is recommended for large-scale research or production of viable microorganisms containing recombinant or synthetic nucleic acid molecules that require BSL-2 containment at the laboratory scale**.**

Note:

i. Indigenous product development, manufacture and marketing of products derived from organisms falling under RG 1 and RG2 are exempted from obtaining approval from competent authority (Vide MoEF&CC notification no. G.S.R.616(E) dated 20th September 2006).

ii. For large scale operations that are not covered in the above-mentioned notification, one should seek approval of the competent authority. In order to seek approval it will be necessary to furnish the relevant details in a prescribed format to GEAC for further consideration.

iii. No provisions are made for large-scale research or production of viable microorganisms containing recombinant or synthetic nucleic acid molecules that require BSL-3 or BSL-4 containment at the laboratory scale. If necessary, these requirements will be established by competent authority on a case-by-case basis. These large scale facilities are not appropriate for housing/keeping/rearing of animals or aquatic organisms or growing of plants.

iv. For all large scale operations, 'principles of containment' shall be applicable.

3.2.1. REQUIREMENTS OF BSL-1 LARGE SCALE FACILITY

i. The facility to be certified must be a fully enclosable space bounded by walls, doors, windows, floors and ceilings, to prevent the release of any genetically engineered (GE) microorganisms.

ii. Any openings in the walls, ceiling or roof, such as air vents, must be screened with insect proof mesh.

iii. Surfaces like walls, floors, benches, ceilings must be smooth, impermeable to water, cleanable and resistant to damage by the cleaning agents and/or disinfectants.

iv. If the facility has floor drainage exits, all effluent from these drains must be decontaminated by heat treatment or chemical treatment before being discharged. If the facility has a sink, then all liquid effluent must be decontaminated prior to discharge down the sink.

v. Open spaces between and under benches, cabinets and equipment must be accessible for cleaning.

vi. The facility must contain either a wash basin fitted with hands-free tap(s) and supplied with potable water, or some other means of decontaminating hands.

vii. Eyewash equipment must be provided within the facility.

viii. Potable water supplied to the facility must be provided with backflow prevention by a registered testable device for protection against both back-pressure and back-siphonage.

ix. Designated storage or hanging provisions for protective clothing must be available in the facility.

x. Cultures of viable microorganisms containing recombinant DNA molecules shall be handled in a closed system (e.g. closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g. biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable microorganisms.

xi. Cultures fluid shall not be removed from a closed system or other primary containment equipment unless the viable microorganism containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the microorganism that will serve as the host for propagating the recombinant DNA molecules.

xii. Sample collection from a closed system, the addition of materials to a closed system and the transfer of culture fluids from one closed system to another shall be done in a manner which minimizes the release of aerosols and contamination of exposed surfaces. When procedures in the facility will produce aerosols containing genetically engineered microorganisms, then the facility must contain a biological safety cabinet or other equipment designed to contain aerosols.

xiii. Other equipments such as centrifuges, filtration systems which are used to process genetically engineered microorganisms must be designed to contain the microorganisms.

xiv. Secondary containment, such as bunding, must be provided to retain any leakage from the primary vessel or closed system. It must be of sufficient capacity to retain:

   a. The maximum volume of fluid in the closed system.

   b. The volume of any disinfectant that might be used.

   c. With additional capacity to prevent any expected general fluid movement from breaching the secondary containment.

xv. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to HEPA filters or by other equivalent procedures (e.g. incineration) to minimise the release of viable microorganisms containing recombinant DNA molecules to the environment.

xvi. A closed system or other primary containment equipment that has viable microorganisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilised by a validated sterilisation procedure. A validated sterilisation procedure is one which has been demonstrated to be effective using the microorganism that will serve as the host for propagating the recombinant DNA molecules.

xvii. Spills and accidents which result in over exposures to microorganisms containing recombinant or synthetic nucleic acid molecules are immediately reported to the Biosafety Officer, IBSC and other appropriate authorities (if applicable). The facilities must have procedures and the means in place to clean up any spills in the facility including large spills, involving genetically engineered microorganisms.

xviii. Emergency plans as and when required shall include methods and procedures for handling large losses of cultures on an emergency basis as recommended by IBSC and approved by the competent authority.

xix. Access of facility must be restricted to authorized persons.

xx. The facility personnel must be trained in the equipment and procedures used in the facility. Records of the training must be kept and made available to the regulator if requested.

xxi. The facility must be kept free of pests. A record of pest prevention/control/eradication must be kept and made available to the regulator if requested.

xxii. The facility must be inspected at least once every 12 months. The inspection report must detail the extent of compliance with the conditions of certification and a copy of the most recent inspection report must be provided to the Regulator, if requested.

xxiii. The following personal protective clothing must be worn by personnel performing procedures in the facility: A laboratory coat or gown, or equivalent, to protect the arms and front part of the body from spills or any other source of contamination.

xxiv. Personal protective clothing and equipment must be removed before leaving the facility and stored in designated storage or hanging provisions or disposed off.

xxv. Facility doors must remain closed when laboratory procedures are in progress and must be locked when the facility is unattended.

xxvi. All GE microorganisms, and waste contaminated with GE microorganisms, being transported out of the facility must be transported in accordance with relevant guidelines, as in force from time to time, issued by the Competent Authority(ies).

xxvii. Transport of GE microorganisms between the certified facility and the storage unit must be in accordance with relevant guidelines.

3.2.2. REQUIREMENTS OF BSL-2 LARGE SCALE FACILITY

In addition to the requirement specified for BSL-1 large scale facility(ies), following practices should be considered:

i. Cultures of viable microorganisms containing recombinant or synthetic nucleic acid molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable microorganisms.

ii. Biological safety cabinet must pass tests for containment efficiency and a certificate summarizing the test results and the date of the next test, must be affixed to the cabinet.

iii. Culture fluids that contain viable microorganisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

iv. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable microorganisms shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

v. A closed system must be used for the propagation and growth of viable microorganisms. It should be tested for integrity of the containment features using the microorganism that will serve as the host for propagating recombinant or synthetic nucleic acid molecules. Testing, and

modification or replacement of essential containment features shall be accomplished prior to the introduction of viable microorganisms. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test microorganism. Records of tests and results shall be maintained on file.

vi. A closed system used for the propagation and growth of viable microorganisms containing recombinant or synthetic nucleic acid molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable microorganisms.

vii. The universal biosafety sign shall be pasted on each closed system and primary containment equipment when used to contain viable microorganisms containing recombinant or synthetic nucleic acid molecules.

viii. Emergency plans shall also include methods and procedures for handling large losses of culture on an emergency basis.

ix. Spills and accidents which result in over exposures to microorganisms are immediately reported to the Biosafety Officer, Institutional Biosafety Committee, and other appropriate authorities (if applicable). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

x. Emergency drench showers and eyewash equipment must be provided within the facility.

xi. The following personal protective clothing must be worn by personnel performing procedures in the facility: (a) Laboratory coat or gown, or equivalent, to protect the arms and front part of the body from spills or any other source of contamination; and (b) Appropriate gloves (while performing procedures that might lead to contamination of the hands).

Note: Assessment should be made of the need to wear face shields when working with closed systems.

3.3. ANIMAL BIOSAFETY LEVEL FACILITIES

3.3.1. PURPOSE

This type of facility houses animals for research purposes that include testing of chemical drugs or risk-inherent microorganisms or its products on laboratory animals. In addition, animals housed at the facility but not as part of ongoing research should also be protected. It should be ensured that:

i. Healthy animals are not acquiring infection leading to clinical disease or mortality.

ii. Infections are not spreading to other animals housed within the same facility.

iii. Preventing zoonosis. Infections are not transmitting to laboratory workers from the infected animals from bites, scratches and inhalations of aerosols.

Note: In experimental and diagnostic purposes, working with animals should first fulfil legislative obligations set in Prevention of Cruelty to Animals Act, 1960 and Breeding of and Experiments on Animals (Control & Supervision) Rules of 1998, 2001 and 2006. Accordingly, approval from Institutional Animal Ethical Committee (IAEC) will be mandatory to initiate any experiments on animals. Also it is a moral obligation to take care of animal in every step to avoid causing them unnecessary pain or suffering. Animals must be provided with comfortable, hygienic housing and adequate wholesome food and water. During experiment, effort should be made to reduce pain or suffering and at the end of the experiment they must be dealt with a humane manner.

3.3.2. TYPES OF ANIMAL BIOSAFETY LEVEL FACILITIES

3.3.2.1. Animal Biosafety Level 1 (ABSL-1):

Suitable for:

i. Maintenance of most stock animals after quarantine (except nonhuman primates, regarding which appropriate national authorities should be consulted).

ii. Breeding, housing and experiments with animals that are deliberately inoculated with RG 1 microorganism.

iii. **Category I genetic engineering experiments on animals:**

This category includes experiments which generally do not pose significant risks to laboratory workers, community or the environment. Examples are:

a. Breeding of GE animals transformed with sequences of viral vector belonging to RG 1.

b. Breeding, housing and experiments of gene 'knockout' in rodents, independent of whether the mice carry a selectable marker gene, provided that the marker gene does not confer any selective advantage to the animal. If further genetic manipulations are performed on these 'knockout' mice, containment should be decided following thorough risk assessment.

c. Research involving the introduction of nucleic acids into animals provided that the nucleic acid does not give rise to any infectious agent.

d. Work involving the introduction of genetically manipulated somatic cells into animals, unless they are able to give rise to infectious agents.

Before commencement of Category I GE experiments, the investigator should intimate the IBSC of the objective and experimental design of the study along with organisms involved. IBSC should review the same as and when convened for record or action if any.

It is desirable to designate a separate area in the facility with proper labelling for Category I GE experiments to avoid any chances of contamination.

3.3.2.2. Animal Biosafety Level 2 (ABSL-2):

Suitable for:

i. Experiments with animals that are deliberately inoculated with RG 2 microorganism.

ii. **Category II genetic engineering experiments on animal:**

These experiments may pose low-level risks to laboratory workers, community or the environment. Examples are:

a. Experiments with GE animal and associated materials, harbouring DNA from a RG 2 microorganism.

b. Experiments with whole animals (including non-vertebrates) which involve stable genetic manipulation of oocytes, zygotes or early embryos to produce a novel organism.

c. Experiments with animals infected with GE microorganism(s) that fall under RG 2.

All category II GE experiments require prior authorization from IBSC before the commencement of the experiments through submission of information in the prescribed proforma.

It is desirable to designate a separate area in the facility with proper labelling for Category II GE experiments to avoid any chances of contamination.

3.3.2.3. Animal Biosafety Level 3 (ABSL-3):

Suitable for:

i. Experiments with animals that are deliberately inoculated with RG 3 microorganism.

ii. **Category III and above genetic engineering experiments on animal:**

These experiments pose moderate to high risks to laboratory workers, community or the environment. Examples are:

a. Experiments with animals infected with GE microorganisms that fall under RG 3.

b. Experiments involving the use of infectious or defective RG 3 viruses in the presence of helper virus.

c. Experiments on animals using DNA which encodes a vertebrate toxin.

d. Experiments using viral vectors whose host range includes human, and where the viral vectors contain one or more inserted DNA sequences coding for a product known; to play a role in the regulation of cell growth; or to be toxic to human cells.

e. Experiments using defective vector/helper virus combinations which have the potential to regenerate non-defective recombinant virus.

f. Introduction of pathogenicity genes into microorganisms other than the host organisms listed in Annexure 3. This category includes those genes whose products are suspected of, or have a risk of initiating autoimmune diseases.

g. Cloning or transfer of entire viral genome, viroids, or fragments of a genome capable of giving rise to infectious agent with the capacity to infect human, animal or plant.

h. Experiments involving recombination between entire viral genomes, viroids and/or complementary fragments of these genomes, where one or more fragments encode virulence or pathogenic determinants. The experiments that could alter the host range of pathogens or increase pathogen virulence or infectivity.

i. Experiments where a fragment of or the entire genome of a virus is injected into an embryo to produce a transgenic animal which secretes or produces infectious viral particles.

j. Experiments with animal infected with GE microorganism(s) that fall under RG 3.

All category III and above GE experiments require prior authorization from IBSC and subsequent approval from RCGM before commencement of the experiments through submission of information in the prescribed proforma.

3.3.2.4. Animal Biosafety Level 4 (ABSL-4):

Suitable for:

i. Experiments with animals that are deliberately inoculated with RG 4 microorganism.

ii. Category III and above genetic engineering on animal involving GE microorganisms that fall under RG 4.

Note:

i. ABSL facilities should not be used for:

a. Other than animals that are not used in the intended experiments.

b. Housing/keeping/rearing of any plants, arthropods or aquatic organisms, unless they are integral to the intended activity in the contained facility.

ii. **Genetic engineering experiments on animals not covered under any of the above categories will require case by case evaluation for selection of appropriate containment levels. Approval from IBSC and RCGM shall be required prior to initiate such experiments.**

iii. All existing ABSL-3 and 4 facilities must be certified by RCGM. A format for certification is available in this guideline.

iv. The new ABSL-3 and 4 facilities shall require certification at the time of commissioning operations as per the format.

3.3.3. OPERATIONAL GUIDE FOR ABSL FACILITIES

A) Facility design

ABSL-1	ABSL-2	ABSL-3	ABSL-4
In addition to those mentioned in BSL-1: i. Doors to areas where infectious materials and/ or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically. ii. The facilities should be separated from the general traffic patterns of the building and restricted as appropriate. iii. Entrances to all animal areas must have an "Admittance to Authorized Personnel Only" label. This label contains appropriate	In addition to the facility design features specified for ABSL-1, the following features are essential: i. Anteroom: The facility must have an anteroom. ii. Note: If no dedicated anteroom is present, then an adjacent room, either uncertified or certified may act as an anteroom subject to approval by IBSC. IBSC may attach conditions to the room acting as the anteroom to the animal facility. iii. If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must be available for hand washing at the exit from each segregated area. Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of pests. iv. The direction of airflow into the animal facility should be inward; animal rooms maintain inward directional airflow	Same as ABSL-2 and BSL-3. In addition: i. Animals in the facility must be housed in primary containment devices within the work area. Primary containment devices must be fitted with exhaust HEPA filters; either as Individually Ventilated Cages (IVC) or within HEPA filtered ventilated enclosures. Exhaust systems on the primary containment devices must be sealed to prevent escape of GE microorganisms. In normal operation, all exhaust air from the cages must be contained and filtered to a standard that is equivalent to HEPA filtration. Air must be drawn through the primary containment devices to remove aerosols. Safety mechanisms must be in place that prevent the primary containment devices and exhaust air paths from becoming positively pressured	Same as ABSL-3 and BSL-4

contact information for general and emergency entrance to the lab. Additionally, the lab entrance must be labelled with: personal protective equipment requirements, contact information for the person responsible, as well as any specific procedures for entering and exiting the area.	compared to adjoining hallways. A ducted exhaust air ventilation system to be provided. Exhaust air may be recirculated into the animal facility only. v. Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process. vi. The cage wash area may be designed to accommodate the use of high-pressure spray systems, humidity, strong chemical disinfectants and 180°F water temperatures during the cage/equipment cleaning process. vii. Biosafety Cabinets should be located away from doors, and other possible airflow disruptions. viii. The international biohazard warning symbol and sign along with information on (i) microorganism(s) being handled, (ii) the animal species being handled, (iii) the name and telephone number of the Animal Facility In-charge or other responsible individual, and (iv) Any special requirements for entering the laboratory should be displayed on entry doors.	relative to the surrounding area in the event of failure of the exhaust fan. The system must also be alarmed to indicate where operational malfunctions occur.	

B) Safety Equipment

ABSL-1	ABSL-2	ABSL-3	ABSL-4
As in BSL-1	As in BSL-2	Same as BSL-3	As in BSL-4

C) Personal Protective Equipment

ABSL-1	ABSL-2	ABSL-3	ABSL-4
Same as BSL-1 although care should be taken to prevent animal biting during handling.	As in BSL-2	As in BSL-3	As in BSL-4

D) Procedures

ABSL-1	ABSL-2	ABSL-3	ABSL-4
As in BSL-1, plus i. All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from non-transgenic animals.	As in ABSL-1, plus: i. Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the organism used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of	As in ABSL-2 and BSL-3, plus i. Consideration should be given to the use of containment caging systems to reduce the risk of infectious aerosols from animals and bedding. ii. Caging systems must be ventilated to prevent escape of microbes from the cage. Animals in the facility must be housed	Same as ABSL-3 and BSL-4, plus: i. All handling of organisms, infected animals and housing of infected animals must be carried out in Class III

ii. If the animal involved in the dealings escape within the facility, trapping devices must be used to capture the animal and the animal must be returned to its container or cage or euthanised. iii. A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used. iv. All procedures prior to initiation of the work involving animals must be approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals **(CPCSEA)** as per clause 15 of PCA act. v. Proper pest management program should be adopted and constantly monitored. vi. Identification of specific infectious organisms are recommended when more than one organism is being used within an animal room.	transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented. ii. If arthropods are used in the experiment or the organism under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent. iii. Animals those are not associated with the work should not be kept in the same laboratory.	in primary containment devices within the work area. Primary containment devices must be fitted with exhaust HEPA filters, either as individually ventilated cages (IVC) or within HEPA filtered ventilated enclosures.	biological safety cabinet.

E) Laboratory monitoring

ABSL-1	ABSL-2	ABSL-3	ABSL-4
i. The containment area shall be patrolled or monitored at frequent intervals. ii. The facility in-charge must ensure that all personnel receive site-specific training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious organisms, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates or additional training when procedures or policies change.	Same as BSL-2 and ABSL-1.	The section on Lab monitoring applies as ABSL-2 and BSL-3.	The section on Lab monitoring applies as ABSL-1 and BSL-4.

F) Waste management

ABSL-1	ABSL-2	ABSL-3	ABSL-4
i. Cages should be autoclaved or otherwise decontaminated prior to washing. Mechanical cage washer should have a final rinse temperature of at least 180°F. ii. The volume of the effluent generated from ABSL facilities including solid wastes such as the dung, washed away bedding materials etc., must be taken into account while designing the effluent treatment facility. iii. The effluent treatment plant must be located below the animal facility to allow the waste flow to be taken care by gravity and not require any energy intensive methods.	Same as ABSL-1 and BSL-2.	Same in ABSL-2 and BSL-3.	Same as BSL-4 and ABSL-1.

iv. A macerator that breaks up solid masses into small pieces for efficient decontamination should be used wherever necessary. v. Heat treatment (autoclaving) is the preferred method for decontaminating effluents. vi. Incineration can be used for disposing of animal carcasses and other wastes, with or without prior decontamination.			

G) Health and medical surveillance

ABSL-1	ABSL-2	ABSL-3	ABSL-4
Same as BSL-1	same as BSL-2	Same as BSL-3	Same as BSL-4

H) Emergency procedures

ABSL-1	ABSL-2	ABSL-3	ABSL-4
Same as BSL-1	Same as BSL-2	Same as BSL-3	Same as BSL-4

3.4. PLANT BIOSAFETY LEVEL FACILITIES

3.4.1. PURPOSE

The purpose of plant containment is to avoid:

i. The unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material.

ii. Release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants, and

iii. Escape and establishment of GE plant into natural environment.

iv. The following guidelines specifies the physical containment and work practices suitable to conduct experiments with plants containing rDNA [Genetically Engineered (GE) plants] and plants associated with GE microorganisms, small animals or arthropods. Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoa and algae that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant disease. Plant-associated small animals include arthropods that are in obligate association with plants, plant pests, plant pollinators, nematodes and those that transmit plant disease, for which tests of biological properties necessitate the use of plants.

3.4.2. TYPES OF PLANT BIOSAFETY LEVEL FACILITIES

For experiments on plants, four biosafety levels laboratories are specified depending on nature of works. **The levels, PBSL-1-4, include structures comprising greenhouses, screen houses and flexible film plastic structures.**

3.4.2.1. Plant Biosafety Level 1 (PBSL-1):

The PBSL-1 applies to structures comprising greenhouses, screen houses and flexible plastic film structures for:

i. Experiments on plants involving RG 1 organism. For example, experiments on plants with non-pathogenic nitrogen fixing bacteria and *Agrobacterium* spp.

ii. **Category I genetic engineering on plants:**

This category includes experiments which generally do not pose significant risks to laboratory workers, community or the environment. Examples are:

a. Research involving model plants such as Arabidopsis, Tobacco, and Chlamydomonas with the introduction of DNA from other plants with a proven history of safe consumption and RG 1 organisms, but do not code for any known toxins/allergens.

b. Working with plants for the development/improvement of transformation protocols with well-known and characterized marker genes such as npt II, HYG, etc. and reporter genes such as *uidA* (GUS), GFP, luciferase, etc and their molecular characterization.

c. Maintenance of GE plants modified with genes from other plants that have no known invasive trait and microorganisms that fall under RG 1.

d. Experiments involving genome editing leading to Site Directed Nucleases (SDN) 1 type mutations that are genetically indistinguishable from organisms which could have occurred naturally.

Before commencement of Category I GE experiments, the investigator should intimate the IBSC of the objective and experimental design of the study along with organisms involved. IBSC should review the same as and when convened for record or action if any.

It is desirable to designate a separate area in the facility with proper labelling for Category I GE experiments to avoid any chances of contamination.

3.4.2.2. Plant Biosafety Level 2 (PBSL-2):

The PBSL-2 applies to structures comprising greenhouses, screen houses and flexible film plastic structures for:

i. Experiments on plants involving RG 2 organisms. The primary exposure hazards associated with organisms requiring PBSL-2 are those that can enter through the ingestion, inoculation and mucous membrane route. Organisms requiring PBSL-2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands) or splashes.

ii. Experiments using plant associated transgenic insects or small animals as long as they pose no threat to managed or natural ecosystems.

iii. **Category II genetic engineering on plants:**

These experiments may pose low-level risks to laboratory workers, community or the environment. Examples are:

a. Research, development, and maintenance of GE plants harbouring DNA from RG 2 microorganism.

b. Experiments on non-GE plants involving GE organisms that falls under RG 1/RG 2, GE arthropods and GE nematodes.

c. Research and development work involving GE plants that may exhibit weediness characteristics or that may be capable of interbreeding with weeds.

d. Experiments on GE plants conferring herbicide tolerance or pathogen resistance.

e. Research and development work with plants expressing heterologous genes which confer resistance to biotic and abiotic stresses.

f. Research and development work on plants for gene or promoter tagging in crop species or model species.

g. Experiments involving genome editing leading to SDN 2 and 3 type modifications.

All category II GE experiments require prior authorization from IBSC before the commencement of the experiments through submission of information in the prescribed proforma.

It is desirable to designate a separate area in the facility with proper labelling for Category II GE experiments to avoid any chances of contamination.

3.4.2.3. Plant Biosafety Level 3 (PBSL-3):

The PBSL-3 applies to greenhouses only for:

i. Experiments on plants involving RG 3 organisms.

ii. **Category III and above genetic engineering on plants:**

These experiments may pose moderate to high risks to laboratory workers, community or the environment. Examples are:

a. Growing genetically modified plants containing genes from microorganisms that fall under RG 3.

b. Experiments with microbial pathogens of insects or small animals associated with plants if the organism has a recognized potential for serious and detrimental impact on managed or natural ecosystems.

c. Experiments with plant associated GE organisms or plants infected with these GE organisms that fall under RG 3.

d. Experiments involving GE plants containing genes directly involved in the production of toxins/allergens or part of their biosynthetic pathway that could harm the humans if established in environment.

All category III and above GE experiments require prior authorization from IBSC and subsequent approval from RCGM before commencement of the experiments through submission of information in the prescribed proforma.

3.4.2.4. Plant Biosafety Level 4 (PBSL-4):

The PBSL-4 applies to greenhouses only for:

i. Experiments which involve certain exotic, readily transmissible infectious organisms or potentially serious pathogens of major Indian crops and these experiments are performed in the presence of their arthropod vector.

ii. Category III and above genetic engineering on plants involving biopharming experiments in which bioactive compounds (e.g., vaccines) are produced in GE plants.

iii. Experiments with plants using GE organism of RG 4 is not permitted.

Note: PBSL facilities shall not be used for:

i. Activity with any organism and related material other than plants unless they are part of the experiments.

ii. Housing/keeping/rearing of any animals, arthropods or aquatic organisms unless they are integral to the activity in the contained facility.

iii. Genetic engineering experiments on plant not covered under any of the above categories will require case by case evaluation for selection of appropriate containment levels. Approval from IBSC and RCGM will be required prior to initiate the experiment.

iv. All existing PBSL-3 and 4 facilities must be certified by RCGM. A format for certification is available in this guideline.

v. The new PBSL-3 and 4 facilities shall require certification at the time of commissioning operations as per the format.

3.4.3. OPERATIONAL GUIDE FOR PBSL FACILITIES

A) Facility design

PBSL-1	PBSL-2	PBSL-3	PBSL-4
i. Floor should be composed of gravel or other porous material and walkways are of an impervious material (e.g., concrete) ii. The walls and roof should be constructed of impact resistant, ISI branded transparent or translucent material to allow passage of sunlight for plant growth. Suitable materials include glass, mesh (40x40), polycarbonate and flexible film plastics such as polythene or screens. iii. Windows and other opening in the walls and roof of the facility may be open for ventilation however screens are recommended to contain or exclude pollen, microorganisms or small	Same as PBSL-1, plus: i. Any openings in the walls or roof (e.g. windows, vents, and air supply and exhaust inlets and outlets) should be screened with fine screens (thirty–gauge 30/32 mesh wire gauze). ii. If the plant facility is an isolated unit, it should have an anteroom for entry and exit. An anteroom is not necessary if the plant facility connects directly with a certified small or large scale containment facility. iii. Gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable	Same as PBSL-2, plus: i. The facility should be constructed with a rigid reinforced frame with walls, floors and glazing forming a shell. Floors should be slip resistant. Transparent section should be made of impact resistant material such as methyl-acrylate (Perspex). ii. Additional protection such as physical screen should be provided to protect against extreme situation (storm, wild animals). iii. Joints between any structural components should be sealed and mechanically strong and durable. iv. The anteroom should allow materials, equipment, trolleys to pass through ensuring one door can	In addition to the facility design features specified for PBSL-3 and BSL4, the following additional features are essential: i. This level of containment represents an isolated unit, functionally and, when necessary, structurally independent of other areas. ii. PBSL-4 emphasizes maximum containment by complete sealing of the facility perimeter with confirmation by pressure decay testing.

flying animals (e.g. arthropods and birds). iv. There will be double door at the entrance and must have proper door sealing. v. An internal small room for keeping small implements, threshing data recording etc. should be provided. vi. Entrances to the plant facility should be posted with an appropriate signage identifying the type of plant facility and listing the procedures applicable, including emergency and maintenance procedures, person to be contacted in case of emergency. vii. All surfaces should be cleanable in accordance with the requirements for research and maintenance of healthy plants. viii. Plant facility should contain a sink for hand-washing.	unless propagules of experimental organisms are readily disseminated. iv. Screens must be placed on windows and openings to exclude birds and arthropods v. Attempt should be made to minimize the ingress of arthropods through intake fans. vi. Containment can be satisfied by using a growth chamber or growth room within a building. vii. If the plant facility is an isolated unit, it should have an anteroom for entry and exit. The anteroom should be fitted with a sticky pest strip or electric insect-control unit to trap arthropods which may gain entry. An anteroom is not necessary if the plant facility that connects directly with a RCGM certified small or large scale containment facility.	be closed at all time. The facility should be provided with a footbath containing a suitable disinfectant v. An autoclave for decontamination of plant facility waste should be provided in the laboratory preferably located in the barrier wall of the PBSL-3 but not located in the anteroom. If located within the barrier wall, it should be accessible for maintenance from outside the laboratory. For larger plants and trees, disinfectant dunk tank can be used for decontamination. vi. A universal hazard warning sign should be displayed on the doors, identifying the organisms, the name of the laboratory supervisor and other responsible person(s) and indicating any special	

		conditions of entry into the area (immunizations, etc.) (See Figure 3). vii. For studies involving genetic engineering, utmost care should be taken to prevent any escape of pollen, viable plant materials by any means. The strategies for isolation, storage and waste disposal should be documented properly.	

B) Safety Instrument

PBSL-1	**PBSL-2**	**PBSL-3**	**PBSL-4**
Same as BSL-1	Same as BSL-2	Same as BSL-3	Same as BSL-4

C) Personal Protective Equipment

PBSL-1	**PBSL-2**	**PBSL-3**	**PBSL-4**
Same as BSL-1	Same as BSL-2 although care should be taken to prevent pollen transmission from laboratory to environment.	Same as BSL-3	Same as BSL-4

D) Procedures

PBSL-1	PBSL-2	PBSL-3	PBSL-4
Same as BSL-1 plus: i. An institutional PBSL-1 practices and procedures manual should be adopted for use. ii. A programme should be implemented to control undesired species (e.g. weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with national regulations. iii. Plants associated organisms which are integral to the activity should be housed in appropriate cages. If these organisms (e.g. flying arthropods or nematodes) are released within the facility, precaution should be taken to minimise escape from the plant facility. iv. Experiments involving other organisms (e.g. handling of non pathogenic *E.coli*) that require basic containment may be conducted in the PBSL-1 concurrently with experiments that require PBSL-1 containment provided that all work is conducted in accordance with PBSL-1 practices. v. Materials containing experimental microorganisms, which are brought into or removed from the facility in a viable or intact state, shall be transferred in a closed non-breakable container. Alternatively, there should be additional provisions in PBSL-1 facility for maintaining of risk group 1 microorganisms.	Same as PBSL-1 and BSL-2.	Same as PBSL-1 and BSL-3 plus i. Plants that are not related to the experiment should not be placed in one containment.	Same as BSL-4 and PBSL-3, plus: **Transfer of Materials:** Experimental materials that are brought into or removed from the facility in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose. Supplies and materials shall be brought into the facility through a double door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use.

E) Laboratory monitoring

PBSL-1	PBSL-2	PBSL-3	PBSL-4
Same as BSL-1	Same as BSL-2	Same as BSL-3	Same as BSL-4

F) Waste management

PBSL-1	PBSL-2	PBSL-3	PBSL-4
i. PBSL-1 experimental plants and soil must be rendered biologically inactive before final disposal. These materials can be rendered inactive by desiccation, steam treatment, chemical treatment, freezing, or by a validated autoclave. ii. Contaminated materials are to be decontaminated away from the laboratory and placed in a durable leak-proof container that is covered before being removed from the laboratory. iii. If viable PBSL-1 transgenic materials must be transferred to another facility for inactivation,	Same as BSL-2, plus: i. After plant materials are inactivated using validated parameters, they may be disposed of in the regular trash. Contaminated materials are to be decontaminated away from the laboratory and placed in a durable leak-proof container that is covered before being removed from the laboratory. ii. If part of the facility is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate,	Same as BSL-3, plus i. Where propagules (such as seeds, pollen or arthropod life stages) could potentially survive extended emersion under water, liquid waste outlet should be fitted with strainers or adequate fine mesh/gauge to prevent escape. The floor of the facility should be designed such that all effluents are collected, treated and drained appropriately. ii. Materials and equipment taken into or out of the plant facility should be treated by an appropriate technique to destroy or remove all other organisms (including all stages of its life-cycle). This requirement applies to soil substitutes and where feasible to soil. Soil substitutes which can be readily decontaminated should be used whenever possible. A system is established for the reporting of accidents, incidents, exposures and for the medical surveillance of potential laboratory associated illnesses.	Same as BSL-4 and PBSL-3

a transportation containment SOP must be reviewed and approved by the IBSC.	or render inactive, any organisms potentially entrapped by the gravel.	reporting of accidents, incidents, exposures and for the medical surveillance of potential laboratory associated illnesses. iii. If plant materials contain rDNA that may harm humans, a biohazard symbol must be present on the outside of the plastic bag prior to treatment. After treating the plant materials using validated parameters, the biohazard symbol must be covered (i.e. place in non-see-through trash bag) prior to final disposal in the regular trash. iv. Experimental materials that are brought into or removed from the facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vectors are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. v. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism	

G) Health and medical Surveillance

PBSL-1	PBSL-2	PBSL-3	PBSL-4
Same as BSL-1	Same as BSL-2	Same as BSL-3	Same as BSL-4

H) Emergency Procedures

PBSL-1	PBSL-2	PBSL-3	PBSL-4
Same as BSL-1	Same as BSL-2	Same as BSL-3	Same as BSL-4

3.5. INSECT BIOSAFETY LEVEL FACILITIES

3.5.1. PURPOSE

The purpose of establishment of Insect Biosafety Level (IBSL) Facilities is to prevent escape and establishment of the experimental arthropods into the natural environment and ensure the safety of the laboratory personnel in the facility.

Arthropods to be considered include following but not limited to: Insects (Lepidopterans; Coleopterans; Dipterae eg. mosquitoes, fruit flies, tse tse flies, black flies, sand flies and midges; Hemipterae eg. reduvids, Anoplurae eg. Lice; Siphonapterae fleas); Blattodea and Arachnids (ticks and mites). All stages of life-cycle (eggs, larvae, nymphs, pupae and adults) should be handled within the appropriate Insect Biosafety level facility.

The IBSL should ensure safety to laboratory personnel and outside environment from:

i. Arthropods that transmit pathogens of public health importance or become the crucial link in completing the transmission cycle for a disease.

ii. Arthropods that could cause economic damage to crop plants, or local environment.

iii. Uninfected arthropods and those carrying infectious agents

iv. GE arthropods or non-GE arthropods but challenged/ infected with GE organisms.

3.5.2. TYPES OF INSECT BIOSAFETY LEVEL FACILITIES

3.5.2.1. Insect Biosafety Level 1 (IBSL-1):

Suitable for maintenance, rearing and to conduct laboratory level experiments with terrestrial arthropods that are:

i. Uninfected by infectious agents and are present in the same geographic area.

ii. Genetically engineered arthropods with genes from RG 1 microorganisms and other non pathogenic organisms provided the genetic engineering process has no, or only negative effects on viability, survivorship, host range, or vector capacity.

iii. Challenged or infected with GE microorganisms that fall under RG 1.

3.5.2.2. Insect Biosafety Level 2 (IBSL-2):

Suitable for maintenance, rearing and experiments with terrestrial arthropods that are:

i. Infected with or suspected to be infected with RG 2 microorganisms or other pathogenic organisms that may cause animal and/or human diseases.

ii. Genetically engineered arthropods with genes from RG 2 microorganisms and other non pathogenic organisms provided the genetic engineering process has no, or only negative effects on viability, survivorship, host range, or vector capacity.

iii. Challenged or infected with GE microorganisms that fall under RG 2.

3.5.2.3. Insect Biosafety Level 3 (IBSL-3):

Suitable for maintenance, rearing and experiments with terrestrial and exotic arthropods that are:

i. Infected with or suspected to be infected with RG 3 microorganisms or other pathogenic organisms that cause animal and/or human diseases.

ii. Genetically engineered arthropods with genes from RG 3 microorganisms and other pathogenic organisms.

iii. Genetically engineered to contain genes from RG 2 microorganisms where the genetic engineering process could positively affect viability, survivorship, host range, or vector capacity.

iv. Challenged or infected with GE microorganisms that fall under RG3.

3.5.2.4. Insect Biosafety Level 4 (IBSL-4):

i. IBSL-4 shall be suitable for maintenance, rearing and experiments with terrestrial and exotic arthropods that are infected with or suspected to be infected with RG 4 microorganisms.

ii. Unless notified, genetic engineering of arthropods with DNA from RG 4 microorganisms and any such exotic pathogens is not permitted currently in India. Similarly, no challenge/ infection studies on arthropods (both GE and non-GE) with RG 4 or exotic pathogens are permitted. Permission may be obtained on case-by-case basis.

Note: IBSL facilities shall not be used for:

i. Activity with any organism and related material other than insects unless they are part of the experiments.

ii. Housing/keeping/rearing of any animals, plants, microbes or aquatic organisms unless they are integral to the activity in the contained facility.

iii. Genetic engineering experiments on insects not covered under any of the above categories will require case by case evaluation for selection of appropriate containment levels. Approval from IBSC and RCGM will be required prior to initiate the experiment.

iv. All existing IBSL-3 and 4 facilities must be certified by RCGM. A format for certification is available in this guideline.

v. The new IBSL-3 and 4 facilities shall require certification at the time of commissioning operations as per the format.

3.5.3. OPERATIONAL GUIDE FOR IBSL FACILITIES

A) Facility design

IBSL-1	IBSL-2	IBSL-3	IBSL-4
Following in addition to BSL-1: i. Facility should be located out of the flow of general traffic, avoiding hallways. ii. Arthropods must be placed in appropriate closets. iii. The facility must be maintained to allow detection of escaped arthropods. This could be achieved by: a. Avoiding all materials that are unrelated to arthropod rearing and experimentation e.g., plants, unused containers, boxes, cabinets etc. b. Walls of the facility should be painted white or with a contrasting colour to the arthropod. iv. Door openings should be covered by rigid panels, glass, screens, plastic sheets or cloth to minimise escape and entry of arthropods. v. Any opening like ventilation area, AC pipes, and drainage exit should be covered with suitable-sized mesh.	Following in addition to IBSL-1 and BSL-2: i. Entry to the facility should be through double self-closing door that provides a seal sufficient to contain the arthropod species under study. For example, the two contiguous doors must not be opened simultaneously. Internal doors may open outwards or be sliding, but are self-closing, and are kept closed when arthropods are present. ii. The facility should also be free of other possible escape routes e.g. false ceilings.	Following in addition to IBSL-2 and BSL-3: i. 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 a ultra-violet insect zapper. ii. Access room doors should be sealed to be arthropod-proof. iii. 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	Same as IBSL-3 and BSL-4

vi. Entrance door should be posted with appropriate signage identifying the type of arthropod facility and make aware of the presence of arthropod vectors. The contact information of the laboratory supervisor or other responsible persons should be listed. vii. Facility should be fitted with a suitable electric insect-control unit or an appropriate insect trap. viii. Windows should be covered with mesh or screen to effectively prevent escape of the smallest arthropods contained within.	iii. Windows are not recommended, but if present cannot be opened and are well sealed. Windows must be resistant to breakage (e.g., double paned or wire-reinforced). iv. Additional barriers (e.g., screened partitions, hanging curtains) are highly recommended.	arthropods, eggs or larvae from their personnel clothing/ belonging before leaving the facility.	

B) Safety Instrument

IBSL-1	IBSL-2	IBSL-3	IBSL-4
Following in addition to BSL-1 : i. Autoclaving or incinerator: for killing of arthropods and their life stages infected with a non-pathogen is recommended. ii. Non-breakable Cages used to hold arthropods effectively prevent escape of all stages. Screened mesh, if used, is durable and of a size appropriate to prevent escape.	Same as IBSL-1 and BSL-2	Same as IBSL-2 and BSL-3	Same as IBSL-3 and BSL-4

C) Personal Protective Equipment

IBSL-1	IBSL-2	IBSL-3	IBSL-4
i. Gloves: Appropriate gloves are worn when handling host animals or blood used to feed the arthropods. ii. Torso Apparel: White laboratory coats, gowns, and/or uniforms are worn at all times in the facility when handling blood and animals. iii. Arthropod-Specific Personal Protective Equipment: Personal protective equipment is worn as appropriate e.g., respirators for arthropod-associated allergies, particle masks, head covers.	Same as IBSL-1	Same as BSL-3	Same as BSL-4

D) Procedures

IBSL-1	IBSL-2	IBSL-3	IBSL-4
Following in addition to BSL-1: i. An institutional biosafety manual describing arthropod facility practices and SOP should be prepared and adopted. ii. All arthropods should be kept in suitable containers. All containers should be clearly labelled giving species, strain/origin, date of collection, responsible investigator. A central logbook for maintenance of stocks must be kept in the facility. iii. Practices should be in place such that arthropods in primary containers do not escape by inadvertent disposal. Cages and other culture containers should be appropriately cleaned to prevent arthropod survival and escape (e.g. heated to or chilled below the lethal temperature).	Following in addition to IBSL-1 and BSL-2: i. All supplies for insect maintenance should be located in a designated area and not on open shelves. It is recommended that a closed storage room, cabinets with tight-fitting doors or drawers be used. Doors and drawers are opened only for access. Insect diet should be kept in sealed containers.	Following in addition to IBSL-1 and BSL-3 : i. Materials taken into and out of the arthropod facility should be suitably treated for destroying or removing all stages of the life-cycle of arthropods and their pathogens. This requirement applies to soil	Same as IBSL-3 and BSL-4

iv. Living arthropods should not be taken out from the facility except when they are being transferred to another containment facility or to an approved release site. Arthropods taken into or out of the facility should be carried in non-breakable secure containers. v. Animals used as hosts or blood sources may be housed within the facility but should be adequately protected from access by escaped arthropods. vi. Arthropods fed on host animals should be prevented from accidental transfer to host cages. vii. When handling/removing animals after exposure to arthropods, precautions should be taken to prevent arthropod escape through screens, covers, and by flying. Host animals are inspected closely (e.g. concealment in fur, ears, crevices) and the primary container is sufficiently robust to prevent escape during feeding. viii. Mechanism should be adopted to recover escaped arthropods.	ii. Spread of RG 2 microorganism to uninfected arthropods is prevented. Generally this is accomplished by isolating infected material in a separate room. iii. Care not to disperse viable life stages into the drainage system. iv. Infected arthropods must not be killed with bare hands, and must be transferred using filtered mechanical or vacuum aspirators.	substitutes and soil. Soil substitutes which can be readily decontaminated should be used in preference to soil.	

E) Laboratory monitoring

IBSL-1	IBSL-2	IBSL-3	IBSL-4
Same as BSL-1	Following in addition to BSL-2: i. An effective arthropod trapping program to be adopted to monitor the escape prevention program. Oviposition traps, ground-level flea traps, oil-filled channels surrounding tick colonies, light traps for mosquitoes and so on are recommended. Particularly in the case when exotic arthropods are used, exterior monitoring is recommended. Records of exterior captures are maintained. ii. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.	Same as BSL-3 and IBSL-2	Same as BSL-4 and IBSL-3

F) Waste management

IBSL-1	IBSL-2	IBSL-3	IBSL-4
Following in addition to BSL-1: i. Living arthropods are to be disposed of only after proper decontamination. ii. All wastes from the facility (including arthropod carcasses, and rearing medium) are transported in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional or local requirements.	Following in addition to BSL-2: i. Containers are disinfected chemically and/or autoclaved if used for infected material. Autoclaving or incineration of primary containers is recommended for containers holding uninfected material.	Same as BSL-3 and IBSL-2	Same as BSL-4 and IBSL-3

iii. All stages of arthropods are killed before disposal. iv. Autoclaving or incineration of material is recommended. Material may be killed with hot water or freezing before flushing down drains. v. Escaped arthropods should be recovered and rendered nonviable before proper disposal.	ii. Autoclaving or incineration of arthropod materials is recommended. Infected arthropods are autoclaved or incinerated.		

G) Health and medical Surveillance

IBSL-1	IBSL-2	IBSL-3	IBSL-4
Same as BSL-1	Same as BSL-2	Same as BSL-3	Same as BSL-4

H) Emergency Procedures

IBSL-1	IBSL-2	IBSL-3	IBSL-4
Same as BSL-1	Same as BSL-2	Same as BSL-3	Same as BSL-4

3.6. AQUATIC ORGANISM BIOSAFETY LEVEL FACILITIES

3.6.1. PURPOSE

The purpose of establishment of Aquatic Organism Biosafety Level (AqBSL) Facilities is primarily to prevent escape of **aquatic animal pathogens** into the natural aquatic environment so as to protect the environment and prevent spread of infectious diseases to vulnerable aquatic animal populations in India. In addition, such containment will also offer protection to human from aquatic animal pathogens that are considered to be zoonotic or opportunistic in nature. The document sets forth the minimum physical and operational requirements for facilities **working on aquatic organisms and related pathogens** to prevent the accidental release of potentially harmful pathogens into the aquatic environment. The containment level that is required depends on the biology of the specific pathogens involved and the impact that a release of the pathogens might have on the Indian environment.

3.6.2. TYPES OF AQUATIC ORGANISM BIOSAFETY LEVEL FACILITIES

3.6.2.1. Aquatic Organism Biosafety Level 1 (AqBSL-1):

Storing, rearing and experiments with aquatic organisms that are:

i. Uninfected and does not pose health threat to humans.

ii. Genetically engineered to contain genes from RG 1 microorganisms.

iii. Challenged or infected with GE microorganisms that fall under RG 1.

iv. Challenged or infected with pathogens specific for aquatic organisms that are not considered a risk to aquatic organisms or to the aquatic environment and are non-pathogenic to humans.

3.6.2.2 Aquatic Organism Biosafety Level 2 (AqBSL-2):

Storing, rearing and experiments with aquatic organisms that are:

i. Infected with or suspected to be infected with RG 2 microorganisms or aquatic organism pathogens.

ii. Genetically engineered to contain genes from RG 2 microorganisms and other non pathogenic organisms provided the genetic engineering process does not increase virulence and environmental fitness of the organism.

iii. Challenged or infected with GE microorganisms that fall under RG 2.

3.6.2.3 Aquatic Organism Biosafety Level 3 (AqBSL-3):

Storing, rearing and experiments with aquatic organisms that are:

i. Challenged, infected or suspected to be infected with RG 3 microorganisms.

ii. Challenged, infected or suspected to be infected with aquatic organism pathogens that could harm aquatic environment, if released.

iii. Genetically engineered to contain genes from RG 2 microorganisms where the genetic engineering positively affects environmental fitness and virulence.

Note:

i. At this time, there are no pathogens requiring BSL-4 type containment for aquatic organisms i.e. AqBSL-4; however, the decision to designate a pathogen as requiring AqBSL-4 level will be made on a case-by-case basis.

ii. All existing AqBSL-3 facilities must be certified by RCGM. A format for certification is available in this guideline.

iii. The new AqBSL-3 facilities shall require certification at the time of commissioning operations as per the format.

iv. AqBSL facilities shall not be used for:

   a. Activity with any organism and related material other than aquatic organisms unless they are part of the experiments.

   b. Housing/keeping/rearing of any animals, plants, arthropods and aquatic organisms unless they are integral to the activity in the contained facility.

3.6.3. OPERATIONAL GUIDE FOR AqBSL FACILITIES

A) Facility design

AqBSL-1	AqBSL-2	AqBSL-3
Following in addition to BSL-1: i. Facility rearing area should be located out of the flow of general traffic, avoiding hallways and preferably lockable. ii. Aquatic organisms must be placed in appropriate tanks that are leak proof and sturdy. iii. Entrance door should be posted with appropriate signage identifying the type of aquatic facility. The contact information of the laboratory supervisor or other responsible persons should be listed. iv. Drainage system should be protected with at least two screens or filters of appropriate size. v. The facility must be designed at a sufficient elevation to preclude flooding or unintentional escape of these aquatic organisms.	Following in addition to AqBSL-1 and BSL-2: i. Appropriate signage indicating the nature of the aquatic animal pathogens being used (e.g. type and containment level) should be posted on the entry door to each laboratory. ii. All penetrations of the containment perimeter, including all conduits and wiring, to be sealed with non-shrinking sealant. iii. All animal holding units to be provided with covers or equivalent strategies to prevent splashing transfer between tanks and reduce room humidity. iv. Live aquatic animal entry to the holding facility to be provided in a manner that prevents breach of containment. v. Drains from live animal holding tanks, sinks, sumps, showers, or drainage in contact with contaminated materials to be connected to an effluent treatment system. vi. Drains connected to effluent treatment systems to be sloped towards the decontamination system to ensure gravity flow; consideration should be given to installing valves to isolate sections for decontamination. vii. The following provisions apply to the room housing a completely closed and contained liquid effluent treatment system:	As per AqBSL-2 and BSL-3 facility design.

vi. Doors, frames, casework and bench-tops and all material supporting animal holding units (i.e., tanks and equivalent structures) to be non-absorbent (wood surfaces are not permitted).	• Doors must be kept locked at all times. • Doors must have appropriate signage. • Room must accommodate the volume capacity of the effluent treatment system. • Floor surfaces must be sealed. • Floor drains must be sealed or re-routed to the effluent treatment system. viii. A dedicated area or necropsy room for experimental activities such as animal necropsy, tissue manipulations and surgical preparation to be provided within the containment zone ix. Backsplashes, if installed tight to wall, to be sealed at wall-bench junction. x. Waste decontamination processes (heat, chemical, etc.) must be equipped with an appropriate monitoring and recording system in order to capture critical operational parameters such as date, cycle number, time, temperature, chemical concentration and pressure. xi. Water decontamination processes (chlorine, ultra violet, heat, ozone injection, etc.) must be equipped with a monitoring and log recording system to record critical operational parameters.	

B) Safety Instrument

AqBSL-1	AqBSL-2	AqBSL-3
Following in addition to BSL-1 : i. Autoclaving: for disposal of all biological materials including contaminated liquids. ii. Non-breakable tanks to be used to hold all aquatic organisms. Tanks must be appropriately covered to prevent splash and escape of aquatic organisms.	Same as AqBSL-1 and BSL-2	Same as AqBSL-2 and BSL-3

C) Personal Protective Equipment

AqBSL-1	AqBSL-2	AqBSL-3
i. Gloves: Appropriate gloves are worn when handling ii. Torso Apparel: White laboratory coats, gowns, and/ or uniforms are worn	Follow in addition to AqBSL-1: Other Personal Protective Equipment: as appropriate e.g., respirators	Same as BSL-3

D) Procedures

AqBSL-1	AqBSL-2	AqBSL-3
Following in addition to BSL-1: i. An institutional biosafety manual describing aquatic facility practices and SOP should be prepared and adopted. ii. The screens at drainage system should be cleared regularly to prevent blockage and overflow. iii. All aquatic organisms should be kept in suitable tanks. It should be clearly labelled giving species, strain/origin, date of collection, responsible investigator. A central logbook for maintenance of stocks must be kept in the facility. iv. Living aquatic organisms should not be taken out from the facility except when they are being transferred to another containment facility or to an approved release site. Aquatic organisms taken into or out of the facility should be carried in non-breakable secure containers.	Following in addition to AqBSL-1 and BSL-2: i. Aquatic organism ingested with pathogens must be kept separate. ii. Care to be taken not to disperse viable life stages into the drainage system.	Same as BSL-3.

E) Laboratory monitoring

AqBSL-1	AqBSL-2	AqBSL-3
Same as BSL-1	Following in addition to BSL-2: i. Periodic inspections of the facility should be made to check for inward directional airflow (if applicable), faults and deterioration (e.g. deteriorated door seals). Corrective action should be taken. ii. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.	Same as BSL-3 and AqBSL-2

F) Waste management

AqBSL-1	AqBSL-2	AqBSL-3
Following in addition to BSL-1: i. Living organisms are to be disposed of only after proper decontamination. ii. All wastes from the facility (including carcasses, and rearing medium) are transported in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional or local requirements. iii. Autoclaving or incineration of material is recommended.	Following in addition to BSL-2: i. Containers are disinfected chemically and/or autoclaved if used for infected material. ii. Aquatic organism carcasses and tissues must be incinerated or processed using technology proven to effectively decontaminate all tissues. Where such materials should be transported for decontamination outside the facility, this should be done using leak-proof and impact resistant containers labelled appropriately.	Same as BSL-3 and AqBSL-2

G) Health and medical Surveillance

AqBSL-1	AqBSL-2	AqBSL-3
Same as BSL-1	Same as BSL-2	Same as BSL-3

H) Emergency Procedures

AqBSL-1	AqBSL-2	AqBSL-3
Same as BSL-1	Same as BSL-2	Same as BSL-3

CHAPTER 4

CONTAINMENT REQUIREMENT FOR IMPORT, EXPORT AND EXCHANGE

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CHAPTER 4
CONTAINMENT REQUIREMENT FOR IMPORT, EXPORT AND EXCHANGE

The handling, transfer and shipment of improperly packed specimens and hazardous microorganisms, genetically engineered organisms or cells and products thereof may carry a risk of infection/hazard to all people directly engaged in, or in contact with, any part of the process. Improper handling within the laboratory endangers not only the immediate staff but also administrative, secretarial and other support personnel. Transfer of materials between laboratories or institutions widens the scope of risk to the public and environment.

4.1. INTERNAL HANDLING PROCEDURES

Specimen containers: Specimen containers should be leak proof. No material should remain on the outside after the cap has been closed.

Transport: To avoid accidental leakage or spillage into the environment, special secondary containers should be provided for the transport of specimens between wards or departments and laboratories. These should be of metal or plastic.

Receipt of specimens: Where large numbers of specimens are received, a separate room should be provided for their receipt. In a small facility, this may be part of the laboratory room.

Opening of packages: Ideally, all packages received via mail or airfreight or other common carrier should be opened in a biological safety cabinet keeping in view of the risk associated with received material(s).

4.2. SHIPMENT BY MAIL, AIRFREIGHT OR OTHER COMMON CARRIER

The United Nations Committee of Experts on the Transport of Dangerous Goods (UNCETDG), the International Air Transport Association (IATA), the Universal Postal Union (UPU), the International Civil Aviation Organization (ICAO) and the World Health Organization (WHO) have developed agreed common definitions, packaging, and labelling requirements on dangerous goods.

4.2.1. DEFINITIONS

The definitions adopted for application as from United Nations Model Regulations are as follows (Text reproduced from the United Nations Model Regulations is italicized):

i. "*Infectious substances (or infectious materials) are defined as substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganisms*

(including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals".

ii. Based on the nature of hazard posed by the infectious substances, it has been classified into two categories:

   a. Category A: "*An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.*"

   b. Category B: "*An infectious substance which does not meet the criteria for inclusion in Category A*."

iii. *"Patient specimens are human or animal materials, collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention*."

iv. *"Biological products are those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines."*

v. *"Genetically modified microorganisms (GMMOs) not meeting the definition of infectious substance are classified in Class 9 (Miscellaneous dangerous substances and articles, including environmentally hazardous substances). GMMOs and GMOs are not subject to dangerous goods regulations when authorized for use by the competent authorities of the countries of origin, transit and destination. Genetically modified live animals shall be transported under terms and conditions of the competent authorities of the countries of origin and destination."*

4.2.2. THE PACKAGING REQUIREMENTS OF BIOLOGICAL MATERIALS

As per UN model regulation, biological products are divided into two groups for the purpose of transport:

i. **Substances that are not subject to dangerous goods regulations:** Substances that are manufactured and packaged in accordance with the requirements of appropriate national authorities and transported for the purposes of final packaging or distribution, and use for personal health care by medical professionals or individuals.

ii. **Substances covered under dangerous goods regulations:** It include two categories of infectious substances and are assigned with specific UN numbers for the purpose of shipment-

a. **Category A infectious substances:** The proper shipping name for UN 2814 is INFECTIOUS SUBSTANCE, AFFECTING HUMANS. The proper shipping name for UN 2900 is INFECTIOUS SUBSTANCE, AFFECTING ANIMALS only.

b. **Category B infectious substances:** The proper shipping name of UN 3373 is "BIOLOGICAL SUBSTANCE, CATEGORY B".

Note:

i. Substances that are not covered under dangerous goods regulations are mentioned in Guidance on regulations for the Transport of Infectious Substances 2015–2016 prepared by WHO.

ii. Indicative examples of substances that meet these criteria are given in the table in Annex 2 of "Guidance on regulations for the Transport of Infectious Substances, 2015–2016" prepared by WHO. The packaging requirements are determined by UNCETDG and are set out as Packing Instructions P620 and P650 available in Annexes 3 and 4, respectively of Guidance on regulations for the Transport of Infectious Substances 2015–2016 prepared by WHO.

iii. Some licensed biological products may present a biohazard only in certain parts of the world. In that case, competent authorities may require these biological products to be in compliance with local requirements for infectious substances or may impose other restrictions.

4.2.3. INSTRUCTIONS ON PACKAGING

4.2.3.1. For Substances that are not subject to Dangerous Goods Regulations

i. The packaging should consist of three components:

a. a leak-proof primary receptacle(s);

b. a leak-proof secondary packaging; and

c. An outer packaging of adequate strength for its capacity, mass and intended use, and with at least one surface having minimum dimensions of 100 mm × 100 mm.

ii. For liquids, absorbent material in sufficient quantity to absorb the entire contents should be placed between the primary receptacle(s) and the secondary packaging so that, during transport, any release or leak of a liquid substance will not reach the outer packaging and will not compromise the integrity of the cushioning material;

iii. When multiple fragile primary receptacles are placed in a single secondary packaging, they should be either individually wrapped or separated to prevent contact between them.

4.2.3.2. For Substances Covered under Dangerous Goods Regulations

For shipment of infectious substances, it should be ensured that packages are prepared in such a manner that they arrive at their destination in good condition and will not present any hazard to persons or animals during transport.

For such, there are variations in the packaging, labelling and documentation requirements for by Category A infectious substances (UN 2814 and UN 2900) and Category B infectious substances (UN 3373). Senders must follow the Packing Instructions P620 and P650 as determined by UNCETDG for category A and category B respectively. The overall design of packaging and labelling of category A and B infectious substances are presented in Fig.4A and 4B respectively.

In general, a triple layer packaging system (Fig. 4) is mandatory for all infectious substances: (a) a primary watertight receptacle containing the specimen; (b) a secondary watertight receptacle enclosing enough absorptive material between it and the primary receptacle to absorb all of the

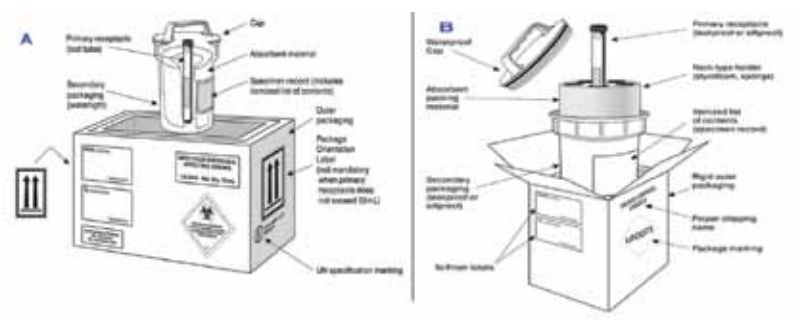

Fig. 4. Example of triple packaging system for the packaging and labelling of (A) Category A and (B) Category B infectious substances. For details please see Guidance on regulations for the Transport of Infectious Substances 2015–2016 prepared by WHO.

fluid in the specimen in case of leakage; and (c) an outer package which is intended to protect the secondary package from outside influence such as physical damage and water, while in transit. It is important to tape securely on the outside of the secondary container one copy of the specimen data forms, letters and other information that identifies or describes the specimen. (Another copy should be sent by airmail to the receiving laboratory and a third copy retained by the sender). In this manner, the receiving laboratory can identify the specimen and make the decision regarding safe internal handling and examination.

The IATA Shipper's Declaration for Dangerous Goods must also be completed for shipment by either airfreight or airmail.

The Universal Postal Union (UPU) requires that containers for international shipment of non-infectious diagnostic specimens and other biologicals materials bear the standard international violet-coloured "matieres biologiques perissables" (perishable biological substances) label (Fig. 5).

Fig. 5

4.2.4. ADDITIONAL REQUIREMENT FOR PACKAGING AND SHIPMENT OF GE ORGANISM OR THEIR DERIVED LIVING PRODUCTS (PROPAGULES, SEEDS ETC.)

i. Additional care should be taken on case by case basis to efficiently contain the GE organisms during transport and to prevent their escape into environment. In such cases, IBSC may evaluate the risks associated with GE organism and prescribe additional measures on packaging and transport.

ii. In addition to above labelling, the container containing GE organism should also be labelled indicating presence of GE organism, Biosafety level, Type and amount, contact details of person in event of unintentional release.

iii. All containers used should be sanitised prior to filling and after the GE organism have been removed, if intended to be re-used. Alternatively, containers should be destroyed after use by autoclaving or burning. Any residual materials recovered during the process of sanitisation should be rendered non-viable.

iv. If an unintentional release of GE organism during transport occurs, all attempts should be made to recover as much of the materials as possible. The location should be marked and treated in a manner that ensures that no additional release of materials occurs. Any corrective actions taken should be documented and the regulatory authorities notified.

v. After a corrective action is taken to address a compliance infraction, the authorised party should undertake a timely review of the situation to identify its cause(s) and then institute any changes in management practices or additional training of personnel to ensure that the situation is not repeated.

vi. Adequate records of the transport of GE organism as they move between research facilities, storage facilities and field trial sites should be maintained by IBSC to ensure an adequate system is in place for tracking the movement of this material.

vii. The shipper should notify the recipient of the date, kind and amount of material that will be sent before shipped. Upon receiving the material, the recipient should confirm that the shipment has arrived intact and that no material has been lost.

4.3. IMPORT AND SHIPMENT

The import, export, and exchange (within the country) of GE organisms, non-GE hazardous microorganisms and products thereof, and vectors of disease or their carriers are subject to the approval from competent regulatory authorities. Relevant permits for such activities shall be issued by IBSC, RCGM or GEAC depending on the purpose:

i. For research purpose:

   a. In order to facilitate R&D related permission for GE organisms and products thereof the RCGM had issued "Simplified Procedures & Guidelines on Exchange, Import & Export of GE organisms & Products thereof for R&D purpose" (DBT vide OM dated 22.9.2015) (Annexure 4). The guideline has empowered IBSCs to consider and approve exchange, import or export of prescribed quantities of polynucleotides, proteins, non living plant materials, GE microorganisms and cell lines that could be handled at biosafety level 1 facilities. The same shall be applicable for laboratory use of model organisms where it may carry routine and standard experimental mutations/insertions and do not carry foreign gene-insertions from non-model organisms. Import, export and exchange of above than the specified quantity of above-mentioned materials and those that require higher containment levels (2 and above) shall require permission/ approval by RCGM Secretariat. In addition, GE Organisms and product(s) thereof not covered in the said guideline shall also require approval by RCGM Secretariat. The applicant shall submit the application in requisite proforma i.e. Form B1, B3, B5 and B7 along with checklist (Annexure 5) to IBSC for its consideration and getting subsequent approval from RCGM. On request RCGM Secretariat will issue NOC/ Permit to facilitate custom clearance, in case of import.

   b. Import of GM plants and planting materials requires a permit to be issued by the Director, National Bureau of Plant Genetic Resources, New Delhi (NBPGR) of the Indian Council of Agricultural Research. The permit is issued subject to the issue of Import authorization letter by the Review Committee on Genetic Manipulation (RCGM). The authorization letter clearly specifies the scope of the import and required safety measures to be adopted during R&D work. The import consignment shall enter through Delhi Airport only where customs shall verify the documents and hand it over to NBPGR. NBPGR shall verify the contents and the accompanying documents (Import permit, Phytosanitary Certificate, Supplier Declaration that the GM material does not contain any embryogenesis deactivator gene). NBPGR also tests for the absence of diseases, pests and other undesirable material, absence of embryogenesis deactivator gene and for the presence of the declared transgene. Subject to satisfactory outcome of the tests, NBPGR shall hand over the consignment to the importer. Parts of the material (5% or 5 to 50 seeds whichever is less) will be kept at

NBPGR in a double lock system in the presence of importer. This lot of seed will act as source material in case of any legal dispute.

ii. Large scale imports for commercial/industrial use are regulated by Genetic Engineering Appraisal Committee (GEAC). However, import and marketing of products derived from living modified organisms (LMO) as Drugs and Pharmaceuticals in bulk and/or finished formulation where the end product being imported is not a LMO shall be exempted from obtaining approval from GEAC (Vide MoEF&CC notification G.S.R.616(E) dated 20th September 2006).

Note:

i. The regulations on import, export and exchange shall not override any other existing regulations or guidelines, unless specified here.

ii. In case of export of biological materials belonging to SCOMET category, the applicant needs to apply to Directorate General of Foreign Trade (DGFT), Min. of Commerce in addition to permission from IBSC. Information is available on www.dgft.gov.in.

iii. Access to biological resources and / or associated knowledge for research, bio-survey and bio-utilization, commercial utilization, obtaining Intellectual Property Rights, transfer of results of research and transfer of accessed biological resources shall also fulfil the regulatory criteria as prescribed by National Biodiversity Authority.

iv. The import and export of any biological materials shall also meet the quarantine regulations, wherever applicable.

v. Approval procedures for import and export of GE plants and planting materials are described in the 'Procedure of Import and Export of GM Plants & Planting Material' published by MoEF&CC (http://www.geacindia.gov.in/resource-documents.aspx).

4.4. STORAGE OF GE ORGANISMS AND RELATED MATERIALS

i. GE organism should be clearly labelled and stored in isolation in such a way that it could not be mixed with other GE and non-GE organisms or conventional materials (e.g. filing cabinet, refrigerator, office, closet, cold room).

ii. It is preferable to use separate cabinet/ room/ refrigerator to store GE organism. This will be mandatory for organisms belonging to Risk Group 3 and above. All storage areas should be clearly labelled at the point of access as containing GE organism and access should be limited to authorised personnel only. All personnel who have access to the storage areas should be adequately trained on the labelling, storage and disposal procedures.

iii. Where a storage area is used to store multiple samples of GE organism, each item should be

stored separately in a sealed, labelled container such as a primary container for shipment.

iv. Proper care should be taken to maintain appropriate storage condition including temperature while storage of GE organism.

v. Appropriate pest control should be implemented to ensure that pests do not damage storage containers, mix or remove GE organism and related material from the storage facility.

vi. Storage areas should be cleaned prior to and immediately following the period of storage. Any residue or other material recovered during cleaning or any material removed from storage for disposal, should be rendered non-viable.

vii. Access to the area for the purpose of inspection should be provided to regulatory officials upon request, provided they present official identification documents and the inspection is undertaken at a reasonable time.

viii. In the event of any suspected unintentional release of GE organism from storage, emergency action plans should be adopted and competent authority should be informed.

ix. An inventory of all GE organisms in storage should be maintained. Sub-samples that may be removed from storage when required for experimental or other purposes should be recorded in the inventory list.

x. The storage area should be checked and maintained at regular intervals to avoid unintentional release of GE organism into the environment and such inspections should be recorded. These inspections should include checks on the integrity of material packaging that may have been deployed.

GLOSSARY

Biosafety	The maintenance of safe conditions in biological research to prevent harm to workers, non-laboratory organisms and the environment.
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 .
Goods	Tangible products, including animals and plants, transported on an international voyage, including those for utilization on board a conveyance.
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.
Indicator	It is a variable that can be measured repeatedly (directly or indirectly) over time to reveal change in a system. It can be qualitative or quantitative, allowing the objective measurement.
Isolation	Separation of ill or contaminated persons or affected baggage, containers, conveyances, goods or postal parcels from others in such a manner as to prevent the spread of infection or contamination.
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.

Quarantine	The restriction of activities and/or separation from others of suspect persons who are not ill.
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.
Specimen	It refers to biological materials taken by sampling, from an individual or animal for laboratory analysis to diagnose some medical conditions including a disease process. Common examples include throat swabs, sputum, urine, blood, surgical drain fluids and tissue biopsies.
Surveillance	The systematic ongoing collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health response as necessary.
Warning system	A specific procedure to detect and report any abnormal occurrences as early as possible.
Zoonosis	Any infection or infectious disease that is naturally transmissible from vertebrate animals to humans.

OTHER APPLICABLE POLICIES

i. Bio-medical Waste Management Rules, 2016.

ii. Biological Diversity Act, 2002.

iii. Disaster Management Act, 2005.

iv. Food Safety and Standards Act, 2006.

v. Hazardous Waste Rules, 2016.

vi. Industries (Development & Regulation) Act, 1951 - New Industrial Policy & Procedures, 1991.

vii. Plant Quarantine (Regulation of Import into India) Order, 2003.

viii. Protection of Plant Varieties and Farmers' Rights Act 2001, PPV & FR Regulations 2006.

ix. Seeds Act, 1966; Seeds Rules, 1968; Seeds (Control) Order, 1983; Seeds Policy 1988 & 2002.

REFERRED INTERNATIONAL GUIDELINES

i. WHO guideline on Laboratory Biosafety manual, 3rd Edition (2004).

ii. NIH guidelines for research involving recombinant or synthetic nucleic acid molecules (2016).

iii. ACGM guidelines (http://www.gla.ac.uk/media/media_81030_en.pdf;http://www.gla.ac.uk/media/media_81041_en.pdf; and http://www.gla.ac.uk/media/media_81044_en.pdf).

iv. The Approved List of biological agents prepared by Advisory Committee on Dangerous Pathogens (ACDP) (http://www.hse.gov.uk/pubns/misc208.pdf).

v. The Centers for Disease Control and Prevention (CDC) guideline on Biosafety in Microbiological and Biomedical Laboratories 5th Edition (2009).

vi. A Practical Guide to Containment: Plant biosafety research in greenhouse (http://www.uab.cat/doc/guiesref_plantcontainment_2008).

vii. The Genetically Modified Organisms (Contained Use) Regulations, 2014 (http://www.hse.gov.uk/pubns/books/l29.htm).

viii. OGTR (2015) Application to certify facilities (http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/certifications-1).

ix. WHO (2015) Guidance on regulations for the Transport of Infectious Substances 2015–2016.

x. Ministry of Natural Resources and Environment, Malaysia (2012) Biosafety Guidelines: Risk Assessment of Genetically Modified Microorganisms.

xi. Ministry of Natural Resources and Environment Malaysia (2010) Biosafety Guidelines for Contained Use Activity of LMO.

xii. GMAC, Singapore (2006) The Singapore Biosafety Guidelines for Research on Genetically Modified Organisms (GMOs)

xiii. OGTR (2011) Dealings exempt from licensing (http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/exemptdealings-1Sept2011-htm/$FILE/exemptdealings-1sept2011.pdf)

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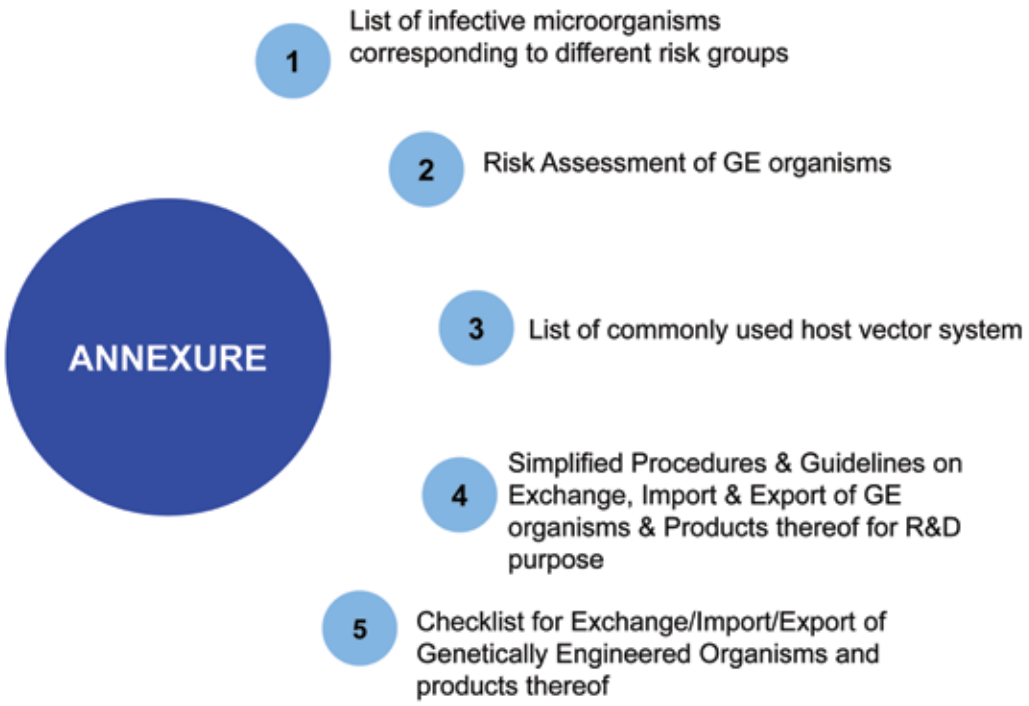
ANNEXURE
1
List of infective microorganisms corresponding to different risk groups
2
Risk Assessment of GE organisms
3
List of commonly used host vector system
4
Simplified Procedures & Guidelines on Exchange, Import & Export of GE organisms & Products thereof for R&D purpose
5
Checklist for Exchange/Import/Export of Genetically Engineered Organisms and products thereof

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ANNEXURE 1: LIST OF INFECTIVE MICROORGANISMS CORRESPONDING TO DIFFERENT RISK GROUPS

i. The list is indicative but not exhaustive and will be updated periodically.

ii. For working with organisms not listed here, investigators should determine the appropriate containment level with consultation with IBSC.

iii. Depending upon the work envisaged, organisms listed in higher risk groups (RG 2/3) may be handled in lower containment laboratory provided, if the work does not involve genes known to be involved in pathogenesis/carcinogenesis/production of toxins, etc. Accordingly, lower containment facility may be used with prior approval from IBSC and information to RCGM.

A. List of Risk Group 1 microorganisms

Bacteria

- *Acetobacter spp.*
- *Actinoplanes spp.*
- *Agrobacterium spp.*
- *Alcaligenes aquamarinus*
- *Aquaspirillum spp.*
- *Arthrobacter spp.*
- *Azotobacter spp.*
- *Bacillus spp., except cereus and anthracis*
- *Bifidobacterium.spp., except dentium*
- *Bradyrhizobium spp.*
- *Brevibacterium spp.*
- *Caryophanon spp.*
- *Clostridium spp. i.e. C. aceticum, C. acetobutylicum, C. acidiuric, C. cellobiparum, C. kluyveri, C. thermoaceticum, C. thermocellum, C. thermosulfurogenes*
- *Corynebacterium spp. i.e. C. glutomicum, C. lilium*
- *Enterococcus facium*
- *Erwinia spp. except E. chrysanthemi, E. amylovora and E. herbicola*
- *Gluconobacter spp.*
- *Klebsiella planticola*
- *Lactobacillus spp. i.e. L. acidophilus, L. bauaricus, L. breuis, L. bucneri, L. casei, L. cellobiosis, L. fermentum, L. helveticus, L. sake*
- *Lactococcus lactis*
- *Leuconostoc spp.*
- *Lysobacter spp*
- *Methanobacter spp.*
- *Methylomonas spp.*
- *Micrococcus spp.*
- Nonpathogenic *Escherichia coli* e.g. ATCC 9637, NCIB 8743, K12 and derivatives
- *Pediococcus spp.*
- *Pseudomonas spp. i.e. P. fluorescens, P. gladioli, P. syringae*
- *Ralstonia spp.*
- *Rhizobium spp.*
- *Rhodobacter spp.*

- *Rhodopseudomonas spp.*
- *Rickettsiella spp.*
- *Staphylococcus carnosus*
- *Streptococcus salivarius*
- *Streptomyces spp*
- *Thermobacteroides spp.*
- *Thermus spp.*
- *Thiobacillus spp.*
- *Vibrio spp. i.e V. fischeri, V. diazotrophicus*

Fungi

- *Agaricus bisporus*
- *Acremonium spp. i.e. A. chrysogenum, A. elegans,*
- *Actinomucor elegans*
- *Ashbya gossypii*
- *Aspergillus oryzae*
- *Aureobasidum pullulans*
- *Blakeslea trispora*
- *Brettanomyces bruxellensis*
- *Candida spp. i.e. C. boindinii, C. utilis*
- *Chaetomium globosum*
- *Cladosporium cladosporioides*
- *Claviceps spp. i.e. C. purpurea,C. paspali*
- *Coprinus cinereus*
- *Cunninghamella spp. i.e. C. blakesleana, C. elegans*
- *Cyathus stercoreus*
- *Dacrymyces deliquescens*
- *Debaryomyces hansenii*
- *Engyodontium album*
- *Hansenula spp. i.e. H. anomala, H. polymorpha*
- *Hypholama spp. i.e. H. fasciculare, H. roseonigra*
- *Lentinus edodes*
- *Lipomyces lipofer*
- *Metarhizium anisopliae*
- *Monascus pupureus*
- *Moniliella suaveolens*
- *Mortierella vinacea*
- *Mucor spp. i.e, M. mucedo, M. plumbeus, M. rouxii*
- *Neurospora spp. i.e. N. crassa, N. sitophilla*
- *Nigrospora sphaerica*
- *Oxyporus populinus*
- *Pachysolen tannophilus*
- *Paecilomyces varioti*
- *Penicillium spp. i.e. P. funiculosum, P. camemberti, P. chrysogenum*
- *Phycomyces blakesleanus*
- *Pichia spp. i.e. P. membranae faciens, P. farinosa, P. guilliermondii, P. stipitis*
- *Pleurotus ostreatus*
- *Rhizoctonia solani*
- *Rhodosporidum toruloides*
- *Rhodotorula glutinis*
- *Saccharomyces cerevisiae*
- *Schizosaccharomyces pombe*
- *Schwanniomyces occidentalis*
- *Sordaria macrosopra*
- *Thanatephorus cucumeris*

- *Trametes versicolor*
- *Trichoderma spp. i.e. T. harzianum, T. viride*
- *Trigonopsis variabilis*
- *Verticillium lecanii*
- *Volvariella volvacea*
- *Wallernia sebi*
- *Xeromyces bisporus*
- *Zygorhynus moelleri*
- *Zygosaccharomyces spp. i.e. Z. bailii, Z. rouxii*

Virus

- Apathogenic, endogeneous, animal retroviruses
- Attenuated viral strains which are accepted vaccines. Only a limited number of passages in defined cell culture or host-systems are allowed
- Baculoviruses of insects
- Newcastle disease virus - strains licensed for vaccine use
- Influenza virus A/PR/8/34
- Poikilothermal vertebrate retrovirus
- Rinderpest - attenuated virus strain (e.g. Kabatte-O) licensed for vaccine use.
- Viral strains from fungal or bacterial systems, provided they do not contain virulence-factors and are described as apathogenic for higher animals and human beings

B. List of Risk Group 2 microorganisms

Bacteria

- *Acinetobacter spp. i.e., A. calcoaceticus, A. lwoffii*
- *Actinobacillus spp.*
- *Actinomadura spp. i.e., A. madurae, A. pelletieri*
- *Actinomyces spp. i.e., A. israelii, A. bovis*
- *Aeromonas hydrophila*
- *Afipia spp*
- *Aggregatibacter actinomycetemcomitans*
- *Agrobacterium radiobacter*
- *Alcaligenes spp.*
- *Amycolata autotrophica*
- *Anaplasma spp.*
- *Arachnia propionica*
- *Archanobacterium haemolyticum*
- *Arizona hinshawii - all serotypes*
- *Bacillus spp. i.e., B. anthracis, B. cereus*
- *Bacteroides spp.*
- *Bartonella spp. i.e., B. bacilliformis, B. henselae*
- *Bifidobacterium dentium*
- *Bordetella spp. i.e., B. avium, B. bronchiseptica, B. parapertussis, B. pertussis, B.quintana, B.vinsonii*
- *Borrelia spp. i.e., B. burgdorferi, B.recurrentis, B.duttonii, B.vicenti*
- *Brucella ovis*
- *Burkholderia spp. i.e., B. cepacia B.mallei (Pseudomonas mallei)*

- *Campylobacter spp. i.e., C. coli, C. fetus subsp. fetus, C. jejuni*
- *Cardiobacterium hominis*
- *Chlamydia spp. i.e., C. pneumonia, C. trachomatis*
- *Chlamydophila pneumonia*
- *Chlamydia trachomatis*
- *Citrobacter spp.*
- *Cladosporium (Xylohypha) trichoides*
- *Clostridium spp. i.e., C. chauvoei, C. difficle, C. fallax, C.haemolyticum, C. histolyticum, C.novyi, C. perfringens, C. septicum*
- *Corynebacterium spp. i.e., C. diphtheriae , C. minutissimum, C. pseudotuberculosis, C. renale, C. ulcerans*
- *Coxiella burnetii* - specifically the Phase II, Nine Mile strain, plaque purified, clone 4
- *Cytophaga spp. pathogenic to animals*
- *Dermatophilus congolensis*
- *Edwardsiella tarda*
- *Eikenella coreodens*
- *Enterobacter spp.*
- *Enterococcus faecalis*
- *Eperythrozoon spp.*
- *Erysipelothrix spp. i.e., E. rhusiopathiae, E. tonsillarum*
- *Escherichia coli (excluding non-pathogenic strains*
- *Flavobacterium meningosepticum*
- *Fluoribacter bozemanae*
- *Francisecla tularensis*
- *Fusobacterium spp. including F. necrophorum*
- *Gardnerella vaginalis*
- *Haemophilus spp. i.e.,H. ducreyi, H. influenzae*
- *Helicobacter spp. i.e., H. pylori, H. hepaticus*
- *Klebsiella spp. i.e., K. pneumonia, K. mobilis, K. oxytoca*
- *Legionella spp. i.e., L. pneumophila*
- *Leptospira interrogans - all serotypes*
- *Listeria spp.*
- *Moraxella spp.*
- *Morganella morganii*
- *Mycobacterium BCG vaccine strain*
- *Mycoplasma spp. i.e., M. agalactiae, M. mycoides, M. caviae , M. hominis, M. pneumoniae*
- *Neisseria spp. i.e., N. meningitidis, N. gonorrhoeae*
- *Nocardia spp. i.e., N. otitidiscaviarum, N. brasiliensis, N. farcinica, N. nova, N. otitidiscaviarum, N. asteroids, N. transvalensis*
- *Pantoea agglomerans*
- *Pasteurella* - all species except those listed in RG 3
- *Peptococcus spp.*
- *Peptostreptococcus spp.*
- *Porphyromonas spp.*
- *Prevotella spp.*
- *Proteus spp. i.e., P. mirabilis, P. penneri, P. vulgaris*

- *Providencia alcalifaciens*
- *Pseudomonas aeruginosa*
- *Rhodococcus equi*
- *Salmonella spp. i.e., S. abortusequi, S.abortusovis, S. arizonae, , S. choleraesuis, S.dublin, S. enteritidis, S. gallinarum, S. meleagridis, S. paratyphi, A, B, C, S. typhi, S.pullorum, S. typhimurium*
- *Serpulina spp.*
- *Serratia spp. i.e., S.liquefaciens, S. marcescens*
- *Shigella spp. i.e., S.boydii, S. dysenteriae, S. flexneri, S. sonnei*
- *Sphaerophorus necrophorus*
- *Staphylococcus spp. i.e., S. aureus, S. epidermidis*
- *Streptobacillus moniliformis*
- *Streptococcus spp. i.e., S.agalactiae, S. dysgalactiae, S. pneumoniae, S. pyogenes, S. suis, S. uberis, S. equi*
- *Streptomyces somaliensis*
- *Treponema spp. i.e., T.pertenue, T. carateum,T. pallidum*
- *Ureaplasma urealyticum*
- *Veillonella spp.*
- *Vibrio spp. i.e., V. parahaemolyticus, V. vulnificus, V. cholerae, V. fluvialis, V. metschnikovii, V. mimicus*

Fungi

- *Acremonium spp. i.e., A. kiliense, A. recifei, A. falsiforme, A. strictum*
- *Arthroderma benhamiae/simiti*
- *Aspergillus spp. i.e., A. fumigatus, A. flavus, A. parasiticus*
- *Basidiobolus haptosporus*
- *Blastomyces dermatitidis*
- *Candida spp. i.e., C. albicans, C. tropicalis*
- *Cryptococcus neoformans var gattii (Filobasidiella bacillispora)*
- *Curvularia lunata*
- *Dactylaria galopava*
- *Emmonsia parva var crescens, var parva*
- *Epidermophyton spp. including:E. floccosum*
- *Exophilia spp. i.e., E. castelanii, E. dermatitidis, E. mansonii*
- *Filobasidiella neoformans*
- *Fonsecaea spp. i.e., F. compacta, F. pedrosoi*
- *Fusarium coccophilum*
- *Geotrichum candidum*
- *Histoplasma capsulatum*
- *Hortaea werneckii*
- *Leptosphaeria spp. i.e., L. senegalensis, L. thompkinsii*
- *Loboa loboi*
- *Madurella spp. i.e., M. grisea, M. mycetomi*
- *Microsporum spp. i.e., M. audouinii, M. canis, M. distortum, M. duboisii, M. equinum, M. ferrugineum, M. gallinae, M. gypseum, M. praecox, M. nanum, M. persicolor*
- *Monosporium apiospermum*
- *Myrothecium verrucaria*
- *Nannizzia spp. i.e., N. gypsea, N. obtussa, N. otae*

- *Neotestudina rosatii*
- *Paceilomyces lilacinus*
- *Paracoccidioides brasiliensis*
- *Penicillium marneffei*
- *Phialophora verrucosa*
- *Pseudollescheria boydii*
- *Rhinocladiella spp. i.e., R. compacta, R. pedrosoi, R. spinifera*
- *Rhinosporidium seeberi*
- *Rhizomucor pusillus*
- *Rhizopus spp. i.e., R. cohnii, R. microspous*
- *Scedosporium spp. i.e., S. apiospermum, S. prolificans*
- *Sporothrix schenckii*
- *Trichophyton spp. i.e., T. cocentricum, T. equinum, T. erinacei, T. gourvilli, T. megninii, T. mentagrophytes, T. rubrum, T.schoenleinii, T. smii, T. soudanense, T. tonsurans, T. verrucosum, T. violaceum, T. yaoundei*
- *Xylophora carrionii*

Virus

- Adeno-associated viruses (AAV)
- Aino virus
- All isolates of Orthoreovirus and Orbivirus
- Alphaviruses
- Animal adenoviruses
- Animal papillomaviruses
- Animal papillomaviruses
- Astroviruses
- Aura-virus
- Avian viruses. i.e. adenovirus, encephalomyelitis, enterovirus, influenza, poxvirus, retrovirus, encephalomyelitis virus, reticuloendotheliosis virus, smallpox virus, sarcoma virus
- Barmah forest virus
- Batai virus
- Bebaru virus
- Bern-virus
- BK-virus
- Border disease virus
- Borna virus
- Bovine ephemeral-fever virus
- Bovine foamy virus
- Bovine herpesvirus 2, 3, and 4
- Bovine mucosal disease virus
- Bovine papilloma virus
- Bovine polyomavirus (BPoV)
- Bovine rhinoviruses (types 1-3)
- Breda virus
- Bunyamwera virus
- Cache Valley virus
- Caliciviruses
- California encephalitis virus
- Canine distemper virus
- Canine parvovirus (CPV)
- Chikungunya vaccine strain 181/25
- Chikungunya virus (for studies except vector inoculation, transmission)
- Chimpanzee herpesvirus
- Chuzan virus
- Coltivirus all types including Colorado tick fever virus

- Coxsackie A and B viruses
- Cytomegalovirus
- Dengue virus (for studies except vector inoculation, transmission)
- Drosophila X virus
- Eastern equine encephalomyelitis virus
- Echoviruses - all types
- Ectromelia virus
- Emsliki Forest virus
- Encephalomyocarditis virus (EMC)
- Enterovirus
- Entomopoxviruses
- Equine infectious anemia virus
- Equine influenza virus 1 (H7N7) and 2 (H3N8)
- Equine rhinopneumonitis virus
- Exogenous retroviruses (i.e. murine mammary-tumor virus, feline immuno-deficiency virus)
- Feline calcivirus
- Flanders virus
- Fort morgan virus
- Hart Park virus
- Hepatitis A & D
- Herpes simplex types 1
- Herpes zoster
- Human adenoviruses
- Human herpesvirus types 6 and 7
- Human papillomaviruses (HPV)
- Human parvovirus (B 19)
- Human rhinoviruses
- HVJ virus (Sendai virus)
- Infectious Bovine Rhinotraecheitis virus (IBR)
- Infectious Bursal diseases of poultry
- Infectious Laryngotraechitis (ILT)
- Influenza viruses - all types except A/PR/8/34, which is in RG 1
- JC virus
- Japanese encephalitis virus (for studies except vector inoculation, transmission)
- Rhinoviruses - all types
- Ross river virus
- Rota virus
- Rubella virus
- Sandfly fever virus
- Shope fibroma virus
- Simian foamy virus
- Simian virus 40
- Simian viruses - all types except Herpervirus simiae (Monkey B virus) and Marburg virus which are in RG 4
- Junin virus
- Langat virus
- Lassa virus
- Lumpy Skin Disease (LSD)Virus
- Lumpy skin disease virus
- Lymphocytic choriomeningitis virus (nonneurotropic strains)
- Lymphocytic choriomeningitis virus (non-neurotropic strains)
- Mammalian retrovirus (except HIVm HTLV-1 (ATLV) and HTLV-II)

- Marek's Disease virus
- Measles virus
- Minute virus in mice
- Molluscan contagiosum virus
- Monkey (SV40, SA-12, STMV, LPV)
- Mouse hepatitis virus
- Mouse rotaviruses (EDIM, epizootic diarrhoea of infant mice)
- Mumps virus
- Murine penumoniae virus
- Myxoma virus
- NDV
- O'nyong-nyong virus
- Orbi virus
- Other avipoxviruses
- Parainfluenza viruses
- Paramyxoviruses
- Parvovirus
- Pichinde virus
- Pixuna virus
- Polio viruses-all types, wild and attenuated
- Polyoma virus
- Porcine adenovirus
- Poxviruses- All types except Alastrim, Monkey pox, Sheep pox and White pox
- Pseudorabies virus
- Rabies (fixed, attenuated) virus
- Rat rotavirus
- Reovirus
- Respiratory syncytial virus
- Reticuloendotheliosis viruses (REV)
- Rhabdoviruses
- Shope fibroma virus
- Simbu virus
- Sindbis virus
- Stomatitis papulosa virus
- Swine vesicular disease virus
- Tensaw virus
- Turlock virus
- Una virus
- Uukuniemi virus
- Vaccinia virus
- Varicella virus
- Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
- Vesicular stomatitis virus
- Vesiculovirus
- Yellow fever virus vaccine strain 17D
- Sindibis virus

Parasites

- *Ancylostoma human hookworms*
- *Acanthamoeba spp.*
- *Ancylostoma spp. i.e., A. ceylanicum, A. duodenale*
- *Angiostrongylus spp.*
- *Anisakis simplex*
- *Ascaris lumbricoides*
- *B.microti*
- *Babesia spp. i.e., B. divergens*
- *Balantidium coli*

- *Blastocystis hominis*
- *Brugia filaria worms: B. malayi, and B. timori*
- *Capillaria spp.*
- *Coccidia spp.*
- *Cryptosporidium spp.*
- *Cysticercus cellulosae* (hydatid cyst, larva of T. solium)
- *Dicrocoelium dendriticum*
- *Dientamoeba fragilis*
- *Dracunculus medinensis*
- *Echinococcus spp. i.e., E. granulosus, E. multilocularis, E. vogeli*
- *Entamoeba histolytica*
- *Enterobius spp.*
- *Enterobius vermicularis*
- *Fasciola spp. i.e., F. hepatica, F. gigantica*
- *Giardia lamblia*
- *Heterophyes spp.*
- *Hymenolepis spp. i.e., H. diminuta, H. nana*
- *Isospora spp.*
- *Leishmania spp. i.e., L. braziliensis, L. donovani, L.ethiopia, L. peruvania, L. tropica, L. major, L. mexicana*
- *Loa loa filaria worms*
- *Mansonella spp. i.e., M. ozzardi, M. perstans, M. streptocerca*
- *Metagonimus spp.*
- *Microsporidium*
- *Naegleria spp. i.e., N. fowleri, N. gruberi*
- *Necator americanus*
- *Necator human hookworms*
- *Nosema bombycis*
- *Onchocerca filaria worms*
- *Onchocerca volvulus*
- *Opisthorchis spp. i.e., O. felineus, O. sinensis (Clonorchis sinensis), O. viverrini (Clonorchis viverrini), O. filaria, O. volvulus*
- *Plasmodium spp. i.e., P. cynomolgi, P. falciparum, P. malariae, P. ovale, P. vivax, P. westermani*
- *Sarcocystis sui hominis*
- *Schistosoma spp. i.e., S. mansoni, S. haematobium, S. intercalatum, S. japonicum, S. mekongi*
- *Strongyloides stercoralis*
- *Taenia solium*
- *Toxocara canis*
- *Toxoplasma gondii*
- *Trichinella spp. i.e., T. nativa, T. nelso, T. pseudospiralis, T. spiralis*
- *Trichomonas vaginalis*
- *Trichostrongylus orientalis*
- *Trichuris trichiura*
- *Trypanosoma spp. i.e., T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
- *Wuchereria bancrofti*

Bacteria – plant pathogens

- *Agrobacterium spp. i.e., A. rhizogenes, A. rubi, A. tumefaciens*
- *Clavibacter michiganensis*

- *Erwinia spp. i.e., E. carotovora subsp. betavasculorum, E. chrysanthemi pv. Chrysanthemi*
- *Pseudomonas spp. i.e., P. cichorii, P. fluorescens, P. syringae subsp. syringae*
- *Rhodococcus fascians*
- *Xanthomonas campestris pv. alfalfae*

Fungi – Plant pathogens

- *Alternaria dauci*
- *Botrytis spp. i.e., B. allii, B. elliptica*
- *B. hyacynthi, B. tulipae*
- *Cladosporium spp. i.e., C. phlei, C. variabile*
- *Claviceps purpurea*
- *Fusarium spp. i.e., F. arthrosporioides, F. culmorum, F. graminum, F. oxysporum f. sp. betae, F. oxysporum f. sp. pisi*
- *Glomerella spp. i.e., G. cingulata (anamorph Colletotrichum gloeosporioides), G. graminicola, G.tucamanensis (anamorph Colletotrichum falcatum)*
- *Mucor circnelloides*
- *Penicillium spp. i.e., P. corymbiferum, P. cyclopium, P. digitatum, P. expansum, P. italicum*
- *Phytophthora spp. i.e., P. infestans, P. megasperma*
- *Rhizoctonia spp. i.e., R. carotae, R. fragariae, R. tuliparum*
- *Rhizopus spp. i.e., R. arrhizus, R. stolonifer, R. oryzae*
- *Sclerophthora spp. i.e., S. macrospora, S. graminicola*
- *Sclerotinia minor*
- *Sclerotinia trifoliorum*
- *Septoria spp. i.e., S. azalea, S. lactucae*
- *Trichoderma longibrachaiatum*

Virus – plant pathogens

- Alfalfa mosaic virus
- Apple chlorotic leaf spot virus
- Apple mosaic virus
- Apple stem grooving virus
- Barley yellow mosaic virus
- Beet western yellows virus
- Carnation ringspot virus
- Cucumber mosaic virus
- Hop mosaic virus
- Maize dwarf mosaic virus
- Melon necrotic spot virus
- Papaya ringspot virus
- Pea early-browning virus
- Potato leafroll virus
- Potato virus A, M, S, X, and Y
- Tobacco mosaic virus
- Tobacco necrosis virus
- Tobacco rattle virus
- Tobacco stunt virus
- Tomato mosaic virus
- Tulip breaking virus
- Turnip crinkle virus
- Turnip mosaic virus

C. List of Risk Group 3 microorganisms

Bacteria

- *Actinobacillus mallei*
- *Bacillus anthracis*
- *Bartonella bacilliformis*
- *Bordetella bronchiseptica*
- *Brucella spp. (except B. ovis,* listed in Risk Group 2) *i.e. B. abortus, B.canis, B.melitensis, B.suis*
- *Burkholderia (Pseudomonas) mallei*
- *Campylobacter fetus subsp. venerealis*
- *Chlamydia psittaci (avian strains)*
- *Cladosporium spp. i.e., C. bantianum,C. (Xylohypha) trichoides*
- *Coccidiodes immitis*
- *Clostridium spp. i.e. C. botulinum, C. tetani*
- *Coxiella burnetii*
- *Ehrlichia spp. including E. sennetsu*
- *Francisella tularensis*
- *Mycobacterium spp. i.e. M. marinum, M. scrofuiaceum, M. ulcerans, M. africanum, M. avium, M. bovis (except the BCG strain), M. chelonae, M. leprae, M. tuberculosis, M. fortuitum*
- *Mycoplasma spp.*
- *Pasteurella multocida*
- *Pseudomanas mallei, P.*
- *R. typhi pseudomallei*
- *Yersinia pestis*

Virus

- Alastrim, Monkeypox, Whitepox viruses (when used *in vitro*)
- African Horse Sickness Virus (Attenuated strain except animal passage)
- Akabane virus
- Avian herpesvirus 1 (ILT)
- Avian influenza virus A-Fowl plague
- Avian leucosis viruses (ALV)
- Blood-borne hepatitis viruses
- Blue Tongue virus (only serotypes reported in India)
- Border disease virus
- Borna diseases virus (Gr. Bornaviridae)
- Bovine diarrhoea virus
- Bovine herpesvirus 1
- Bovine immunodeficiency virus (BIV)
- Cabassou virus
- California encephalitis virus
- Chikungunya virus
- Chimeric Viruses
- Classical Swine Fever Virus
- Contagious Bovine Pleuropneumonia Agent
- Contagious Caprine Pleuropneumonia Agent
- Dengue virus type 1-4
- Eastern equine encephalitis virus
- Enzephalitis virus
- Epstein - Barr virus
- Equine arteritis
- Everglades virus
- Feline immunodeficiency virus (FIV)

- Feline Leukemia
- Feline sarcoma virus (FeSV)
- Flexal virus
- Foot and Mouth Disease virus
- Fowl plague virus
- Gibbon Ape Lymphosarcoma
- Hantaan virus (Korean haemorrhagic fever)
- Hazara virus
- Hepatitis virus (type B, C and E)
- Herpes simplex 2
- Herpes simplex saimiri
- Herpes virus ateles
- Herpes virus B
- Hog cholera virus
- Human immunodeficiency viruses (HIV) types 1 & 2
- Human T-cell lymphotropic viruses (HTLV) types 1 & 2
- Infectious Equine Anaemia virus
- Infectious pancreatic necrosis virus (Gr. Birnaviridae)
- Japanese encephalitis virus
- Las Crosse virus
- Leukomogenic murine oncovirus (Murine lymphosarcoma virus: MuLV)
- Lymphocytic choriomeningitis virus (LCM) (neurotropic strains) (Gr. Arenaviridae)
- Lymphosarcoma viruses of nonhuman primates
- Mayaro virus
- Meningitis virus
- Middle East respiratory syndrome coronavirus (MERS-CoV)
- Middelburg virus
- Mobala virus (Gr. Arenaviridae)
- Monkey mammary tumor viruses (MPTV)
- Mucambo virus
- Murine mammary tumor viruses (MMTV)
- Murine sarcoma viruses (MuSV)
- Murray Valley encephalitis virus
- Nairobi sheep disease virus
- Newcastle disease virus (Asiatic strains)
- Israel turkey meningocephalomyelits virus
- Louping ill virus
- Lumpy skin disease virus
- Oropouche virus
- Pappataci-fever virus
- Porcine sarcoma virus
- Pseudorabies virus
- variant of vaccinia
- Rabies street virus, when used inoculations of carnivores
- Rabies virus
- Rat lymphosarcoma virus (Murine lymphosarcoma virus: MuLV)
- *Rickettsia spp. i.e. R. bellii, R. canada, R. conori, R.akari, R. australis, R. montana, R. parkeri, R. sibirica, R. tsutsugamushi*
- Rift Valley fever virus
- SARS-associated coronavirus (SARS-CoV)
- Semliki Forest virus
- Sheep pox (field strain)

- Simian immunodeficiency viruses (SIV)
- Simian sarcoma viruses (SSV)
- St. Louis encephalitis virus
- Swine Fever virus
- Swine vesicular disease virus
- Tacaribe virus
- Vesicular Somatitis virus
- West Nile virus (WNV)
- Wooly Monkey fibrosarcoma
- Wesselsborn disease virus
- Yaba pox virus

Fungi

- *Ajellomyces capsulatus/dermatitides*
- *Coccidioides immitis*
- *Cladosporium trichoides*
- *Histoplasma duboisii*
- *Histoplasma farciminosum*
- *Paracoccidioides brasiliensis*

Parasites

- *Eimeria spp. i.e. E. acervulina, E. burnetti, E. maxima, E. necratix*
- *Theileria spp. i.e. T. annulata, T. hirei, T. parva*
- *Trichomonas spp. i.e. T. foetus, T. brucei*

Prions including Bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), Gerstmann-Sträussler-Scheinker encephalopathy (BSE)

Bacteria –Plant Pathogen(s)

- *Erwinia spp. i.e. E.salicis, E. tracheiphila*
- *Pseudomonas syringae pv. phaseolicola and pv. Pisi*
- *Xanthomonas spp. i.e. X.campestris pv. aberrans, X. populi*

Virus- Plant Pathogen(s)

- Lettuce mosaic virus
- Tobacco streak virus
- Tomato bushy stunt virus
- Tomato yellow leaf curl virus
- Wheat dwarf virus
- Wheat spindle steak mosaic virus

Fungi- Plant Pathogen(s)

- *Alternaria solani*
- *Botrytis fabae*
- *Claviceps gigantea*
- *Fusarium spp. i.e. F. coeruleum, F. oxysporum f. sp. lycopersici, F.oxysporum f. sp. trifolii, F.solani f. sp. cucurbitae, F.solani f. sp. phaseoli, F.solani f. sp. pisi*
- *Mucor spp. i.e. M. circinelloides, M. piriformis, M. racemosus, M. strictus*
- *Septoria spp. i.e. S.apiicola, S.chrysanthemella, S.lycopersici var. lycopersici*

Parasites- Plant Pathogen(s)

- *Heterodera glycines*

D. List of Risk Group 4 microorganisms

- Alastrim, Monkeypox, Whitepox viruses (when used for transmission and animal inoculation experiments)
- African horse sickness virus (serotype not reported in India and challenge strains)
- African swine fever virus (Gr. Adenoviridae)
- All Hemorrhagic fever agents
- *Besnoitia besnoiti*
- Crimean-Congo hemorrhagic fever virus (Gr. Bunyaviridae)
- Ebola fever virus
- Ephemeral fever virus
- Equine morbillivirus (Hendra virus)
- FMD virus (Exotic types)
- Guanarito virus
- Hanzalova virus
- Herpesvirus simiae (Herpes B or Monkey B virus)
- Junin virus (Gr. Arenaviridae)
- Kyasanur forest virus
- Lassa virus (Gr. Arenaviridae)
- Machupo virus (Gr. Arenaviridae)
- Marburg virus
- Mopeia viruses (Gr. Arenaviridae)
- Rift Valley fever virus
- Sabia (Gr. Arenaviridae)
- Tick-borne encephalitis virus complex including Russian spring-summer encephalitis viruses
- Variola (major & minor) virus
- Western equine encephalomyelitis virus
- Yellow fever virus
- Zika virus (Gr. Flavivirida)

ANNEXURE 2: RISK ASSESSMENT OF GE ORGANISMS

Genetic engineering can alter/change the overall risks of an organism on which the engineering is performed. So, re-evaluation of risks associated with the GE organism will be required to assess appropriate risk groups and for the selection of requisite biosafety level facilities.

Risk will be evaluated as a function of consequence of hazard (Table 1) and its possibility of exposure (Table 2) to people and environment. All the risks will be classified into four categories (Fig. 1) depending on severity and appropriate containment level will be selected accordingly.

Table 1: Consequence assessment

Consequences	Definitions relating to the health of people and the environment
Marginal	• Minimal adverse health effects. • Minimal or no damage to the environment or disruption to biological communities.
Minor	• Adverse health effects that is reversible. • Damage to the environment or disruption to biological communities that is reversible and limited in time and space or numbers affected.
Intermediate	• Adverse health effects that is irreversible. • Damage to the environment or disruption to biological communities that is widespread but reversible or of limited severity.
Major	• Adverse health effects those are severe, widespread and irreversible. • Extensive damage to the environment or extensive biological and physical disruption of whole ecosystems, communities or an entire species that persists over time or is not readily reversible.

Table 2: Likelihood assessment

Likelihood	Definitions
Highly unlikely	May occur only in very rare circumstances.
Unlikely	Could occur in some circumstances.
Likely	Could occur in many circumstances.
Highly likely	Is expected to occur in most circumstances.

Likelihood	Consequence			
	Marginal	Minor	Intermediate	Major
Highly likely	Low	Moderate	High	High
Likely	Negligible	Low	High	High
Unlikely	Negligible	Low	Moderate	High
Highly unlikely	Negligible	Negligible	Low	Moderate

Fig. 1. Risk categorization approach

Risks will be evaluated on parameters described below:

A. Assessment of effect on human health

i. Expected toxic or allergenic effects of the GE organism and/or its metabolic products.
ii. Comparison of the GE organism to the recipient or (where appropriate) parental organism.
iii. Pathogenicity.
iv. Capacity for colonisation.
v. Diseases caused and mechanism of transmission including invasiveness and virulence.
vi. Infective dose.
vii. Possible alteration of route of infection or tissue specificity.
viii. Possibility of survival outside human host.
ix. Biological stability.
x. Antibiotic-resistance patterns.
xi. Allergenicity.
xii. Toxigenicity.
xiii. Availability of appropriate therapies and prophylactic measures.

B. Information on recipient organism

i. Taxonomy, identification, source, culture.
ii. Nature of pathogenicity and virulence, infectivity, allergenicity, toxicity and vectors of disease transmission.
iii. Nature of indigenous vectors and adventitious agents, where they could mobilise the modified organism, and the frequency of mobilisation.
iv. Nature and stability of genetic modification, if any.
v. History of prior genetic modification, if any.

vi. Information on natural occurrence.

vii. Information on possible spread of recipient organism and its progeny in the absence of confinement.

viii. Host range (if relevant).

ix. Information on ecological functions of recipient organism.

x. Information on the potential of organism to establish itself in the accessible environment.

xi. Ability to form survival structures (such as spores).

xii. History of safe use, if any.

C. Information on process of genetic modification

i. The nature, function and source of the inserted donor nucleic acid, including regulatory or other elements affecting the function of the DNA and of the vector.

ii. Purpose and method of genetic transformation and procedure followed for selection of the modified organism.

iii. Any possible dual usage that may compromise health and safety of people and environment.

iv. Rate and levels of expression of the introduced genetic material.

v. Function of the expressed protein(s).

vi. The structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism.

vii. Characterisation of the site of modification of the recipient genome and sequence confirmation of the introduced genetic elements for the accuracy of modification and for intended function.

viii. Stability of the genetic modification (inserted DNA).

ix. Similarity of the expressed recombinant protein with potential known toxin and/or allergen.

x. Kinetics and level of expression of inserted genetic material.

D. Assessment of effect on environment

i. Routes by which the GE organism could be released (including waste disposal, equipment failure and human spread).

ii. Potential to infect or colonise animals and plants.

iii. The local environment surrounding the containment facility as well as the wider environment, especially if there is a possibility that the GE organism could survive and disseminate.

iv. Expected survivability, multiplication and extent of dissemination of the GE organism in the identified ecosystems.

v. Anticipated result of interaction between the GE organism and the organisms or microorganisms which might be exposed in case of unintentional release into the environment.

vi. Known or predicted effects on humans, plants and animals such as pathogenicity, toxicity, allergenicity, vector for a pathogen, altered antibiotic-resistance patterns, altered tropism or host specificity, colonisation known or predicted involvement in biogeochemical processes.

Note:

i. Experiments, handling and use of organisms/ products derived from genetic engineering require added precaution for which experiments must be monitored at the institution level by IBSC and other competent regulatory authorities.

ii. All genetic modification risk assessments should be reviewed regularly and be updated in the light of new scientific knowledge or where there has been a change in the nature of the activity (including a change in scale or any new procedures and containment measures).

iii. All experiments on genetic engineering must be properly documented and audited. All data should be recorded and must be available to the competent regulatory agencies whenever asked on demand, for risk assessment. It is the responsibility of investigator to maintain records and later by the institution for at least 6 years after the work has ceased (storage of materials is also considered to be active work in this case).

iv. Efficiency of decontamination and disposal mechanisms should be discussed and adopted accordingly. Specific measures may be required and must be developed on case by case basis over and above those mentioned in this guideline. IBSC will ensure that the measures are sufficient for containment and are recorded and informed to all personnel working in the facility.

ANNEXURE 3: LIST OF COMMONLY USED HOST-VECTOR SYSTEM

Use of the below cited host-vector system in genetic engineering is considered to be safe based on long history of safe use. Therefore its use is not expected to produce any neoplastic effects. This list will be updated time to time with information generated from practical experience.

	Host	**Vector**
Bacteria	*Escherichia coli* K12 or *E. coli* B derivatives or similar non pathogenic lab strains (normal) which do not contain: • conjugation proficient plasmids/ genes • generalised transducing phages	1. Non-conjugative plasmids 2. Bacteriophage: • lambda • lambdoid • Fd or F1 (e.g.M13) 3. None (non vector system)
	Bacillus sp. - with a reversion frequency of less than 10-7: • *B. amyloliquifaciens* • *B. licheniformis* • *B. pumilus* • *B. subtilis* • *B. thuringiensis*	1. Non-conjugative plasmids 2. Indigenous *Bacillus* plasmids and phages whose host range do not include *B. cereus* or *B. anthracis* or any other pathogenic strains. 3. None (non vector system)
	Pseudomonas putida strain KT 2440	1. Non-conjugative plasmids. Certified plasmids i.e. pKT 262, pKT 263, pKT 264 2. None (non vector system)
	Streptomyces specified species: • *S. coelicolor* • *S. lividans* • *S. parvulus* • *S. griseus* • *S. aureofaciens* • *S. cyaneus* • *S. rimosus* • *S. venezulae*	1. Non-conjugative plasmids. 2. Certified plasmids: SCP2, SLP1, SLP2, PIJ101 and derivatives 3. Actinophage phi C31 and derivatives 4. None (no-vector system)

	Agrobacterium specified species: • *A. radiobacter* • *A. rhizogenes*- disarmed • *A. tumefaciens* -disarmed	1. Non tumorogenic disarmed Ti plasmids or Ri plasmids 2. None (no-vector system)
	Other bacterial species: • *Lactobacillus sp.* • *Rhizobium sp.* • *Pediococcus* • *Strepotococcus thermophilus* • Nonpathogenic strains of *Micromonospora* • Strains of *Nocardia mediterranei.* • *Klebsiella pneumoniae* strain M 5 al.	1. Non-conjugative plasmids. 2. None (no-vector system)
Fungi/ Yeast	• *Neurospora crassa*, laboratory strains • *Saccharomyces cerevisiae* • *Pichia pastoris* • *Schizosaccharomyces pombe* • *Kluyveromyces lactis* • *Trichoderma reesei* • *Yarrowia lipolytica* • *Pencillium chrysogenum*	1. All vectors 2. None (non-vector system)
Slime moulds	*Dictyostelium* species	*Dictyostelium* shuttle vectors, including those based on the endogenous plasmids Ddp1 and Ddp2

Tissue cultures	All that do not contain any infectious agent and cannot spontaneously generate whole animal/ organism: • Animal or human cell lines including packaging lines • Isolated cells, tissues • Early non human mammalian embryos cultured *in vitro*.	1. Non-conjugative plasmids. 2. Non-viral vectors or defective viral vectors (including retrovirus or retroviral-helper combinations) that cannot infect human cells. 3. *Baculovirus* (*Autographa californica* nuclear polyhedrosis virus), polyhedron minus. 4. None (no-vector system)
	All that do not contain any infectious agent and are not intended, not likely to vegetative propagate, flower or regenerate into whole plant without human intervention: • Plant cell cultures • Isolated plant tissues or organs	1. Non-tumorigenic disarmed Ti plasmid vectors in *Agrobacterium tumefaciens, A. radiobacter, A. rhizogenes* 2. Non-pathogenic viral vectors. 3. None (no-vector system)

Note: Investigators may request to have new host-vector systems added to the list by making a detailed submission to competent regulatory authorities through IBSC.

ANNEXURE 4: SIMPLIFIED PROCEDURES & GUIDELINES ON EXCHANGE, IMPORT AND EXPORT OF GE ORGANISMS AND PRODUCTS THEREOF FOR R&D PURPOSE

1. Exchange/Import/Export of following items (GE Organisms and products thereof) for research purpose will require IBSC Approval			
Category	**Containment**	**Quantity Permissible**	**Approval Procedure and conditions**
1.1 Polynucleotides (of natural or synthetic or recombinant origin) a. Polynucleotides/ plasmids/ genetic constructs that cannot produce infectious forms of any biological agent (for eg. viruses) by itself when introduced into an animal or permissive cell or host or any other in vitro system with or without the introduction of rescue plasmids or other exogenous factors. b. These nucleic acids/ polynucleotides upon translated *in vivo* or *in vitro*, in a vector or recombinant host genome do not produce functional form of toxin that is lethal for vertebrates at LD_{50} of less than 1 microgram per kilogram body weight. c. These nucleic acids/ polynucleotides have not been modified or manipulated (e.g., encapsulated into synthetic or natural vehicles) to render them capable of penetrating cellular membranes.	BL1	100 µg	Approval by Sponsor's IBSC after examination of the information submitted by applicant to IBSC in prescribed proforma. Applicant should provide a certificate to IBSC stating that the polynucleotide preparation does not contain any living microorganisms or cell Subsequent applications should accompany a Statement on the Utilization of the earlier received material. IBSC shall have to submit annual report of transactions in prescribed proforma to RCGM. Transfer of above than the specified quantity will require approval by RCGM Secretariat / RCGM. On request RCGM Secretariat will issue NOC/Permit to facilitate custom clearance, in case of import.

1.2 Proteins (including pure plant proteins) a. These proteins are not toxic at LD_{50} of less than or than equal to 1 microgram per kilogram body weight.	BL 1	20 g	
1.3 Non-living plant material	BL 1	20 g	
1.4 GM Microorganisms and Cell lines which can be handled at BL 1 Containment a. Risk group 1 microorganisms/ cell lines b. DNA from Risk Group 2 and above agents is transferred into lower risk group microorganisms /cell lines which can be handled at BL 1 Containment	BL1	20 vials (1-5 mL, 10^{6-8} cells/ vial)	
1.5 Model organisms: Plants (such as *Arabidopsis*), common laboratory models (such as *Ceanorhabditis, Drosophila*, *Danio* etc) and other model organisms (such as S*accharomyces, Schizosaccharomyces, E. coli, Pichia* and other model organisms) which are routinely used in laboratories globally. The IBSC will certify that the plants/model organisms carry routine and standard experimental mutations/ insertions and do not carry foreign gene-insertions from non-model organisms.	BL1-N/BL1-P/ BL 1	For laboratory use only	

2. Exchange/Import/Export of following items (GE Organisms and products thereof) for research purpose will require permission from RCGM Secretariat			
2.1 Polynucleotides (of natural or synthetic or recombinant origin) a. Nucleic acids/ polynucleotides/ plasmids/genetic constructs that can produce infectious forms of any biological agent (for eg viruses) when introduced into an animal or permissive cell or host or any other in vitro system with or without the introduction of rescue plasmids or other exogenous factors. b. These nucleic acids/ polynucleotides upon translated *in vivo* or *in vitro*, in a vector or recombinant host genome produce a toxin that is lethal for vertebrates at LD_{50} of less than 1 microgram per kilogram body weight.	BL2-BL4	The applications will be examined on case to case basis considering the permissible quantity of respective item mentioned at S. No. 1	After IBSC Approval applicant has to apply RCGM Secretariat for NOC/Permit. RCGM Secretariat will process the case in consultation with 02 Experts and NOC/Permit will be issued on the basis of expert consultations In cases of export of GE Organisms and Toxins belonging to the category 2 of SCOMET items, permission to export from DGFT shall also be required.
2.2 Proteins a. The toxin proteins that is lethal for vertebrates at LD_{50} of less than 1 microgram per kilogram body weight.	BL2-BL4		

2.3 GM Microorganisms/ Cell lines a. Microorganisms/cell lines belonging to risk group 2 and above b. Any manipulation of lower risk group microorganisms/ cell lines which rendered them as risk group 2 and above. c. Transfer of drug resistant trait or virulence modification trait or changing the host immune response trait or the traits that are not known to be acquired naturally, if such acquisition could compromise the ability to control disease agents in humans, animals or agriculture	BL2-BL4		
Import/Export of GE Organisms and product(s) thereof not covered in above table will require approval by RCGM Secretariat/ RCGM.			
RCGM Secretariat after examination of applications belonging to S. No. 2 above may ask applicant to seek approval from RCGM Committee on case to case basis.			

ANNEXURE 5: CHECKLIST FOR EXCHANGE/ IMPORT/ EXPORT OF GE ORGANISMS AND PRODUCTS THEREOF

S.No.	Information type	Information provided (Yes/No)	Comments, if any
General Information			
1	Name of IBSC, Registration No. and date		
2	Minutes and Date of IBSC Meeting		
3	Consignor's Name & Address		
4	Consignee's Name & Address		
5	Activity for which approval is sought (Import/Export/Exchange)		
6	Material Transfer Agreement duly signed by both parties		
7	If the proposed GE Organisms /LMOs was imported/exported earlier, provide the copy of issued permit and quantity imported		
8	Utilization certificate		
9	If the end user is different from the consignee, give details and justify		
10	Whether the GE organisms /LMO/toxin is belonging to SCOMET* list of DGFT *If yes, applicant has to apply to DGFT in cases of export		
Scientific Information			
11	Title of Proposal		
12	Objectives of proposal		
13	Material Transported (give detailed Scientific description, Quantity)		

14	Quantity to be Exchanged/Imported/Exported (if it is more than the quantity prescribed in guidelines for category 1 regulated biological materials, please submit the application to RCGM)		
15	Molecular Biology information a. Source of nucleic acid/Inserted DNA /Protein b. Sequence of Nucleic acid/Protein (FASTA format with accession number) c. Vector information (Physical map of vector, vector sequence, table identifying each genetic component of vector along with size, origin and intended function) d. Manipulative procedures used		
16	Biochemical Characterization a. Whether there is transfer of drug resistant trait to microorganism which is not known to acquire the trait naturally b. Toxicity (Give LD_{50}) and Allergenicity of protein/nucleic acid		
17	Information of GE Organisms/LMO a. Host carrying the vector- Taxonomy, history of safe use b. Pathogenicity, if any c. Risk group/category		
18	Certificate of analysis, if applicable		
19	Containment facility a. Containment facility recommended as per the these guidelines 2017 b. Proposed Containment facility c. Proposed decontamination, disposal mechanism & risk management measures		
20	Summary of proposed activity		

CERTIFICATION OF CONTAINMENT LEVEL 3 AND 4 FACILITIES

Application for certification

This application is for the certification of a facility to the specified containment levels (3 and 4). 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 audit. 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: _____

Facility type

Please select the facility type for which certification will be provided.

You are welcome to contact the RCGM if you wish to clarify your choice of containment level / facility type.

(At present no AqBSL facilities are envisioned as AqBSL-4. However, the decision to designate a pathogen as requiring AqBSL-4 level will be made on case-by-case basis.)

BSL-3	☐	BSL-4	☐
ABSL-3	☐	ABSL-4	☐
PBSL-3	☐	PBSL-4	☐
IBSL-3	☐	IBSL-4	☐
AqBSL-3	☐		

Application type

New ☐ Renew ☐

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

_____ _____

Other Certification

Is this facility currently certified by other agencies?

If YES, please indicate certification number, containment level and facility type.

Certification agency: _____
Other certification number: _____
Containment level & facility type: _____

2. Facility Inspection

The facility shall be inspected by an RCGM nominated expert(s) who has acquired thorough training, qualifications or experience, or a combination of these, the knowledge and skill enabling that person to assess compliance with the requirements for certification of a physical containment facility.

Appropriate inspection checklist for evaluation of facility design and operational practices within the facility should be filled at the time of inspection. Laboratory supervisor or person in-charge of laboratory must be present at the time of inspection. The filled checklist duly signed by inspector must be sent 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.

-------Enclose separate sheet, if required-------

c. Please provide any other information that may assist the RCGM in making a decision about this application.

-------Enclose separate sheet, if required-------

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 Inspector on VERIFICATION

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 **Name of Inspector with complete designation**

1. Application Checklist for BSL-3/ABSL-3/PBSL-3/IBSL-3/AqBSL-3 Facility

1.1 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 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-siphonage. • 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 biohazardous 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.			

1.2 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. Eating and drinking was not observed and no food/drinks stored in work areas.			
4. Personal protective equipment is clean, available and used appropriately; not worn outside of lab.			
5. Biosafety cabinets (BSCs) are field tested and certified annually. Date of last certification: _____			
6. Autoclaves are maintained, calibrated and tested. Date of last calibration: _____			
7. Aerosol generating activities (sonication, vortexing, homogenizing) are performed inside BSC for risk group 2 microorganisms.			
8. Centrifuge safety cups or sealed rotors are used to centrifuge RG 2 microorganisms.			
9. Personnel employ safe handling of sharps.			
10. Work areas are decontaminated regularly after work and after known contamination.			
11. Personnel know how to clean up a spill.			
12. All biohazardous waste containers are closed or covered when not actively adding waste.			
13. Autoclave bags with biohazard symbol are available and used for decontamination.			
14. Lab personnel are up to date on required safety training and lab specific training.			
15. Personnel know symptoms associated with organisms used in the lab.			
16. Personnel know how to handle exposures and to report accidents immediately.			
17. Appropriate laboratory operation manual is accessible to personnel.			
18. Record of work is duly registered in the register available.			
19. Personnel know the process of registering and reporting in case of accidents			
20. A training program is available for fresh candidate.			
21. Record of medical examination of all laboratory personnel including past medical history is available.			

22. Baseline serum sample are stored for future reference. 23. Immunocompromised personnel are not employed. 24. Medical contact card is available for all personnel. 25. Laboratory monitoring plan is available and working including periodic surveillance. 26. Proper waste management plan available and is adopted. 27. All instructions related to waste management are posted inside and outside of laboratory and must be visible clearly. **Additional requirements for ABSL** 1. Personnel are trained to handle animals. 2. GE animals are properly marked and separately handled from non-GE animals. 3. All activities are duly permitted by CPCSEA. **Additional requirements for PBSL** 1. No undesirable species are present and growing. 2. Institutional practice manual available. 3. Proper non breakable containers are being used for material transport. 4. All transgenic biological materials are transported according to guideline 5. Materials and equipment taken into or out of the plant facility are treated by an appropriate technique to destroy or remove all other organisms (including all stages of its life-cycle).			

2. Application Checklist for BSL-4/ABSL-4/PBSL-4 Facility

2.1 Checklist for evaluation of Facility design

S.No.	Requirements of Facilities	Yes	No	Remarks
Cabinet laboratory				
1	The BSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Full access to all exterior surfaces of the facility and service penetrations must be provided to facilitate integrity testing.			
2	The facility must be a sealed internal shell bounded by walls, doors, windows, floors and ceilings, which allows gaseous decontamination and operation of the facility under negative pressure. Seals at all facility junctions must be continuous. Interior surfaces must be gastight to prevent transfer of any gases. All penetrations must be sealed. Doors must be lockable.			
3	All windows in the facility must be closed and sealed.			
4	The entrance to work area must be through an outer and inner change room separated by shower airlock. The shower airlock must be interlocked to prevent simultaneous opening of both doors. 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.			
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 with filter with pore size of less than or equal to 0.2 µm. These vents must also be able to isolated or decontaminated.			
7	The cabinet room housing Class III biological safety cabinet should be separated from outside environment by two air lock entry doors. Handling of risk group 4 microorganisms should be performed in this room and nothing must be taken out of the room. All required instruments for study must be placed inside the cabinet room.			

8	Where the facility shares an airlock with a ABSL-3 or IBSL-3, 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.			
9	Provision must be made for viewing of work areas from outside the facility.			
10	Walls, ceiling, and floors are smooth, easily cleanable, impermeable to liquids, and resistant to the chemicals and disinfectants.			
11	Adequate illumination is ensured for carrying out all activities.			
12	Laboratory furniture is sturdy, and open spaces between and under benches, cabinets, and equipment is accessible for cleaning.			
13	Bench tops is impervious to water and resistant to disinfectants, acids, alkalis, organic solvents, and moderate heat.			
14	Biological safety cabinets for handling of infectious microorganisms of risk group 3 are available. BSCs must be tested, commissioned and results documented before use.			
15	Piped gas supplies to the facility must have reverse flow prevention on outlets located within the BSC.			
16	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.			
17	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. There must be a pressure differential of at least 25 Pa between each room to achieve air flow direction within work area.			
18	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.			

19	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 15 Pa above the set point for 2 min.			
20	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.			
21	Supply or replacement air to the facility is HEPA filtered.			
22	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.			
23	Supply and exhaust HEPA filters must be mounted in a gas tight housing. The sealed access doors and the ductwork between the facilities must also be gas tight.			
24	Access to the laboratory area should be designed to prevent entrance of free-living arthropods and other vermin.			
25	Wash-basin is provided in each laboratory or any other means of decontamination of hands provided.			
26	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-siphonage. i. Laboratory sinks outlets. ii. Outlets within a BSC or other aerosol containment equipment. iii. Direct connections to an autoclave. Backflow prevention must isolate the facility to the exclusion of all other areas. There must be an isolation valve immediately outside the facility.			

27	An autoclave must be provided within the work area. It must be of double ended type with interlocked doors. The inner door must open internally to the facility and the outer door must open externally to the facility. The interlock must prevent simultaneous opening of both doors. Maintenance must be achievable from outside the facility.			
28	Designated storage or hanging provisions for personal protective equipment available in facility.			
29	Eyewash equipment is provided.			
30	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.			
31	Two alternate communication systems must be provided for contact between persons inside and outside the facility. At least one of these systems must operate in case of power failure.			
32	The facility must have an automatic changeover emergency power source that is activated in event of power failure. The emergency power source must ensure continuing operation of ventilations systems, biosafety cabinets, flexible film isolators, shower control, emergency lightening, control systems associated with ventilation, effluent and waste management.			
Suit Laboratory				
33	Exit is through a chemical shower airlock fitted with airtight doors. The chemical shower must incorporate disinfectant that is effective against microorganisms dealt within suit area. Alternate method of suit decontamination is available.			
34	A protective suit laboratory is designed and maintained to provide personnel protection equivalent to that provided by Class III biological safety cabinets			
35	All procedures must be conducted by a person wearing one piece positive pressured suit ventilated by a life support system.			
36	It should be attached with outer and inner changing rooms fitted with shower.			

37	An automatic second exhaust fan must be provided to re-establish the negative pressure of the suit area in the event of exhaust fan failure.			
38	The personnel breathing air source must be connected with an uninterrupted power supply.			
39	Air flow in the supply and exhaust components of the ventilating system must be monitored and an appropriate system of controls must be used to prevent pressurization of the suit laboratory. HEPA-filtered supply air must be provided to the suit area, decontamination shower and decontamination airlocks or chambers.			
40	Exhaust air from the suit laboratory must be passed through a series of two HEPA filters prior to release outdoors.			
41	Proper wastewater treatment facility available, working properly and record maintained.			
42	Provisions for autosensing alarm for fire and other emergencies that evacuation.			
43	Backup power source in event of power failure.			

2.2 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. Eating and drinking was not observed and no food/drinks stored in work areas.			
4. Personal protective equipment is clean, available and used appropriately; not worn outside of lab.			
5. Life support system that is used in suit is working properly.			
6. Biosafety cabinets (BSCs) are certified annually. Date of last certification: _____			
7. Autoclaves are maintained, calibrated and tested. Date of last calibration: _____			
8. Aerosol generating activities (sonication, vortexing, homogenizing) are performed inside BSC for risk group 2 microorganisms.			
9. Centrifuge safety cups or sealed rotors are used to centrifuge RG 2 microorganisms.			
10. Personnel employ safe handling of sharps.			
11. Work areas are decontaminated regularly after work and after known contamination.			
12. Personnel know how to clean up a spill.			
13. All biohazardous waste containers are closed or covered when not actively adding waste.			
14. Autoclave bags with biohazard symbol are available and used for decontamination.			
15. Lab personnel are up to date on required safety training and lab specific training.			
16. Personnel know symptoms associated with organisms used in the lab.			
17. Personnel know how to handle exposures and to report accidents immediately.			
18. Appropriate laboratory operation manual is accessible to personnel.			
19. Record of work is duly registered in the register available.			

20. Personnel know the process of registering and reporting in case of accidents 21. A training program is available for fresh candidate. 22. Record of medical examination of all laboratory personnel including past medical history is available. 23. Baseline serum sample are stored for future reference. 24. Immunocompromised personnel are not employed. 25. Medical contact card is available for all personnel. 26. Laboratory monitoring plan is available and working including periodic surveillance 27. All effluents from the maximum containment laboratory are rendered safe, including the shower water. 28. Proper waste management plan available and is adopted. 29. All instructions related to waste management are posted inside and outside of laboratory and must be visible clearly. 30. active cooperation with national and local health authorities **Additional requirements for ABSL** 1. Personnel are trained to handle animals. 2. GE animals are properly marked and separately handled. 3. All activities are duly permitted by CPCSEA. 4. Handling of infected animals and housing of infected animals are to be carried out in Class III biological safety cabinet **Additional requirements for PBSL** 1. No undesirable species are present and growing. 2. Institutional practice manual available. 3. Proper biohazard containers are being used for material transport. 4. All materials to transport outside lab are rendered safe. 5. All transgenic biological materials are transported according to guideline			

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