Bangladesh Biosafety and Biosecurity Guidelines

for

Handling and Disposal of Biohazardous Materials

First Edition

September 2019

Bangladesh Biosafety and Biosecurity Society

Bangladesh Biosafety and Biosecurity Guidelines

for

Handling and Disposal of Biohazardous Materials

First Edition

September 2019

Bangladesh Biosafety and Biosecurity Society

Editors

Dr Asadulghani (MSc, MPhil, PhD) Head of Biosafety, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka, Bangladesh and President, Bangladesh Biosafety and Biosecurity Society

Dr Md. Giasuddin (DVM, MSC, PhD) Director, National Reference Laboratory for Avian Influenza, Bangladesh Livestock Research Institute, Savar and Vice President, Bangladesh Biosafety and Biosecurity Society

Dr. Latiful Bari (MSc, PhD) Head of Food Analysis and Research Laboratory, Center for Advanced Research in Sciences, University of Dhaka and Vice President, Bangladesh Biosafety and Biosecurity Society

Professor Dr. Md. Shahidul Islam (MS, PhD) Head, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh and General Secretary, Bangladesh Biosafety and Biosecurity Society

Dr. Zhahirul Islam (MSc, PhD) Scientist and Head, Laboratory of Gut Brain Signaling, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali Dhaka, Bangladesh and Treasurer, Bangladesh Biosafety and Biosecurity Society

Professor Dr. Md. Rakibul Islam (MS, PhD) Professor, Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh and Joint Secretary, Bangladesh Biosafety and Biosecurity Society

Dr. Md. Salimullah (MSc, MPhil, PhD) Director General, National Institute of Biotechnology, Savar, Dhaka, Bangladesh and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Professor Dr. Kazi Nadim Hasan (MSc, MPhil, PhD) Professor, Department of Biology and Chemistry, School of Life Sciences North South University, Dhaka, Bangladesh and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Dr. Chandan Kumar Roy (MBBS, MPhil, FCPS) Associate Professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Lt Col (Dr) Jamal Pasha Chowdhury (MBBS, DCP, FCPS) Classified Specialist in Pathology, Combined Military Hospital, Rangpur Cantonment, Rangpur and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Dr. Tasnim Farzana (MSc, MPhil, PhD) Principal Scientific Officer, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Dr. Mohammad Aminul Islam (MSc, PhD) Assistant Professor, Paul G Allen School for Global Animal Health, College of Veterinary Medicine, Washington State University, Pullman, USA and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Dr. Munawar Sultana (MSc, PhD) Associate Professor, Department of Microbiology, University of Dhaka, Dhaka Bangladesh and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Professor Dr. A.H.M. Nurun Nabi (MSc, PhD) Professor, Department of Biochemistry & Molecular, University of Dhaka, Dhaka, Bangladesh and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Dr. Abdul Jabbar (DVM, MSc) Shikdar Director Admin, Department of Livestock Services, Ministry of Fisheries and Livestock, Farmgate Khamarbari Sarak, Dhaka and Executive Committee Member, Bangladesh Biosafety and Biosecurity Society

Professor Dr Khondoker Moazzem (MSc, PhD) Department of Biotechnology and Genetic Engineering, Khulna University and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Professor Dr Mohammad Riazul Islam (MSc, PhD) Department of Biochemistry and Molecular Biology, University of Dhaka and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Professor Dr KHM Nazmul Hussain Nazir (MSc, PhD) Department of Microbiology and Hygiene, Bangladesh Agricultural University and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Salma Ahmed (MBBS, MPH, MPhil, PhD) Associate Professor, Department of Microbiology, Mymensingh Medical College and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Monira Pervin (MBBS, MPhil, MPH) Associate Professor, Department of Virology, Dhaka Medical College and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Professor Dr Mohammad Hafizur Rahman (MBBS,MPhil) Department of Biochemistry, Mugda Medical College and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Md. Ziaul Amin (MSc, PhD) Associate Professor, Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr S M Rashed-ul Islam (MBBS, MPhil) Assistant Professor, Department of Virology, Bangabandhu Sheikh Mujib Medical University and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Professor Dr AKM Mostafa Anower (MSc, PhD) Department of Microbiology and Public Health, Faculty of Animal Science and Veterinary Medicine and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Khondokar Nasirujjaman (MSc, PhD) Department of Genetic Engineering and Biotechnology, University of Rajshshi and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Md. Atiar Rahman (MSc, PhD) Associate Professor, Department of Biochemistry and Molecular Biology, Chittagong University and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Jabin Akhter (MBBS, MPhil) Assistant Professor, National Institute of Preventive and Social Medicine and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Hafiza Sultana (MBBS, MPhil) Assistant Professor, Department of Microbiology, Rangpur Medical College and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr Shamim Ahmed (MSc, PhD) Head, Department of Biochemistry and Molecular Biology, Shahjalal University of Science and technology, Sylhet, Bangladesh and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Professor Dr Mahbub Robbani (MSc, PhD) Department of Horticulture, Patuakhali Science and Technology University, Barisal, Bangladesh and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Dr. S M Shahidul Islam (MBBS, PPM) Superintendent of Police, Human Resource, Central Police Hospital, Dhaka and Divisional Coordinator, Bangladesh Biosafety and Biosecurity Society

Supported by:

Core Committee:

(The Core Committee meetings were held on: 11 and 25 July 2015)

Professor *Dr. Be-Nazir Ahmed*, Director, Disease Control, Line Director, Communicable Disease Control, National Focal Point, International Health Regulations, Directorate General of Health Services, Ministry of Health and Family Welfare

Professor *Dr. Md. Anwar Hossain*, Professor, Department of Microbiology, University of Dhaka

Professor *Dr. Sharif Akhtaruzzaman,* Department of Biochemistry and Molecular Biology, University of Dhaka and National Advisor, National Forensic DNA Profiling Laboratory, Dhaka Medical College

Professor *Dr. Rakha Hari Sarker*, Department of Botany, University of Dhaka, Dhaka

Dr. Md. Giasuddin, Laboratory in Charge, National Reference Laboratory for Avian Influenza, Bangladesh Livestock Research Institute; Savar, Dhaka

Dr. Md. Salimullah, Director General, National Institute of Biotechnology, Savar, Dhaka

Dr. Asadulghani, Head, Biosafety and Biosafety Level 3 Laboratory, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka

Note: Affiliations are mentioned as of the date of the program

Major Contributors:

(Consultative workshops developing and reviewing the guidelines was organized on: 22 August 2015, 2 April 2016, and 24 September 2016)

Professor *Dr. Md. Imdadul Hoque,* Dean, Faculty of Biological Science, University of Dhaka

Professor *Dr. Md. Anwar Hossain*, Department of Microbiology, University of Dhaka

Professor Dr. Rakha Hari Sarker, Department of Botany, University of Dhaka, Dhaka

Professor *Dr. Sharif Akhtaruzzaman*, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka and National Advisor, National Forensic DNA Profiling Laboratory, Dhaka Medical College

Professor *Dr. AKM Shamsuzzaman,* Director Disease Control and Line Director Communicable Disease Control, Directorate General of Health Services

Md. Solaiman Haider, Director (Planning), Deptment of Environment, Government of Bangladesh

Dr. Md. Salimullah, Director General, National Institute of Biotechnology, Savar, Dhaka

Dr. Md. Giasuddin, Laboratory in Charge, National Reference Laboratory for Avian Influenza Bangladesh Livestock Research Institute, Savar, Dhaka

Dr. Latiful Bari, Principal Scientist, Center for Advanced Research in Sciences, University of Dhaka, Dhaka

Dr. Chandan K. Roy, Associate Professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Shahbagh, Dhaka

Professor Dr. Md. Shahidul Islam**,** Department of Biotechnology, Bangladesh Agricultural University, Mymensingh

Dr. Zhahirul Islam, Associate Scientist, Enteric Microbiology Laboratory, Laboratory Sciences and Services Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka

Dr. Aminul Islam, Associate Scientist, Food Microbiology Laboratory, Laboratory Sciences and Services Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka

Dr. Abdul Khaleque**,** Associate Professor, Department of Biochemistry and Microbiology, School of Life Sciences, North South University, Bashundhara, Dhaka

Dr. Kazi Nadim Hasan**,** Associate Professor and Chairman, Department of Biochemistry and Microbiology, School of Life Sciences, North South University, Bashundhara, Dhaka, Bangladesh

Dr. Khandaker Shadia, Assistant Professor, Department of Microbiology, Ibrahim Medical College Hospital (On Doctoral Fellowship)

Dr. Md. Hossain Sohrab**,** Senior scientific Officer, Chemical Research Division, BCSIR Laboratories, Dhaka

Professor Dr. Md. Nazibur Rahman, Department of Biochemistry and Microbiology, Jahangirnagar University, Savar, Dhaka

Dr. Monira Pervin, Associate Professor, Department of Virology, Dhaka Medical College Hospital, Dhaka

Professor Dr. A.H.M. Nurun Nabi**,** Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka

Dr. Munawar Sultana, Assistant Professor, Department of Microbiology, University of Dhaka, Dhaka

Professor Dr. Md. Reazul Islam, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka (On Postdoctoral Fellowship)

Brigadier General (Dr) Md. Saidur Rahman**,** Director, Kurmitoral General Hospital, Dhaka

Lt. Col. (Dr) Abdullah-Al-Baki**,** Assistant Professor, Armed Forces Medical School, Dhaka Cantonment, Dhaka

Lt. Col. (Dr) Jamal Pasha Choudhury**,** Classified Specialist in Microbiology, Armed Forces Institute of Pathology, Dhaka Cantonment, Dhaka

Dr. Nikhat Ara, Consultant Microbiologist and Infection Control, Appolo Hospital, Dhaka

Dr. Abdul Jabbar Shikdar, Principal Scientific Officer, Field Disease Investigation Laboratory, Department of Livestock Services, Barisal

Dr. Md. Ziaul Amin, Associate Professor Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Jessore

Dr. Md. Rafiqul Islam, Associate Professor and Chairman, Department of Genetic Engineering and Biotechnology, university of Chittagong

Dr. Muhammad Ali Siddiquee**,** Chief Scientific Officer and Head, Grain Quality and Nutrition Division, BRRI, Gazipur, Dhaka

Dr. Md. Al-Amin**,** Scientific Officer, Central Disease Investigation Laboratory, Dhaka

Dr. Forhadul Alam**,** Senior Scientific Officer, Department of Livestock Services, Dhaka

Dr. Shamim Ahmed**,** Head, Department of Biochemistry and Molecular Biology, Shahjalal University of Science and Technology, Sylhet

Dr. Sharmina Aftab**,** Assistant Professor, Department of Microbiology, Rajshahi Medical College, Rajshahi

Dr. Khondokar Nasirujjaman**,** Associate Professor, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi

Dr. Mohammad Showkat Mahmud**,** Senior Scientific Officer, Research on Foot and Mouth Disease and Peste des Petits Ruminant in Bangladesh, Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka

Dr. Afroza Akhter Sweety**,** Lecturer, Department of Virology, Dhaka Medical College, Dhaka

Dr. Fahmida Khanam**,** Assistant Professor, Department of Virology, National Institute of Preventive and Social Medicine, Dhaka

Dr. Jabin Akhter , Assistant surgeon, Bangladesh Institute of Tropical and Infectious Disease, Fouzderhat, Chittagong

Dr. Hafiza Sultana, Assistant Professor, Department of Microbiology, Rangpur Medical College, Rangpur

Dr. Atiar Rahman**,** Associate Professor, Biochemistry and Molecular Biology Department, University of Chittagong, Chittagong

Dr. Salma Ahmed**,** Associate Professor of Microbiology, Mymensingh Medical College, Mymensingh

Dr. M.M. Daulatana, Deputy Director, Livestock Medicine store, Kazi Alauddin Road, Dhaka

Sardar Tanzir Hossain, Challenge Tuberculosis, Bangladesh Management Sciences for Health

Dr. Md Aminul Islam, Upazilla Live Stock Officer, Feni, Department of Livestock Services

Professor Dr. Md. Hasibur Rahman, Microbiology Department, Jahangirnagar University

Professor Dr. Md. Ariful Islam, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh

Professor Dr. Reza Md Shahjahan, Department of Zoology, University of Dhaka

Dr. Md. Rezaul Karim, Scientific Office, Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh

Dr. Rubena Haque, Assistant Professor, Biochemistry, Sir Salimullah Medical College, Dhaka

Dr. Md. Sadequl Islam, Deputy Director, Rajshahi Medical College Hospital, Rajshahi

Professor Dr. Abdul Ahad, Department of Microbiology and Veterinary Public Health, Chittagong Veterinary and Animal Sciences University, Chittagong

Dr. Md. Sadequl Islam, Assistant Professor and Chairman, Department of Anatomy and Histology, Hajee Mohammad Danesh science and Technology University, Dinajpur

Professor Dr. Md. Aktar Hossain, Department of Environmental Sciences, Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong

Mr. Keshob Chandra Das, Senior Scientific Officer and Head, Molecular Biotechnology Division, National Institute of Biotechnology, Savar, Dhaka

Dr. A.K.M Mostafa Anowar, Associate Professor and Chairman, Department of Microbiology and Public Health, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University (Barisal Campus), Khanpura, Babugonj, Barisal

Dr. Md. Rakibul Islam, Associate Professor, Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh

Dr. Arifur Rahman, Assistant Professor, Department of Microbiology, TMSS Medical College, Bogra

Dr. Mohammad Hafizur Rahman, Associate Professor, Department of Biochemistry, Dhaka Medical College, Dhaka

Dr. Md. Khaled Hossain, Associate Professor, Dept. of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur

Mr. Md. Kamal Uddin, Lecturer, Department of Microbiology, University of Chittagong, Chittagong, Bangladesh

Dr. Md. Abdul Alim Al-Bari, Associate Professor, Department of Pharmacy, University of Rajshahi, Rajshahi

Dr. Nasima Pervin, Bacteriologist and Government Analyst, National Control Laboratory, Directorate General of Drug Administration, Mohakhali, Dhaka

Dr. Syed Ahmed Abdullah, Lecturer, Dental Unit, Department of Microbiology, Shaheed Sohrawardi Medical College, Dhaka

Dr. Md. Afazuddin Miah, Department of Livestock Services, Deputy Curator, Bangladesh National Zoo, Dhaka

Dr. Md. Nazrul Islam, Upazila Livestock Officer, Department of Livestock Services, On deputation- Public Health Section, Livestock Research Institute, Mohakhali, Dhaka

Dr. Abdullah Mohammad Shohael, Associate Professor of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka

Dr. Md. Iqbal Kabir Jahid, Associate Professor, Department of Microbiology, Jessore University of Science and Technology, Jessore

Dr. K.H.M. Nazmul Hussain Nazir, Associate Professor, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh

Dr. Sahida Abedin, Lecturer, Department of Virology, Mymensingh Medical College, Mymensingh

Dr. Syeda Anjuman Nasreen, Associate Professor, Department of Microbiology, Jamalpur Medical College

Dr. Tasnim Farzana, Principal Scientific Officer, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh

Dr. Md. Morshed Alam, Assistant professor, Department of Biochemistry, National Institute of Kidney Diseases and Urology, Sher-E-Bangla Nagar, Dhaka

Professor *Dr. Md. Morsaline Billah,* Department of Biotechnology and Genetic Engineering, Khulna University

Dr. Mohammad Sahedrur Rahman, Associate Professor, Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Dhaka

Mr. Abu Hashem, Senior Scientific Officer and Head, Microbial Biotechnology Division, National Institute of Biotechnology, Savar, Dhaka

Note: Affiliations are mentioned as of the date of the program.

FOREWORD

Vice-Chancellor
University of Dhaka

University of Dhaka established in 1921 during the British Raj is the oldest University in Bangladesh. It has made significant contributions to the modern history of Bangladesh. Under the Dean of Biological Sciences, seven among the ten departments in this University are conducting basic and advanced research in the field of biological sciences. Thus, biological safety and biological security are the top most priorities of this University and we are mentoring the Biosafety and Biosecurity program organized by Bangladesh Biosafety and Biosecurity Society since its foundation.

I am indeed very happy to learn that the Society is now going to publish the first edition of the Bangladesh Biosafety and Biosecurity Guidelines for Handling and Disposal of Biological Hazards. I am delighted to know that our academicians from the department of Botany, Biochemistry and Molecular Biology, Biotechnology and Genetic Engineering, Microbiology, and Zoology substantially contributed in the development of these guidelines.

I highly appreciate the support from the Disease Control Division of the Directorate General of Health Services, Department of Livestock Services, Bangladesh Livestock Research Institute, National Institute of Biotechnology and funding agencies for their support on developing guidelines.

Long live Dhaka University, Long live Bangladesh.

Professor Dr. Md. Akhtaruzzaman

Vice-Chancellor In-Charge
Rajshahi University

University of Rajshahi is the finest and second largest of its kind in Bangladesh. It began academic program in 1953. Now it has established itself as a center of excellence in quality higher education and research in Bangladesh. The University bears a distinct significance due to its location in Rajshahi, the centre of the northern part of the country. Under the Faculty of Life & Earth Sciences, there are seven Biological Science disciplines working actively in offering Masters, MPhil and PhD degrees in the fields concerned. Biosafety and biosecurity are the most significant areas in those fields.

I am glad to know that faculties of our University are already actively involved in programs on strengthening the capacity of biosafety and biosecurity in Bangladesh. It is also a matter of great pleasure that under the leadership of a faculty member of our University, 16 resource persons are working in those fields in Rajshahi Division.

Finally, I would like to thank the resource persons of the University of Rajshahi for their contribution in the development of the Bangladesh Biosafety and Biosecurity Guidelines. I am hopeful that the country will be largely benefitted by implementing biosafety and biosecurity policies and procedures.

Professor Dr. Choudhury M. Zakaria

Vice-Chancellor Bangladesh Agricultural University

Established in 18 August 1961 The Bangladesh Agricultural University campus is situated at the western side of the old Brahmaputra river covering an area of 1200 acre, 3 kilometers away south of Mymensingh town. All together in the Faculty of Veterinary Science, Faculty of Agriculture, Faculty of Animal Husbandry, Faculty of Agricultural Economics & Rural Sociology, Faculty of Agricultural Engineering & Technology, and Faculty of Fisheries there are 44 departments and from among them all are involved in research with biological materials where encountering with more or less biohazardous materials is inevitable. Thus, Biosafety and Biosecurity are the paramount consideration for my faculties.

I am pleased to know that my University is participating in all the different Biosafety and Biosecurity programs organized by Bangladesh Biosafety and Biosecurity Society. I am very happy to know that Faculties of my University are leading the Biosafety and Biosecurity Society in Bangladesh and substantially contributing in the sustainable development in this area since the initiation of the program. At this stage, regarding the development of the First Edition of the Bangladesh Biosafety and Biosecurity Guidelines, I would like to congratulate the contributors for the indispensible contribution to guide the researchers to follow rules and regulations for establishing best practices in Biorisk Management.

I wish every success of the biosafety and biosecurity program in Bangladesh.

Lutful Hassan

Professor Dr. Lutful Hassan

Vice-Chancellor (In-Charge)
University of Chittagong

The academic activities of the university formally began on 18 November, 1966. Chittagong University is a significant landmark in the academic heritage of its proprietor, the Bangladesh Convention. Students who attend Chittagong University find it to be a place where they can grow academically, morally and spiritually, in a novel environment.

The University is supporting Biosafety and Biosecurity program in Bangladesh since its foundation. Currently there are 15 Biosafety and Biosecurity resource persons trained and working under the leadership of Chittagong University. I am indeed happy to know that these resource persons have significantly contribute in developing the first Edition of the Bangladesh Biosafety and Biosecurity Guidelines for handling and disposal of biological hazards, which is going to be published in September 2019.

I am hopeful that researchers and faculties working in the field of biological sciences will find these guidelines as useful document to establish biosafety and biosecurity principles and practices in the field of diagnosis and research with biological hazards.

I wish all the success of the Biosafety and Biosecurity program in Bangladesh.

Professor Dr Shireen Akhter

Vice-Chancellor
Shahjalal University of Science and Technology

Shahjalal University of Science and Technology (SUST) was established in 1986. This is a renowned university with the mark of its magnificent progress. Research activities have earned fame of the university at both home and aboard. Among the six Schools under the School of Agricultural and Mineral Sciences, Applied Sciences and Technology, and Life Sciences students and faculties are conducting basic and advanced in the field of biological sciences.

As the University is conducting research in the field of biological sciences the University is mentoring the Biosafety and Biosecurity program since its initiation. Under the leadership of a Faculty Member of this University, Biosafety and Biosecurity program of Sylhet division is being organized and the University hosted a few such programs on its ground. I am indeed very happy to know that five faculties of my university are working as the resource persons in the Nationwide Biosafety and Biosecurity program organized by Bangladesh Biosafety and Biosecurity Society.

I am very much happy to know that the Society is going to publish the first edition of the Bangladesh Biosafety and Biosecurity Guidelines for handling and Disposal of Biohazardous Materials and my Faculty Members also contribute in it s development.

I am indeed hopeful that these guidelines will be practically helpful for implementing biosafety and biosecurity policies and procedures in Sylhet Division to contribute to the sustainable development in health and agriculture.

Professor Farid Uddin Ahmed

Vice-Chancellor
Khulna University

The academic programs of Khulna University started on 31 August 1991 with 80 students in four disciplines. Khulna University is the first public university to have acted as a powerful performer in the economic and cultural life of the southwestern region of Bangladesh. The university has a highly qualified faculty imparting instructions and providing persistent supervision to the students in the fields of Science, Engineering and Technology, Life Science, Business Studies, Social Sciences, Arts and Humanities and Fine Arts.

School of Life Sciences considering Biosafety and Biosecurity as the integral part of Biological Sciences and accordingly mentoring and even hosting sensitization and training program under the Bangladesh Biosafety and Biosecurity Society Since 2015. I am indeed very happy to know that faculties of my University have been contributing in the development of the First Edition of the Bangladesh Biosafety and Biosecurity Guidelines for handling and disposal of Biohazards and even contributing in the Society Council for organizing nationwide biosafety and Biosecurity Program.

I hope the First Edition of the Guidelines will provide timely support to develop and implement policies and procedures of Biosafety and Biosecurity in Biotechnology and Genetic Engineering and in all the other areas of Biological Sconces, as well.

Prof. Dr. Mohammad Fayekuzzaman

Vice-Chancellor
Bangabandhu Sheikh Mujib Medical University

Bangabandhu Sheikh Mujib Medical University is the first public medical university in Bangladesh established in 1998. The university offers courses like MD, PhD, MS, MPhil, MDS, Diploma and FCPS. There is no undergraduate medical or dental course. As the leading Medical University Biosafety and Biosecurity is prioritized in this University in academic courses and research and diagnostic activities. The University is the pioneer in introducing Biosafety and Biosecurity in academia in 2011.

As a medical University, Biosafety and Biosecurity is the integral part of the management system in diagnosis, research and clinical care. We are in a continuous development. The University is mentoring and supporting the biosafety and biosecurity program since it was initiated in 2011. The faculties of this university are actively involved in this program and playing leading role contributing to the development in this area.

I am indeed very happy to know that the Society has developed and going to publish the first edition of the Bangladesh Biosafety and Biosecurity Guidelines for researchers and Biosafety professionals. I would like to thank the Faculties of my university to significantly contribute in the development of these guidelines. I hope these guidelines will be very much useful in designing and implementing policies and procedures of Biosafety and Biosecurity in the field of diagnostics, research and clinical care in Bangladesh.

Professor Dr. Kanak Kanti Barua

Vice-Chancellor
Hajee Mohammad Danesh Science and Technology University

Hajee Mohammad Danesh Science & Technology University Started on 11 September 1999. The University is the first Science and Technology University in the northern region & the second oldest Science and Technology University of Bangladesh. Under the Faculty of Agriculture, Fisheries and Veterinary and Animal Science a total of 23 departments are working in the field of Biological Sciences conducting research with Biological materials and offering MSc and PhD Degrees.

I am indeed very happy to know that Bangladesh Biosafety and Biosecurity Society has developed its own Guidelines on Biosafety and Biosecurity and offering it for the Nation. On this regard I would like to congratulate the Society members for their contribution in developing these guidelines.

I am highly pleased to know that among the 14 resource persons working under Rangpur Division, 7 are from my University and actively contributing in strengthening biosafety and biosecurity capacity in Bangladesh. I myself also witnessed their activity organized in my University on 11-12 March 2018 inviting top management, mid level staff members, and the bench workers including faculties from Medical College.

I am hopeful that the guidelines will fulfill the long lasting desire of the scientists and researchers to contribute to the sustainable development through development and implementation of biosafety and biosecurity policies and procedures.

Professor Dr. M. Abul Kashem

Vice-Chancellor Patuakhali Science and Technology University

The Patuakhali Science and Technology University was inaugurated on 08 July 2000 by the then Honorable Prime Minister, Government of the Peoples Republic of Bangladesh, Her Excellency Sheikh Hasina. Among the eight Faculties in this University, Agriculture, Fisheries, Nutrition and Food Science, and Animal Science and Veterinary Medicine are the Faculties having 37 Departments working in the fiend of biological sciences research and offering MS and PhD degrees.

I am indeed delighted to know that Bangladesh Biosafety and Biosecurity Society already included this University under the Nationwide Strengthening Biosafety and Biosecurity Status Program in Bangladesh. Biosafety and biosecurity programs were organized not only in PSTU main campus but also at Barisal campus inviting all the faculty members working in the field of biological sciences.

As a leading University operating in the southern region, Biosafety and Biosecurity should be the paramount practice for the researchers working in the field of biological sciences. I am happy to know that the faculty members of PSTU have already been trained and included in the Council of the Society and they have started practicing Biosafety and Biosecurity at their work.

It is a great pleasure to know that the Society is going to publish the First Edition of the Bangladesh Biosafety and Biosecurity Guidelines where faculty members of PSTU also contributed for its development. I hope these guidelines will indeed help to attain the sustainable development goals in health and agricultural sectors in Bangladesh.

Professor Dr. Md. Harun-Or- Rashid

Vice-Chancellor Jessore University of Science and Technology

With a view to imparting science and technology oriented education in Bangladesh, the Jashore University of Science & Technology was established in 2007 by the Shadhinota Shorok (Independence Road) in Jashore district. The University started four-year undergraduate courses from the 2009–2010 session.

Under the Faculty of Biological Sciences and Technology, the University is operating four departments where students and faculties are conducting basic and advanced research in the field biological sciences and obtaining Masters and PhD degrees with significant contributing in the advancement of Science and Technology. Overall the University encourages practicing of safe techniques for advancing research science and thus new laboratories are being developed with the incorporation of essential safety and security features.

Bangladeshi Biological Scientists have been waiting for a long time for Biosafety and Biosecurity Guidelines as a single reference document, for facilities handling biohazards originated from humans, animals, environment and plants, to implement guidance of biosafety principles and practices, facility design and construction and dispose biohazards. I am indeed delighted to know that the Society is going to publish the First Edition of the Bangladesh Biosafety and Biosecurity Guidelines for Handling and disposal of biohazardous materials that included all the areas of our desire.

As a member of the 'Core Committee" for initiating the development of the guidelines, I would like to congratulate all the contributors for their contribution in developing these guidelines and hope that the document will substantially help us to develop and implement policies procedures of Biosafety and Biosecurity for biotechnological and microbiological research and diagnostic activities in Bangladesh.

Ahossain

Professor Dr. Md. Anwar Hossain

Director General

Directorate General of Health Services
Ministry of Health and Family Welfare

I am highly pleased to know that the Bangladesh Biosafety and Biosecurity Guidelines are developed in the context of the handling and disposal of biohazardous materials. The guidelines included all issues of biological hazards originated from microbiological and biomedical research and diagnostics. It was a long cry of the researchers working in the field of biological sciences to have a single document guiding on all the aspects of principles and practices of biosafety and biosecurity, construction of laboratory facilities, and disposal of waste generated for health care, to be followed to implement biosafety and biosecurity policies and procedures in biochemical and microbiological research and diagnostics. Thus, for inclusion of all the chapters in a thoughtful manner was indeed a big challenge for the core committee. On this regard I would like to congratulate all the contributors and the core committee members to fulfill of their jobs, at the completion of the guidelines.

I am thankful to the Centers for Disease Control and Prevention (CDC), Atlanta, USA, and the Biosecurity Program (BEP) of the United States Department of State for the support for developing the guidelines. I am thankful to Biosafety Office of icddr,b for substantial support for developing these guidelines and taking forward the Biosafety and Biosecurity activities in Bangladesh.

I would like to thank Professor **Dr. Be-Nazir Ahmed**, Professor **Dr. AKM Shamsuzzaman**, and Professor **Dr. Sanya Tahmina Jhora** to guide the Society from its inception.

I am hopeful that researchers and biosafety professionals in Bangladesh will enjoy the full benefit of these guidelines.

Professor Dr Abul Kalam Azad

Director General
Department of Livestock Services
Ministry of Fisheries and Livestock

Department of Livestock Services, under the Ministry of Fisheries and Livestock, operating Central Disease Investigation Laboratory (CDIL), eight Field Disease Investigation Laboratories (FDILs), Nutrition Laboratory, Veterinary Public Health Laboratory, Quality Control Laboratory and the Livestock Research Institute (LIR). All these facilities are dealing with biological hazard and spontaneously Biosafety and Biosecurity considerations are paramount for the Department. And thus Biosafety and Biosecurity Programs organized by Bangladesh Biosafety and Biosecurity Society are highly appreciated, well accepted, and fully participated by the Department since the first program organized in 2011.

I am indeed delighted to know that among the 130 total resource persons working in Bangladesh in the field of Biosafety and Biosecurity, 34 are from the DLS. These resource persons, as the members of the Society, are substantially contributing in the advancement of Biosafety and Biosecurity Program in the country. All together, the contribution of these resource persons has established the Society as the strong one-health platform, which is essentially needed for a robust biosafety and biosecurity program nationwide in a country like Bangladesh.

I am very happy to know that the Society has developed the First Edition of the Biosafety and Biosecurity and Biosecurity Guidelines for the one-health platform taking into consideration of the origination biohazards from the activities with human health, animal health, agriculture and the environment. I believe that these guidelines will be fully utilized by the DLS to contribute to the sustainable in health and agriculture in Bangladesh.

Dr. Hiresh Ranjan Bhowmik

Director Disease Control

Directorate General of Health Services
Ministry of Health and Family Welfare

Disease Control Division of the Directorate General of Health Services has been closely mentoring the Biosafety and Biosecurity program organized by the Bangladesh Biosafety and Biosecurity Society since 2011. Under the guidance of the Director Disease Control, the Society is working for strengthening biosafety and biosecurity status in Bangladesh since 2013 supported by Centers for Disease Control and Prevention (CDC), Atlanta, USA and the Biosecurity Engagement Program (BEP) of the United States Department of State. Under this program resource persons were developed to work in all the eight divisions in Bangladesh in the field of biosafety and biosecurity. Considering the necessity of time and recognizing the gaps due to the absence of a pertinent guidelines, the biosafety and biosecurity guidelines for handling and disposal of biohazardous materials first outlined by the five member core committee leaded by the Director Disease Control and line Director Communicable Disease Control, Directorate General of Health Services, Ministry of Health and Family Welfare on 11 July 2015. I am indeed happy to know that finally the guideline is available for the researchers and biosafety professionals in Bangladesh to implement biosafety and biosecurity policies and procedures in diagnostic and research activities in Bangladesh.

I would like to thank CDC, BEP, and Biosafety Office of icddr,b for the support for organizing biosafety and biosecurity program in Bangladesh. I am grateful to all the members of the executive committee the Society, divisional coordinator, and resource persons for their hard work to contribute to these guidelines to make it a complete guiding document.

Professor Dr. Sanya Tahmina Jhora

Acknowledgements

We wish to emphasize that the 1st edition of the Bangladesh Biosafety and Biosecurity Guidelines for Handling and Disposal of Biohazards will be the document recommending best practices for biorisk management in agricultural, biomedical, clinical, microbiological, plant, or animal sciences research and diagnostics or field activities from biosafety and biosecurity perspectives. This is not a regulatory document; however, regulators in Bangladesh may like to follow this document to formulate rules and establish regulations on biosafety and biosecurity concerns and thus, we recognize that it will be used in that way by the regulators.

Biosafety is to protect Earth's entire biosphere from unintentional release of biohazards and biosecurity is to limit access to biological materials to protect the biosphere from intentional release of biohazards. Taking into consideration of these universal definitions of biosafety and biosecurity, these guidelines are concentrated on mitigating biorisk in diagnostics and research activities in Bangladesh in all the areas of biological sciences.

After the first training of the trainer program organized by Biosafety Office, icddr,b, utilizing the platform of Bangladesh Biosafety and Biosecurity Society, development of Bangladesh Biosafety and Biosecurity Guidelines for researcher and biosafety professionals became a priority responsibility for the Society to guide the resource persons and the society members to follow and establish principles and practices of biosafety and biosecurity. Accordingly, the core committee was formed to determine the structure and content of the guidelines. We thank the core committee for their valuable support and guidance.

The guidelines include sections suggested and included after vetting by the experts. The content of the guidelines was developed/collected/incorporated by the resource persons, experts and faculty members from the Universities, Medical Colleges and Research Organizations from all over Bangladesh during several consultative workshop. During the final consultative workshop, we worked to harmonize the recommendations received from the previous workshop and included in the final version. We thank the contributors for their hard work to complete the content of this document.

The main intent of developing the guidelines was to generate a single document as a reference for developing safe facilities, managing biosafety and biosecurity program, and safely disposing biological waste. We looked for the best available guidelines like CDC/NIH, WHO guidelines, Canadian Biosafety Guidelines, Singapore Biosafety Guidelines for GMO, Arthropod Containment Guidelines of American Society of Tropical Medicine and Hygiene, NIH recombinant DNA guidelines, and collected and adapted content of this guidelines following those standard documents. We thank all the original contributors for their work that made our path easy to confidently develop these guidelines.

We thank Disease Control Division of the Directorate General of Health Services for continuously supporting Biosafety and Biosecurity Program in Bangladesh organized by Bangladesh Biosafety and Biosecurity Society. We are thankful to Department of Livestock Services and the Department of Environment for the continuous support. We are thankful to Vice-Chancellors University of Dhaka, University of Rajshahi, Bangladesh Agricultural University, University of Chittagong, Shahjalal University of Science and Technology, Khulna University, Bangabandhu Sheikh Mujib Medical University, Hajee Mohammad Danesh Science and Technology University, Patuakhali Science and Technology University and Jessore University of Science and Technology for hosting our sensitization and training programs. We are also thankful to Executive Director of icddr,b for continuous and substantial support for the nationwide strengthening biosafety and biosecurity program.

We thank Centers for Disease Control and Prevention, Atlanta, USA and Biosecurity Engagement Program of the United States Department of State for the continuous support from 2013 to 2018 for Strengthening Biosafety and Biosecurity Status in Bangladesh and Promoting Culture Free Disease Diagnosis and Effective Disease Detection and Control Strategies in Bangladesh programs.

I am thankful to all the patrons, advisors and members of the Society for their support.

We hope that the users of these guidelines will find it helpful to implement principles and practices of biosafety and biosecurity in all the areas of biological sciences leading to sustainable development in Health and Agriculture.

Dr. Asadulghani
Chairman, Bangladesh Biosafety and Biosecurity Society

LIST OF ABBREVIATIONS

ABSL	Animal Biosafety Level
ACL	Arthropod Containment Level
AIDS	Acquired immunodeficiency syndrome
ANSI	American National Standards Institute
ATCC	American Tissue Culture Collection
BSC	Biosafety Cabinet
BSL	Biosafety Level
BSO	Biological Safety Officer
CDC	Centers for Disease Control and Pretension, Atlanta, USA
DNA	Deoxyribonucleic acid
GM	Genetic Manipulation
GMO	Genetically Modified Organism
HEPA	High Efficiency Particulate Air Filter
HIV	Human Immunodeficiency Virus
HVAC	Heating Ventilating and Air Conditioning
IATA	International Air Transportation Association
IBC	Institutional Biosafety Committee
ISO	International Organization for Standardization
LAN	Local Area Network
MoEF	Ministry of Environment and Forest
MoHFW	Ministry of Health and Family Welfare
MSDS	Material Safety Data Sheet
NCB	National Committee on Biosafety
NIAID	National Institute of Allergy and Infectious Disease
NIH	National Institute of Health, USA
NSF	National Sanitation Foundation
P-BSL	Plant Biosafety Level
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PPE	Personal Protective Equipment
RG	Risk Group
SARS	Severe acute respiratory syndrome
UV	Ultraviolet
VBM	Valuable biological materials
VHP	Vaporized hydrogen peroxide
WHO	World Health Organization

TABLE OF CONTENTS

SECTION 1.0: RESEARCH AND DIAGNOSTICS WITH BIOHAZARDOUS MATERIALS

Chapter 1.1: Introduction

1.1.1 BACKGROUND

Bangladesh is a country in southern Asia, in the Ganges River delta, on the Bay of Bengal. In recent years laboratory capacity has grown to meet the needs of diagnosing illnesses and conducting research on endemic and emerging pathogens in humans, animals, plants and environmental specimens in Bangladesh. Human Nipah outbreaks in Bangladesh are unlike other Nipah outbreaks as they exhibit person-to-person transmission and recur in a specific region almost annually during the winter and spring. Both the national and international organizations in Bangladesh are working in collaboration to investigate such diseases outbreaks [1,2].

TB isolates are found to be resistant to at least one drug, with significant number of multi-drug resistance since 2005, underscoring the need for appropriate containment and inventory procedures. Other endemic diseases such as *Bacillus anthracis* are transmitted to the human population by contaminated meat, with the most recent outbreak occurring 2010-2011. Like surrounding countries, Bangladesh also has had reported cases of H5N1 in birds that have culminated in human infections. Currently animal diseases are also diagnosed and researched in Bangladesh.

Success with *Bacillus thuringiensis* (*Bt)* brinjal has led Bangladesh to prioritize the field testing of a new late blight resistant potato, which is an important crop occupying 0.5 million hectares in Bangladesh. Once released, the blight resistant (RB) potato will be farmers' answer to late blight, one of the most devastating plant diseases caused by fungal attack. *Bt* cotton is being evaluated in field trials as well as Golden Rice, which could address the prevalent Vitamin A deficiency in the country [3,4].

Genetically modified (GM) crops may have better taste, increased nutrients, resistance to disease and pests, and faster output of crops. It is believed that farmers can grow more food on less land with GM crops. Such animals have certain genes inserted into their genomes and thus they can produce ‘better’ milk, eggs, and meat. These animals are also expected to have a higher resistance to disease and overall better health, with better natural waste management. Theoretically, GM crops and animals will also be more environmentally friendly because they conserve water, soil, and energy. However, there are controversies. One of these controversies are the potential health risks, including allergies, antibiotic resistance, and unknown effects. Genetically modified organisms (GMOs) or living modified organisms (LMOs) are going to be more and more popular in Bangladesh. Thus, this is the time to grow awareness and to formulate and implement policies and procedures on the development and use GMOs and LMOs in Bangladesh [3,4,5].

Thus, considering the above all “Bangladesh Biosafety and Biosecurity Guidelines” for handling and disposal of biohazardous materials in Bangladesh is developed, adhering to applicable guidelines, rules and regulations [5,6,7,8,9].

1.1.2 POLICY

Adequate safety measures will be followed while handling and manipulating all biohazardous materials in Bangladesh. All health care facilities, diagnostic laboratories, research facilities, and academic arena where biohazardous materials are handled (collection, transportation, lab testing, research, storage and disposal) should put into force all the safety sustaining standards of practices mentioned below to minimize possible risks to human health and the environment while extracting maximum benefits from any potential that biological sciences and modern biotechnologies may offer:

1.1.2.1 Biosafety and Biosecurity Program

Risk assessment for biohazardous agent shall be conducted for each diagnostic and research area [7,8,9,10]. To conduct risk assessment, a generalized five-step approach is as follows:

- Identify the biorisk characteristics and doses of biohazardous agents handled by the laboratory.
- Identify laboratory practices that increase exposure risks such as aerosol-generating procedures and the use of sharps.

- Determine the appropriate biosafety level (BSL) and develop a biosafety program that includes the appropriate precautions, practices, personal protective equipment (PPE), safety equipment and facility design [7,8,9].
- Review the risk assessment process and biosafety program with a biosafety professional.
  - Ensure staff knowledge and proficiency regarding the laboratory's biosafety and biosecurity program, including the use of PPE and safety equipment.
  - Based on this risk assessment Identify the laboratory's biosafety level(s).
  - Develop and implement an appropriate biosafety and biosecurity program.
  - Incorporate the use of safety equipment, practices and procedures.

NOTE: Human, animal, and environmental diagnostic laboratories must minimally meet Biosafety Level (BSL) 2 criteria [7,8,9,10]. Teaching laboratories will follow policies and procedures of BSL1 or BSL2 depending on the biohazards handled within [7,8,9,10,11].

1.1.2.2 Biosafety and Biosecurity Training

- All personnel involved with handling biohazardous materials, clinical specimens and other infectious or potentially infectious materials and/or biomedical waste and the concerned top management shall receive training on the laboratory's biosafety and biosecurity program [7,8,9,10,11] including:
  - The potential hazards associated with their work activities and the practices, and
  - Procedures intended to avoid exposure to and/or dissemination of infectious material.
  - Training shall be conducted as part of initial employee training and annually thereafter and shall be documented.
  - Training should include familiarization with the laboratory's occupational exposure control plan.
  - Training and discussion should be supplemented with ongoing supervisory observation to ensure staff compliance with the laboratory's safety policies and proper use of PPE.

1.1.2.3 Personal Practices

- Eating, drinking, smoking, handling contact lenses, and applying cosmetics or lip balm are prohibited in work areas that present a reasonable likelihood of occupational exposure to hazardous/infectious materials [10].
- Personal electronic devices (e.g. cell phones, beepers) or other personal items should not be handled at the workstation [10].
- No pet animals and aesthetic plants should be allowed to be placed inside laboratory [7,8,9].

1.1.2.4 Biohazard Labels

- Warning labels with the universal biohazard symbol or with the signage "Biohazard" shall be affixed to refrigerators, freezers and incubators containing clinical specimens and other infectious or potentially infectious materials [3,4,5].
- Warning labels with the universal biohazard symbol or with the signage "Biohazard" shall be affixed on the infectious or potentially infectious waste materials' disposal or storage bin [7,8,9]. On the top of the signage "Infectious/Biohazardous Waste" should be clearly written.

1.1.2.5 Food Storage

- Food and drink shall be stored outside the work areas in cabinets or refrigerators designated for this purpose and not in refrigerators or areas where clinical specimens or other infectious or potentially infectious materials may be present [7,8,9,10].

1.1.2.6 Biological Safety Cabinet (BSC)

Laboratories utilizing a BSC shall:

- Decontaminate the BSC with an appropriate disinfectant before and after each use and immediately following a spill or splash [7,8,9,10];
- Monitor the air flow while in use [12];
- Test and certify the BSC *in situ* (within the laboratory) at the time of installation, at any time the BSC is moved, and at least annually thereafter [12];
- Document that all users are trained in the proper use of the BSC and are periodically observed for compliance with defined practices [7,8,9,10]; and
- The need for a class II or higher BSC should be determined based on the laboratory's biohazard risk assessment [7,8,9,10].

1.1.2.7 PPE Availability, Use and Maintenance

The employer shall:

- Provide PPE as appropriate for the type of work performed at no expense to the employee [7,8,9,10];
- Ensure that PPE is accessible at the worksite and properly maintained;
- Provide cleaning, maintenance and/or disposal at no cost to the employee;
- Ensure that employees are trained in the proper use of PPE prior to use, including donning and doffing, which is closely monitored by Biosafety officer;
- Not allow employees to wear PPE outside the work area;
- Not allow employees to remove PPE or laboratory coats from the premises;
- Aprons worn in public areas outside the laboratory or health care facility should not be used as PPE and should be stored in an area clearly delineated from PPE and work stations;
- PPE such as PAPRs (Powered Air Purifying Respirators) or respirators should be examined prior to each use and should be inspected annually. A visual inspection of the hosing, bonnet, and unit as well as a battery check should be performed every time the unit is used; and,
- Annual competency assessment should include the proper use of all PPE.

1.1.2.8 Disposable Gloves

The laboratory's biosafety program shall include a policy regarding use of disposable gloves when handling biohazardous or potentially infectious materials including those gloves [13]:

- May optionally be worn in defined situations in which it is unlikely that handling items will result in direct contact with infectious or potentially infectious materials;
- Must be worn when handling primary specimens. The term "primary specimen" refers to all fresh, frozen or diluted patient specimens, specimens collected from animals or any kind of environmental samples that have not been processed or treated to eliminate infectious risk.
- Must be worn when handling any items for which there is a likelihood that such handling may result in direct contact with infectious or potentially infectious materials;

- Must be worn when the employee has cuts, scratches or other breaks in the skin and is handling infectious or potentially infectious materials, regardless of likelihood of direct exposure;
- Must be removed and discarded immediately upon contamination;
- Must be removed and discarded immediately upon task completion at each work station (e.g. BSC, bench space);
- Must not be washed or reused;
- The laboratory's risk assessment should guide the laboratory head in tailoring a "glove use policy" that is based on the type of work performed by the laboratory and the nature and dose of infectious agents likely to be handled by the laboratory;
- Hands should be washed each time gloves are removed. Chemical disinfectants are not considered an acceptable alternative to soap-and-water hand washing in the clinical laboratory setting;
- Caution should be observed in removing gloves; snapping or stretching the gloves may result in aerosol formation;
- Removing gloves immediately upon leaving each workstation greatly reduces the likelihood for inadvertent contamination of communal and personal objects (e.g. phones, pencils, keyboards, etc).

1.1.2.9 Sharps

The laboratory biosafety and biosecurity program shall include the following practices [14]:

- Training for the safe handling of sharps;
- Needles shall not be recapped, or removed from syringes or other devices, unless it can be demonstrated that no alternative is feasible or that such action is required by a specific procedure (e.g., collection of blood gas specimens); and,
- Used disposable needles shall not be bent, sheared, broken, removed from syringes or otherwise manipulated by hand, but shall be placed in a puncture-proof, leak-proof container used for sharps disposal.
- Sharps container should not be overfilled.

1.1.2.10 Safety Breaches

The laboratory safety manual shall include the procedure for decontaminating spills and splashes of infectious or potentially infectious materials [8]. Such incidents, as well as other safety breaches, shall be:

- Cleaned immediately and surfaces decontaminated using an appropriate disinfectant;
- Immediately reported to the laboratory Head and documented;
- Assessed for the need to implement the employee exposure plan;
- Investigated to identify cause; and
- Followed up with remedial action and retraining as necessary.
- Spill decontamination protocols should be adequate for the spill size, location (e.g. floor, inside BSC) and nature of the spilled materials.
- Procedures should include guidance for safe clean-up and disposal of broken glass and other sharps.

1.1.2.11 Employee Occupational Exposure Plan

The laboratory shall establish an employee biohazardous agent exposure control plan appropriate for the testing and procedures performed by the laboratorians [8]. The plan shall include:

- Immediate notification of the laboratory director or designee of an occupational exposure or of an employee exhibiting symptoms consistent with an occupational exposure;

- Medical risk assessment;
- Diagnostic testing and treatment, as appropriate
- Root cause investigation; and
- Implementation of corrective action and retraining as necessary.
- The employee exposure plan should be developed based on the laboratory's biohazardous agent risk assessment and should take into account the specimen types received and the procedures performed.

1.1.2.12 Facilities

Laboratory facilities shall be designed to ensure that infectious agents cannot be transmitted to workers or the general public [7,8,9,10] and shall include:

- A pest management plan which ensures that pests cannot act as a mechanical vector to spread infectious agents;
- Sufficient space between benches, cabinets and equipment to allow adequate cleaning;
- Flooring and furniture located in the testing laboratory that can be easily cleaned and decontaminated;
- Work surfaces that are impervious to water and resistant to moderate heat and the chemicals used for cleaning and decontamination;
- Adequate hand washing facilities within the laboratory work area;
- Eye wash station;
- Emergency showers, if appropriate; and
- Doors, preferably self-closing, to facilitate access control,
- Emergency exit to evacuate in case of emergency.
- The pest management plan can include mechanical barriers such as screens on the windows to prevent flies from entering the laboratory or visual inspection of the structural integrity of the facility.
- Carpets and rugs shall not be used in the laboratory.
- Chairs and other furniture used in the laboratory work area should be covered with non-fabric materials that can be easily decontaminated.
- Minimally, hands should be washed immediately upon contamination, removing PPE and completing each laboratory task, prior to leaving the laboratory and prior to handling communal objects (e.g. phone, keyboard, etc).
- Chemical disinfectants are not considered an acceptable alternative to soap-and-water hand washing in the clinical laboratory setting. Emergency showers are required where employees may be exposed to caustic or corrosive chemicals.

1.1.2.13 Access to Facilities

Access to the laboratory shall be limited or restricted as required to protect the public and/or employees [7,8,9,10].

- The laboratory head is responsible for defining and approving the levels of access and identifying the laboratory's biosecurity practices, as appropriate for the setting.

1.1.2.14 Work Surface Decontamination

Laboratory work surfaces shall be decontaminated with an appropriate disinfectant following spills of infectious or potentially infectious material, and at the start and completion of work activities [7,8,9,10].

- When using diluted household bleach (5.25% sodium hypochlorite), it is recommended that 1:10 dilutions be prepared daily.

1.1.2.15 Specimen Storage

Any biological materials stored for more than 90 days should be considered as long term storage [7,8,9].

- Complete inventory of the stored materials should be developed for biological specimens and infectious agents.
- The inventory of the stored biological materials should be available in the facility.
- Samples that are unlabelled mislabeled, or for which the origin, type, date of collection, or ownership are unknown, should be inactivated or destroyed following procedures effective method.
- Storage facility can be separated based on the risk group of microorganisms present in the facility.

1.1.2.16 Specimen Packaging and Shipping

The laboratory shall comply with International Air Transport Authority (IATA) regulations and requirements for packaging and shipping of infectious substances [14,15].

- Packaging and shipping requirements vary based on several factors including the type of specimen; likelihood that the specimen contains a category A or Category B [15]; and the type of carrier/shipper being used (e.g. commercial carrier; private ground carrier; air transport) [16].
- Additionally National Institute of Allergy and Infectious Diseases emerging infectious diseases/pathogens category may also be considered that includes Biodefense Research and Additional Emerging Infectious Diseases/Pathogens [17].

1.1.2.17 Chemical Hygiene Plan

Laboratories shall develop and implement a written chemical hygiene plan [8] that shall be available to employees upon request whenever laboratory work involves the use of hazardous chemicals. The plan shall:

- Describe the use of fume hoods or other protective equipment whenever handling hazardous materials;
- Establish procedures for exposure monitoring when permissible exposure levels of hazardous materials are exceeded;
- Describe precautions for handling reagents containing toxic, hazardous or radioactive substances, including methods for their proper labeling and disposal;
- Ensure proper storage of hazardous materials, including the use of a flame proof cabinets, where appropriate;
- Establish a designated area for hazardous chemical storage and disposal;
- Include an action plan for dealing with laboratory accidents;
- Contain a protocol for managing documented exposure to chemical or radiological materials;
- Contain a management protocol for maintenance of chemical and radiological exposure records on each employee;
- Document that employees are provided with training regarding toxic substances in the workplace and use of protective equipment;
- Provide that Material Safety Data Sheets (MSDS) for all chemicals in use are on file in the laboratory and are readily available to all employees at all times.

- The laboratory should have proper ventilation systems to rid the area of fumes created from hazardous materials. Acceptable limits should not be exceeded.
- Minimally, training should be conducted as part of initial employee training and annually thereafter.

1.1.2.18 Radioactive Materials

Laboratories must follow national rules (Nuclear Safety & Radiation Control Rules-1997) related to the handling of radioactive materials:

- Have a license to store radioactive materials;
- Maintain documentation of inspection by Radiation Protection pursuant and ensure ongoing compliance with such regulations; and
- Document that employees are trained in the handling and disposal of radioactive materials.
- Minimally, training should be conducted as part of initial employee training and annually thereafter.

1.1.2.20 Biomedical Waste

Laboratories must follow all the institutional and national rules for the handling of biomedical waste and bags and containers used during the transportation of the waste shall be constructed to prevent release or leakage of potentially infectious materials and liquids [18,19,20,21,22].

- Laboratories must transport sterilized or properly packaged or sealed containers or bags to stop the spreading of biohazardous materials during transportation [19,29,23].
- Laboratories must manage waste containing GMO/LMO following institutional, national and international guidelines [24].

1.1.3 PURPOSE

These guidelines are to establish and to ensure the adequate biorisk assessment, containment, handling, transport, and disposal of biohazardous materials used in various diagnostics and research purposes in Bangladesh.

1.1.4 SCOPE

The scope of the Bangladesh Biosafety and Biosecurity Guidelines for Handling and Disposal Biohazardous Materials that involve diagnostics, research, construction and/or propagation of all biological entities (cells, organisms, prions, viroids or viruses) which have been isolated from source or made by genetic manipulation and are of a novel genotype or which are unlikely to occur naturally or which could cause public health or environmental hazards.

1.1.5 DEFINITIONS

1.1.5.1 Biohazard

A risk or hazard to human or animal health or the environment arising from biological work, especially with microorganisms [8].

1.1.5.2 Biosafety

Control of biohazard to prevent the transmission to workers, other persons or the environment controlling unintentional release. Biosafety protects not just humans from harm but Earth's entire biosphere as well [8].

1.1.5.3 Biosecurity

Security measures to protect the release of a biohazard like microbial agents, biological pathogens, toxins, critical information, pests or diseases as a result of theft or misuse [25].

1.1.5.4 Biorisk

Biorisk encompasses biosafety and biosecurity (accidental infection or unauthorized access, loss, theft, misuse, diversion or intentional release), where the hazards are biological agents and their products [25].

1.1.5.5 Biorisk Assessment

Process of evaluating the biorisk(s) arising from a biohazard(s), taking into account the adequacy of any existing controls, and deciding whether or not the biorisk(s) is acceptable [25].

1.1.5.6 Biosafety Officer/Personal

A professional who develops and participates in Biosafety and Biosecurity programs for microbiological or biomedical research and diagnostics to promote safe practices, procedures, and proper use of containment equipment and facilities, stimulates responsible activities among workers, and provides advice on facilities design where infectious or biohazardous materials are handled, manipulated, or stored [8,9].

1.1.5.7 Containment

The principles of holding or being capable of holding or including within a fixed limit or area, preventing the unintentional release of biological agents through a combination of laboratory practices, containment equipment (primary barrier) and laboratory facility design (secondary barrier) [8,9].

1.1.5.8 Genetically Modified Organism (GMO)

A GMO or transgenic organism (microorganism, plant, vertebrate - e.g. GM mouse, and/or invertebrate - e.g., GM fruit fly) may be defined as one in which the genetic material has been altered in a way that does not occur naturally by mating or natural recombination or by a combination of both [24].

1.1.7.9 Institutional Biosafety Committee (IBC)

The IBC is the cornerstone of institutional oversight of research relating to biohazardous materials or recombinant DNA [8,9,24].

1.1.5.10 Pathogenic Agents

Any microbial agent or biological toxin capable of causing illness in humans, animals or plants [8,9,10].

1.1.5.11 Recombinant or Synthetic Nucleic Acids

Molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids. Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids are the synthetic Nucleic Acids. Molecules that result from the replication of those described earlier are also considered under either of the groups [24].

1.1.5.12 Potentially Infectious Material (PIM)

In 2015, the Global Commission for the Certification of the Eradication of Poliomyelitis certified the eradication of wild poliovirus type 2. The eradication of WPV1 and WPV3, and the elimination of circulating oral polio vaccine-derived polioviruses are anticipated in the near future, along with the gradual disappearance of immunodeficiency-associated VDPV excreters. At that point, the only remaining poliovirus (PV) reservoirs will be the facilities retaining PV infectious or PV potentially infectious materials [19,20].

1.1.6 ROLES AND RESPONSIBILITIES

1.1.6.1 Institutions

Any institution, company or organization that carries out research and diagnosis with infectious biohazardous materials, genetic manipulation in live organisms, imports organisms arising from such work, produces such organisms, or plans to sell or release such organisms into the environment, should abide by all existing legislation and relevant guidelines, especially national biosafety guidelines and rules [7,8,9].

- Institutions, companies or organizations are required to establish an IBC and provide the resources and facilities, which are necessary for safe work in laboratories. The IBC should carry out its duties adequately, and ensure, by recruitment, procedures and other measures that adequate supervision of work occurs.
- Institutions may consider making compliance with the National Committee(s) on Biosafety (NCB) as appropriate. Those Institutions conducting large scale or industrial scale work should have a dedicated BSO.
- Small institutions and companies may have difficulties in establishing an IBC. These institutions and companies can choose to be supervised by another IBC. Such arrangements should be formalized between the institutions concerned, and the NCB notified. A representative of the smaller institution should closely liaise with, or be a member of the IBC.
- Institutions should recognize the essential roles of their IBC and give it the authority and support its needs to undertake its duties.
- The institution should ensure that laboratory staff is informed of hazards and have adequate training to make sure that their work is carried out within these guidelines. The IBC Chair or BSO should be readily accessible to give advice.
- High Containment Facilities (HCF) like Biosafety Level 3 (BSL3) and Biosafety Level 4 (BSL4) facilities (laboratories, animal houses, plant houses, insectaries, bird houses, aquaria) are to be approved by the regulatory agency (ies) and certified by international certifiers. Institutions planning to conduct experiments, which require BSL3 or BSL4 containment, should notify the NCB. The necessary advice regarding the structural requirements about these BSL3 and BSL4 facilities are included in Section 2 of these guidelines. This advice may also be found in the Laboratory Biosafety Manual, 3rd Ed, WHO and Biosafety for Microbiological and Biomedical Research 5th Ed, CDC/NIH.

1.1.6.2 IBC and its Function

IBC is vital for executing guidelines and thus the monitoring and surveillance of handling and manipulation of infectious material and genetic manipulation work. The competence and knowledge of IBC members should be such that it can competently undertake its duties. The IBC chair shall be a senior, knowledgeable person with adequate firsthand experience in biosafety and recombinant DNA technology. The chair shall preferably have clear understanding about laboratory research including recombinant DNA technology, infectious and biohazardous agents. The Biosafety officer, if not its chairman, shall at least be an ex-officio member of this Committee. The Chair of the Committee should be of sufficient standing in the institution for decisions and advice by the IBC to be effectively carried out. Appropriate deputizing arrangements should be made when the Chair is on leave [5,6,7,8,9,24].

- The composition of a basic biosafety committee may include but not limited to:
  - Biosafety officer(s)
  - Scientists
  - Medical personnel
  - Veterinarian(s) (if work with animals is conducted)
  - Representatives of technical staff
  - Representatives of laboratory management.
- One microbiologist and one molecular biologist and/or a geneticist should be included as well as a scientist with expertise relevant to the organisms being studied in the institution. Different disciplines need only be represented when work falling within that area is performed in the institution. For example, an institution working only on plants need not have an animal geneticist. Responsibilities may be combined in the same person if appropriate.
- The IBC should ensure that NCB and its own advice on proposals are received by principal investigator(s) and, if necessary, are acted upon. The IBC should visit laboratories and facilities occasionally to monitor

biosafety aspects and implementation.

- In order to affect the intent of these guidelines, an IBC may draft whatever rules it considers necessary to supplement these guidelines. Furthermore, IBC should have appropriate powers to ensure that all aspects of these guidelines are observed.
- The main functions of the IBC are to:
  - Assess all research proposals it receives, (including changes to BSL1 projects), so as to identify potential hazards to the researchers, the public and the environment. It should also advise the investigator(s) about these hazards and their management;
  - Ascertain the containment level and procedures for experiments falling within Categories A and B, and determine the storage and transportation requirements for genetically manipulated organisms falling within these Guidelines;
  - Send an original typed 'Proposal Form for Assessment of Genetic Manipulation Work', together with the IBC's assessment, to the NCB for assessment of experiments falling under BSL3/4, and make sure that NCB advice is followed. For BSL2, an original typed proposal form, together with the IBC's assessment, may be sent to NCB for suggestion. See Appendix 4.2 for instructions on completing proposal forms;
  - Inspect plant houses, animal houses, bird houses, insectaries and aquaria before they are used for genetic manipulation work. The IBC should also conduct inspections and monitor procedures in all the institution's containment facilities. At least annual inspections of these facilities should be carried out to make sure that they continue to meet the relevant containment standards;
  - Monitor ongoing work within the institution from time to time and make recommendations to investigators, if appropriate;
  - Assess the qualifications and experience of personnel involved in research proposals, to make sure that they are adequate for good microbiological practice and the supervision of junior personnel;
  - Maintain a register of approved projects with their assessment as well as projects exempted under guidelines;
  - Maintain a list of the personnel who work in containment facilities, and ensure that new workers are familiar with the appropriate containment procedures and the correct use of laboratory equipment.
  - Take responsibility for drafting rules and making decisions about specific procedural safety matters. NCB does not need to be consulted about these, as long as they are consistent with these guidelines.
  - When the IBC is being set up it should provide NCB with a completed 'Annual Report by Institutional Biosafety Committee' form.
  - To avoid any potential conflicts of interest, IBC members should not assess their own proposals that they have submitted. The IBC should have sufficient scientifically qualified members to ensure that proposals can be adequately evaluated.
  - IBC members who may have commercial interests on an item of the agenda being assessed should declare their interests and be excluded from the meeting.

1.1.6.3 Biological Safety Officer

To ensure that biosafety policies and programs are followed consistently throughout the laboratory/institute Biological Safety or Biosafety Officer (BSO) is indispensable. This is the responsibility of the BSO to execute these duties on behalf of the head of the institute/laboratory. S/he is an individual with academic degree preferentially Master of Science or higher academic degree in the field of Biological Sciences with technical competence and knowledge in Biochemistry and Molecular Biology, Biotechnology and Genetic Engineering, Microbiology, or Medical Microbiology having in depth knowledge in recombinant DNA technology and possess the professional competence necessary to suggest, review and approve specific activities that follow appropriate biosafety procedures. The BSO should apply relevant national and international rules, regulations and guidelines, as well as assist the laboratory in developing standard operating procedures. The person appointed must have substantial knowledge of laboratory and clinical practices and safety, including

containment equipment and engineering principles, relevant to the design, operation and maintenance of facilities, which is highly desirable. S/he should also be able to communicate effectively with administrative, technical and support personnel [5,6,7,8,9,24].

- The activities of the BSO should include, but not limited to, the following:
  - Biosafety, biosecurity and technical compliance consultations.
  - Periodic internal biosafety audits on technical methods, procedures and protocols, biological agents, materials and equipment.
  - Discussions of violation of biosafety protocols or procedures with the appropriate persons.
  - Verification that all staffs have received appropriate biosafety training.
  - Provision of continuing education in biosafety.
  - Investigation of incidents involving the possible escape of potentially infectious or toxic material, and reporting of findings and recommendations to the laboratory director and biosafety committee.
  - Coordination with medical staff regarding possible laboratory-acquired infections.
  - Ensuring appropriate decontamination following spills or other incidents involving infectious material(s).
  - Ensuring proper waste management.
  - Ensuring appropriate decontamination of any apparatus prior to repair or servicing.
  - Maintaining awareness of community attitudes regarding health and environmental considerations.
  - Establishment of appropriate procedures for import/export of pathogenic material to/from the laboratory, according to national regulations.
  - Reviewing the biosafety aspects of all plans, protocols and operating procedures for research work involving infectious agents prior to the implementation of these activities.
  - Institution of a system to deal with emergencies.
- Institutions should either appoint a BSO, or assign such duties to the IBC. If institutions have more than one officer, for the purposes of these Guidelines, only one name per institution is to be submitted to NCB in the annual reporting requirements. The officer should ideally have experience in working with containment conditions and should be sufficiently trained and competent to offer advice on, or participate in staff training. Suitable deputizing arrangements should be made when the officer is on leave.
- The BSO should act as adviser to the head of the institution or company in all biosafety matters. The BSO or the IBC should carry out regular safety audits and the supervision of a regular evaluation program for relevant pieces of equipment.
- The BSO should also consult the relevant guidelines for additional requirements that need to be fulfilled.

1.1.6.4 Principal Investigators

The Principal Investigator should be thoroughly familiar with the requirements of the guidelines and should ensure that, the guidelines are adhered to, for any project he/she is responsible and for which, involves the use of genetic manipulation [7,8,9,24]. Specifically, he/she should:

- Assess the proposal to decide if it falls within the guidelines. If unsure, the investigator should consult the IBC;
- Provide all information on the proposal that the IBC may need for assessment or monitoring of the proposal;
- Follow IBC's advice and recommendations;
- Fill out a typed original “IBC Research Registration Application” and hand a copy (keeping a second copy) to their IBC, before starting work on any project which falls under these Guidelines, and ensure that work does not commence until approval is granted by the IBC;

- Submit a new proposal form to the IBC before any major change is made to the experimental system of a proposal, which may result in a change of category or which may affect the exempt status of BSL1 projects.
- Conduct experiments under the conditions of physical containment approved by the IBC or as advised by other regulatory authority in the case of BSL3/4 proposals;
- Ensure that students, subordinates, and other co-workers are aware of the nature of potential hazards of the work and have been given relevant training. They should also arrange for training, if needed;
- Inform the IBC of any changes to the project team;
- Promptly report accidents, unexplained illnesses and absences to the IBC;
- Advise the IBC when intending to import biological material(s) falling within these guidelines.

1.1.7 BIOLOGICAL HAZARD AND CLASSIFICATION OF MICROBES

Biohazard is a biological specimen originated from a biological source or a microorganism, virus or toxin (from a biological source) that can affect agriculture, human health, animal health, or the environments. Thus, two groups of biohazards are non-infectious and infectious. Infectious biohazards are the pathogenic microorganisms that are classified into four risk groups (RG).

Table 1.1.7: Classification of Infectious Microorganisms by Risk Group [7,8]

RG	**NIH Guidelines**	**WHO Guidelines**	**Specific Property**
1	Agents not associated with disease in healthy adult humans	A microorganism unlikely to cause human or animal disease.	No or low individual and community risk
2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are *often* available.	A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.	Moderate individual risk; low community risk
3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available	A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.	High individual risk; low community risk
4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available	A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.	High individual and community risk

1.1.7.1 RG 1

This Group consists of biological agents that pose low individual and community risk and are highly unlikely to cause disease in healthy workers or animals. The agents require Biosafety Level 1 containment.

1.1.7.2 RG 2

This Group consists of biological agents that under unusual circumstances can cause human illness. Under normal circumstances, they are unlikely to be a serious hazard to laboratory workers, the community or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive are available and the risk of spread is limited.

1.1.7.3 RG 3

This Group consists of biological agents that usually cause serious human or animal disease, or which can result in serious economic consequences but are not ordinarily spread by casual contact from one individual to another, thus there is little community risk.

1.1.7.4 RG 4

This Group consists of biological agents that usually produce very serious human or animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.

1.1.8 PRIMARY CONTAINMENT: BIOLOGICAL SAFETY CABINET

A wide variety of Biological Safety Cabinet **(**BSC) designed to meet diverse applications in the life science, clinical, pharmaceutical and industrial laboratory are available now a days [7,8,9,12].

1.1.8.1 Classification of BSC

Classification is an important consideration in the selection of any BSC. Over the years, the scientific community has adopted commonly accepted classification criteria to differentiate containment capabilities and performance attributes.
BSCs should be used at all facilities for all aerosol-generating procedures involving biohazardous agent manipulation, any operation where BSC use is required by an outside regulatory agency, or other operations as specified on a case-by-case basis by the institutional biosafety committee.

Table 1.1.8: BSCs are divided into 3 classes

Classification	Biosafety Level	Application
Class I	2, 3	low to moderate risk biological agents
Class II	2, 3	low to moderate risk biological agents
Class III	3, 4	high risk biological agents

1.1.8.2 BSC acquisition

The IBC recommends that principal investigators consult with the Biosafety Office (BSO) prior to purchasing any BSC.

- Any cabinet being considered should meet NSF/ANSI 49 standards.
- Class II, Type A2 BSCs are recommended for general use. Names of a few manufacturers are given below:
  - ESCO
  - Forma
  - Germfree
  - Labsystems
  - Nuaire
  - NUVE
  - Thermo, etc.
- BSC width (four vs. six feet) should be appropriate for the experiments to be conducted in the lab.

1.1.8.3 BSC Installation

- BSCs must be installed in compliance with the provisions of NSF/ANSI Standard 49.
- BSCs should only be moved and installed by those tasked or contracted for moving and installing this type of equipment under the supervision of a trained Engineering.
- BSCs should be installed away from operable windows and doors, entryways, air supply and ventilation registers, and away from high foot traffic areas, following the CDC/NIH guidelines.
- New or relocated BSCs will not be connected or reconnected to laboratory gas lines.

1.1.8.4 BSC Certification and Repair

All BSCs must be certified in the following circumstances:

- Upon initial installation
- Whenever the BSC is moved
- Whenever the BSC has been repaired internally
- Certification must be renewed annually
- The IBC may require a six-month certification interval for BSCs that are used for high hazard work or for BSCs with a history of frequent certification failures
- No BSC may be used for any purpose if its certification has expired or if the cabinet requires repair
- BSCs must be certified by a certifier accredited under NSF/ANSI standard 49
- A current vendor certification label must be affixed to the cabinet and must include the following minimum information:
  - Certifier and vendor names
  - Vendor contact information
  - Certification standard (e.g., NSF/ANSI 49)
  - BSC manufacturer, model number and serial number
  - Certification date
  - Average inflow (face) air velocity at certification
  - Certification differential pressure gauge value (w.g.) (except Class II type B2 BSCs)
- BSCs can be serviced or repaired by individuals accredited under NSF/ANSI standard 49 to certify and service BSCs

1.1.8.5 BSC Use Training

- Anyone utilizing a BSC is required to take training for working in Biosafety Level 2 laboratory. This training is required on an annual basis.
- BSO will provide refresher training to the personnel for proper use of biosafety cabinets.

1.1.8.6 BSC Use Rules

- Only one person at a time may use a BSC, regardless of the cabinet width.
- No volatile chemicals are to be used in Class II Type A1 or A2 BSCs.
- Use of volatile chemicals in Class II Type B1 or B2 cabinets requires approval of the IBC. Sodium Hypochlorite (0.5%) in association with Ethanol (70%) may be utilized in all BSCs for surface disinfection.
- Volatile radionuclides may not be used in any BSC without IBC and Radiation Safety Committee approvals. Non-volatile radionuclides approved for use on the bench top may also be used in Class II BSCs, if properly shielded.

- Use of ultraviolet (UV) light to disinfect the interior cabinet surfaces is discouraged, because the light is of questionable effectiveness and it is a significant hazard to laboratory occupants.

1.1.8.7 BSC Decontamination and Salvage

- The interior surfaces of BSCs used for work involving biological hazards at BSL2 or BSL3 must be decontaminated with an appropriate chemical disinfectant at the end of each work session. Likewise, all items removed from a BSC must first be decontaminated with an appropriate chemical disinfectant. Examples of appropriate chemical disinfectants for both purposes include 70% ethanol in water or 10% household bleach in water. If bleach solutions are used on stainless steel or aluminum surfaces, they should be followed by a 70% ethanol rinse [7,8,9,10,12].
- The interior and exterior surfaces of BSCs used for work at BSL2, other than for culture of infectious agents, must be decontaminated with an appropriate chemical disinfectant before being certified, serviced, or moved [12].
- All BSCs must be gas or VHP (vaporized hydrogen peroxide) decontaminated before being transferred to a new owner in place or in another location, decommissioned, or salvaged [8,9,12].
- BSCs used for biohazardous agent culture at BSL2, diagnostic work involving Risk Group 3 agents (i.e., Mycobacterium tuberculosis), or work at BSL3 must be gas or VHP decontaminated before being transferred to a new owner or project in place, or decommissioned and salvaged.
- Cabinet decontamination must be conducted by an individual accredited under NSF/ANSI standard 49 to field-service BSCs. Biological indicators should be used to verify decontamination [12].
- After decontamination by an approved method, the sash must be closed, if possible, and a sign with the following wording must be affixed to the face above the sash: “This BSC was decontaminated by (vendor’s name) on (date), using (method of decontamination).”

1.1.8.8 Operation of Class II BSCs

Turn on cabinet fan 15 minutes before beginning work [7,8,9,12].

- Disinfect the cabinet work-surface with 70% ethanol or other disinfectant.
- Place supplies in the cabinet. Locate the container for disposal of pipettes inside the cabinet. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.)
- Work as far to the back (beyond the air split) of the BSC workspace as possible.
- Always use mechanical pipetting aids.
- Avoid using open flames inside BSC’s.If a flame is necessary, use a burner with a pilot light and place it to the rear of the workspace. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSC’s.
- Do not work in a BSC while a warning light or alarm is signaling.
- Locate liquid waste traps inside the cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container to prevent spilling.
- Wear gloves when there is potential for skin contact with infectious material.
- Keep the work area of the BSC free of unnecessary equipment or supplies.
- Clutter inside the BSC may affect proper airflow and the level of protection provided. Also, keep the front and rear grilles clear. Adapt a “clean to dirty” pattern for BSC procedures.
- When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow the cabinet to run for 15 minutes.

- Some BSCs are equipped with ultraviolet (UV) lights. If good procedures are followed, UV lights are not needed. If an UV light is used, due to its limited penetrating ability, surfaces must be dust-free and the UV light tube should be wiped frequently with alcohol to remove dust. UV radiation should not take the place of 70% ethanol for disinfection of the cabinet interior.
- The UV lamp should never be on while an operator is working in the cabinet.
- Minimize traffic around the biosafety cabinet and avoid drafts from doors and air conditioning.

1.1.8.9 Operation of Class III BSCs

The Class III BSCs, are designed to provide the highest level of containment when working with hazardous materials. These gas-tight enclosures protect the operator, the environment as well as the work in progress. Class III units are also referred to as gloveboxes, as work is conducted by utilizing arm-length gloves. Samples contained within the enclosure are visible via a completely sealed viewing panel. Strictly follow the manufacturer's instruction for operation of this group of BSCs.

1.1.8.10 Horizontal Laminar Flow Clean Air Benches

These are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a BSC in research laboratories.

1.1.9 SECONDARY CONTAINMENT

In general, secondary barriers (containment) are those features associated with the facility, which surround the primary barriers. The primary function of the facility is to provide a physical environment in which work activity can be undertaken efficiently and safely. A well-designed facility will facilitate good laboratory practice, contain equipment necessary to protect the worker and provide for the protection for those outside of the laboratory or building [7,8,9].

CDC/NIH describes four levels of physical containment or Biosafety Levels (BSL). For each BSL, there are specific supervisory qualifications as assurance that laboratory workers are provided appropriate role models and knowledgeable mentors. The essential elements of the four-biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in Table 2 [7,8,9].

1.1.9.1 Biological Laboratories

Biological laboratories are those that contain biologically active materials or involve the chemical manipulation of these materials. This includes laboratories that support such disciplines as biochemistry, microbiology, cell biology, biotechnology, genomics, immunology, botany, pharmacology, and toxicology. Both chemical fume hoods and biological safety cabinets are commonly installed in biological laboratories. Depending on the risk group (RG 1, 2, 3, or 4) of biological agents to be handled in these laboratories biosafety level will also vary (BSL1, 2, 3, or 4) following the CDC/NIH and WHO guidelines [7,8,9].

1.1.9.2 Clinical Laboratories

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents. Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and identification of isolates can be done safely at standard BSL2 laboratory, the recommended level for work with bloodborne pathogens such as hepatitis B virus, hepatitis C virus and HIV. Enhanced practices (use of N59 masks/powered air purified respirator, face shield, cover all, etc.) may be required according to the assessed risk for diagnosis of suspected cases of highly infectious diseases. This requires the use of specific

precautions with all clinical specimens of blood or other potentially infectious material (Universal or Standard Precautions) [7,8,9,10].

Primary barriers such as BSCs (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. BSCs also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC (Class II) is indicated to protect the integrity of the specimen. The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory head/director [10].

Table 1.1.9: The U.S. Centers for Disease Control and Prevention (CDC) defines four levels of biosafety, which are outlined below in the table [8]:

BSL	Practices	Safety equipment (primary barriers)	Facilities (secondary barriers)
1	Standard BSL1 practices	None required	Laboratory bench and sink
2	Standard BSL1 practices, limited access, Biohazard warning signs, "Sharps" precautions, Manual defining Safety, Security, and any needed waste decon, medical surveillance policies	Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials, laboratory coats and gloves, face protection as needed	BSL1 plus autoclave available
3	All BSL2 practices, controlled access, decon all waste, decon laboratory clothing before laundering	Class I or II BSCs or other physical containment devices used for all open manipulations of agents, Protective lab clothing and gloves Respiratory protection as needed	BSL2 plus pass through, autoclave, physical separation from access corridors Self-closing, double-door access, exhausted air not re-circulated, negative airflow into laboratory
4	All BSL3 practices, clothing change before entering, shower on exit, all material decontaminated on exit from facility	All procedures conducted in Class III BSCs, or Class I or II BSCs in combination with full body air-supplied positive pressure personnel suit	BSL3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decontamination systems Other requirements outlined in the text

A combination of work practices and physical containment requirements (facility and safety equipment) are designed to reduce the risk of laboratory infection when working with biohazardous material. The degree of protection recommended is proportional to the risk associated with an agent and the proposed research operations.

1.1.9.3 Animal Laboratories

Animal laboratories are areas for manipulation, surgical modification, and pharmacological observation of laboratory animals. They also include animal holding rooms, which are similar to laboratories in many of the performance requirements but have an additional subset of requirements. Depending on the risk group (RG 1, 2, 3, or 4) of biological agents to be handled in these laboratories four biosafety levels are also described for activities involving infectious disease work with experimental mammals following the CDC/NIH and WHO guidelines. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, (ABSL1, ABSL2, ABSL3, ABSL4), and provide increasing levels of protection to personnel and the environment, which are parallel to BSL 1-4 [7,8,9].

1.1.9.4 Plant Laboratories

Plant containment levels BSL1-P through BSL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. For experiments in which plants are grown at the BSL1-P – 4-P laboratory settings, containment practices shall

be followed as described in Appendix 4.5, 4.6, 4.7 and 4.8 of this manual. These containment practices is to avoid the unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants [15].

The containment principles are to mitigate overall threat to humans, animals and environment and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility. Four biosafety levels, Biosafety Level (BSL) 1 - Plants (P), BSL2-P, BSL3-P, and BSL4-P, are explained as ***Physical Containment Levels*** are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant or synthetic nucleic acid molecules. These biosafety levels, in conjunction with ***Biological Containment*** conditions provide flexible approaches to ensure the safe conduct of research [15].

1.1.9.5 Arthropod Laboratories

The study of arthropod transmitted disease is important to understanding the relationship between disease transmission via arthropods and its impact on public health. Therefore, the use of arthropods which are intentional carriers of infectious disease has led to the development of guidance on how to safely carry out work within the research setting [19]. Laboratories in which living arthropods are reared and maintained for research purposes have been in existence for decades with few reports of harm to their workers or to the communities in which they are located. The Guidelines provide descriptions (**APPENDIX 4.9**) and levels of containment for arthropod research. These levels are graded from ACL1-4, increasing in severity, and align closely with the concept of biosafety levels 1-4.

1.1.10 LABORATORY PRACTICES

Laboratory practices under the four BSL are adapted from CDC/NIH and WHO guidelines [7,8,9,10].

1.1.10.1 Practices in BSL1

- The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
- Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.
- Precautions, including those listed below, must always be taken with sharp items. These include:
  - Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
- Perform all procedures to minimize the creation of splashes and/or aerosols.

- Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - Materials to be removed from the facility for decontamination must be packed in accordance with applicable national and international regulations.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
- An effective integrated pest management program is required.
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.
- Personnel must receive annual updates or additional training when procedural or policy changes occur.
- Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions.
  - All laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection.
  - Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

1.1.10.2 Practices in BSL2

In addition to the general microbiological practices as in BSL1 lab the following special practices are observed in BSL2 laboratories

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- When appropriate, a baseline serum sample should be stored.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL2 agents.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be

reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.

- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

1.1.10.3 Practices in BSL3

In addition to the microbiological practices as in BSL1 and in BSL2 labs the following special practices are observed in BSL3 laboratories

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
- Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL3 agents.
- Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
    - Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
    - Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

1.1.10.4 Practices in BSL4

In addition to the microbiological practices as in BSL1, BSL2, and BSL3 labs the following special practices are observed in BSL4 laboratories

- All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements in accordance with institutional policies. Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes should be authorized to enter. Entry into the facility must be limited by means of secure, locked doors. A logbook, or other means of documenting the date and time of all persons entering and leaving the laboratory must be maintained. While the laboratory is operational, personnel must enter and exit the laboratory through the clothing

change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. Laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves, must be used by all personnel entering the laboratory. All persons leaving the laboratory must take a personal body shower. Used laboratory clothing must not be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering. After the laboratory has been completely decontaminated, necessary staff may enter and exit without following the clothing change and shower requirements described above.

- Laboratory personnel and support staff must be provided appropriate occupational medical service including medical surveillance and available immunizations for agents handled or potentially present in the laboratory. A system must be established for reporting and documenting laboratory accidents, exposures, employee absenteeism and for the medical surveillance of potential laboratory-associated illnesses. An essential adjunct to such an occupational medical services system is the availability of a facility for the isolation and medical care of personnel with potential or known laboratory acquired infections.
- Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
- A laboratory-specific biosafety manual must be prepared. The biosafety manual must be available, accessible, and followed.
- The laboratory supervisor is responsible for ensuring that laboratory personnel:
  - Demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL4 containment.
  - Receive appropriate training in the practices and operations specific to the laboratory facility.
  - Receive annual updates or additional training when procedural or policy changes occur.
- Removal of biological materials that are to remain in a viable or intact state from the laboratory must be transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container.
- These materials must be transferred through a disinfectant dunk tank, fumigation chamber, or decontamination shower. Once removed, packaged viable material must not be opened outside BSL4 containment unless inactivated by a validated method.
- Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
  - Spills involving infectious materials must be contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with infectious material.
  - A spill procedure must be developed and posted within the laboratory.
  - Equipment must be decontaminated using an effective and validated method before repair, maintenance, or removal from the laboratory.
  - The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.
  - Equipment or material that might be damaged by high temperatures or steam must be decontaminated using an effective and validated procedure such as a gaseous or vapor method in an airlock or chamber designed for this purpose.
- Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All incidents must be reported to the laboratory supervisor, institutional management and appropriate laboratory personnel as defined in the laboratory biosafety manual. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained..

- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- Supplies and materials that are not brought into the BSL4 laboratory through the change room, must be brought in through a previously decontaminated double-door autoclave, fumigation chamber, or airlock. After securing the outer doors, personnel within the laboratory retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors must be secured after materials are brought into the facility. The doors of the autoclave are interlocked in a manner that prevents opening of the outer door unless the autoclave has been operated through a decontamination cycle.
- The doors of a fumigation chamber must be secured in a manner that prevents opening of the outer door unless the fumigation chamber has been operated through a fumigation cycle. Only necessary equipment and supplies should be stored inside the BSL4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the facility.
- Daily inspections of essential containment and life support systems must be completed and documented before laboratory work is initiated to ensure that the laboratory is operating according to established parameters.
- Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies. Training in emergency response procedures must be provided to emergency response personnel and other responsible staff according to institutional policies.

1.1.11 SAFETY EQUIPMENT

All the information provided here adapted from CDC/NIH and WHO guidelines [7,8,9,10].

1.1.11.1 Safety Equipment for BSL1

- Special containment devices or equipment, such as BSCs, are not generally required.
- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials.
- Glove selection should be based on an appropriate risk assessment.
- Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL1 workers should:
  - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
  - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

1.1.11.2 Safety Equipment for BSL2

- Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:
  - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of

infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

- High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

- Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL2 laboratory workers should:
  - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

1.1.11.3 Safety Equipment for BSL3

- All procedures involving the manipulation of infectious materials must be conducted within a BSC **(**preferably Class II), or other physical containment devices.
- Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls is worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.
- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL3 laboratory workers should:
  - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
- Eye, face, and respiratory protection must be used in rooms containing infected animals.

1.1.11.4 BSL4 Safety Equipment: Cabinet Laboratory

- All manipulations of infectious materials within the facility must be conducted in the Class III biological safety cabinet. Double-door, pass through autoclaves must be provided for decontaminating materials passing out of the Class III BSC(s). The autoclave doors must be interlocked so that only one can be opened at any time and be automatically controlled so that the outside door to the autoclave can only be opened after the decontamination cycle has been completed.
  - The Class III cabinet must also have a pass-through dunk tank, fumigation chamber, or equivalent decontamination method so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet. Containment must be maintained at all times.
  - The Class III cabinet must have a HEPA filter on the supply air intake and two HEPA filters in series on the exhaust outlet of the unit.
  - There must be gas tight dampers on the supply and exhaust ducts of the cabinet to permit gas or vapor decontamination of the unit. Ports for injection of test medium must be present on all HEPA filter housings.
  - The interior of the Class III cabinet must be constructed with smooth finishes that can be easily cleaned and decontaminated. All sharp edges on cabinet finishes must be eliminated to reduce the potential for cuts and tears of gloves.
  - Equipment to be placed in the Class III cabinet should also be free of sharp edges or other surfaces that may damage or puncture the cabinet gloves. Class III cabinet gloves must be inspected for leaks periodically and changed if necessary.
  - Gloves should be replaced annually during cabinet recertification. The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, etc.) to be performed from the exterior of the cabinet whenever possible.
  - Manipulation of high concentrations or large volumes of infectious agents within the Class III cabinet should be performed using physical containment devices inside the cabinet whenever practical. Such materials should be centrifuged inside the cabinet using sealed rotor heads or centrifuge safety cups. The Class III cabinet must be certified at least annually.
- Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls must be worn by workers when in the laboratory. No personal clothing, jewelry, or other items except eyeglasses should be taken past the personal shower area. All protective clothing must be removed in the dirty side change room before showering. Reusable clothing must be autoclaved before being laundered.
- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment. Prescription eyeglasses must be decontaminated before removal through the personal body shower.
- Gloves must be worn to protect against breaks or tears in the cabinet gloves. Gloves must not be worn outside the laboratory. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

1.1.11.5 BSL4 Safety Equipment: Suit Laboratory

- All procedures must be conducted by personnel wearing a one-piece positive pressure suit ventilated with a life support system.
- All manipulations of infectious agents must be performed within a BSC or other primary barrier system.
- Equipment that may produce aerosols must be contained in devices that exhaust air through HEPA filtration before being discharged into the laboratory.
- These HEPA filters should be tested annually and replaced as needed. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations.

- Protective laboratory clothing such as scrub suits must be worn by workers before entering the room used for donning positive pressure suits. All protective clothing must be removed in the dirty side change room before entering the personal shower. Reusable laboratory clothing must be autoclaved before being laundered.
- Inner gloves must be worn to protect against break or tears in the outer suit gloves. Disposable gloves must not be worn outside the change area. Alternatives to latex gloves should be available. Do not wash or reuse disposable gloves. Inner gloves must be removed and discarded in the inner change room prior to personal shower. Dispose of used gloves with other contaminated waste.
- Decontamination of outer suit gloves is performed during operations to remove gross contamination and minimize further contamination of the laboratory.

1.1.12 LABORATORY BIOSECURITY

Laboratory Biosafety is to reduce or eliminate accidental exposure to or release of potentially hazardous agents. On the other hand Laboratory Biosecurity is to protect biological agents against theft by those who intend to pursue bioterrorism or biological weapons proliferation. Biological materials that require (according to their owners, users, custodians, caretakers or regulators) administrative oversight, control, accountability, and specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm are known as Valuable biological materials (VBM). VBM may include pathogens and toxins, as well as non-pathogenic organisms, vaccine strains, foods, genetically modified organisms, cell components, genetic elements, and extraterrestrial samples [25].

- A qualitative risk assessment has to be performed to define risks that a security system should protect against.
- Acceptable risks and incidence response planning parameters have to be defined.
- The whole building should be securely locked when unoccupied.
- Doors and windows have to be break-proof.
- Rooms containing hazardous materials and expensive equipment should be locked when unoccupied.
- Access to such rooms, equipment and materials should be appropriately controlled and documented. Accordingly, security measures to protect the release of microbial agents, biological pathogens, toxins, critical information, pests or diseases as a result of theft or misuse are -

1.1.12.1 Physical Security

Access should be limited to authorized and designated employees based on the need to enter sensitive areas. Methods for limiting access could be as simple as locking doors or having a card key system in place. Evaluations of the levels of access should consider all facets of the laboratory's operations and programs (e.g., laboratory entrance requirements, freezer access). The need for entry by visitors, laboratory workers, management officials, students, cleaning/maintenance staff, and emergency response personnel should be considered.

1.1.12.2 Personnel Reliability

Personnel management includes identifying the roles and responsibilities for employees who handle, use, store and transport dangerous pathogens and/or other important assets. The effectiveness of a biosecurity program against identified threats depends, first and foremost, on the integrity of those individuals who have access to pathogens, toxins, sensitive information and/or other assets. Employee screening policies and procedures are used to help evaluate these individuals. Policies should be developed for personnel and visitor identification, visitor management, access procedures, and reporting of security incidents.

1.1.12.3 Scientific and Programmatic Oversight

If a biosecurity plan is implemented, institutional management must support the biosecurity program. Appropriate authority must be delegated for implementation and the necessary resources provided to assure program goals are being met. An organizational structure for the biosecurity program that clearly defines the chain of command, roles, and responsibilities should be distributed to the staff. Program management should ensure that biosecurity plans are created, exercised, and revised as needed. The biosecurity program should be integrated into relevant institutional policies and plans.

1.1.12.4 Biological Specimens/Infectious Agent Accountability

Material accountability procedures should be established to track the inventory, storage, use, transfer and destruction of dangerous biological materials and assets when no longer needed. The objective is to know what agents exist at a facility, where they are located, and who is responsible for them. To achieve this, management should define: 1) the materials (or forms of materials) subject to accountability measures; 2) records to be maintained, update intervals and timelines for record maintenance; 3) operating procedures associated with inventory maintenance (e.g., how material is identified, where it can be used and stored); and 4) documentation and reporting requirements.
It is important to emphasize that microbiological agents are capable of replication and are often expanded to accommodate the nature of the work involving their use. Therefore, knowing the exact “working” quantity of organisms at any given time may be impractical. Depending on the risks associated with a pathogen or toxin, management can designate an individual who is accountable, knowledgeable about the materials in use, and responsible for security of the materials under his or her control.

1.1.12.5 Transportation Security

Procedures and practices to correctly categorize, package, document and safely and securely transport valuable biological materials (VBM) from one place to another, following applicable national and/or international regulations have to be developed. Material transport policies should include accountability measures for the movement of materials within an institution (e.g., between laboratories, during shipping and receiving activities) and outside of the facility (e.g., between institutions or locations). Transport policies should address the need for appropriate documentation and material accountability and control procedures for pathogens in transit between locations. Transport security measures should be instituted to ensure that appropriate authorizations have been received and that adequate communication between facilities has occurred before, during, and after transport of pathogens or other potentially hazardous biological materials. Personnel should be adequately trained and familiar with regulatory and institutional procedures for proper containment, packaging, labeling, documentation and transport of biological materials.

1.1.12.6 Information Security

The objective of an information security program is to protect information from unauthorized release and ensure that the appropriate level of confidentiality is preserved. Facilities should develop policies that govern the identification, marking and handling of sensitive information. The information security program should be tailored to meet the needs of the business environment, support the mission of the organization, and mitigate the identified threats. It is critical that access to sensitive information be controlled. Policies for properly identifying and securing sensitive information including electronic files and removable electronic media (e.g., CDs, computer drives) should be developed.

1.1.13 DETERMINATION OF BIOSAFETY LEVELS FOR DIFFERENT RISK GROUPS

Four biosafety levels (BSL) provide increasing degrees of protection against the risk posed by the infectious agent and at the same time provide right balance of practices & procedures, primary barriers and secondary barriers for a particular agent. Thus, it is crucial to determine the suitable BSL and work practices for a particular biological specimen or isolated infectious agents [7,8,9,10]. However, infectious biological specimens with low or no microorganisms can be analyzed under BSL2 with appropriate protection blocking

all routes of entry for the particular agents. Isolated infectious agents under different risk group should be handled in the respective BSLs as shown in Figure 1.1.13.

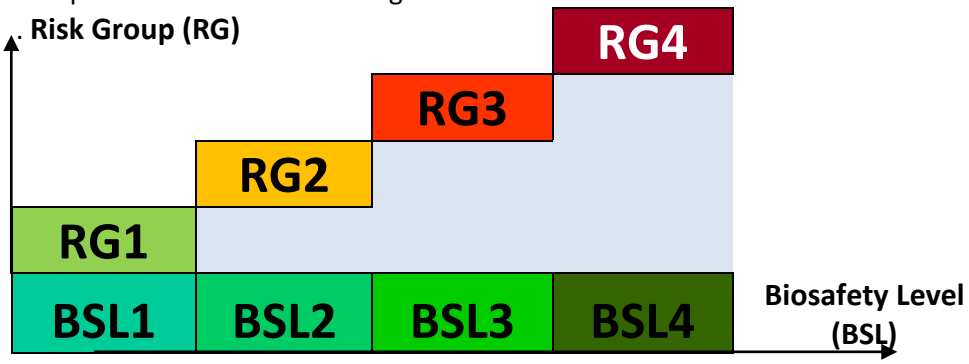


Figure 1.1.13: Risk Group of infectious agents and appropriate biosafety levels for manipulating live agents. It is the responsibility of the laboratory managers/heads and the director of research to establish standard procedures in the laboratory, which realistically address the issue of the infective hazard of clinical specimens. Threats of major infectious diseases for Bangladesh, causative agents and their BSL for diagnostics and research activities are summarized in Table 1.1.13 [7,8,9,10].

Table 1.1.13: Major infectious diseases threats for Bangladesh, RG and their BSL

Diseases	Agent	RG	BSL*
AIDS	HIV	3	2, 3
Anthrax	*Bacillus anthrasis*	3	2, 3
Chikungunya fever	Alpha virus	3	2, 3
Cholera	*Vibrio cholerae*	2	2
Dengue	DEN 1, 2, 3, & 4	2	2
EBOLA	Ebola virus	4	2, 4
Encephalitis	Henipaviruses	4	2, 4
Enteric fever	*Salmonella paratyphi* and *typhi*	3	2, 3
Highly Pathogenic Avian Influenza	H5N1, H7N1 H9N1,	3	2, 3
Influenza	H1N1	2	2
Japanese encephalitis	Japanese encephalitis virus	3	2, 3
Leishmaniasis kala-azar	*L. donovani* complex	2	2
Leptospirosis	*Leptospira interrogans*	2	2
Malaria	*Plasmodium vivax*	2	2
Plague	*Yersinia pestis*	3	2, 3
SARS	*Coronavirus*	3	2, 3
Shigelosis	*Shigella dysenteriae type I*	3	2, 3
Tuberculosis	*Mycobacterium tuberculosis*	3	2, 3
Zika fever	Zika virus	3	2, 3

*Laboratory diagnosis can be conducted at lower BSL with biological specimens; however, to protect the route of transmission, it is necessary to use appropriate personal protective equipment and to practice enhanced policies and procedures that are practices of BSL3 in BSL2. Higher BSL is necessary for concentration, amplification, isolation, and culture of the isolated microorganisms.

1.1.14 TRAINING

Biosafety & Biosecurity training is essential for the successful implementation of a program. Program management should establish training programs that inform and educate individuals regarding their responsibilities within the laboratory and the institution. The institution should ensure that laboratory staff is informed of hazards and have adequate training to make sure that their work is carried out within these guidelines. The IBC Chair or BSO should be readily accessible to give advice [7,8,9,10,11]. Table 1.1.14 indicates the training requirement for working in different laboratories and field activities. Staff members or students must participate in the face-to-face training on biosafety and and biosecurity organize by the institution before the commencement of the work with biohazard/biological materials in a biological,

biotechnological, biomedical or microbiological research or diagnostic laboratories. Once appeared in face-to-face training and certified, annual retraining/refresher training is necessary to review and update the knowledge in the field of biosafety and biosecurity, which is strongly recommended to be practiced by all the institutions involved in research or diagnostic activities with biological materials.

Table 1.1.14: Biosafety related training course summary

Work	Orientation on Lab Safety	Biosafety & Biosecurity for BSL2 Lab	Respiratory Protection Programme	Training for Outbreak investigation	Working safely in BSL3 Lab
Clinical Lab	Need to be completed	Need to be completed	As Needed	NA	NA
BSL2 Lab	Need to be completed	Need to be completed	As Needed	NA	NA
Outbreak investigation	Need to be completed	Need to be completed	Need to be completed	Need to be completed	NA
BSL3 Lab	Need to be completed	Need to be completed	Need to be completed	Need to be completed	Need to be completed

1.1.15 CERTIFICATION OF CONTAINMENT LABORATORIES

BSL3 and BSL4 facilities (laboratories, animal houses, plant houses, insectaries, bird houses, aquaria) are to be approved by the regulatory agency (ies) and certified by professional certifiers. Institutions planning to conduct experiments requiring BSL3 or BSL4 containment are recommended to notify the NCB or equivalent committee. The necessary advice regarding the structural requirements about these BSL3 and BSL4 facilities are detailed in Section 2 of these guidelines [7,8,9, 24].

1.1.16 MEDICAL SURVEILLANCE OF WORKERS

For personnel using BSL2 physical containment facilities, no special arrangements are necessary outside the normal institutional practices for laboratory workers. Institutions doing microbiological research may take baseline serum samples from personnel. Such samples are stored for diagnostic tests on workers exposed to accidents or who develop unexplained illness. For experiments requiring BSL3 or higher, laboratory workers should have an initial medical examination, and other requirements as stipulated by the BSO [6,7,8,9].

1.1.17 ACCIDENTS AND INCIDENTS

The IBC or the BSO should record accident, incident, and near misses and the follow-up action. If the IBC Chair is satisfied that the accident or incident was directly attributable to genetic manipulation work, and was significant, they should make a report to the head of the institution. An example of such an incident could be the intentional failure to comply with these guidelines, or an incident which might have risked human health or the environment [6,7,8,9,10].

Chapter 1.2: Purview – Extent and Exemptions

1.2.1 EXTENT OF GUIDELINES

Experiments that involve biohazardous materials or the construction and/or propagation of cells, prions, viroids, viruses or organisms, or which have been made by genetic manipulation and are of a novel genotype and which are unlikely to occur naturally, or which could cause public health or environmental hazards, are covered under these guidelines [24,26].

The categories of experiments, which fall under these guidelines, are described in Chapter 1.3.

- Intentional or unintentional release of biohazardous or infectious or genetically manipulated organisms must adhere to these Guidelines.
- An investigator should submit a description of their proposed research, in writing, to their Institutional Biosafety Committee(s) (IBC) for clarification and to be sure whether of their research proposal falls within these guidelines, before the commencement.

1.2.2 EXEMPTIONS

- Experiments are exempt from the guidelines unless they fall under the group of biohazardous materials [15,16].
  - Experiments involving non-pathogenic organisms or the fusion of mammalian cells which generate a non-viable organism, for example, the construction of hybridomas to make monoclonal antibodies.
  - Fusion of protoplasts between non-pathogenic microorganisms.
  - Protoplast fusion, embryo-rescue, *in vitro* fertilization or zygote implantation in plant cells.
  - Experiments involving the breeding or use of genetically modified model organisms, namely mice, rats, zebrafish, *Xenopus* spp. and *Arabidopsis* spp. in which:
    i. The genetic modification involves knock-out, deletion and inactivation of genes; and/or
    ii. The genetic modification using knock-in, activation, gene substitution, and activation of genes in which the outcome does not confer any survival advantage to the animal and cause subsequent detrimental effects in human health and environment through the expression of toxins and/or human pathogens. These may include selectable reporter/marker genes such as fluorescent proteins.
    iii. If further manipulations are performed on any of the above-described transgenic organisms, they may not be automatically exempted from the guidelines and could fall within group of biohazardous materials and risk has to be assessed separately.
  - Research involving the introduction of naked nucleic acids into plants or animals (other than humans), unless the nucleic acid is both recombinant and able to give rise to infectious agents.
  - Work involving the introduction of genetically manipulated somatic cells into animals, unless they are able to give rise to infectious agents.
  - Experiments involving approved host/vector systems provided that the donor DNA:
    i. is not derived from plant or animal pathogens and that the DNA to be introduced is characterized fully and will not increase host or vector virulence;
    ii. is derived from mammalian sources and is used to construct shotgun libraries in an approved host/vector system
    iii. does not code for a vertebrate toxin having a LD50 of less than 100 µg/kg;
    iv. does not represent or comprise more than two-thirds of a viral genome, and is not being used in any experiment in which missing segments of the viral genome that are essential for infection are available in the host cell or will become available by further breeding processes.
  - All experiments, whether exempt or not, should be carried out under conditions of standard microbiological laboratory practice. When pathogenic organisms are used, appropriate containment levels should be used and the personnel should be properly trained and have had the recommended vaccinations as stipulated in the guidelines issued or recommended by regulatory authorities such as Ministry of Health and Family Welfare (MoHFW) and Ministry of Environment and Forest (MoEF).

- Exemption from these guidelines does not equal exemption from statutory provisions applying to any aspect of a project involving genetic manipulation (e.g. importation, quarantine legislation).

1.2.3 WORK SUBJECTED TO OTHER AND/OR ADDITIONAL REGULATORY REQUIREMENTS

- Work with GMOs derived from biological agents and toxins known to be hazardous to human health are regulated under Bangladesh Biosafety Rules 2012 [5] and Biosafety Guideline of Bangladesh [6]. Large-scale production of GMOs derived from biological agents and toxins known to be hazardous to human health must be regulated under the relevant Guidelines [5,8,9,24]. Large-scale production refers to the production of 10 or more liters of biological agent at any one time [6,7,8].
- Work in the field of human health such as gene therapy, or other genetic manipulations on humans involving stem cells, whole organs or individuals will be assessed by its designated authority [24]. These investigations include the introduction of nucleic acids (genetically manipulated or chemically synthesized and their derivates), or genetically manipulated microorganisms, or cells into human subjects for the purposes of gene therapy, cell marking, or for stimulating an immune response against a subject's own cells, as used for the treatment of some cancers [8,9,24].
- Research proposals where the introduction into human subjects of nucleic acids (genetically manipulated or chemically synthesized), or genetically manipulated microorganisms, or cells, is designed to stimulate an immune response to antigenic determinants of infectious agents, as in the case of classical vaccine, should be submitted to the appropriate Bioethics committees.
- Vaccines that have been approved for use in Bangladesh, as well as the transfer of non-genetically manipulated autologous host cells, organ and tissue transplants are subjected to other relevant authority requirements.

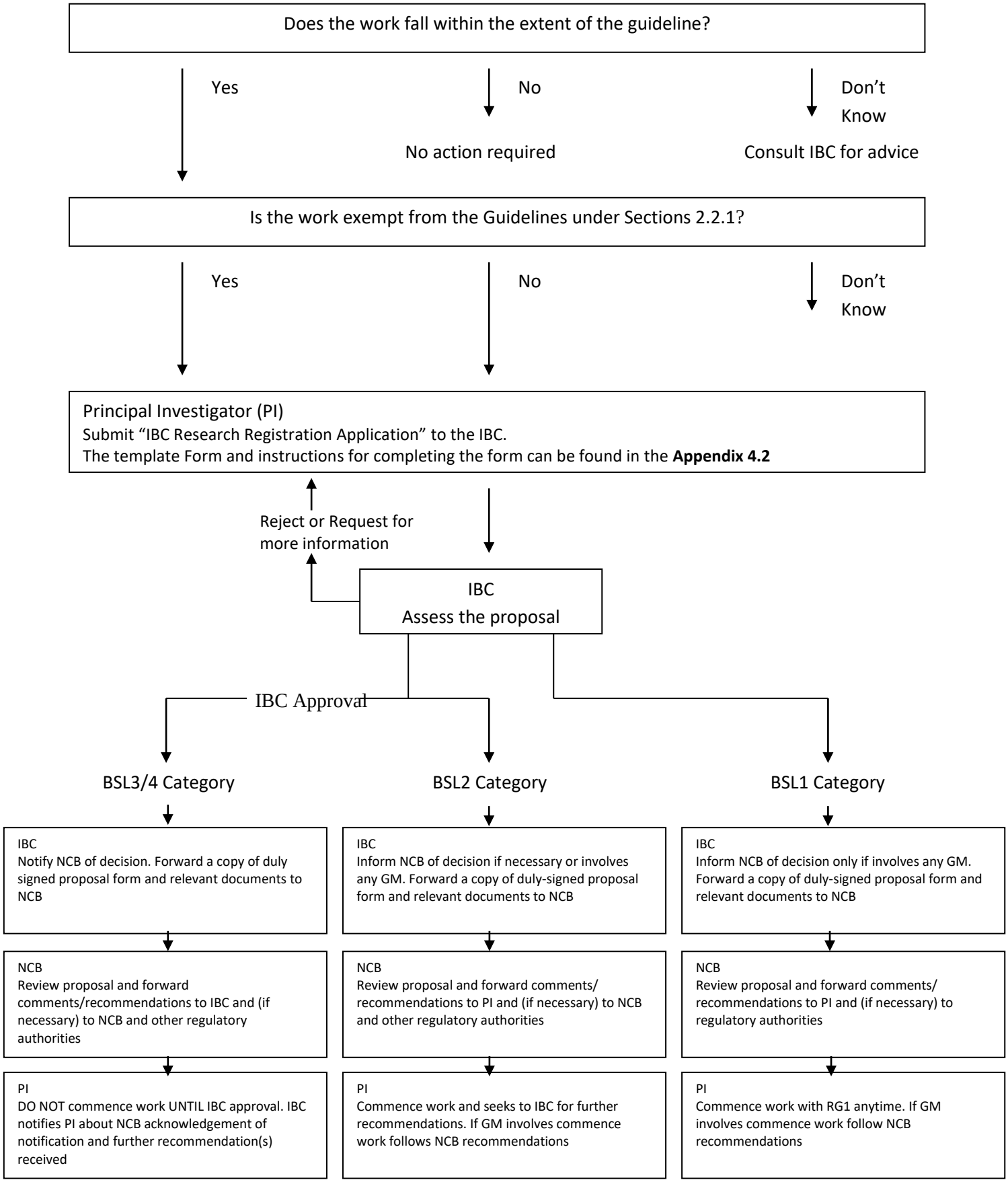
1.2.4 REGULATORY AUTHORITIES

The national authorities responsible for legislating the various aspects of technology and activities pertaining to genetic modification technology and genetically modified organisms in Bangladesh are:

- Ministry of Environment and Forest (MoEF)
- Ministry of Health and Family Welfare (MoHFW)
- Ministry of Agriculture (MoA)
- Ministry of Fisheries and Livestock (MoFL)
- Ministry of Science and Technology (MoST)
- Add all as discussed

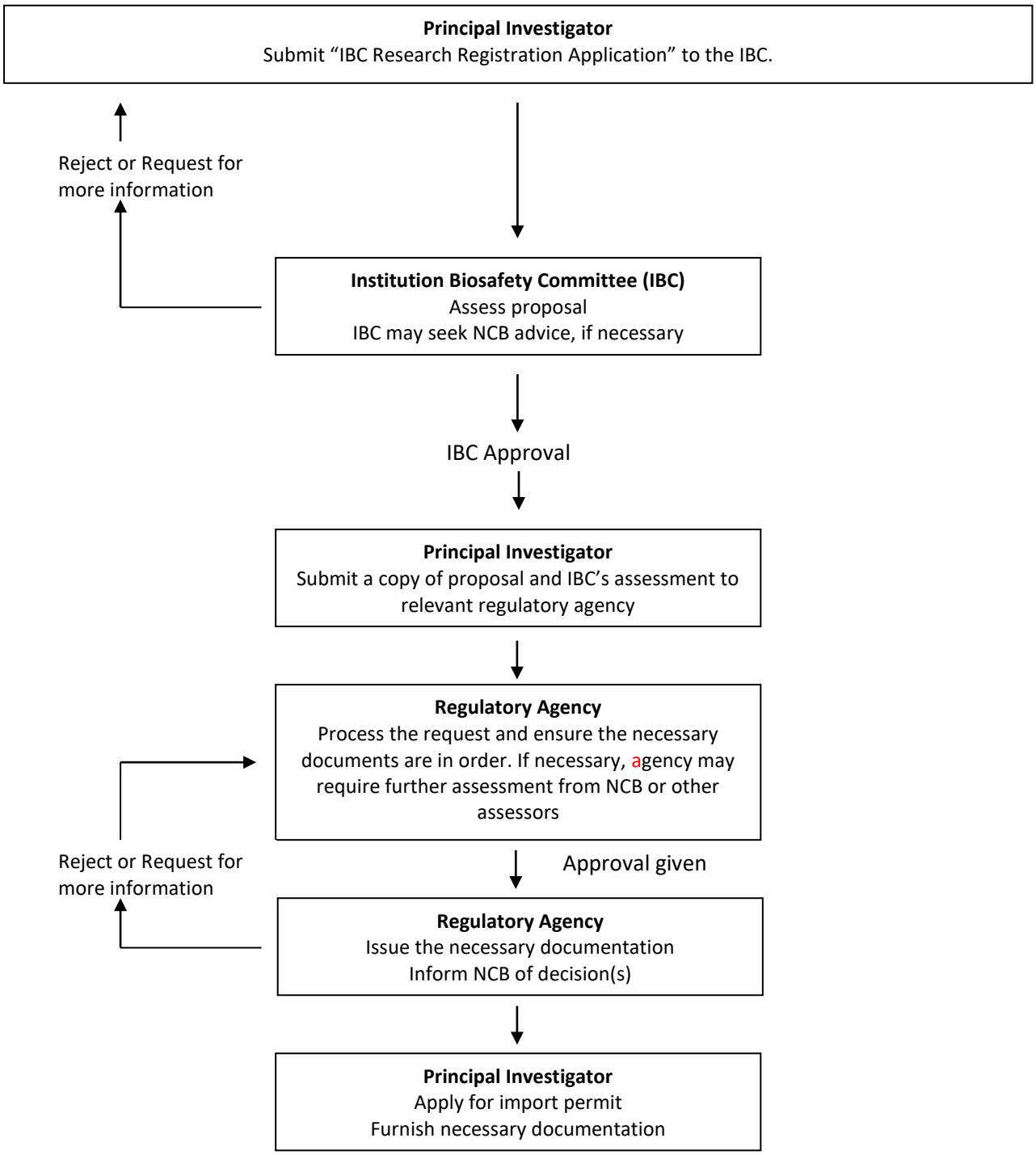
Chapter 1.3: Procedures

1.3.1 DECISION FLOW CHART FOR ASSESSMENT AND NOTIFICATION OF RESEARCH WORK



1.3.2 FLOW CHART FOR IMPORTATION OF BIOHAZARDOUS MATERIALS FOR RESEARCH

Importation or procurement to import specific biological agents and toxins, which are capable of causing death, disease or malfunction in a humans and animals, should be regulated under the Bangladesh Biosafety Rules 2012 [5].



1.3.3 EXPERIMENTS EXEMPT FROM THE GUIDELINES

Experiments under BSL1 are exempt from the guidelines and therefore do not require NCB notification, unless otherwise involves GM. PIs who are unsure if their work falls under the exemptions should submit a proposal on an "IBC Research Registration Application" to their respective IBCs for assessment.

- The IBC shall assess the proposal and determine the appropriate categorization. PIs are encouraged to inform NCB of their decision by forwarding a duly signed proposal form and relevant documents.

1.3.4 BSL2 EXPERIMENTS REQUIRING IBC APPROVAL

Experiments in this category may require assessment by the IBC prior to commencement. PIs may commence BSL2 experiments if s/he has conducted biological risk assessment and decided the work is safe to conduct with infectious/biohazardous materials and any genetic manipulation (GM) work is not involved. PIs intending to conduct experiments classified as BSL2 should submit an "IBC Research Registration Application" to their respective IBCs for assessment. The IBC shall assess the proposal and determine the appropriate working and containment measures and facilities necessary for the work planned.

- The IBC shall forward a copy of the approved proposal form, together with a summary of the IBC's recommendations, to the NCB, if the work involves GM. NCB will review and forward comments/ recommendations to IBC and if necessary and to relevant regulatory authorities.
- The NCB will return a copy of "IBC Research Registration Application", carrying a NCB case reference number, to the IBC within 10 working days to acknowledge receipt of the proposal. The case reference number should be quoted in all future correspondences relating to the proposal. The acknowledgment of receipt does not imply NCB's acknowledgement or endorsement of IBC's decision. PIs can commence work concurrently with IBC notification. NCB will normally not advise the IBC on BSL2 proposals, unless necessary and involves any GM work.

1.3.5 BSL3/4 EXPERIMENTS REQUIRING IBC APPROVAL

Experiments in this category require both IBC assessment and NCB notification. PIs should not commence work on proposals assessed as BSL3/4 work until advised by their IBC.

- PIs intending to conduct experiments classified as BSL3/4 should submit a proposal form for assessment of research and/or diagnostic activity to their respective IBC for assessment. The IBC shall assess the proposal and determine the appropriate working and containment measures and facilities necessary.
- A proposal in this category should be submitted by the PIs to the IBC for assessment on an "IBC Research Registration Application". The IBC should assess the proposal and determine the appropriate working and containment conditions. Upon approval, the IBC should forward the proposal to the NCB secretariat, together with a summary of the IBC's recommendations or comments, for notification.
- When completing the forms and assessing the experiments, the IBC and the investigator should identify potential hazards and their types, and decide upon any special procedures necessary for the proposed experiments.
- The NCB Secretariat will return a copy of the IBC Research Registration Application, carrying a NCB case reference number, to the IBC within 10 working days to acknowledge receipt of the proposal. The case reference number should be quoted in all future correspondences relating to the proposal. The acknowledgment of receipt does not imply NCB's acknowledgement or endorsement of IBC's decision. PIs may commence work concurrently with IBC notification. However, NCB will advise the IBC on BSL3/4 proposals if IBC assessed risk is not appropriate according to NCB assessment. NCB will assess risk on both BSL3/4 agents and GM work and accordingly appreciates that IBC and PIs will comply with NCB suggestions and recommendations.

NOTE: Currently an NCB is functional under Ministry of Environment, Forest and Climate Change to review and approve research relating to Plant Biotechnology and Agriculture, GMO and LMO. Until an appropriate NCB is functional, each institution is responsible to have its own IBC reviewed and approved research with infectious materials. However, it is still advisable to communicate with the NCB if the work involves a GM to comply with the Biosafety Guidelines of Bangladesh 2008 [6] and the Biosafety Rules [5].

Chapter 1.4: Experiments Covered by the Guidelines

1.4.1 BSL1

This is the category includes experiments with non-biohazardous or non-pathogenic materials, which do not pose significant risks to laboratory workers, the community or the environment [7,8,9,10,24,26].

- Principal Investigators who are unsure of the categorization of their experiments are required to seek advice from their respective IBCs, by submitting a 'Proposal Form for Assessment of Genetic Manipulation Work'. The IBC shall assess the proposal and determine the appropriate categorization status.

1.4.2 BSL2

This category includes experiments with biological materials of RG2 infectious agents, which may pose low-level risks to laboratory workers, the community or the environment. These experiments require at least Biosafety Level 2 physical containment (laboratory, plant house, animal house, insectary, bird house or aquarium), as determined by the IBC and with reference to the Biosafety level detailed in the guidelines [7,8,9,10,16,19]. Some experiments may require additional precautions or higher containment because the donor DNA or its components are hazardous or infectious, for example special containment features are needed for the housing of transgenic animals. Recommendations for procedures for BSL2 and other containment levels are listed under 1.1.9 laboratory practices [7,8,9,10,24,26].

- IBC assessment is required before work begins on this category of experiments. Principal investigators should not commence work on proposals assessed as BSL2 until specifically advised by the IBC.
- If the proposed experiments should fall within both BSL3/4 and 2, BSL3/4 classification shall take precedence i.e. BSL3/4 conditions shall be applied.
- The following classes of experiments fall within BSL2:
  - Experiments with whole animals (including non-vertebrates) which involve genetic manipulation of oocytes, zygotes or early embryos to produce a novel organism. For transgenic animal work, prior approval from the institution's bioethics committee is needed.
  - Genetic manipulation experiments involving the production of modified whole plants.
  - Work with non-approved host/vector systems where the host or vector either:
  - Does not usually cause disease in plants, humans or animals; 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 with approved host/vector systems, in which the gene inserted is:
  - A pathogenic determinant; or
  - DNA that is not fully characterized from microorganisms which are able to cause disease in humans, animals or plants; or
  - An oncogene.

Shot-gun cloning of mammalian DNA in approved host/vector systems does not fall into this category.

Note: that experiments not falling within BSL2 or in BSL3/4 Category, but falling under the Extent in this Section, require NCB advice and IBC approval.

1.4.3 BSL3/4

This category includes experiments, which may pose high risks to laboratory workers, the community or the environment. This category also includes experiments for which the type or level of hazard is unclear. The level of containment required will vary depending on the kind of experiments and their assessed hazard [7,8,9,10,24,26].

- In general, experiments involving biological specimens or toxins that are classified as Risk Group 3 and 4 of the CDC/NIH and WHO Laboratory Biosafety Manual generally fall under BSL3 and 4, respectively. A

facility with practices of, at least, BSL2 (as determined by the IBC and with reference to the Biosafety level detailed by CDC/NIH) is required is required with enhanced practices for unknown biological specimens on the basis of risk assessment.

- This category of work requires IBC assessment and approval, followed by NCB notification before work begins. Principal investigators should not commence work on proposals assessed as BSL3/4 until advised by the IBC, following IBC's receipt of NCB acknowledgement of notification. Please refer to Section **1.3.5** for procedures for submitting proposal forms and obtaining NCB advice. The following classes of experiments fall within this category:
- Experiments with toxin producers:
  - Experiments using DNA which encodes a vertebrate toxin having an LD50 of less than 100 µg/kg.
  - Experiments in which toxin genes are expressed at a high-level, even if the LD50 is greater than 100 µg/kg. Experiments using uncharacterized DNA from toxin-producing organisms and, which therefore could contain toxin sequences also fall under this subcategory. However, experiments using DNA which has been fully characterized and shown not to code for a toxin, from a toxin-producing organism as donor, is not included in this sub-category.
- Experiments using viral vectors whose host range includes human cells, 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. (Special conditions for working with viral vectors encoding oncogenes are given in Appendices 5 and 6.)
- Experiments involving introduction of DNA into microorganisms, which can cause plant or animal (including human) diseases when used as host or vector, except:
  - Microorganisms used as hosts or vectors; or
  - If the DNA will not increase the virulence of the host or vector and the DNA is fully characterized, in which case it is classified as BSL2
- This sub-category does not include experiments using replication defective viruses as host or vector. However, experiments using defective vector/helper virus combinations, which have the potential to regenerate non-defective recombinant virus, are included in this subcategory.
- Introduction of pathogenicity genes into microorganisms other than the approved hosts included in. This sub-category includes those genes whose products are suspected of, or have a risk of initiating autoimmune diseases.
- Cloning or transfer of entire viral genomes, viroids, or fragments of a genome capable of giving rise to infectious particles with the capacity to infect human, animal or plant cells. Experiments involving cloning of less than two-thirds of an entire viral genome do not fall within this subcategory. Cloning of a viral genome, which lacks a vital component of its replication or packaging activity that is not supplied by the experimental system, also does not fall within this sub-category.
- 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. This sub-category includes experiments that could alter the host range of pathogens or increase pathogen virulence or infectivity.
- 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.
- Experiments not falling within the BSL3/4 category listed above or into BSL2, but which fall within the extent of the guidelines.

Chapter 1.5: Import, Export and Transport of biohazardous Materials

1.5.1 GENERAL CONSIDERATIONS FOR TRANSPORT AND PACKAGING OF MATERIAL

1.5.1.1 Basic Requirements

Biological specimens, infectious agents or viable genetically modified organisms should be of minimal risk to humans, animal, plants and the environment while transportation [7,8,9,15,16].

Risk is minimized through-

- Proper packaging of the material following WHO guidance on regulation for the transportation of infectious substances [15];
- Proper labeling of the package with required signage [15,16] to alert the workers in the transportation chain to the hazardous contents of the package;
- The information about the hazardous contents of the package should properly documented so that the necessary information is available in an emergency situation;
- Essential training of workers in the transportation chain to be able to respond appropriately to emergency situations; and
- The samples should be transported in packaging on the basis of risk categories [15,16]. The recipients should have facilities to contain the organisms at the required level.

1.5.1.2 Transport Requirements

- Triple packaging in sealed unbreakable containers or bags is necessary for categories A and B [15,16] to transfer out of a containment laboratory. Standard operating procedures (SOP) should be implemented by the management that should include risk assessment and packaging according to risk category for transport within an institution.
- Procedures must have been set up for the safe transport of biological materials by air, rail and road. Packaging and transport arrangement should correspond to its risk level. Risk levels are categorized according to WHO guidance [15].
- The sender and the recipient must follow all packaging and transport regulations. Biological materials transportation is controlled by the IATA Dangerous Good Regulations [16].

1.5.1.3 Documentation

Shipper's declaration for dangerous goods must be completed, when infectious material is being transported. The documentation must indicate information including origin, contents and date of dispatch, and should be placed in a separate leak-proof bag so as to protect the declaration form from potential contamination by the contents of the package. Recipients should be informed about all known hazards associated with the material before delivery [15,16].

1.5.1.4 Labeling

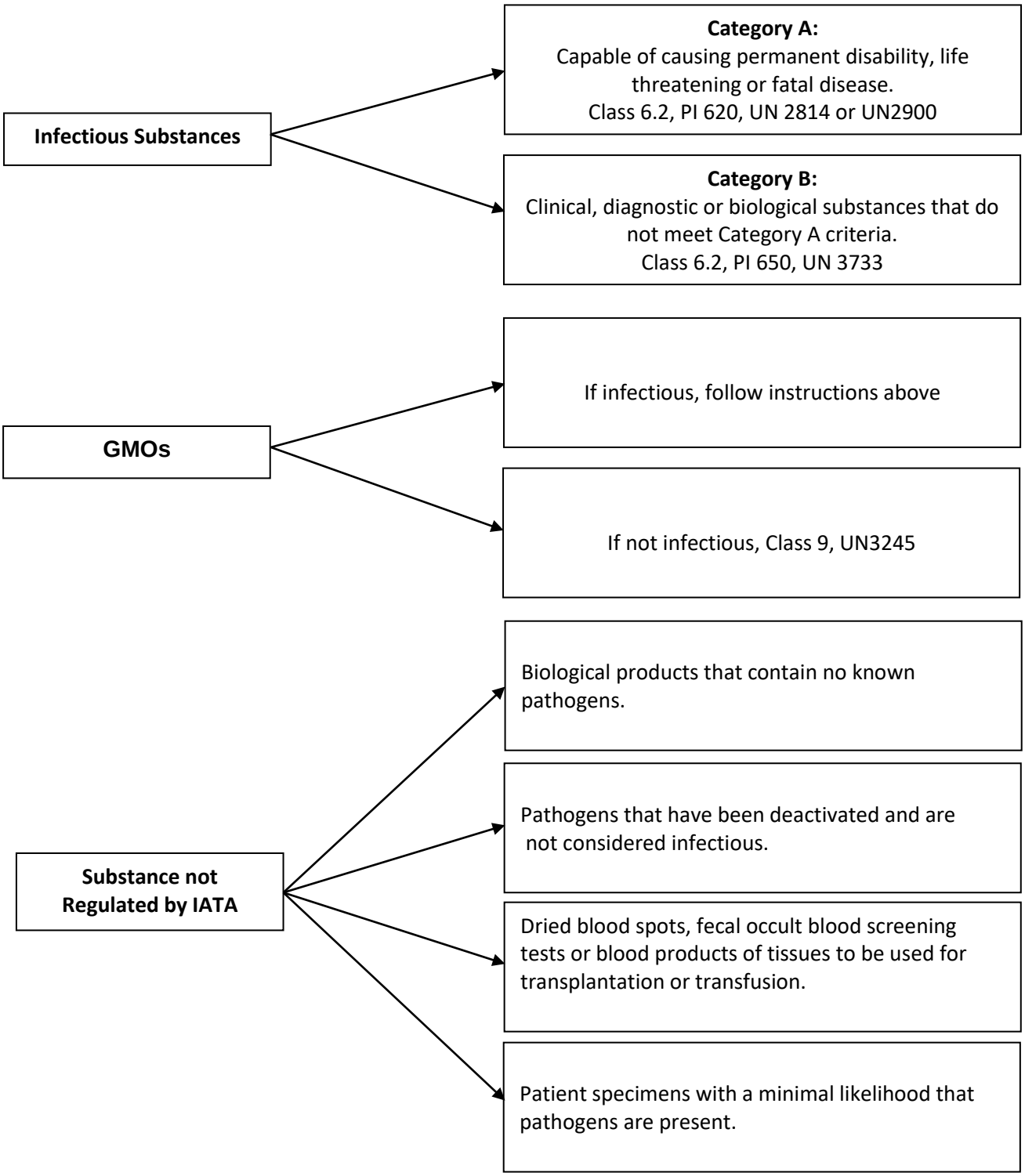
The package must be labeled (according to IATA standards) to clearly show the name, address and contact details of the persons responsible for the materials, so that the person can be contacted should the package be lost, damaged or misdirected [16].

1.5.1.5 General Considerations

- Only trained personnel may undertake the packaging for transport which should be done according to the above regulations.
- Facilities should be provided for after-hours delivery of samples, and all staff including night staff should be warned of any hazards.
- Procedures and precautions for unpacking should be appropriate to the type of package being unpacked.

- When infectious waste is removed from a laboratory, waste should be disposed of by authorized waste disposal facility.

1.5.2 FLOWCHART TO DETERMINE IF YOUR BIOLOGICAL SAMPLE IS CATEGORY A, CATEGORY B, OR EXEMPT



1.5.3 TRANSPORT OF GENETICALLY MODIFIED MICROORGANISMS

1.5.3.1 General Considerations

- All genetically modified microorganisms should undergo risk assessment
- Transport of biological agents via air shall adhere to the specified packaging under the IATA Dangerous Good Regulations.
- Both sender and addressee need to ensure that no sample vials/canisters containing biohazardous materials are missing in the delivery process. It is the responsibility for both sender and addressee to exercise diligence respectively in checking the number of sample vials/canisters containing the biohazardous materials tally at the point of delivery to that received

1.5.3.2 Packaging and Transport Requirements

- Biohazardous materials to be transported must be wholly contained inside a watertight, leak-proof, sealed, unbreakable primary container packed in a secondary container and finally packed into a rigid outer container.
- Sufficient quality of absorbent material should be wrapped around the primary container to absorb all fluids (if any) that may emanate from the biological agent in the event of a breakage of or leakage from the primary containers.
- The packaging should be sufficiently strong to withstand any impact which the package would normally be subject to during the transportation, loading and unloading.

1.5.3.3 Labeling

- The outermost container must be labeled to clearly show the species/origin of the biological material, the name, address and contact details of the person responsible for the dealings, so that the person can be contacted should the package be lost, damaged or misdirected.
- A biohazard label must be attached to at least the outermost container holding any biohazardous material which fit into the classification of Cat A and B [15,16].

1.5.3.4 Treatment of Containers After Transport

Any materials transported with the biohazard must be either retained with the organisms under containment or decontaminated after transport has occurred.

1.5.4 TRANSPORT OF TRANSGENIC ANIMALS

1.5.4.1 General Considerations

In making transport arrangement for transgenic animals, two principles are paramount:

- The need to prevent the animals from escaping, to ensure that transgenic animals will not interbreed with feral populations; all reasonable scenarios such as accidents should be considered;

- The need to ensure that the animals are properly identified, that they arrive at the intended destination, and that a competent biologist with experience in handling transgenic animals takes delivery of them. Accounting procedures should be instigated to make sure that all animals sent are delivered - whether dead or alive.

1.5.4.2 Packaging and Transport Requirements

- The IBC should formulate rules it considers essential to meet these conditions. It may be necessary for the IBC to inspect the transport arrangements to determine that the above principles are complied with and that any additional conditions, which the IBC considers appropriate, have been met.

- Animal boxes should comply with IATA standards. Modifications can be made to the boxes especially for pathogen-free animals. The boxes must be escape-proof and allow easy observation during an import inspection without opening the box [16].

- Transport of all animals should also adhere to the relevant Guidelines on the Care and Use of Animals for Scientific Purposes [16].

SECTION 2: DESIGN REQUIREMENTS FOR BIOMEDICAL RESEARCH AND DIAGNOSTIC LABORATORY

Chapter 2.1: General Requirements

2.1.1 LABORATORY DOOR

Laboratory doors shall be of adequate size to install and remove all equipment, including oversized equipment. In addition to general requirements, the following requirements shall apply to laboratory doors [27].

- Laboratory should have a lockable door and an emergency exit door.
- Each laboratory room shall be served by at least one 1.1 m (3 ft. 6 in.) wide single leaf, or 1.2 m (4 ft.) wide unequal pair doors (914 mm [3 ft.] active/305 mm [1 ft.] inactive) for delivery of equipment and supplies. Doors serving administrative offices within laboratory suites may be 914 mm (3 ft.)wide.
- Laboratories with unusually large equipment shall have doors of adequate size to move such equipment.
- Vision panels are recommended for all laboratory doors. In laboratories where the use of larger equipment is anticipated, wider/higher doors should be considered.
- Laboratory doors should be recessed and swing in the direction of egress. Doors should be self-closing.
- Emergency exit doors are recommended to be operable from inside of the laboratory only. Laboratory doors are considered high-use doors.
- All hardware should be appropriately specified to withstand the type of use.
- Light commercial grade hardware will not be specified.
- All appropriate hardware to meet security, accessibility, and life safety requirements should be provided.

2.1.2 FLOOR AND BASE MATERIALS

Utilize resilient flooring in break areas, pantries, and other locations subject to spills or staining. Where appropriate, use sustainable products such as linoleum or rubber. Minimum thickness shall be 3 mm (1/8 in.) [27].

- Floor materials should be non-absorbent, skid-proof, resistant to wear, and resistant to the adverse effects of acids, solvents, and detergents.
- Materials may be monolithic or have no/a minimal number of joints. Provide slip-resistant floor surface.
- Floor materials should be installed to allow for decontamination with liquid disinfectants and to minimize the potential spread of spills.
- Consideration should be given to use laboratory grade self leveled epoxy resin flooring materials with coving at the junction of the floor and the wall. Sheet flooring seams shall be heat welded.
- Install floor finishes wall-to-wall, extending under casework and equipment.
- Carpeting is not permitted in any area of the laboratory, including office areas that can only be accessed by passing through a laboratory.

2.1.3 WALLS

Low-luster acrylic enamel paint shall be used as the primary interior partition finish. Paint for general interior applications shall be solvent free, water-based, latex paint, and primer. Application shall be a threecoat system including one primer coat and two finish coats unless specified otherwise. The final coat shall provide a semigloss or eggshell finish, except where more durable finishes are required for functional reasons. Epoxy paint and other specialized coatings are required in areas subject to high humidity, frequent decontamination, impact and wear, and other conditions specified by program requirements [27].

- Wall surfaces should be free from cracks, unsealed penetrations, and imperfect junctions with ceiling and floors.
- Materials should be capable of withstanding washing with strong detergents and disinfectants and be capable of withstanding the impact of normal traffic.

- Wall should be painted with epoxy paint.

2.1.4 CEILINGS

Minimum ceiling height shall be 2.7 m (9 ft.). Optimal ceiling height is 2.9 m (9 ft. 6 in.). Unusual ceiling height requirements have to be prior confirmed [27].

- Washable ceilings (such as lay-in acoustical tiles) should be provided for most laboratory spaces.
- Open ceilings are acceptable provided minimal ducting and piping is present and all exposed surfaces are smooth and cleanable. Open ceilings may be acceptable in laboratories under the following conditions**:**
  - The ceiling structure is concrete or another material that is smooth and uniform and can be painted.
  - The height of the ceiling structure will result in an acoustical tile ceiling that is unacceptably low.
  - Ductwork, conduit and other ceiling-mounted mechanical and electrical items can be minimized, and configured in a way that is neat and visually organized.
  - Acoustics are not a factor or are affectively addressed.
  - The use and function of the laboratory is consistent with an open ceiling.

2.1.5 WINDOWS AND WINDOW TREATMENT

Operable windows cause uncontrolled fluctuations of the air in a room, which can be detrimental to the operation of BSCs, fume hoods and other equipment. Fluctuations are also detrimental to maintaining pressurization and directional air flow and controlling air temperature and humidity [27].

- Windows should be non-operable and should be sealed and caulked.
- Window systems that use energy-efficient glass are recommended.
- Treatments should meet all functional and aesthetic needs and standards.

2.1.6 EQUIPMENT PATHWAY

- The potential routing or pathway for the addition or relocation of heavy equipment should be reviewed and identified during the design phase.

2.1.7 HAZARD COMMUNICATION SIGNAGE

- Each laboratory should have a signage holder for prominently displaying hazard communication information at the entrance door.
- Individual labs should have signage holders that are consistent with the type used by other laboratories within each laboratory/unit.

2.1.8 LABORATORY BENCHES, CHAIRS, AND FURNITURE

- All laboratory work surfaces shall be impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment. Chair covers shall be nonporous and easily cleanable.
- Lab benches shall be 3’-0” height, 9’-0” wide, and 2’-6” '(single) or 5’-0” (double) depth. Typically, one double lab bench shall have 6 persons working space (3’-0” height, 9’-0” wide, and 5’-0”depth) with or without a laboratory sink attached to it. In case if multiple laboratory benches are there in a laboratory, to increase bench area laboratory sinks can be placed attached to alternative lab benches. Other benches for placing laboratory equipment shall be 3’-0” height and 1’-8’’ to 2’-6’’ in depth. Length can be considered depending on the need, available space and any assessed risk/inconvenience associated. Adjustable height of the chairs shall be 2’-0” to 2”-4”.

- Provide for at least 3'-4" to 4'-0" inches of clearance between lab benches and everything else in the lab, e.g., other lab benches, walls, and fumehoods.
- Epoxy resin/painted counter tops and Lab grade laminate can be used.
- Provide Stainless Steel counters at locations with radioactive materials.
- Laboratory furniture shall be capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

2.1.9 CASEWORK

- Laboratory casework should be easily cleanable, and finishes should be compatible with materials used for cleaning and disinfection.
- Fixed casework and countertops should be sealed to walls and floors during installation to minimize harborage of pests and provide a cleanable joint. Countertop materials will vary depending on usage.
- Traditional materials such as chemical-resistant plastic laminates may be appropriate for some applications.
- Epoxy resin will apply to most applications where corrosive chemicals are used or where sinks or heavy water usage occurs.
- Stainless steel should be used for glassware wash areas, cold rooms, and other areas as necessary based on usage.

2.1.10 ELECTRICAL AND UTILITIES CONSIDERATIONS

- Electrical wiring and circuits: The laboratory shall be fitted with electrical circuits and receptacles that can accommodate existing requirements plus an additional 30% to 40% capacity. Electrical receptacles above counter tops within six feet of sinks, safety showers, or other sources of water, recommended to have Earth Leakage Circuit Breaker (ELCB) circuit protection system. It is recommended to provide all electrical wiring dropping from the ceiling into the laboratory bench instead of running through the area of traffic movement or through the laboratory walls (to minimize making pores/penetrations on the wall).
- Lighting: Laboratory research and diagnostics require high-quality lighting for close work, in terms of both brightness and uniformity. Fixtures should be positioned to provide uniform, shadow-free and glare-free illumination of the laboratory bench top. General lighting for laboratories should be fluorescent fixtures. Fluorescent light fixtures should be directly above and parallel to the front edge of the laboratory bench to prevent shadows. Local wall switches should control light fixtures.
- Alarm and Monitoring Systems: The increasing sophistication and fine control of laboratory instruments and the unique quality of many experiments demand closely monitored and alarmed systems that can be connected to individual pieces of equipment or temperature-controlled rooms. Several excellent monitoring systems are available for this purpose. They can be connected to a central monitoring facility at several levels of observation or can be used internally within a laboratory setting. Architects and engineers should consult with the Biosafety Office to evaluate the need for alarms and monitoring systems.
- Back-up Electricity: Based on the type of work, laboratory should have the provision of online UPS or back-up electricity to ensure uninterrupted work, especially while working/ handling hazardous materials and aerosols inside the BSC.

2.1.11 VENTILATION REQUIREMENTS

- The general ventilation shall be used for input to local ventilation devices; not be relied on for protection from toxic substances released into the lab; it is recommended to ensure that lab air is continually

replaced, preventing increase of air concentrations of toxic substances during the working day; direct air flow into the lab from non-lab areas and out to the exterior of the building.

- When local exhaust systems such as hoods are used as the primary method of control, six to twelve room air changes per hour is recommended (ten to twelve room air changes per hour for moderate to high-risk laboratories).

2.1.12 VENTILATION DEVICES

- Fume hoods shall be located 10 feet (minimum) from exit doors.
- Exhaust air from glove boxes and isolation rooms shall be passed through scrubbers or other treatment before release into the regular exhaust system.
- Biological Safety Cabinets must be selected, installed and tested in accordance with the guidelines mentioned above. Contact BIOSAFETY OFFICER for further detail about other acceptable international guidelines and standards as needed.

2.1.13 FIRE EXTINGUISHER SMOKE DETECTOR

- The distribution of fire extinguishers is specified by fire code. For example, a fire extinguisher must be within 30 feet of a flammable liquid storage area.
- Extinguishers should be conspicuously located where they will be readily accessible in the event of fire. They should be located close to the exits from an area and along normal paths of travel.
- Fire/smoke detector should be plenty in numbers. Fire protection and fire detection equipment should not be obstructed. Architects and engineers should consult with Fire Safety Personnel regarding questions on the placement of fire extinguishers in laboratories.

2.1.14 LABORATORY SINK

- Each laboratory shall contain at a minimum a sink (preferably hands-free) dedicated for hand washing.
- If the laboratory has multiple processing rooms, then each room should have one sink for hand washing.

2.1.15 EMERGENCY SHOWERS AND EYEWASHES

- The valve shall be designed so that the water flow remains "on" without requiring the use of the operator's hands (hands-free) and shall remain activated until intentionally shut off.
- Valve shall be simple to operate and go from "off" to "on" in 1 second or less.
- Equipment shall be in accessible locations that require no more than 10 seconds to reach from any laboratory work area.
- Each location shall be identified with a highly visible sign.
- Water shall be tepid, moderately warm.
- Only potable water shall be used for eyewashes and showers.
- The shower shall be located so that a water column is provided that is not less than 82 inches nor more that 96 inches in height from the surface on which the user stands.
- Often, the best location for a shower is in the main corridor.
- The center of the spray pattern shall be located at least 16 inches from any obstruction.
- The shower shall be capable of delivering a minimum of 20 gallons of water per minute.
- An eyewash facility shall be readily available to all labs.

- Eyewashes shall be installed close to the showers (in addition to other laboratory locations) so that, if necessary, the eyes can be washed while the body is showered.
- The eyewash shall be positioned with the water nozzles 33 inches to 45 inches from the surface on which the user stands and 6 inches minimum from the wall or nearest obstruction.
- There shall be no sharp projections anywhere in the operating area of the unit.
- Eyewashes shall be designed to provide water to both eyes simultaneously, be a continuous flow design, and operate hands-free (although they can be actuated by hand).
- Eyewashes shall be capable of delivering to the eyes not less than 0.4 gallons of water per minute.
- Eyewash nozzles shall be protected from airborne contaminants and the protector cap's removal shall not require a separate motion by the operator.
- Eyewashes shall be drained or be of the swivel type that allows the water to run directly into the sink. (Eyewashes shall be activated weekly to purge bacteria and debris from the water.)

2.1.16 COMPRESSED GAS CYLINDER

- If gas cylinders are to be placed in the lab, they should be properly secured to a vertical surface or counter out of the way of traffic in the space.
- Appropriate space for such cylinders should be provided within the laboratory to minimize potential hazards associated with the use of these cylinders and to maximize usable laboratory space.

2.1.17 STORAGE UNITS

- **Flammable Chemicals and Waste:** Flammable-chemical storage cabinets may be placed in each laboratory and meet applicable fire safety requirements as needed. Flammable storage cabinets should not be located near exit doorways, stairways, or in a location that would impede egress. Space should be allocated in each laboratory for storage of chemical waste.
- **Corrosive Cabinets:** Corrosive cabinets may be provided for labs that need to store corrosive material.
- **Base Cabinets for Fumehoods:** Wherever possible, flammable storage cabinets shall be used as the base cabinets for fumehoods. If flammable storage cabinets are not used as the base cabinet, then the cabinet shall be constructed to withstand fire conditions so that the hood remains supported. If vented storage cabinets are provided under the hood, they shall be vented without cutting the work surface for venting the base cabinets. Vent through the side wall of the hood with 1.5 to 2 inch national pipe thread (npt) galvanized pipe. The tap into the exhaust shall be done above the hood. The tap from the back of the cabinet shall be at the lower part of the cabinet and as supplied by the cabinet manufacturer.
- **Freezers/Refrigerators:** Freezers/refrigerators will be kept in the dedicated space/room. Segregation is recommended on the basis of Risk Group (RG) of infectious agents. RG3 agents may be stored under BSL3 containment. For particular requirement of the storage area, contact Biosafety Office for recommendation. Hallway or corridor will not allow storing any such storage equipment. All such storage equipment should be labeled no food and drink inside. Freezers/refrigerators are recommended to be manufactured as "lab safe," but need not to be "explosion-proof."

2.1.18 COLD ROOMS

- A cold room is an environmentally controlled prefabricated unit usually operated at $4^{O}C$. Controlled environment rooms are available with variable temperature ranges and can be adjusted for use. The prefabricated unit may require that the floor be a depressed slab or a ramp may be required to access the room. The whole unit may be installed inside a room of a building or there may be the provision of an anteroom in front of the environmentally controlled prefabricated unit. This room will provide, at a minimum, facilities for temporary holding of the specimens, samples, equipments, or any materials

before entering into the cold room; and the facilities for holding entry/exit log, hand sanitization and hand washing, changing into required personal protective equipment before entry, as well. Just outside this room there may be features for biosecurity like access control, CCTV, etc., as required to maintain if the cold room is an independent unit, not installed inside a laboratory.

- Controlled environment rooms should have stainless steel counters on legs, wire shelves, and a stainless steel sink. Utilities may include power, vacuum, and mechanical ventilation, filtered water, and fire alarm strobe light. Natural gas may be required based on program needs. If natural gas is provided, the environmental room must be supplied with supply and exhaust ventilation. Requirements for compressed air, gas and vacuum shall be verified. A sink is sometimes required.
- Temperature controlled room shall be lockable and all mechanical components shall be accessible and serviceable from the outside. A high and low temperature monitoring and alarm system shall be connected to a central equipment alarm system. All gaps between the room and adjacent construction shall be sealed. Provide emergency exhaust capability. A manual door release must be provided inside the room.

2.1.19 WARM ROOMS

- A warm room is an environmentally controlled prefabricated unit often used for growing cell cultures, usually at 37^{o}C at a constant temperature and humidity, may have similar safety and security features mentioned above for cold rooms.

Chapter 2.2: Requirements for Microbiological and Biomedical Laboratory

2.2.1 LABORATORY FACILITIES FOR LABS AT A BIOSAFETY LEVEL 1

- Each Biosafety Level 1 (BSL1) laboratory shall contain a sink for hand washing. The sink may be manually, hands-free, or automatically operated. Laboratories should have the sink located near the exit door. If the facility has multiple rooms for laboratory activity, each laboratory room shall contain a sink for hand washing.
- An eyewash facility shall be readily available.
- Illumination shall be adequate for all activities, avoiding reflections and glare that could impede vision.
- The laboratory shall be designed so that it can be easily cleaned.
- Bench tops shall be impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
- Laboratory furniture shall be capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Chairs and other furniture used in laboratory work shall be covered with a non-fabric material that can be easily decontaminated.
- If the laboratory has windows that open to the exterior, they shall be fitted with fly screens.

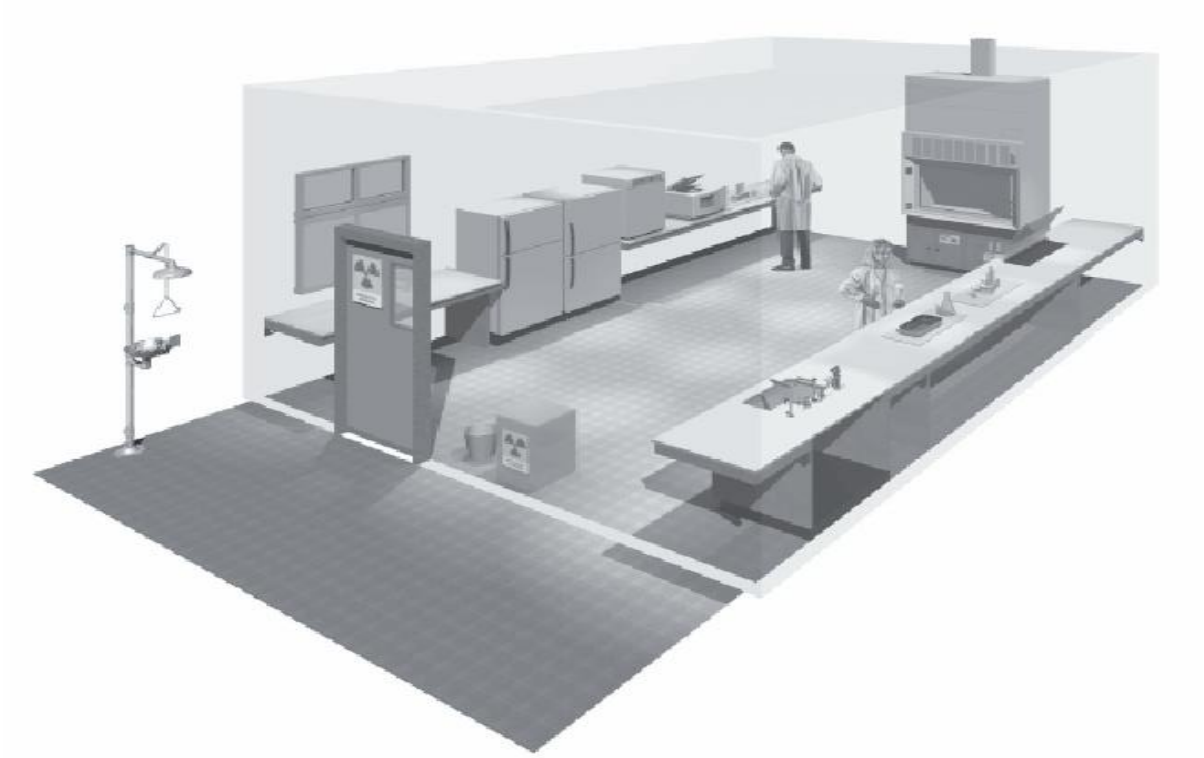


Figure: 2.2.1: BSL1 laboratory [7,8]

2.2.2 LABORATORY FACILITIES FOR LABS AT A BIOSAFETY LEVEL 2

All laboratories dealing with biohazardous materials will be constructed to fulfill the standard criteria of a Biosafety Level 2 (BSL 2) laboratory or above at a minimum. These are in addition to all of the requirements for BSL 1.

- Each laboratory shall have an anteroom for storing, donning, and doffing personal protective equipment (PPE) for entering into the laboratory area from the separated office area.
- Each laboratory shall contain a sink for hand washing as in BSL 1 laboratory. When a separate tissue culture room is located within a main lab room, there should be a hand washing sink located inside the tissue culture room.

- Class II (A2) Biological Safety Cabinets (BSC II (A2)) may be required. If BSCs are used, install them in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.

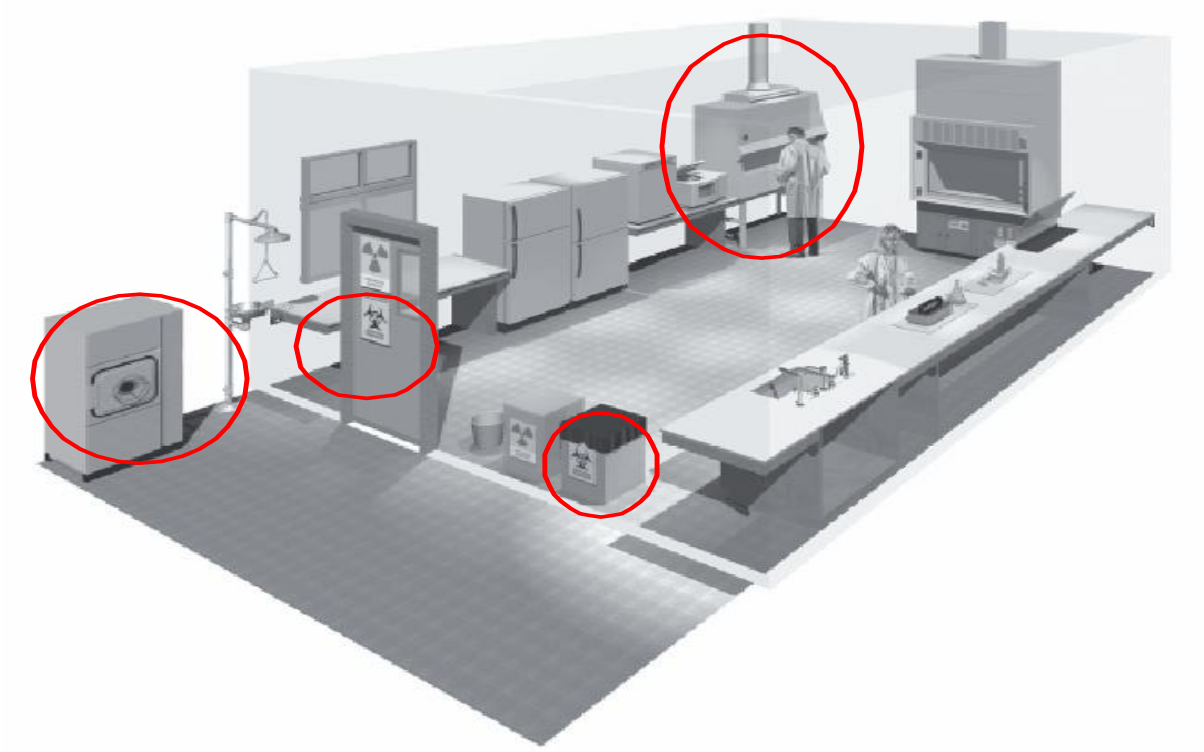


Figure: 2.2.2: BSL2 laboratory [7,8]

- A method for decontaminating all laboratory wastes will be available in the facility. Steam sterilization by an autoclave will be the major means of decontamination of biological waste. Autoclave space will be in a convenient location of the building as large amount of microbiological research is performed in the facility.
- There shall be an inward flow of air without recirculation to spaces outside the laboratory.
- There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

2.2.3 LABORATORY FACILITIES FOR LABS AT A BIOSAFETY LEVEL 3

These are in addition to all of the requirements for BSL 1 and BSL 2.

- The laboratory shall be separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors in the basic requirement for entry into the laboratory from access corridors. A clothes change room (shower optional) may be included in the passageway.

- Each laboratory room shall contain a sink for hand-washing as in BSL 1 and 2; howeverhe sink is preferably hands-free or automatically operated.

- The interior surfaces of walls, floors, and ceilings of areas where BSL3 agents are handled shall be constructed for easy cleaning and decontamination. Seams, if present, shall be sealed. Walls, ceilings, and floors shall be smooth, impermeable to liquids and resistant to the chemicals and disinfectants

normally used in the laboratory. Floors shall be monolithic and slip-resistant. Use coved floor coverings. Penetrations in floors, walls, and ceiling surfaces shall be sealed. Openings such as around ducts and the spaces between doors and frames shall be capable of being sealed to facilitate decontamination.

- Windows in the laboratory shall be closed and sealed.
- A method for decontaminating all laboratory wastes shall be available in the facility and utilized, preferably within the laboratory (i.e., autoclave, chemical disinfection, incineration, or other approved decontamination methods).

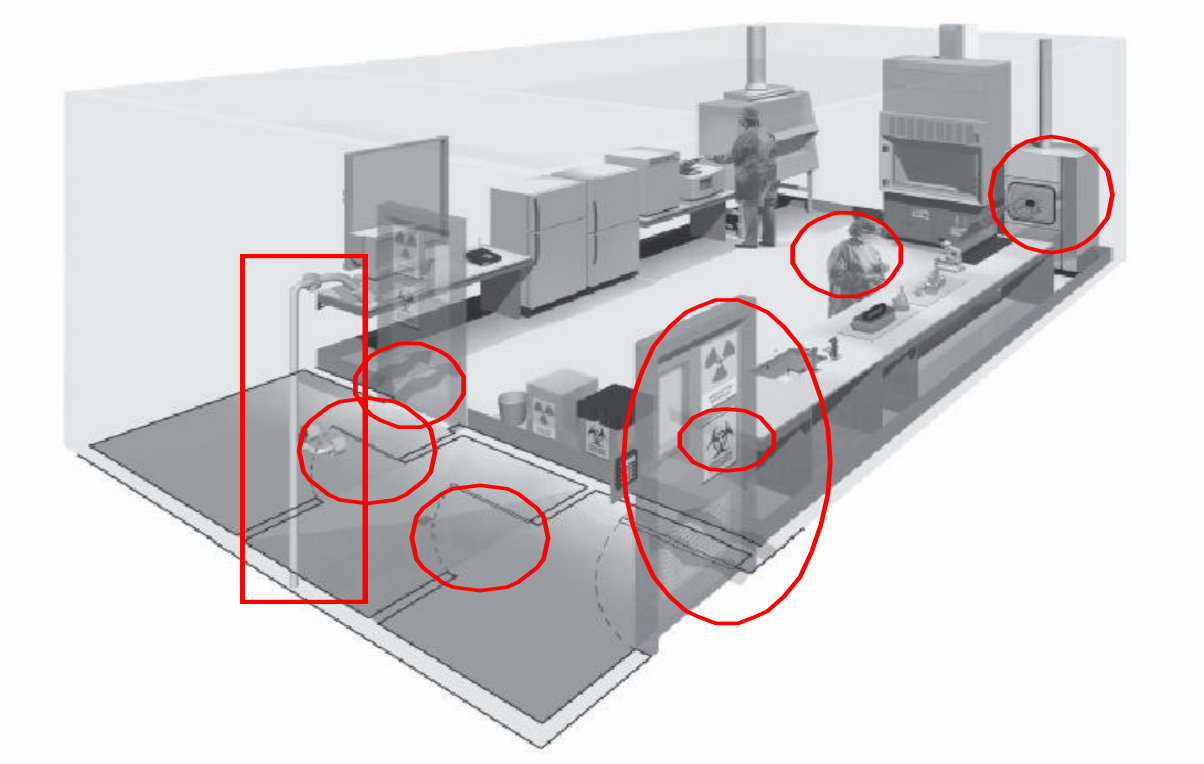


Figure: 2.2.3: BSL3 laboratory [7,8]

- Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily-traveled laboratory areas.
- A ducted exhaust air ventilation system shall be provided. This system shall create directional airflow which draws air into the laboratory from “clean” areas and toward "contaminated" areas. The exhaust air shall not be recirculated to any other area of the building. Filtration and other treatments of the exhaust air are not required, but may be considered based on site requirements, and specific agent manipulations and use conditions. The outside exhaust shall be dispersed away from occupied areas and air intakes, or the exhaust shall be HEPA-filtered. A visual monitoring device that indicates and confirms directional inward airflow shall be provided at the lab entry. Consideration shall be given to installing an HVAC control system to prevent sustained positive pressurization of the lab. Audible alarms shall be considered to notify personnel of HVAC system failure.
- HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets shall be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system (e.g., an air gap between the cabinet exhaust and the exhaust duct). When Class III biological safety cabinets are used they shall be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets.

- Centrifuges or other equipment that may produce aerosols are contained in devices that exhaust air through HEPA filters before discharge into the laboratory. These HEPA systems are tested at least annually. Alternatively, the exhaust from such equipment may be vented to the outside if it is dispersed away from occupied areas and air intakes.

- The laboratory shall have uninterrupted power supply.

- The Biosafety Level 3 facility design and operational procedures shall be documented. The facility shall be tested for verification that the design and operational parameters have been met prior to operation. Facilities shall be re-verified, at least annually, against these procedures as modified by operational experience.

2.2.4 LABORATORY FACILITIES FOR LABS AT A BIOSAFETY LEVEL 4

The features of a containment laboratory – Biosafety Level 3 also apply to a maximum containment laboratory – Biosafety Level 4 with the addition of the following.

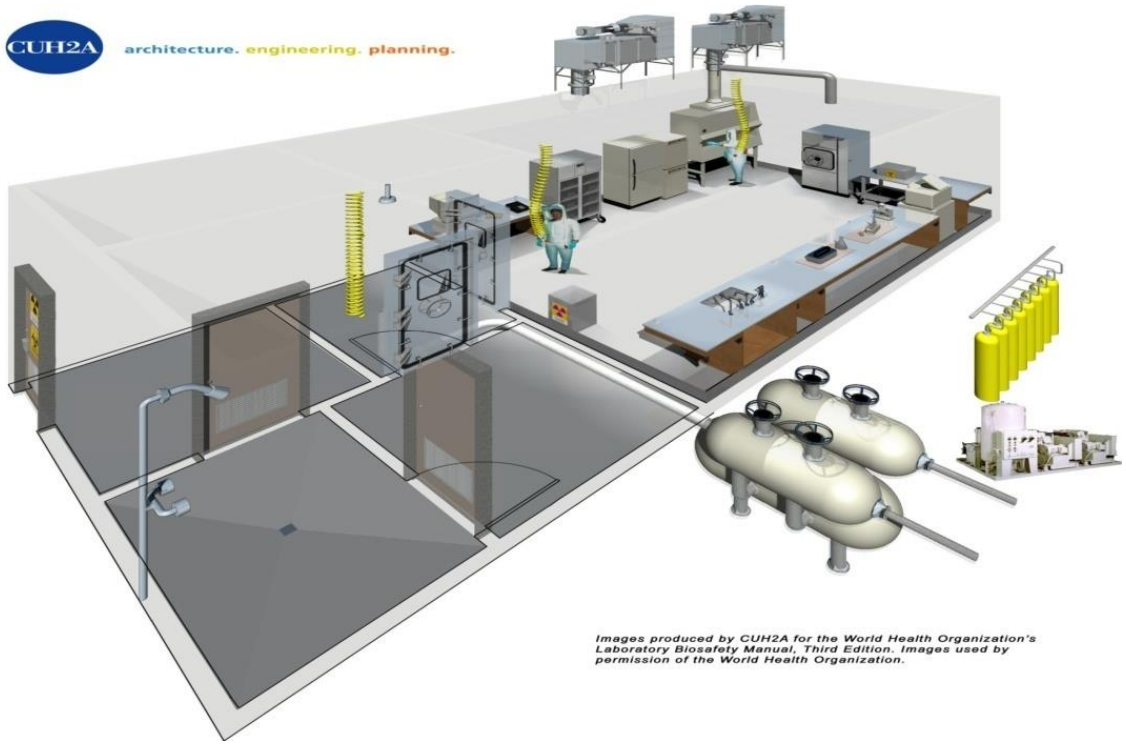


Figure: 2.2.4: BSL4 laboratory [7,8]

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

  - *Class III cabinet laboratory*: Passage through a minimum of two doors prior to entering the rooms containing the Class III biological safety cabinet(s) (cabinet room) is required. In this laboratory configuration the Class III biological safety cabinet provides the primary containment. A personnel shower with inner and outer changing rooms is necessary. Supplies and materials that are not brought into the cabinet room through the changing area are 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.
  - *Suit laboratory*: A protective suit laboratory with self-contained breathing apparatus differs significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biological safety cabinets. The rooms in the protective suit laboratory are arranged so as to direct

personnel through the changing and decontamination areas prior to entering areas where infectious materials are manipulated. A suit decontamination shower must be provided and used by personnel leaving the containment laboratory area. A separate personnel shower with inner and outer changing rooms is also provided. Personnel who enter the suit area are required to don a one-piece, positively pressurized, HEPA-filtered, 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. Entry into the suit laboratory is through an airlock fitted with airtight doors. 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.

- The maximum containment laboratory – Biosafety Level 4 must be located in a separate building or in a clearly delineated zone within a secure building. Entry and exit of personnel and supplies must be through an airlock or pass-through system. On entering, personnel must put on a complete change of clothing; before leaving, they should shower before putting on their street clothing.

- Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. There are significant differences in the ventilating systems of the Class III cabinet laboratory and suit laboratory:

    - *Class III cabinet laboratory*: 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. 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. A dedicated non-re-circulating ventilating system for the cabinet laboratory is required.
    - *Suit laboratory*: Dedicated room air supply and exhaust systems are required. The supply and exhaust components of the ventilating system are balanced to provide directional airflow within the suit area from the area of least hazard to the area(s) of greatest potential hazard. Redundant exhaust fans are required to ensure that the facility remains under negative pressure at all times. The differential pressures within the suit laboratory and between the suit laboratory and adjacent areas must be monitored. Airflow 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. Exhaust air from the suit laboratory must be passed through a series of two HEPA filters prior to release outdoors. Alternatively, after double HEPA filtration, exhaust air may be re-circulated, but only within the suit laboratory. Under no circumstances shall the exhaust air from the Biosafety Level 4 suit laboratory be re-circulated to other areas. Extreme caution must be exercised if recirculation of air within the suit laboratory is elected. Consideration must be given to the types of research conducted, equipment, chemicals and other materials used in the suit laboratory, as well as animal species that may be involved in the research.

- All effluents from the suit area, decontamination chamber, decontamination shower, or Class III biological safety cabinet must be decontaminated before final discharge. Heat treatment is the preferred method.

- Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment.

- A double-door, pass-through autoclave must be available in the laboratory area.

- Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization.

- Airlock entry ports for specimens, materials and animals must be provided.

- Emergency power and dedicated power supply line(s) must be provided.

- Containment drain(s) must be installed.

2.2.5 CLINICAL LABORATORY

- Clinical laboratories are often unique environments that may pose identifiable infectious disease risks to persons in or near them. Laboratory acquired infections have been recognized for many years. Properly designed facility is fundamental to the safety of laboratory workers, hospital staff, and patients. The following are the suggestions to consider designing or renovating of the clinical diagnostic laboratories [10].

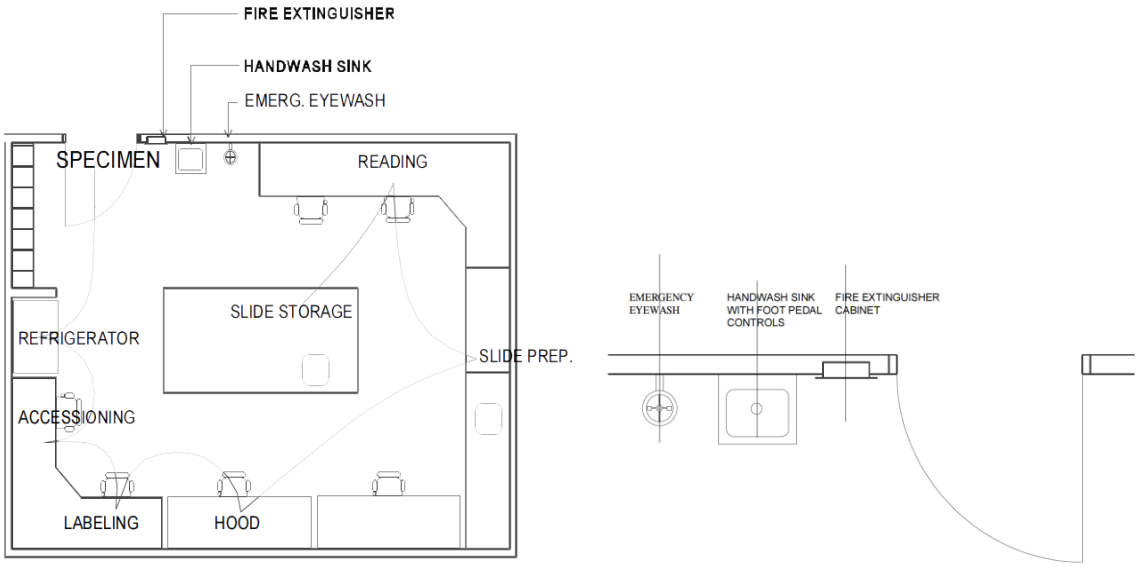


Figure: 2.2.5: Minimal clinical diagnostic laboratory and its safety module.

Design options for the clinical diagnostic laboratory should include but not limited to-

- An enclosed component of the overall laboratory, separated by closable doors from other laboratory sections of other areas in a building [10].
    - It is recommended to have directional inward airflow from the main laboratory into the microbiology laboratory. Mechanical ventilation is also recommended in newly constructed diagnostic laboratories.
    - If the facility is an open design and has no drop ceiling, the microbiology laboratory can have clear glass or Plexiglas walls, which give an appearance of openness but provide a floor-to-ceiling safety barrier from possible aerosol exposures.
    - If a drop ceiling is in place, the clear wall needs to penetrate the deck beyond the ceiling to seal the area.
    - In a previously constructed laboratory without directional room air, the continual operation of biological safety cabinets (BSCs) is encouraged to provide some direction to potential aerosols.
    - Directional air is encouraged to provide zones of containment that proceed with increasing negative pressure toward work spaces in which higher-risk laboratory procedures are conducted.
    - An air handling systems within the microbiology laboratory suite must be able to be adjusted and balanced with directional airflow from the corridor into the microbiology laboratory and from the general microbiology laboratory into separate and enclosed tuberculosis, mycology, and virology specialty laboratories.
    - For human laboratories, the separate tuberculosis and virology laboratories that manipulate cultures for identification and characterization would ideally meet BSL3 requirements.

- For animal diagnostic virology laboratories in which most manipulated viruses are not human pathogens, the practice is to meet BSL2 requirements unless a risk analysis indicates a high probability that an agent in a specimen needs BSL3 containment. Risk assessments should be performed on each facility to include consideration of the specific risks encountered in each laboratory.
- Equipped sample collection room separated from mail lab should be placed.
- Biosafety laboratory should be designated according to the tier (primary, secondary and tertiary) to BSL 1, BSL 2 and BSL 3 lab and guideline should be accordingly implimented.
- Any lab can be upgraded to superior level with proper planning and approval.

- The receiving and set-up areas in microbiology laboratories should be designed with sufficient space to accommodate the greatest number of specimens anticipated [10].
  - This area needs a Class IIA2 BSC, a sink for hand washing, and an emergency eye wash station.
  - Telephone jacks, computer jacks, and electrical outlets should be built into the module (use of wireless technologies can reduce the need for telephone or computer wiring in each module) along with refrigerator space for one or two side-by-side refrigerators or one double refrigerator to enable easy access by the set-up staff.
- The general laboratory should contain sit-down work spaces designed with adequate space for a computer at each station [10].
  - Work benches that have storage shelves above the center of the bench might be preferred; these would provide space for supplies without cluttering the work area.
  - Storage shelves need a 1-cm (1/2-inch) lip to ensure chemicals cannot slide off a shelf.
  - Under-shelf lighting is best to illuminate the work area. For convenience, electrical outlets are recommended at each workstation, along with telephone and computer jacks.
  - Gas burners are no longer universally recommended.
- If possible, locate carbon dioxide and anaerobic gas tanks outside the actual laboratory (preferably shielded or even installed outside the walls of the building) [10].
  - Placing the tanks outside the laboratory or the building in a locked area will allow easy access for exchange of tanks. Where appropriate, lines that connect gas tanks to specific areas of the laboratory should be made of synthetic tubing to allow future moving if necessary.
  - Accommodations need to be made for daily reading of the gauges in the laboratory unless alarms can be installed. Gas tanks should be individually secured.
- If waste will be decontaminated on-site before disposal, the laboratory must have an autoclave large enough to handle its needs. Locate the autoclave in a well-ventilated area, or ensure it is exhausted through a capture hood above it [10].
  - Ideally, the Mycobacteriology laboratory will have its own autoclave. Double-door autoclaves can be installed so that one side opens into the Mycobacteriology laboratory and the other side opens into a disposal area used by the laboratory for disposing of other waste.
  - Validation of the autoclave cycles for effective decontamination of the projected loads is recommended in addition to a regular maintenance and quality-assurance program.
- Optimally, the diagnostic laboratory would plan for

- A general microbiology laboratory area able to be closed off from the main laboratory, i.e., from other laboratory disciplines;
- Separate Mycobacteriology, virology, and mycology rooms (under negative pressure relative to the general laboratory with a Class IIA2 BSC) with telephone and computer jacks;
- Adequate space or separate rooms for quality control testing, receipt of supplies, and record storage;
- An extra room for future expansion to offer more services, e.g., molecular or virology testing. The room might need to be renovated to accommodate a Class IIA2 BSC, directional airflow, telephone jacks, and communication devices such as intercoms. The telephone jacks and communication devices should be in all such rooms.
- Ensure that current and future microbiology space is designed for an adequate number of blood culture instruments, automated identification instruments, automated enzyme immunoassays, nucleic acid extraction and testing platforms, and pipetting instruments; refrigerators; automated Gram stainers; automated plate streakers; BSCs; freezers; and additional computer stations for optional use. Some identification instruments require at least 8 feet of footprint space for the unit, printer, and modules. If the laboratory will provide the service, it should plan for a medium-sized anaerobe chamber, about 6 feet of footprint. Risk assessments must include evaluation of the infectious aerosols that might be produced by automated procedural equipment to determine whether containment ventilation is recommended [10].

- For microbiology laboratories, it is critical that the supervisor and laboratory director, along with a biosafety professional, provide input regarding the special needs of a new laboratory facility [10].
  - Access into the microbiology section must be restricted to staff only.
  - The microbiology section must have a decontamination facility or have a medical waste contract in place, and it must provide a sink for hand washing.
  - Hands-free sinks (foot-pedal operated) are required for biosafety level (BSL)-3 facilities and are recommended for BSL2 facilities.
  - Bench-tops must be constructed of impervious materials; laminate materials can delaminate and become difficult to disinfect.
  - For BSCs that vent to the outside, air handling should be planned carefully to ensure that the air is vented to the outside after filtration and that the outside vents are placed away from the facility's air intake units.
  - For laboratories that contain multiple classes of BSCs, the hazards that are permitted to be manipulated within the specific unit need to be clearly indicated (by label) to staff.
  - The general human and animal clinical diagnostic laboratory should be BSL2/ABSL2.

Chapter 2.3: Commissioning and Certification

.

2.3.1 AUTOCLAVE – TESTING

2.3.1.1 Physical Indicators

Pressure and temperature recording devices are available. Thermocouples can be placed inside the load to determine the temperature achieved in the bag itself [23].

2.3.1.2 Chemical Indicators

These indicators change color after being exposed to specific temperatures, for example: heat sensitive tape. Upon exposure to the given temperature the change will occur; it is not time related. Therefore these indicators can only attest to the temperature attained and not to exposure time and hence success of sterilization [23].

2.3.1.3 Biological Indicators

Biological indicators should be used in the efficacy testing of the autoclave process to effectively sterilize the contents being treated. *Geobacillus stearothermophilus* spores are used, as they are the most resistant organism to steam autoclaving. To determine the effectiveness of the autoclave process the biological indicator must be placed in a typical test load (solid or liquid) and exposed to the typical cycle conditions. This is the standard method of validating the effectiveness of autoclave procedures. Testing using a biological indicator must be undertaken once in a week, unless otherwise authorized by Biosafety Office [23].

2.3.2 FIRE EXTINGUISHER & SMOKE DETECTOR - TESTING

2.3.2.1 Fire Extinguisher

Portable fire extinguishers are required to be visually inspected when initially placed in service and at least monthly thereafter. These visual inspections, intended to help ensure that each extinguisher is in its designated place and will operate if needed, can be performed by facility staff. A pressure gauge in the "normal" or "operable" range is not a 100 percent guaranty that the extinguisher will perform as intended. A broken seal, for example, may be an indication that someone has used the extinguisher and discharged a portion of the contents too small to affect the pressure gauge. Therefore, it requires that the monthly inspection verify a number of things including [27]:

- Extinguishers are shall be located in the corridors or in their designated places
- The maximum travel distance to an extinguisher shall be 15 m (50 ft.).
- For open labs, the fire extinguisher shall be located as closely as possible to the exit access doors. There are no obstructions to access or visibility
- Safety seals are not broken or missing
- There is no evidence of physical damage, corrosion, leakage or clogged nozzle
- Pressure gauge readings are in the proper range or position
- Operating instructions are legible and facing outward
- Fullness – confirmed by weighing or lifting

2.3.2.2 Smoke Detector

Smoke detector should be tested for three related, but separate, tests-Visual Inspection, Smoke Entry, and Sensitivity testing.

2.3.3 EMERGENCY SHOWERS - TESTING

- When the shower is installed, test it whether it is correctly connected to the water source and the valve(s) closed, visually check the piping for leaks.

- Open the valve to the full open position. The valve shall remain open without requiring further use of the operator's hands.
- Measure the shower. The face of the showerhead shall be not less than 82 inches or more than 96 inches from the surface on which the user stands.
- With the valve in the "full on" position, measure the diameter of the spray pattern. It shall be a minimum of 20 inches at 60 inches above the standing surface. The center of the spray shall be at least 16 inches from any obstructions.
- Delivered water temperature shall be tepid.

2.3.3 EYEWASHES - TESTING

- When the eyewash is installed, the valve shall be operated to determine that both eyes would be washed simultaneously at a velocity low enough to be non-injurious to the user.
- Delivered water temperature shall be tepid.

2.3.4 LABORATORY/FACILITY COMMISSIONING

Laboratory/facility commissioning may be defined as the systematic review and documentation process signifying that specified laboratory structural components, systems and/or system components have been installed, inspected, functionally tested and verified to meet national or international standards, as appropriate. The following is a list of laboratory systems and components that may be included in a commissioning plan for functional testing, depending on the containment level of the facility being renovated or constructed. The list is not exhaustive. Obviously, the actual commissioning plan will reflect the complexity of the laboratory being planned [27,28].

- Building automation systems including links to remote monitoring and control sites
- Electronic surveillance and detection systems
- Electronic security locks and proximity device readers
- Heating, ventilation (supply and exhaust) and air-conditioning (HVAC) systems
- High-efficiency particulate air (HEPA) filtration systems
- HEPA decontamination systems
- HVAC and exhaust air system controls and control interlocks
- Airtight isolation dampers
- Laboratory refrigeration systems
- Boilers and steam systems
- Fire detection, suppression and alarm systems
- Domestic water backflow prevention devices
- Processed water systems (i.e. reverse osmosis, distilled water)
- Liquid effluent treatment and neutralization systems
- Plumbing drain primer systems
- Chemical decontaminant systems
- Medical laboratory gas systems
- Breathing air systems
- Service and instrument air systems
- Cascading pressure differential verification of laboratories and support areas

- Local area network (LAN) and computer data systems
- Normal power systems
- Emergency power systems
- Uninterruptible power systems
- Emergency lighting systems
- Lighting fixture penetration seals
- Electrical and mechanical penetration seals
- Telephone systems
- Airlock door control interlocks
- Airtight door seals
- Window and vision-panel penetration seals
- Barrier pass-through penetration
- Structural integrity verification: concrete floors, walls and ceilings
- Barrier coating verification: floors, walls and ceilings
- Biosafety Level 4 containment envelope pressurization and isolation functions
- Biological safety cabinets
- Autoclaves
- Liquid nitrogen system and alarms
- Water detection systems (e.g. in case of flooding inside containment zone)
- Decontamination shower and chemical additive systems
- Cage-wash and neutralization systems
- Waste management

2.3.5 BIOSAFETY CABINET - CERTIFICATION

- Biological Safety Cabinet should be certified by NSF accredited field certifier at installation, annually, when move, when change filter, when repair, or modify [12].

- Use of gas burner inside BSC is strongly discouraged. Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air being supplied to the work surface [12].

- HEPA-filtered exhaust air from a Class II A2 biological safety cabinet can be recalculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside through the building exhaust air system, the cabinets shall be connected in a manner that avoids any interference with the air balance of the cabinets [12].

2.3.6 LABORATORY/FACILITY CERTIFICATION

Laboratory certification is the systematic examination of all safety features and processes within the laboratory (engineering controls, personal protective equipment and administrative controls). Biosafety practices and procedures are also examined. Adequately trained safety and health or biosafety professionals may conduct laboratory certification activities. Institutions may employ personnel having the appropriate

skill-set required for conducting audits, surveys or inspections associated with the certification process. However, institutions may consider engaging or be required to engage a third party to provide these services. Laboratory certification is an on-going quality and safety assurance activity that should take place on a regular basis [27,28]. Laboratory certification helps to ensure that:

- Proper engineering controls are being used and are functioning adequately as designed.
- Appropriate site and protocol specific administrative controls are in place.
- Personal protective equipment is appropriate for the tasks being performed.
- Decontamination of waste and materials has been adequately considered and proper waste management procedures are in place.
- Proper procedures for general laboratory safety, including physical, electrical and chemical safety are in place.

SECTION 3.0: WASTE MANAGEMENT

Chapter 3.1: Definitions

3.1.1 INFECTIOUS/BIOHAZARDOUS WASTE

Infectious/biohazardous wastes include biological agents with potentials to contribute to an infection [7,8].

- Biohazard: Biological agents and materials that are potentially infectious or hazardous in any other way to humans, animals, or plants are classified as biohazard. Biohazards generally arise from inadvertent human biological processes (e.g. accidental inoculation, needlestick injury).

- Biohazardous/biological/infectious/biomedical waste: Waste generated in any laboratory facility containing microorganisms or biological material is considered as biohazardous waste. The following materials are defined as infectious/biomedical wastes:

  - Cultures and Stocks of Etiologic Agents and Associated Biologicals: This includes, but is not limited to, specimens, cultures and stocks of etiologic agents and agents requiring biosafety level (BSL) 2 and 3 containment, wastes from production of biologicals and serums, and discarded live and attenuated vaccines.
  - Laboratory waste which has come in contact with a biohazard: This includes, but is not limited to, disposable laboratory personal protective equipment (gloves, gowns, shoe covers, masks etc), disposable laboratory plastic ware (culture dishes, plates and flasks, pipettes, and pipette tips), blood specimen tubes, devices used to transfer, inoculate and mix cultures; and paper and cloth which have come into contact with cultures and stocks of etiologic agents.
  - Used, absorbent materials saturated with blood, blood products, body fluids, or excretions or secretions contaminated with visible blood; and absorbent materials saturated with blood or blood products that have dried.
  - Sharp waste**:** All hypodermic needles, syringes with needles attached, IV tubing with needles attached, scalpel blades, and lancets that are capable of puncturing, lacerating, or otherwise penetrating the skin and have been removed from the original package whether contaminated or not with any infectious or biohazardous material.
  - Human Body Fluids: This includes, but is not limited to, blood and blood products, serum and plasma, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, pus etc when they are in free-flowing form.
  - Animal Waste: This includes, but is not limited to carcasses, body parts, and bedding of animals that are known to be infected with, or that have been inoculated with, microorganisms pathogenic/infectious to humans and animals.
  - Recombinant DNA: All contaminated liquids or solid wastes from research activities involving risk group one (RG1), RG2, and RG3 agents.
  - Vaccine: Biological preparations that improve immunity against a particular disease. A vaccine typically contains an antigen/agent that resembles a disease-causing microorganism, and is often made from killed or weakened forms of the microbe, its toxins or one of its surface proteins.

3.1.2 NON-BIOHAZARDOUS WASTE:

Non-biohazardous waste include materials posing no risk to humans, animals or to the environment, and any other materials listed below that are subjected to disposal from biomedical laboratories after use [7,8].:

- Materials that are not saturated with infectious agents**:** Not saturated or dripped with blood or body fluids; not covered with blood clot or caked with dried blood.
- Paper products**:** Non-recyclable paper, paper plates, cups, plastic cutleries, shoes, head covers, aprons, gowns, masks that did not come in contact with biohazardous agents.
- Liquid biohazardous waste that has been decontaminated by approved chemical disinfectants: Liquid biohazardous waste can be decontaminated by chemical disinfectants. Density of the waste in the liquid and the active concentration of the chemical for treatment have to be adjusted properly for successful decontamination.

- Autoclaved items where biological indicator or indicator tape has been used to indicate autoclaved waste: The use of the indicator tape confirms that the material has been appropriately autoclaved; and the use of biological indicator (BI) ensures that the autoclave is performing well and the material is rendered sterilized.
- Waste materials that have been incinerated: Materials incinerated are for disposal as non-biohazardous waste; which however, could be dumped into the ground through approved vendors.

3.1.3 BIOMEDICAL WASTE GENERATOR

A facility or a person has been producing biomedical waste, which includes but not limited to hospitals and clinics, health maintenance organizations, medical buildings, physician's offices, laboratories, veterinary facilities, etc.

3.1.4 PUNCTURE RESISTANT

A substance that is able to withstand punctures from contained sharps during normal usage and handling.

3.1.5 SHARPS CONTAINERS

A rigid, leak and puncture resistant container, well fitted lid, designed primarily for the containment of sharps, clearly labeled with the phrase and the international biological hazard symbol.

3.1.6 WASTE TREATMENT

Processes, including steam, chemicals, microwave shredding, or incineration that changes the character or composition of biomedical waste to render it noninfectious and non-hazardous for its safe disposal.

3.1.7 HAZARDOUS WASTE:

A chemical waste that exhibits one or more of the following characteristics: ignitability, corrosivity, reactivity, and toxicity.

3.1.8 RADIOACTIVE WASTE

Waste materials containing radioactive chemicals or those came in contact with radioactive waste is considered as radioactive waste. All such wastes generated in laboratories or in health care facilities containing radioactive material are thus radioactive wastes.

3.1.9 SATELLITE WASTE ACCUMULATION AREAS (SWAA)

An area designated for waste collection and temporary storage until disposal or predisposal treatment near the point of generation.

3.1.10 COMMON BIOMEDICAL WASTE TREATMENT FACILITY (CBWTF)

A CBWTF is a set up where biomedical waste, generated from a number of healthcare units, is imparted for necessary treatment to reduce adverse effects that this waste may pose to the surrounding environment. The treated waste may finally be sent for disposal in a landfill or for recycling purposes. Installation of individual treatment facilities by small healthcare units requires comparatively high capital investment. In addition, it requires separate power and adequate fuel supply, manpower, and infrastructure development for proper operation and maintenance of treatment systems. CBWTF concept not only addresses such problems but also prevents proliferation of treatment equipment in a city. In turn it reduces the monitoring pressure on regulatory agencies. By running the treatment equipment at CBWTF to its full capacity, the cost of treatment of per kilogram gets significantly reduced. Its considerable advantages have made CBWTF popular and proven concept in many developed countries. It is strongly recommended to transport biohazardous waste after onsite sterilization to CBWTF for incineration or final disposal [21,22].

Chapter 3.2: Waste Segregation

3.2.1 SEGREGATION OF WASTE GENERATED IN LABORATORIES AND HEALTH CARE FACILITIES

Waste segregation is the most important step in waste management processes, and a major contributor to the success of the program. Laboratory or health care wastes that are infectious or hazardous must be segregated from general wastes using color bins at the source. Segregation minimizes the amount of waste requiring special handling and disposal procedures, and reduces the overall disposal costs. Considerable cost offsets can be achieved if the entire waste stream does not have to be treated as biomedical waste. In fact, only a small proportion of all the wastes generated at a health care or similar facility are biomedical waste. Further segregation of biomedical waste into the following types allows for cost effective disposal [21,22]:

SL	Type	Segregation
1	Sharps	Biohazardous and Non- biohazardous
2	Cultures or platess	RG1, RG2, RG3, and RG4
3	Tips, pipettes and disposables	Biohazardous and Non- biohazardous
4	Glass	Biohazardous and Non- biohazardous
5	Blood and body fluids	Human blood, animal blood, blood elements, other body fluids, liquid and semi-liquid materials
6	Vaccine and diluents	Live attenuated vaccine, preservative free whole cell killed vaccine, protein subunit vaccine, empty vaccine vial, and vaccine diluents
7	Animal waste	Animal bedding, Contaminated carcasses, uncontaminated carcasses, and liquid and solid waste
8	GMO/LMO	GMM, GM plants, GM animals
9	Chemicals	Acid, base, solvent, hazardous, non-hazardous, radioactive waste, and Ethidium Bromide (EtBr)

3.2.2 SEGREGATION OF WASTE GENERATED IN HEALTHCARE FACILITIES:

Various biohazardous wastes will be segregated following the criteria described above. The other hospital or health care wastes are listed below [21,22]:

- Hospital bed and mattress
- Stool and related waste, such as, vomitus, fomites.
- Kitchen waste
- Expired medicine/chemicals/ reagents

Chapter 3.3: Packaging and Labeling

3.3.1 GENERAL INSTRUCTIONS

Containers for biomedical waste must be appropriate for its contents. Different types of containers and bags are available for containment and disposal of biomedical wastes. Double bag should be used to prevent perforation, when necessary, and absorbent material should be used in dealing with large volumes of liquid, ensured that the bags are well sealed. The containers/bags should not be overfilled. If the outside of the bag is/likely to be contaminated, double bag should be used. Secondary containment should also be labeled with the biohazard symbol. The types of packaging and associated labeling used for various biomedical wastes are outlined below. All containers for biomedical waste must display the biohazard symbol and the words 'Biohazard' in a color contrasting the container. The different waste groups may have different colors for the containers and bags for the identification according to the hazards and applied throughout the complete disposal chain (segregation, collection, storage, transport, and disposal). The color coding makes the process understandable even for low-skilled workers with language and read problems [21,22].

- Warning colors for hazardous waste (Red, yellow, orange)
- Positive colors for recycling (Blue, green, etc.)
- Neutral colors for normal waste (Black, etc.)

3.3.1.1 Sharps

Must be rigid, leak proof, puncture resistant and sealable.

3.3.1.2 Solid biohazardous waste

3.3.1.3 Should be collected in an autoclavable biohazard bag.

3.3.1.3 Liquid biohazardous waste

Leak-proof containers should be used that are able to withstand thermal or chemical treatment.

3.3.1.4 Vials

Glass vials **s**hould be deposited in approved sharps container; however, plastic vials that may not be broken leading to generate sharps can be disposed in biohazard bags.

3.3.1.5 Fluids

Rigid leak-proof container should be used.

3.3.1.6 Saturated Items

Should be collected in an autoclavable biohazard bag.

3.3.1.7 Anatomical/Animal Waste

3.3.1.8 Should be placed in an autoclavable biohazard bag or burn box lined with biohazard bags.

3.3.1.8 GMO and related waste

Solid waste **s**hould be placed in an autoclavable biohazard bag or liquid waste in appropriate containers for autoclaving. Waste that may not be suitable for autoclaving should be directly incinerated after secured packaging in biohazard bags or burn box. Sharps contaminated with infectious GMM should be disposed in sharps containers.

3.3.1.9 Chemicals

Chemical wastes generated in laboratories are diverse in types. The laboratory workers should segregate their wastes according to the type and compatibility, and plan for final pre-disposal treatment and final disposal. Chemical wastes should be properly packaged, labeled and stored temporarily at the producer's premises before collection for treatment and/or disposal. Appropriately segregated chemical waste should be labeled with their respective chemical hazard-warning symbol following the Material Safety Data Sheet (MSDS). The label should include the name, address and contact telephone number of the waste producer. Acid and base should not be placed closely and be kept in separate rack and to be disposed off separately.

- **Radioactive waste:** Radioactive wastes should be stored by encapsulation for decaying action. Radioactive containers such as glass bottles should be destroyed before disposal for avoidance of public access. All waste materials should be disposed following national/institutional guideline.

- **Ethidium bromide (EtBr):** EtBr is not a regulated waste *per se*, due to its properties; however, it is a potential mutagen and presents hazard if it is poured down the drain or placed untreated in the regular garbage. Correct procedures for the disposal of EtBr depend on the nature of the waste materials and the concentration of EtBr that they contain. Therefore, due to its many uses, it is not possible to have one mode of disposal. All solid wastes containing EtBr will be segregated, labeled, and contained in a well-protected container in a polythene/polypropylene bag for incineration. Liquid waste will be decontaminated with the use of activated charcoal in tea bags. EtBr is not advisable to use. It is better to use non-hazardous gel.

3.3.2 WASTE ACCUMULATION AT SATELLITE WASTE ACCUMULATION AREA (SWAA)

All properly segregated and/or packaged waste for disposal or treatment will be collected from the waste generators and stored, consolidated, and further packaged if necessary, at SWAA before sending treatment and final disposal. This should be a secured area marked with signs indicating all the hazards (biological or chemical) present, and is inspected weekly [21,22].

Chapter 3.4: Treatment and Disposal of Waste

3.4.1 GENERAL INSTRUCTIONS

All biological wastes should be decontaminated prior to their disposal, which results in the destruction or reduction of microorganisms to a much lower level such that there is no danger of infection to others. Decontaminated or disinfected wastes are no longer considered biomedical waste. Therefore, once the waste has been treated, it can be disposed of in the regular waste stream. The main choices for waste decontamination are autoclaving (preferred), chemical disinfection, and incineration. Any waste that cannot be treated with autoclave or chemical treatment (i.e. sharps, carcasses, tissues and body parts etc) will be regarded as biomedical waste and must be incinerated. The chosen method of inactivation (e.g. autoclaving or chemical inactivation) must be validated annually under normal working conditions. A copy of the validation protocol and the results of the validation exercise must be retained by the user [21,22].

3.4.1.1 Chemical disinfection

Chemical disinfection is achieved by using sodium hypochlorite solution to kill microorganisms. Commercially available 5.23% bleach should be mixed with liquid biohazardous waste to be inactivated to achieve a final dilution of 1:10 in the liquid [final active solution = 0.523 % bleach] and left for a minimum of 30 minutes or as long as 8 hours, covered, and then disposed down the sanitary sewer, followed by flushing with cold water for a minimum of 10 minutes. Other work specific chemical disinfectants (70% Ethanol, Phenyle, Dettol, etc.) can also be used.

3.4.1.2 Autoclave

Autoclaving or steam sterilization is one of the most effective methods for decontaminating biohazardous material. Autoclaves use saturated steam under high pressure to decontaminate infectious materials, i.e., cultures, cells, contaminated glassware, pipettes, etc. Autoclaving is the preferred method for treating biohazardous waste to render non-biohazardous. Biohazardous materials that are autoclaved should be discarded as non-contaminated laboratory waste and can be placed in the regular trash [23].

3.4.1.3 Incineration

This involves combustion of the organic substances contained in waste materials, which reduces the original solid mass by 80–85% and the volume by 95-96%, depending on composition and degree of recovery of materials such as metals from the ash for recycling. Any waste not suitable for autoclaving is generally recommended for incineration [21,22].

3.4.1.4 Effluent Treatment Plant (ETP)

This type of waste water treatment method is particularly designed to purify waste water for reuse. The aim of this treatment is to release safe water to environment from the harmful effect caused by the effluent. According to the Environment Conservation Rules, 1997, environmental clearance of proposed health care facility requires design and planning of ETP. An existing facility requires submission of effectiveness of ETP [21,22].

3.4.2 TREATMENT AND DISPOSAL OF BIOHAZARDOUS WASTE

Common waste treatment facility will arrange waste pick-up from the storage area, and additionally perform activities for arranging and maintaining transport van, verify waste material labelling, monitoring, and paper works [21,22].

3.4.2.1 Sharps

Sharps waste disposed in sharps containers should be incinerated in biomedical waste incinerator.

3.4.2.2 Solid biohazardous

Solid waste disposed in autoclavable biohazardous bags should be autoclaved before disposal [20,24,25].

3.4.2.3 Autoclavable liquid waste

This waste should be autoclaved before disposing in to the sink.

3.4.2.4 Un-autoclavable solid waste

Waste that cannot be autoclaved or the organization chooses not to autoclave should be disposed into contaminated materials containers or burn box for incineration.

3.4.2.5 RG 1, 2, 3 and 4 wastes

RG 1 may not be necessary to be inactivated before disposal. RG 2 must be inactivated on the same site as the contained use activity is taking place. RG 3 must be inactivated in an autoclave that is situated within the laboratory and it must not be removed from the laboratory for the purpose of inactivation. RG 4 must be inactivated in a double ended autoclave. Once autoclaved, Class 3 and 4 materials are recommended to be incinerated before final disposal.

3.4.3 TREATMENT AND DISPOSAL OF ANATOMICAL/ANIMAL WASTE

This waste should be properly packaged in biohazard bags and incinerated. Waste should be incinerated everyday and if required can be stored as freezing temperature until incineration. Contaminated waste with different RG of infectious agents should be treated following the policies and procedures of the respective biosafety level [7,8,21,22].

3.4.4 TREATMENT AND DISPOSAL OF GMO AND RELATED WASTE

Irrespective of the class of activity, all material and waste contaminated with GMMs must be inactivated by validated means before disposal [24,26,29,30].

3.4.4.1 RG 1 GMM

This has to be inactivated before disposal. RG 2 GMM must be inactivated on the same site as the contained use activity is taking place. RG 3 GMM must be inactivated in an autoclave that is situated within the laboratory and it must not be removed from the laboratory for the purpose of inactivation. RG 4 GMM must be inactivated in a double ended autoclave. Once autoclaved, Class 3 and 4 material are recommended to be incinerated before final disposal.

3.4.4.2 All GM plants, GM/non-GM plants inoculated with GMMs

Pots, trays, compost, soil or other growth media must be decontaminated by validated means, on the same site as the contained use activity is taking place, prior to cleaning and / or disposal.

3.4.4.3 GM animal

This type of waste presents no greater risk to human health or the environment than a non-GM animal; however, in the interest of public perception GM animal carcasses should be decontaminated prior to disposal.

3.4.5 RECYCLING OF STERILIZE MATERIALS

Sterilized materials can be reused (reusable plastic waste containers, glass ware, test tubes, culture flasks, etc.,) or recycled as appropriate and recommended by the IBC. Virtually, all plastic wastes can be recycled under environmentally sound conditions [24,26,29,30].

3.4.6 TREATMENT AND DISPOSAL OF NON-BIOHAZARDOUS WASTE

Non-biohazardous waste will be directly disposed as appropriate [7,8].

3.4.7 DIRECT DISPOSAL OF LABORATORY WASTE

Liquid culture of RG1 microbes should be directly disposed into the sanitary sewer. Liquid culture of RG2 microbes, should be rendered non-biohazardous by treating with chemical disinfectants or by steam sterilization, and then directly disposed into the sanitary sewer as non-biohazardous waste.

3.4.8 DIRECT DISPOSAL OF OTHERS WASTE

Other non-biohazardous waste will be directly disposed through contracted and authorized vendors.

3.4.9 TREATMENT AND DISPOSAL OF HAZARDOUS CHEMICAL WASTE

Generally, laboratories can arrange treatment or disposal of their chemical wastes following MSDS.

3.4.10 TREATMENT AND DISPOSAL OF LABORATORY EQUIPMENTS

Laboratory equipment may be contaminated with hazardous materials, posing safety threat to anyone handling or using them. Items of concern include, but are not limited to, refrigerators, freezers, incubators, storage cabinets, glassware, water baths, shakers, analytical instruments, etc. Laboratory equipment to be disposed of must first be cleaned and decontaminated by the disposing lab/ facility and inspected by Biosafety Office. Equipment to be abandoned should not be on docks, or in storage areas or exterior spaces, creating hazard or nuisance. A material is considered abandoned if it is no longer suitable for its intended purpose, poses an immediate hazard, or is not intended for use in a reasonable amount of time. Facilities Management Services (FMS), working with general services department will remove abandoned materials. The ways to dispose such materials include [7,8]:

a) Recycle of laboratory equipment and spares

b) Donate workable laboratory equipment, if not in use

c) Discard laboratory equipment

3.4.11 TREATMENT AND DISPOSAL OF LABORATORY FURNITURE

Laboratory furniture to be disposed of must first be cleaned and decontaminated by the disposing lab/ facility and inspected by Biosafety Office. Furniture to be abandoned should not be kept on docks, or in storage areas or exterior spaces, or elsewhere creating a hazard or nuisance. A material is considered abandoned if it is no longer suitable for its intended purpose, poses an immediate hazard, or is not intended for use in a reasonable amount of time. Abandoned furniture will be removed by FMS, working with general services department. The ways to dispose such materials include [7,8]:

a) Recycle of laboratory furniture

b) Donate laboratory furniture to other laboratories

c) Discard laboratory furniture

3.4.15 TREATMENT AND DISPOSAL OF RADIOACTIVE WASTE

All radioactive wastes should be stored in large storage containers at designated radioactive waste storage area for recommended period of time. Radiation count should be monitored during storage and ultimately disposed following the guidelines provided by Bangladesh Atomic Energy Commission (BAEC).

3.4.16 TREATMENT AND DISPOSAL OF OTHER HOSPITAL WASTE

Liquid waste from hospital facilities will be decontaminated, when indicated, using appropriate chemical disinfectants (e.g., sodium hypochlorite). Solid waste materials will also be appropriately treated before disposal. Chemical waste from the hospital will be disposed according to the chemical waste disposal guidelines [21,22].

3.4.17 WASTE MINIMIZATION PLAN

Waste Minimization Plan will include detailed methods to reduce the volume of waste generated, and the plan will be updated annually. Minimizing biomedical waste is the first step in managing wastes safely, responsibly and in a cost effective manner. This management step makes use of the principles of reducing, reusing and recycling. Possible ways to minimize the amount of both general waste and biomedical wastes will include-

3.4.17.1 Waste Segregation

Segregation minimizes the amount of waste requiring special handling and disposal procedures and reduces the overall costs of disposal.

3.4.18 Product Substitution/Process

Product substitution is another means to reduce the amount of wastes generated by a health care or related facility. The potential for substitution of single use/ disposable supplies with reusable ones need to be periodically re-evaluates. Use of reusable plastic cans instead of Biohazard bag should be considered whenever possible. Priority should be given to suppliers/ companies that have a policy of receiving/ recycling used goods.

3.4.19 Minimize the use of hazardous waste by the use of alternative method and generate low or no hazardous waste

Substitution of the method that uses less or no hazardous materials in research is a means to reduce the amount of hazardous wastes generation. Each facility should have a procurement plan that prevents procuring excessive amount that may overstay and expire to generate waste as well as waste money.

3.4.20 STORAGE OF BIOHAZARDOUS WASTE

Biomedical waste should be treated as promptly as possible; however, they may be contained and temporarily held at the waste generator site for a specified period of time (not exceeding 48 hours) []. Treatable waste should not be allowed to accumulate. Waste for off-site disposal may be stored in designated areas, which are secured with controlled access to only the delegated staff.

3.4.21 DISPOSAL IN PITS

If incineration or other complete destruction method is not available, safe burial into a pit would be a best alternative. A waste pit should be located downhill from nearby wells, in an area where the groundwater is not near the surface, and at least 50 meters from rivers, streams, springs, and other water sources. Pit sides and bottoms should be lined with clay to prevent liquids from passing into the soil and groundwater. The pit should be well-marked and have a fence around it to keep people and animals out. Drop sharps into a sharps pit or solid biohazardous waste into a safe burial pit and cover with soil. When almost full, seal the pit with concrete [26].

Chapter 3.5: Training, Audit and Record Keeping

3.5.1 TRAINING & CERTIFICATION

Biosafety Office or respective department will provide theoretical and hands-on training to all staff, and trainees and students who may generate waste; and will also organize annual refresher training. Biosafety office will organize specific safety and operational training on personal protection and safety techniques for the waste treatment plants. This will include CBWTF and staffs involved in waste management. Refresher training sessions will also be organized with relevant updates in the field for continuous development [11].

3.5.2 ANNUAL AUDIT

Biosafety Office should conduct safety audit of all facilities and personals generating, packaging, handling, and treating waste for disposal [7,8].

3.5.3 SAFETY AUDIT FOR WASTE GENERATORS

Safety audit will be conducted at all labs/ units, generating waste, using pre-defined checklist. Considerations will be given to their adherence to SOPs regarding the practices on waste segregation, packaging, labeling, and transportation. Suggestions will be provided continuous improvement and, accordingly, training and retraining will be conducted for the staffs generating and handling waste.

3.5.4 SAFETY AUDIT FOR THE WASTE TREATMENT FACILITIES

Safety audit will be conducted for the waste treatment facilities to ensure their adherence to SOPs regarding transportation, treatment, and final disposal of wastes. Special consideration should be given to the adherence to use of PPE while handling waste. A trained auditor with expertise in this area will visit both autoclave and incineration facilities to conduct annual safety audit. Opportunities to develop and make corrective actions may be suggested in case of non-compliance to facilitate and maintain the required level of safety and security standard.

3.5.5 RECORD KEEPING

The following records should be preserved for future documentation-

- All permits
- Infectious materials inactivation record
- Licenses
- All waste shipping documents
- Inspection logs
- training records
- Regulatory agency correspondence
- Log of total waste generation and treatment

These documents will be kept on file for a minimum of three years and as a soft copy of selective documents may be saved for archiving for longer time.

SECTION 4.0: APPENDIX

Appendix 4.1 National Biosafety and Biosecurity Advisory Committees

National Committee on Biosafety (NCB) should be formed combining members from relevant Ministries and department as required to work as advisory body on biosafety and biosecurity. A Biosafety Core Committee (BCC) shall be working to assist, accelerate, follow-up the functions of NCB and implementation of its mandate. In order to ensure safe management of Biosafety and Biosecurity activities in laboratories and in the fields, there shall be committees under NCB, such as Institutional Biosafety Committee (IBC), Field Level Biosafety Committee (FBC) and also there will be designated BSOs in each biological sciences research establishment in the country [6]. The composition and function of NCB and BCC are given in the following:

Composition of NCB

The NCB shall be composed of members as shown below [6]. The scientists and or experts shall be nominated by the responsible authority. The committee shall be chaired by a senior scientist with substantial experience of conducting research in biomedical or microbiological research and diagnostic laboratories. A knowledgeable individual with substantial expertise in the field molecular biology and recombinant DNA technology with maximum academic degree in the field of biological sciences with firsthand experience of working in the field of biosafety and biosecurity and operating biosafety program and institutional biosafety committee shall be appointed as the member secretary for NCB. The committee can be composed of, but not limited to, the representatives as follows:

1. Ministry of Health and Family Welfare (MoHFW)
2. Ministry of Environment and Forests (MoEF)
3. Ministry of Science and Technology (MoST
4. Ministry of Agriculture (MoA)
5. Ministry of Fisheries and Livestock (MoFL)
6. Directorate General of Health Services
7. Department of Livestock Services
8. Department of Environment
9. Department of Food
10. National Institute of Biotechnology (NIB)
11. Bangladesh Medical Research Council (BMRC)
12. Bangladesh Agricultural Research Council (BARC)
13. Member (relevant), National Board of Revenue
14. Bangladesh Fisheries Research Institute (BFRI)
15. Bangladesh Livestock Research Institute (BLRI)
16. Bangladesh Agriculture Research Institute (BARI)
17. Bangladesh Rice Research Institute (BRRI)
18. Bangladesh Jute Research Institute (BJRI)
19. Bangladesh Forest Research Institute (BFRI)
20. One representative from NGO working with environment related issues (to be nominated by NGO bureau).
21. One representative from NGO working with legal aspects related to environment (to be nominated by NGO bureau).

Note: Depending on the area of biological sciences of consideration, subcommittees can be formed to deal with the day to day activities. Terms of reference can be developed for the subcommittee function on the

subject matters and accordingly, instead of a large body above, the subcommittee can be composed of the essential members from above list, but not limited to, may essentially require running the NCB activities. Subcommittee can be formed taking into consideration of the area of interest like human health, animal health or agriculture.

Functions of IBC but not limited to:

1. Formulate, review, update or amend national policies, guidelines, acts and rules on Biosafety and Biosecurity and shall supervise risk assessment, risk management and the implementation activities thereof.
2. Review all existing bilateral and multilateral projects, and all research that are now being undertaken under private arrangements among universities, research organizations and NGOs.
3. Review, monitor and recommend measures to minimize potential risks that may result from, import, contained use, field trial and release or introduction of new species, strains or biological materials.
4. Facilitate the institutes/faculties/companies already working with biohazardous materials to obtain necessary permission or clearance in favor of their activities.
5. NCB shall examine the application submitted by any university, department and division of a research institute or a private company within a specified time frame and shall approve or disapprove the application on case-by-case basis. MOEF will notify the decision of NCB to the applicant.
6. Instruct the respective authority to ensure implementation of Biosafety and Biosecurity measures at on-site (field), laboratory, during trans-boundary movement, use, handling and release in the market.
7. Prepare different forms e.g. project proposal forms for permission to undertake laboratory genetic manipulation work; for field trial; and for commercial release of GMOs with adequate instructions for filling up of such forms for permission. At the same time forms for assessment and evaluation with adequate instruction will be prepared for various committees overseeing such work.
8. Publish the results of internal deliberations and agency reviews of the Committee.
9. Hold public deliberations on proposed national policies, guidelines and other Biosafety and Biosecurity issues.
10. Review the appointment of the members of the Institutional Biosafety Committee (IBC) upon recommendation of respective heads of institutions/Universities.
11. Hold discussions on the comparative ecological, economic and social impacts of alternative approaches to attain the purposes/objectives of the proposed genetic modification products and/or services.
12. Assess and identify priorities in human resource development in Biosafety and in the related capacity building (including infrastructural) requirements.
13. Coordinate the activities of institutional and field level Biosafety committees.
14. NCB can co-opt relevant experts/officers as and when it will be required for.

Responsibilities

1. Shall consider only those applications where the origin of biological materials used in research is clearly stated.
2. Advise relevant authorities to develop adequate technical, legal and institutional capacity on provisions of all pertinent internationals agreements.
3. Provide advice and assistance to the IBC and other relevant committees on the risk and safety aspects of their work.
4. Suggest practical alternatives, if any to high-risk laboratory works.
5. Provide various forms/formats for application, assessment and evaluation, Biosafety and Biosecurity Guidelines and other documents to relevant committees in different organizations.

6. Inform the various institutions engaged in biomedical and microbiological research and/or diagnostics or work with GMO about new developments in the field of biosafety and biosecurity to avoid unnecessary risk.
7. Coordinate biosafety and biosecurity aspects in public private sector partnership to ensure safety level in all biotechnological, biomedical and microbiological research work being carried out under such partnership.
8. Maintain confidentiality of information of commercial importance upon request from the researcher.
9. Inspect and certify all laboratories and facilities engaged in high-risk genetic manipulation work.
10. Consult with other national bodies and institutions dealing with the safety of human being and the environment and inform these bodies all conceivable developments in the field of modern biotechnology.
11. Cooperate with other national authorities dealing with import of live organisms, to formulate uniform guidelines for identification, inspection and regulation of transgenic species, exotic organisms and others.
12. Advise various research institutions and regulatory agencies in setting up appropriate experimental conditions for genetic manipulation work avoiding potential hazard.

Biosafety Core Committee (BCC) and Composition:

As mentioned earlier that the BCC shall be working to assist, accelerate, follow-up the functions of NCB and implementation of its mandate, the BCC shall be formed under each department with anticipated risk of biohazards with the intention of biorisk assessment and management [6]. The Biosafety Core Committee (BCC) shall be composed of at least eight members. The structure of BCC shall be as follows:

1. Director General
2. Directors of Relevant Departments
3. Biosafety and Biosecurity Personal/Expert with in-depth knowledge on biorisk management
4. Four Bio-scientists to be nominated by the respective ministry
5. One Physician with training and in-depth knowledge on biosafety and biosecurity

Note: Bio-scientists will be chosen from the following areas accordingly: Biotechnology, Molecular Biology, rDNA, Immunology, Tissue Culture, Plant and Animal Breeding, Microbiology, Entomology, Biochemistry, Pathology, Food Science and Nutrition

Functions of BCC but not limited to:

The BCC shall perform the following functions:

1. Monitor the implementation of Biosafety guidelines, policies, acts and rules as complementary to the NCB
2. Examine application for any permit/license of import of biohazardous materials for contained use, field trial and field release and forward recommendations to the NCB for its consideration.
3. Provide technical comments or recommendations to NCB or the government on policy, legal and technical issues of Biosafety and Biosecurity as and when requested for.
4. BCC will meet, as and when necessary, preferably on quarterly basis.
5. BCC shall arrange annual inspection and evaluation of performance of all the laboratories under the department engaged in diagnostic and research with biological materials.
6. BCC may co-opt experts related to specific issues regarding Biosafety, Biosecurity, Biological Waste Management, or on Overall Biorisk Management as and when required.

Appendix 4.2: IBC Research Registration Application

(Submit full document electronically for review and a hardcopy of the signed first page for the IBC record)

SECTION 1- BACKGROUND INFO

***Institution*_____*Phone:* _____**

Lab/Unit:

_____

***Principal Investigator:*_____**

ProjectTitle: _____

Type of Application:	**YES**	**NO**	**Protocol Number/ID**
New Protocol	☐	☐	
Renewal of Existing Protocol	☐	☐	
Modification of Existing Protocol	☐	☐	

SECTION 2- ABSTRACT

Abstract: Please include an overview of the research and a brief description of the procedures to deal with biohazard. OR If this is a renewal or modification of existing, summarize any changes from the previous approval	

I certify that to the best of my knowledge, the information provided in this application is complete and correct. I am familiar with, and agree to abide by the provisions and guidelines established by the IBC and NIH/CDC that pertain to the research project described in this application.

Signature: _____ Date: _____

Principal Investigator/ Safety Coordinator

SECTION 3- IBC REMARKS

Comments:

Approval: _____ Date: _____

Signature of IBC Chair

Approved Protocol Number _____ Valid until: _____

SECTION 3 - APPLICATION FOR USE OF MICROBIAL AGENTS, INFECTIOUS AGENTS OR TOXINS

1. DESCRIPTION OF AGENT.

Questions may be directed to the Biosafety Office (BSO). Supervisors are advised to consult the current version of the institute's Biosafety & Biosecurity Programme Manual and NIH Guidelines to determine if their experiments require IBC/BSO approval or if they are exempt.

A. Name the agent to be used in the project.				
B. What risk group (RG) and policies and procedures of Biosafety level (BSL) with additional precautionary measures has this agent been assigned?				
RG	☐ RG1	☐ RG2	☐ RG3	☐ RG4
BSL	☐ BSL1	☐ BSL2	☐ BSL3	☐ BSL4
C. Is this agent classified as a Select Agent by the CDC?			☐ YES	☐ NO
Select agents and toxins are regulated, please see the current list of agents and toxins.https://www.selectagents.gov/SelectAgentsandToxinsList.html				
What select agent or toxin?				

2. SOURCES AND TRANSPORTATION

How is the material delivered to you?	☐ ***Commercial carrier***	☐ ***Carried across campus***	☐ ***From field sites***	☐ ***Others***
How do you transport the material around campus, including between laboratories	☐ ***On Foot***	☐ ***In primary container***		
	☐ ***Safety Carrier***	☐ ***In secondary container place the primary container***		
	☐ ***Trolley***	☐ ***Use label on secondary container***		
	☐ ***As recommended by Biosafety***	☐ ***As recommended by Biosafety***		
What is the anticipated date material will arrive or work will begin?				

Will you be shipping or transporting this agent to or from the lab? ☐ ***YES*** ☐ ***NO***
If yes where?

3. ***APPLICATION OF AGENT***

A. Will the agent be genetically modified (mutagenesis, insertion of genes etc.) in this protocol ☐ YES ☐ NO
If yes, briefly explain the nature of the intended modifications.

Will these genetic modifications increase the virulence or expand the host range of the agent ☐ YES ☐ NO
If this agent is a BSL3 agent, will any biological material be removed from the BSL3 laboratory? ☐ YES ☐ NO
If yes, briefly describe what method will be used to decontaminate samples and test for viability. Where will the samples be stored until decontamination has been confirmed?

B. Will you be administering this agent (in modified or unmodified form) to microbes/vertebrates? ☐ YES ☐ NO
If yes, what species?

What is the status or protocol no. of the relevant committee review*?*

How will the agent be administered?

Will aerosols be generated during the experiment? ☐ YES ☐ NO

If yes, what aerosol confinement is planned to be in place?

Where will the animals be housed?
How will infected animals and animal waste be disposed? (Briefly describe the specific protocol.)

C. ***Will you be using vertebrate tissue infected with this agent****?*	☐ YES	☐ NO
If yes, what is the tissue to be used?		
Where will this tissue be acquired?		
Will this tissue contain any other known infectious agents?	☐ YES	☐ NO
If yes, what is the agent?		

SECTION 4 - APPLICATION FOR USE OF RECOMBINANT DNA

NOTE: If this protocol will produce material that has the potential to be infectious, then Section 3 of this application must also be completed.

1. DESCRIPTION OF DNA INSERTS

Gene name(s) and acronym(s) (If appropriate)		
Biological source/origin (mouse, virus, bacteria, etc)		
Pertinent biological activities of the encoded protein(s) (normal biological function, oncogenic potential, toxicity, etc.).		
Is the expressed protein a toxin known to affect humans and/or animals?	☐ YES	☐ NO
If yes, is the toxin on the CDC Select Agent List?		

2. DESCRIPTION OF VECTOR.

Will recombinant DNA be inserted into a virus, replicon, bacterial plasmid, BAC or other vector?	☐ YES	☐ NO	
If yes, identify the vector.			
What containment level will be used for experiments involving this vector?			
☐ BSL1	☐ BSL2	☐ BSL3	☐ BSL4
If the vector is a virus, is the vector replication-competent?	☐ YES	☐ NO	
If no, will a packaging or helper system be used?	☐ YES	☐ NO	
If yes, describe the packaging/helper system to be used.			

3. DESCRIPTION OF HOST

Will recombinant DNA molecules be inserted into a bacterial or eukaryotic host cell? (e.g. *E. coli*, yeast, eukaryotic cell line)?	☐ YES	☐ NO
If yes, identify the host organism or cell type/line.		
What containment level will be used for experiments involving this host?		
☐ BSL1	☐ BSL2	☐ BSL3	☐ BSL4
Will cultures be grown in amounts of 10 liters or more?	☐ YES	☐ NO

4. SPECIAL SAFETY CONSIDERATIONS

Are there any special safety considerations associated with the use of any of the recombinant DNA molecules, gene products, vectors, or hosts used in this research project?	☐ YES	☐ NO
If yes, specify.		
Will you be shipping or transporting these recombinant DNA molecules to or from the lab?	☐ YES	☐ NO
If yes, please describe the procedure.		

SECTION 5- APPLICATION for use of Vertebrate Tissue including Human or other Primate Cell Lines

NOTE: If the tissue contains a known infectious agent, then Section 3 of this application must also be completed.

1. DESCRIPTION OF VERTEBRATE TISSUE

Name the tissue or cell line to be used in the project and the species from which it is derived.		
Will this tissue contain a known infectious agent?	☐ YES	☐ NO
If yes, what is the agent?		
How will this tissue be acquired?		
Is institutional review board approval required for this protocol?	☐ YES	☐ NO
If yes, what is the protocol no. or status of that application?		
Is …….(IAEC) approval required for this protocol?	☐ YES	☐ NO
If yes, what is the protocol no. or status of that application?		
How will the tissue be disposed?		
Will this tissue be transported to or from the lab?	☐ YES	☐ NO
If yes, please describe the procedure.		

What safety procedures should the personnel take to protect them from this material?
Will universal precautions be taken and have personnel received Blood borne Pathogen Training?

SECTION 6 - PERFORMANCE SITE

Identify the location of the research laboratories, equipment, common use facilities, and any other area in which the infectious agents, recombinant DNA or vertebrate tissue will be used, for this research project.			
Labs	Equipment	Common facilities	Others
1.			
2.			
3.			
4.			
5.			
6.			
Where will the infectious agents, recombinant DNA or vertebrate tissue be stored?			
Are these facilities properly designed and equipped for performing research under the BSL conditions required for this research project?			
How will infectious agents, recombinant DNA or vertebrate tissue be disposed?			

SECTION 7 - TRAINING REQUIREMENT

SL NO	TRAINING ISSUE	REQUIRED		COMPLETED	
		YES	NO	YES	NO
1	GENERAL LABORATORY SAFETY	☐	☐	☐	☐
2	CHEMICAL SAFETY	☐	☐	☐	☐
3	BIOSAFETY & BIOSECURITY	☐	☐	☐	☐
4	RESPIRATORY PROTECCTION	☐	☐	☐	☐
5	BIOLOGICAL WASTE MANAGEMENT	☐	☐	☐	☐
6	BIOSAFETY & BIOSECURITY FOR OUTBREAK INVESTIGATION	☐	☐	☐	☐
7	BIOSAFETY & BIOSECURITY FOR BSL 3 LAB	☐	☐	☐	☐
8	* ADDITIONAL TRAINING AS NEEDED	☐	☐	☐	☐

All laboratory personnel must complete the institutional Biosafety & Biosecurity training courses SL NO 1-5 before participating in laboratory research. Those who will start working in the field for outbreak investigation must complete SL NO. 3-6. Users of BSL3 must complete SL. NO. 1 through 5, and 7. Individuals received full training this year must update the training participating in the refresher courses next year.

*Indicate if individuals have received additional training required specific to the project from the PI, at SL. NO. 8 and so on, adding new SL. NO.

SECTION 8 - PERSONNEL IMMUNIZATION STATUS

Prior immunization required to work with this agent	☐ YES	☐ NO
Laboratory personnel Immunization status updated and monitored	☐ YES	☐ NO

SECTION 9 - LABORATORY PERSONNEL

SL NO	Name	Position	Training Completed		Project Participant	
			YES	NO	YES	NO
			☐	☐	☐	☐
			☐	☐	☐	☐
			☐	☐	☐	☐

			☐	☐	☐	☐
			☐	☐	☐	☐
			☐	☐	☐	☐
			☐	☐	☐	☐
			☐	☐	☐	☐
			☐	☐	☐	☐

SECTION 10 - IBC APPROVAL

Approved by IBC	YES ☐	NO ☐
IBC recommendation:		

IBC approval document was sent to the PI	YES ☐	NO ☐

SECTION 11 - GENERAL INSTRUCTIONS

Biosafety Office should organize training on how to fill out the form and be willing to substantially help to fill this form. Please follow the instruction given below for each section-

SECTION 1- BACKGROUND INFO

Have to be filled out by all.

SECTION 2- ABSTRACT

Have to be filled out by all. This seems to be a cut and pest from the original document.

SECTION 3 - APPLICATION FOR USE OF MICROBIAL AGENTS, INFECTIOUS AGENTS OR TOXINS

Have to be filled out for using infectious agents or toxins in research.

SECTION 4 - APPLICATION FOR USE OF RECOMBINANT DNA

Have to be filled out by only those projects involving rDNA research.

SECTION 5 - APPLICATION FOR USE OF VERTEBRATE TISSUE INCLUDING HUMAN OR OTHER PRIMATE CELL LINES

Have to be filled out by only those projects require vertebrate tissue including human or other cell lines for research.

SECTION 6 - PERFORMANCE SITE

Have to be filled by all. Multiple labs may have to be used and many types of equipment may be required for the project.

SECTION 7 - TRAINING REQUIREMENT

This training requirement is actually be set by the supervisors. Biosafety Office will be organizing all the training on a routine basis to keep all the units/labs/RGs up to date regarding the training needs; however, this section is for mentioning basic and additional training requirement by the PIs and at the same time to indicate that all the staffs have all the required training completed. If additional training is needed in the field of Biosafety, the office is bound to develop, plan, and organize the required training.

SECTION 8 - PERSONNEL IMMUNIZATION STATUS

In general, it is considered that those who are working with BSL3 agents or in the areas with bloodborne pathogens hazard are the most suitable candidates to consider for vaccination to be required individuals with available vaccines. Other than that vaccination is required on the basis of risk assessment or any kind of requirement considering the health and safety of the workers.

SECTION 9 - LABORATORY PERSONNEL

Have to be filled out by all. Each project has its assigned staffs. This section is to enlist the name and position of the staffs will be working in that particular project.

SECTION 10 - IBC APPROVAL

Biosafety Office will complete all the preparation for IBC approval within the 5 working days after submission of the completed application. IBC approval will take place on the following IBC meeting.

DEFINITIONS

Select Agent: Under United States law "Biological Select Agents or Toxins" (BSATs) — or simply **Select Agents** for short — are biological agents which since 1997 have been declared by the U.S. Department of Health and Human Services (HHS) or by the U.S. Department of Agriculture (USDA) to have the "potential to pose a severe threat to public health and safety". These bio-agents are divided into three broad categories:

(1) **HHS select agents and toxins** (affecting humans);

(2) **USDA select agents and toxins** (affecting agriculture); and

(3) **Overlap select agents and toxins** (affecting both).

Appendix 4.3: GUIDANCE FOR WORK INVOLVING GM VIRUSES FOR GENE TRANSFER INTO ANIMAL AND HUMAN CELLS IN A LABORATORY SETTING

1. General Considerations

Genetic modification of viruses is a common practice in medical research laboratories to study the biology of the viruses. Viruses are also used as efficient vehicles for gene transfer into animal and human cells. The hazards associated with the use of genetically-modified viruses depend on the following [24]:

(a) The virus host range;

(b) Its ability for repeated rounds of infection;

(c) Its competence for replication inside the cell;

(d) The possibility of generating replication-competent virus from replication-defective vectors by recombination (e.g. in the case of retroviruses);

(e) The ability of the genetic material of the virus to be integrated into the chromosome of the infected cell;

(f) The stability of the virus inside the cell and exposed in the environment;

(g) The means of transmission of the virus (e.g. through aerosol or skin abrasions and other physical contact);

(h) The nature of the introduced DNA sequences or its encoded protein.

2. Approvals and Consultation

Principal Investigators should seek the clearance from IBC prior to the start of any experimentation with genetically modified viruses. The Principal Investigator prior to the start of the project shall obtain exhaustive references for the Biosafety Level and Guidelines with regards to the use of specific animal and human-infectious viruses. The following organizations provide good references for specific animal and human viruses:

(a) American Biological Safety Association (ABSA)

www.absa.org

(b) Center for Disease Control (CDC)

www.cdc.gov

(c) American Tissue Culture Collection (ATCC)

www.atcc.org

3. Procedures for Handling Rodent and Other Non-human Viruses

Viruses that are capable of infecting ONLY animal (non-human and non-primate) species are not considered to be associated with any hazard to the manipulator or other laboratory personnel. Practices associated with good tissue culture technique will be adequate in containing and handling these viruses. While the risks associated with the use of these viruses are considered negligible, solutions and contaminated cells shall be decontaminated or autoclaved before disposal to prevent accidental infection of other animal cell lines.

4. Procedures for Handling Viruses That Can Potentially Infect Human Cells ('human infectious' viruses)

The primary hazard associated with the use of live recombinant viruses that have the capacity or that could potentially infect human cells lies on the type of the viruses and the nature of the introduced genetic sequences. Primate-infectious viruses shall be considered "potentially human-infectious". For all human-infectious viruses handled in a class II biological safety cabinet, the primary hazard to the scientist is the possibility of infection by viruses through broken skin brought about by needles and other commonly used sharps such as pipettes. The potential danger to other laboratory personnel depends on the stability and infectivity of the virus in the extracellular environment and the nature of the introduced genetic material. For all human-infectious or potentially human-infectious viruses, the major requirement is for good

virological and tissue culture practice on the part of the scientist with regard to the following precautions:

(a) A facility of containment of level BSL2 or higher (as determined by the IBC and with reference to the Biosafety level detailed by the ATCC and CDC) will be required. All manipulations shall be conducted in a class II biological safety cabinet or equivalent. Only one individual shall use the cabinet at any one time.

(b) Laboratory gowns, gloves and facemask shall be worn, as appropriate PPE during manipulations with recombinant human-infectious viruses.

(c) Dishes and plates of cells containing human-infectious viruses shall be handled in larger plates (or inverted lids) to provide traps for accidental spills.

(d) All pipettes, glassware and plastic ware shall be decontaminated with an efficacious chemical disinfectant or autoclaved before disposal, taking into considerations sharps disposal requirements.

(e) For viruses which are able to persist in the environment (e.g. adenovirus, vaccinia virus, hepatitis virus, papillomavirus), decontamination and bagging of waste should be done within the biosafety cabinet prior to removal and autoclaving. Care should be taken to ensure that the amount of material held in the biosafety cabinet is minimized, in order to avoid interference with the air flow in the cabinet.

(f) Mouth pipetting is strictly prohibited.

(g) Open flames that could interrupt the air-flow in the biosafety cabinet should be avoided.

(h) The use of sharp instruments (sharps) such as syringe needles, glass pipettes, razors, scissors and surgical knives, wherever possible, should be avoided, since skin abrasions represent the most likely portal of entry to the body. Where the use of sharps is unavoidable, these instruments shall be placed in separate biological disposal receptacles and sterilized before disposal.

(i) Tissue cultures infected with human-infectious or potentially human-infectious viruses shall be kept in especially dedicated incubators.

(j) Likewise, frozen stocks of human-infectious or potentially human-infectious viruses should be kept in specially designated and clearly marked liquid nitrogen tanks and freezers. Laboratory personnel who leave the laboratory for new employment shall ensure that these materials are either discarded or entrusted to another worker. A central register shall be maintained which includes a record of stored cell lines and human-infectious viruses. Principal Investigators are responsible for providing information for the register and maintaining a record of the viruses and infected cell lines used in their laboratories. The maintenance of a central register for the institution/ company/organization is the responsibility of the IBC.

(k) Great care shall be taken to decontaminate spills immediately. The correct disinfectant to use in any given situation depends on the organism being handled and is the responsibility of the Principal Investigator in charge of the work to select an appropriate disinfectant. After each session, work surfaces shall be wiped down with an appropriate disinfectant. Where a biosafety cabinet/laminar flow hood has been used for handling amphotropic retroviruses, subsequent use with non-amphotropic viruses can be undertaken following decontamination with an appropriate disinfectant and ultraviolet decontamination.

(l) Only trained individuals shall be permitted to handle human-infectious recombinant viruses. It is the responsibility of the Principal Investigator to ensure the proper training of personnel in consultation with the IBC.

(m) Under no circumstances should investigators be infecting cultures of their own cells, or of their immediate relatives, or those of other members of the laboratory.

(n) Before beginning work with human-infectious (genetically manipulated) viruses where vaccination with the corresponding virus is regarded as an effective means of preventing subsequent infection (e.g. vaccinia, hepatitis), investigators shall be vaccinated.

5. Infection of Animals with Recombinant Viruses

(a) Infectious animal viruses unable to infect human cells Viruses of this group are not considered hazardous and accordingly good animal handling practices are appropriate. Infected animals shall be kept in separate

cages and be held in the biohazard room separate from non-infected animals. Infected animals should be clearly marked. If possible, the use of micro-isolators should be encouraged to prevent cross-infection. Precautions should be taken to avoid animals escaping and coming into contact with other animals. All waste generated from animals of this group shall be autoclaved before disposal.

(b) Viruses with the capacity to infect human cells Animals infected with human-infectious viruses shall be kept in a separate cage which is clearly labeled as containing the particular virus in question. They should be kept in a separate biohazard room from non-infected animals. The main risk is to the handler who shall take great care to avoid being scratched, bitten or exposed to aerosols. Gloves, face mask and protective clothing must be worn to avoid direct contact with tissue and body fluids. Work place should be covered with protective paper, which shall be changed regularly. Only trained staff shall handle these infected animals under the supervision of the Principal Investigator. Precautions should be taken to avoid animals escaping and coming into contact with other animals. All waste should be autoclaved prior to disposal.

6. Gene Therapy

In projects where viruses are being used to deliver genes to human subjects (gene therapy), the approval from the relevant institutional ethics committees and other relevant committee should be obtained, unless the therapy has already been established by the MOH as an approved treatment. Separate guidelines govern the control of virus production and safety testing procedures in gene therapy experiments.

Appendix 4.4: PROCEDURES FOR WORK WITH HAZARDOUS FRAGMENTS OF DNA

When working with isolated DNA molecules or amplifying DNA molecules using techniques such as the polymerase chain reaction (PCR), there are some cases where caution is warranted. Some degree of risk may exist and the extent of this is uncertain.
Such cases include:
1. DNA which encodes an active oncogene product or tumor suppressor gene product, particularly when this is associated with a gene promoter with high activity in human cells. DNA containing more than one active oncogene is associated with increased risk.
2. DNA encoding growth factors, their receptors or other substances that might directly or indirectly alter the growth patterns of human cells.
3. DNA or RNA representing complete viral genomes or fragments with the potential to regenerate live virus. Complete genomes for HIV or papilloma viruses, for example, warrant careful handling.
There is some risk that such molecules could enter the cells of the operator, the principal routes of entry being through breaks in the skin. It is therefore recommended that work of this type be carried out using gloves in order to avoid skin contact. Special care shall be taken when using needles or other sharp instruments. Precautions for handling genetically manipulated viruses with the capacity to infect human cells are described in Appendix 4.3.

Appendix 4.5: BIOSAFETY LEVEL 1 - PLANTS (BSL1-P)

Greenhouse Access (BSL1-P)

- Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress [24]
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BSL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism [24].

Records (BSL1-P)

- A record shall be kept of experiments currently in progress in the greenhouse facility [24].

Decontamination and Inactivation (BSL1-P)

- Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility [24]

Control of Undesired Species and Motile Macro-organisms (BSL1-P)

- A program shall 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 applicable state and Federal laws [24]
- Arthropods and other motile macro-organisms shall be housed in appropriate cages. If macro-organisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Concurrent Experiments Conducted in the Greenhouse (BSL1-P)

- Experiments involving other organisms that require containment level lower than BSL1-P may be conducted in the greenhouse concurrently with experiments that require BSL1-P containment, provided that all work is conducted in accordance with BSL1-P greenhouse practices [24]

Definitions (BSL1-P)

- The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
- The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area [24].

Greenhouse Design (BSL1-P)

- The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.
- Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended [24].

Appendix 4.6: BIOSAFETY LEVEL 2 - PLANTS (BSL2-P)

Greenhouse Access (BSL2-P)

- Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.
- Personnel shall be required to read and follow instructions on BSL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms [24]

Records (BSL2-P)

- A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
- A record shall be kept of experiments currently in progress in the greenhouse facility.
- The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NCB and other appropriate authorities immediately (if applicable). Reports to NCB shall be sent by e-mail. Documentation of any such accident shall be prepared and maintained [24]

Decontamination and Inactivation (BSL2-P)

- Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.
- Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel [24]

Control of Undesired Species and Motile Macro-organisms (BSL2-P)

- A program shall 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 applicable state and Federal laws.
- Arthropods and other motile macro-organisms shall be housed in appropriate cages. If macro-organisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility [35]

Concurrent Experiments Conducted in the Greenhouse (BSL2-P)

- Experiments involving other organisms that require containment level lower than BSL2-P may be conducted in the greenhouse concurrently with experiments that require BSL2-P containment provided that all work is conducted in accordance with BSL2-P greenhouse practices [24].

Signs (BSL2-P)

- A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following:
    - the name of the responsible individual,
    - the plants in use, and
    - any special requirements for using the area.
- If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
- If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol [24].

Transfer of Materials (BSL2-P)

- Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non- breakable container [24].

Greenhouse Practices Manual (BSL2-P)

- A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms [24].

Definitions (BSL2-P)

- The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
- The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area [24].

Greenhouse Design (BSL2-P)

- A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.
- Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds). [24]

Autoclaves (BSL2-P)

- An autoclave shall be available for the treatment of contaminated greenhouse materials [11].

Supply and Exhaust Air Ventilation Systems (BSL2-P)

- If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Other (BSL2-P)

BSL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macro-organisms in a manner that satisfies the intent of the foregoing clauses.

Appendix 4.7: BIOSAFETY LEVEL 3 - PLANTS (BSL3-P)

Greenhouse Access (BSL3-P)

- Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BSL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Records (BSL3-P)

- A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
- A record shall be kept of experiments currently in progress in the greenhouse facility.
- The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NCB, and other appropriate authorities immediately (if applicable). Reports to NCB shall be sent preferably by e-mail. Documentation of any such accident shall be prepared and maintained.

Decontamination and Inactivation (BSL3-P)

- All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Control of Undesired Species and Motile Macro-organisms (BSL3-P)

- A program shall 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 applicable state and Federal laws.
- Arthropods and other motile macro-organisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Concurrent Experiments Conducted in the Greenhouse (BSL3-P)

- Experiments involving organisms that require containment level lower than BSL3-P may be conducted in the greenhouse concurrently with experiments that require BSL3-P containment provided that all work is conducted in accordance with BSL3-P greenhouse practices.

Signs (BSL3-P)

- A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following:
    - the name of the responsible individual,
    - the plants in use, and
    - any special requirements for using the area.
- If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.
- If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Transfer of Materials (BSL3-P)

- Experimental materials that are brought into or removed from the greenhouse 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. 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.

Greenhouse Practices Manual (BSL3-P)

- A greenhouse practices manual shall be prepared or adopted. This manual shall:
  - advise personnel of the potential consequences if such practices are not followed, and
  - outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Protective Clothing (BSL3-P)

- Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.
- Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Other (BSL3-P)

- Personnel are required to thoroughly wash their hands upon exiting the greenhouse.
- All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Facilities (BSL3-P)

Definitions (BSL3-P)

- The term "greenhouse" refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
- The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Greenhouse Design (BSL3-P)

- The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.
- Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double- pane tempered glass or equivalent).
- The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.
- The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.
- Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

- Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Autoclaves (BSL3-P)

- An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Supply and Exhaust Air Ventilation Systems (BSL3-P)

- An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.
- The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Other (BSL3-P)

- BSL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps [24].

Appendix 4.8: BIOSAFETY LEVEL 4 - PLANTS (BSL4-P)

Greenhouse Access (BSL4-P)

- Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.
- Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.
- Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.
- Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.
- Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BSL4-P practices and procedures [24]

Records (BSL4-P)

- A record shall be kept of all experimental materials brought into or removed from the greenhouse.
- A record shall be kept of experiments currently in progress in the greenhouse facility.
- A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.
- The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NCB, and other appropriate authorities immediately (if applicable). Documentation of any such accident shall be prepared and maintained.

Decontamination and Inactivation (BSL4-P)

- All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.
- Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.
- Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal [24]

Control of Undesired Species and Motile Macro-organisms (BSL4-P)

- A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.
- Arthropods and other motile macro-organisms used in conjunction with experiments requiring BSL4-P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms [24].

Concurrent Experiments Conducted in the Greenhouse (BSL4-P)

- Experiments involving organisms that require containment level lower than BSL4-P may be conducted in the greenhouse concurrently with experiments that require BSL4-P containment provided that all work is conducted in accordance with BSL4-P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BSL4-P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested [24]

Signs (BSL4-P)

- A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area [24]
- If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors [24]
- If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Transfer of Materials (BSL4-P)

- Experimental materials that are brought into or removed from the greenhouse 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 greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.
- Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility [24]

Greenhouse Practices Manual (BSL4-P)

- A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms [24].

Protective Clothing (BSL4-P)

- Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.
- Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.
- All laboratory clothing shall be autoclaved before laundering.

Greenhouse Design (BSL4-P)

- The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse facility.
- Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.
- Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).
- Access doors to the greenhouse shall be self-closing and locking.

- The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.
- The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.
- Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room [24]

Autoclaves (BSL4-P)

- A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

Supply and Exhaust Air Ventilation Systems (BSL4-P)

- An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.
- Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow *in situ* decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually [24]

Other (BSL4-P)

- Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.
- A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.
- Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.
- If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum

service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent backflow. HEPA filters shall be certified annually [24].

- **Appendix 4.9: BIOLOGICAL CONTAINMENT PRACTICES**

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems [24]

Biological Containment Practices (Plants)

- Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures:
    - Cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity;
    - Remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage;
    - Ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or
    - Ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Biological Containment Practices (Microorganisms)

- Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures:
    - Confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces;
    - Ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated;
    - Conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection;
    - Use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors;
    - use microorganisms that have an obligate association with the plant; or
    - Use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Biological Containment Practices (Macro-organisms)

- Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures:
    - Use non-flying, flight-impaired, or sterile arthropods;
    - Use non-motile or sterile strains of small animals;
    - Conduct experiments at a time of year that precludes the survival of escaping organisms;
    - Use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or

- Prevent the escape of organisms present in run-off water by chemical treatment or evaporation of runoff water.

Appendix 4.10: BIOSAFETY LEVEL 1 - ANIMALS (ABSL1)

Animal Facility Access (ABSL1)

- The containment area shall be locked.
- Access to the containment area shall be limited or restricted when experimental animals are being held.
- The containment area shall be patrolled or monitored at frequent intervals.

Other (ABSL1)

- 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 among non-transgenic animals.
- 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.
- The containment area shall be in accordance with state and Federal laws and animal care requirements [7,8].

Animal Facilities (ABSL1)

Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

Appendix 4.11: BIOSAFETY LEVEL 2 - ANIMALS (ABSL2)

Animal Facility Access (ABSL2)

- The containment area shall be locked.
- The containment area shall be patrolled or monitored at frequent intervals.
- The containment building shall be controlled and have a locking access.
- The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., accination) may enter the laboratory or animal rooms.
- Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Decontamination and Inactivation (ABSL2)

- Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.
- Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Signs (ABSL2)

- When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Protective Clothing (ABSL2)

- Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.
- Special care shall be taken to avoid skin contamination with microorganisms containing recombinant or synthetic nucleic acid molecules. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Records (ABSL2)

- Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NCB, and other appropriate authorities (if applicable). Reports to the NCB shall be sent by e-mail. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.
- When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

Transfer of Materials (ABSL2)

- Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Other (ABSL2)

- 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 among non-transgenic animals. Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
- Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent 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 transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.
- Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.
- Individuals who handle materials and animals containing recombinant or synthetic nucleic acid molecules shall be required to wash their hands before exiting the containment area.
- 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.
- The containment area shall be in accordance with state and Federal laws and animal care requirements.
- A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Animal Facilities (ABSL2)

- Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

- Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- The animal containment area shall be designed so that it can be easily cleaned.
- Windows that open shall be fitted with fly screens.
- An autoclave shall be available for decontamination of laboratory wastes.
- If arthropods are used in the experiment or the agent 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.

APPENDIX 4.12: BIOSAFETY LEVEL 3 - ANIMALS (ABSL3)

Animal Facility Access (ABSL3)

- The containment area shall be locked.
- The containment area shall be patrolled or monitored at frequent intervals.
- The containment building shall be controlled and have a locking access.
- The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.
- Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

Decontamination and Inactivation (ABSL3)

- The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant or synthetic nucleic acid molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.
- All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.
- Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
- Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a ABSL3 animal facility to a facility with a lower containment classification.
- Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism. More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC.

Signs (ABSL3)

- When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate:
  - the agent,
  - the animal species,
  - the name and telephone number of the Animal Facility Director or other responsible individual, and
  - any special requirements for entering the laboratory.

Protective Clothing (ABSL3)

- Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the ABSL3 area and wearing of personal clothing.
- Special care shall be taken to avoid skin contamination with microorganisms containing recombinant or synthetic nucleic acid molecules. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.
- Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Records (ABSL3)

- Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.
- Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules shall be

reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NCB, and other appropriate authorities (if applicable). Reports to the NCB shall be sent by e-mail. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

- When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

Transfer of Materials (ABSL3)

- Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.
- Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a ABSL3 animal facility to a facility with a lower containment classification.

Other (ABSL3)

- 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 among non-transgenic animals.
- Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the 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 transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.
- Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.
- Individuals who handle materials and animals containing recombinant or synthetic nucleic acid molecules shall be required to wash their hands before exiting the containment area.
- Experiments involving other organisms that require containment levels lower than ABSL3 may be conducted in the same area concurrently with experiments requiring ABSL3 containment provided that they are conducted in accordance with ABSL3 practices.
- Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.
- All procedures shall be performed carefully to minimize the creation of aerosols.
- 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.
- The containment area shall be in accordance with state and Federal laws and animal care requirements.
- All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.
- Personnel shall be required to shower before exiting the ABSL3 area and wearing personal clothing.
- Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.
- Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use

and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

- A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Animal Facilities (ABSL3)

- Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.
- The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.
- Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.
- An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.
- If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area 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, or the equivalent of access doors.
- Access doors to the containment area shall be self-closing.
- The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.
- Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism. More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC.
- An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.
- If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.
- Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.
- In lieu of open housing in the special animal room, animals held in a ABSL3 area may be housed
- in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.
- Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing.
- The sink shall be located near the exit door.
- Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

APPENDIX 4.13: BIOSAFETY LEVEL 4 - ANIMALS (ABSL4)

Animal Facility Access (ABSL4)

- Individuals under 16 years of age shall not be permitted to enter the animal area.
- The containment area shall be locked.
- The containment area shall be patrolled or monitored at frequent intervals.
- The containment building shall be controlled and have a locking access.
- The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.
- Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.
- Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.
- Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

Decontamination and Inactivation (ABSL4)

- All contaminated liquid or solid wastes shall be decontaminated before disposal.
- The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant or synthetic nucleic acid molecules is finished. Where feasible, plastic backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.
- All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.
- No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated.
- Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.
- When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.
- Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
- Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.
- An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.
- Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. If required by design, regulation, local ordinance or policy, liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism.
- More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC. If required by design, regulation, local ordinance or policy, liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Signs (ABSL4)

- When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate:
  - the agent,
  - the animal species,
  - the name and telephone number of the Animal Facility Director, or other responsible individual, and
  - any special requirements for entering the laboratory.

Protective Clothing (ABSL4)

- Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the ABSL4 area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.
- A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a lifesupport system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate.
- When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system.
- Entry to this area shall be through an airlock fitted with airtight doors.
- Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Records (ABSL4)

- Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.
- A system shall be established for: (i) reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant or synthetic nucleic acid molecules, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant or synthetic nucleic acid molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NCB and other appropriate authorities (if applicable). Reports to the NCB shall be sent by e-mail. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.
- When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.
- A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

Transfer of Materials (ABSL4)

- No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated.
- Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.
- Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a

disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another ABSL4 animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a ABSL4 animal facility to one with a lower containment classification.

- Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

Other (ABSL4)

- 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 among non-transgenic animals.
- Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.
- Individuals who handle materials and animals containing recombinant or synthetic nucleic acid molecules shall be required to wash their hands before exiting the containment area.
- Experiments involving other organisms that require containment levels lower than ABSL4 may be conducted in the same area concurrently with experiments requiring ABSL4 containment provided that they are conducted in accordance with ABSL4 practices.
- Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.
- All procedures shall be performed carefully to minimize the creation of aerosols.
- A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.
- The containment area shall be in accordance with state and Federal laws and animal care requirements.
- The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.
- Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant or synthetic nucleic acid molecules. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
- An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.
- A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.
- Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Animal Facilities (ABSL4)

- Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.
- The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.
- Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).
- An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.
- Access doors to the containment area shall be self-closing.
- All perimeter joints and openings shall be sealed to form an arthropod-proof structure.
- The ABSL4 laboratory provides a double barrier to prevent the release of recombinant or synthetic nucleic acid molecule containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.
- A necropsy room shall be provided within the ABSL4 containment area.
- Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. If required by design, regulation, local ordinance or policy, liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated at minimum on a yearly basis with an indicator organism.
- More frequent validation, based on the amount of use or other safety factors, shall be left to the discretion of the IBC. If required by design, regulation, local ordinance or policy, liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.
- A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.
- Exhaust air from ABSL4 containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere.
- Double HEPA filters shall be required for the supply air system in a ABSL4 containment area.
- All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctyl phthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least 1x10-3 micrograms per liter for 0.3 micrometer diameter dioctyl phthalate particles and a challenge concentration of 80-120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).
- If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally 1x108 organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then

assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are *Bacillus subtilis* var. *niger* or *Bacillus stearothermophilus*. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

- All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.
- Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.
- Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.
- The supply water distribution system shall be fitted with a back-flow preventer or break tank.
- All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.
- Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

APPENDIX 4.14: ARTHROPOD CONTAINMENT LEVEL 1 (ACL-1)

ACL1 is suitable for work with uninfected arthropod vectors or those infected with a non-pathogen including [31]:

- Arthropods that are already present in the local geographic region regardless of whether there is active vector borne disease transmission in the locale, and
- Exotic arthropods that upon escape would be inviable or become only temporarily established in areas not having active vector borne disease transmission. This category would include most educational use of arthropods.

Standard Practices

- Location of Arthropods: Furniture and incubators containing arthropods are located in such a way that accidental contact and release is minimized. This may be achieved by locating arthropods out of the flow of general traffic, avoiding hallways, or placing them in closets.
- Supply Storage: The area is maintained to allow detection of escaped arthropods. For example, materials unrelated to arthropod rearing and experimentation (e.g., plants, unused containers, clutter) that provides breeding sites and harborages are minimized.
- General Arthropod Elimination: Accidental sources of arthropods from within the insectary are eliminated. This may be accomplished by cleaning work surfaces after a spill of materials, including soil or water that might contain viable eggs. Pools of water are mopped up immediately.
- Primary Container Cleaning and Disinfection: Practices should be in place such that arthropods do not escape by inadvertent disposal in primary containers. Cages and other culture containers are appropriately cleaned to prevent arthropod survival and escape (e.g., heated to over the lethal temperature or killed by freezing).
- Primary Container Construction: 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. Non-breakable cages are recommended. Bags, rearing trays and so on effectively prevent leakage and escape.
- Disposal of Arthropods: Living arthropods are not to be disposed of. All wastes from the insectary (including arthropod carcasses, and rearing medium) are transported from the insectary in leak-proof, sealed containers for appropriate disposal in compliance with applicable institutional or local requirements. All stages of arthropods are killed before disposal. Autoclaving or incineration of material infected with a non-pathogen is recommended. Material may be killed with hot water or freezing before flushing down drains.
- Primary Container Identification and Labeling: Arthropods are identified adequately. Labels giving species, strain/origin, date of collection, responsible investigator, and so on are firmly attached to the container (and cover if removable). Vessels containing stages with limited mobility (e.g., eggs, pupae, hibernating adults) are securely stored.
- Prevention of Accidental Dispersal on Persons or via Sewer: Personnel take appropriate precautions to prevent transport or dissemination of arthropods from the insectary on their persons or via the sewer.
- Pest Exclusion Program: A program to prevent the entrance of wild arthropods (e.g., houseflies, cockroaches, spiders) and rodents effectively precludes predation, contamination, and possible inadvertent infection.
- Escaped Arthropod Monitoring: Investigators assess whether escapes are occurring. An effective arthropod trapping program is recommended to monitor the escape prevention program.
- Source and Harborage Reduction: Harborage and breeding areas are reduced as appropriate. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods.

- Microbiological and Medical Sharps: Syringes that re-sheath the needle, needle-less systems, and other safe devices are used when appropriate. Plastic-ware is substituted for glassware whenever possible.
- Notification and Signage: Persons entering the area are aware of the presence of arthropod vectors.

Special Practices

- IACUC and IBC Approval: IACUC approval is required for use of vertebrate animals used as hosts. IBC approval is required for non-exempt recombinant DNA protocols.
- Housing of Non-Arthropod Animals: Animals not necessary for culture of the arthropods are not accessible to the arthropods. Animals used as hosts or blood sources may be housed within the insectary but are adequately protected from access by escaped arthropods. Protocols for vertebrate animal use are approved by the local IACUC.
- Containment During Blood-Feeding: Arthropods fed on host animals are prevented from accidental transfer to host cages. When handling/removing animals after exposure to arthropods, precautions must 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.
- Blood Source: The blood source is considered as a source of inadvertent arthropod infection and transmission. Measures are implemented to prevent such an event. Use of sterile blood or blood from sources known to be pathogen-free is recommended. In contrast, use of blood from animals or humans whose disease status is uncertain is to be avoided.
- Escaped Arthropod Handling: Escaped arthropods are killed or collected and properly disposed of.
- Accidental Release Reporting: The insectary director is notified promptly of accidental release of vectors.

Safety Equipment (Primary Barriers)

- Gloves: Gloves are worn when handling host animals or blood used to feed the arthropods.
- Torso Apparel: White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood and vertebrate animals.
- Arthropod-Specific Personal Protective Equipment: Personal protective equipment is worn as appropriate e.g., respirators for arthropod-associated allergies, particle masks, head covers.

Facilities (Secondary Barriers)

- Location of Insectary: The insectary area is separated from areas that are used for general traffic within the building.
- Insectary Doors: Doors openings, whether covered by rigid panels, glass, screens, plastic sheets or cloth, minimize escape and entrance of arthropods.
- Insectary Windows: Windows, if present, effectively prevent escape of the smallest arthropods contained within.

APPENDIX 4.15: ARTHROPOD CONTAINMENT LEVEL 2 (ACL-2)

ACL-2 must be practiced if working with exotic and indigenous arthropods infected with BSL-2 agents associated with animal and/or human disease, or that are suspected of being infected with such agents. *Uninfected genetically modified arthropod vectors also fall under this level provided the modification has no, or only negative effects on viability, survivorship, host range, or vector capacity*. ACL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ACL-1. It is more stringent in the physical containment, disposal, and facilities design. Moreover, access is more restricted than ACL-1. The decision to cultivate infected exotic arthropods under ACL-2 conditions in active transmission areas or in cases in which establishment is a possibility requires that measures that otherwise would only be recommended or preferred must be met [31].

ACL-2 Standard Practices

Location of Arthropods: Furniture and incubators containing arthropods are located in such a way that accidental contact and release by laboratorians, custodians, and service persons is unlikely. This may be achieved by locating arthropods in dedicated rooms, closets, incubators located out of the traffic flow or similar measures.

Supply Storage: The area is designed and maintained to enhance detection of escaped arthropods. Equipment and supplies not required for operation of the insectary should not be located in the insectary. All supplies for insect maintenance that must be kept within the insectary are 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.

General Arthropod Elimination: As in ACL-1

Primary Container Cleaning and Disinfestation: In addition to cleaning cages and culture containers to prevent arthropod escape as in ACL-1, 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.

Primary Container Construction: Cages used to hold arthropods are non-breakable and screened with mesh of a size to prevent escape. Containers are preferably autoclavable or disposable. Openings designed to prevent escape during removal and introduction of arthropods are recommended.

Disposal of Arthropods: In addition to standard ACL-1 disposal practices, autoclaving or incineration of arthropod materials is recommended. Infected arthropods are autoclaved or incinerated.

Isolation of Uninfected Arthropods: Spread of agents to uninfected arthropods is prevented. Generally this is accomplished by isolating infected material in a separate room.

Primary Container Identification and labeling: As in ACL-1

Prevention of Accidental Dispersal on Persons or via Sewer: Before leaving the insectary and after handling cultures and infected arthropods, personnel wash their hands, taking care not to disperse viable life stages into the drainage system. No infected material is disposed of through the sewer. If uninfected materials are disposed of via the sewer, all material is destroyed by heat or freezing and preferably by autoclaving or incineration. Air curtains are recommended as appropriate.

Pest Exclusion Program: ACL-1

Escaped Arthropod Monitoring: Investigators assess whether escapes are occurring by instituting an effective arthropod trapping program 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.

Source and Harborage Reduction: Harborage and breeding areas are eliminated. Furniture and racks are minimized and can be easily moved to permit cleaning and location of escaped arthropods. Equipment in

which water is stored or might accumulate (e.g., humidifiers) is screened to prevent arthropod access, or contains chemicals to prevent arthropod survival.

Microbiological and Medical Sharps: ACL-1

Arthropod Sharps: In addition to minimizing arthropod sharps, these are restricted for use in the insectary if infected materials are used.

Routine Decontamination: Equipment and work surfaces in the insectary are routinely decontaminated with an effective chemical or by radiation (e.g., heat) after actual or potential contact with an infectious agent, and especially after overt spills and splashes of viable materials (including soil or water that might contain infectious agents or eggs).

Notification and Signage: Persons entering the area are aware of the presence of arthropod vectors. If infected material is present, a BSL-2 biohazard sign is posted on the entrance to the insectary listing all species handled within and is updated whenever new species are introduced or pathogenic infectious agents are present. The hazard warning sign identifies the arthropod species, agent(s) known or suspected to be present, lists the name and telephone number of the responsible person(s), and indicates any special requirements for entering the insectary (e.g., the need for immunizations or respirators).

Procedure Design: All procedures are carefully designed and performed to prevent arthropod escape

Safety Manual: A safety manual is prepared, approved by the IBC, and adopted. The manual contains emergency procedures, standard operating procedures, waste disposal and other information necessary to inform personnel of the methods for safe maintenance and operation of the insectary.

Training: Laboratory personnel are advised of special hazards and are required to follow instructions on practices and procedures contained in the safety manual. Adherence to established safety procedures and policies is made a condition of employment and is part of the annual performance review of every employee. Personnel receive annual updates and additional training as necessary for procedural or policy changes. Records of all training are maintained.

Medical Surveillance: An appropriate medical surveillance program is in place. All personnel receive appropriate immunizations or tests for the agents handled or likely to be present. When appropriate, a serum surveillance system is implemented (see BMBL for guidance). Personnel are aware of the symptoms of infection and the procedure to follow in reporting these. In general, persons who may be at increased risk of acquiring infection, or for whom infection may be unusually hazardous (e.g., immunocompromised), are not allowed in the insectary unless special personal protection procedures are in place to eliminate extra risk.

Access Restrictions: Routine access is limited to trained persons and accompanied guests. Service persons are made aware of the hazards present and the consequences of arthropod release and contact with agents that may be present.

Special Arthropod Handling Containers and Areas: Infected arthropods are prevented from release into the laboratory area. This may be accomplished by secure glove boxes, biosafety cabinets, custom handling trays etc. These may vary from BSL recommendations insofar as necessary to safely contain both the arthropod and any agent. Such modifications should be made only in consultation with experts in handling the specific types of infected arthropods and biosafety experts. A dedicated area for handling infected material is recommended. This is preferably a separate cubicle, walk-in-incubator, or screen room.

Safe Transport in the Laboratory: All infectious and potentially infectious samples are collected, labeled, transported, and processed in a manner that contains and prevents transmission of the agent(s). Transfer of arthropods between manipulation and holding areas is in non-breakable secure containers.

ACL-2 Special Practices

IACUC and IBC Approval: IBC approval is required and IACUC if vertebrates are used as hosts.

Housing of Non-Arthropod Animals: Other animals are not accessible to the arthropods. Animals used as hosts or blood sources generally are not housed with arthropods. If present, they are adequately protected

from access by escaped arthropods, and protocols are approved by the IBC and IAUCUC. Containment During Blood-Feeding. Recommendations for ACL-1 containment of arthropods during blood-feeding are more stringently assured by special practices and container design.

Blood Source: As in ACL-1

Escaped Arthropod Handling: Loose arthropods must be killed and disposed of, or recaptured and returned to the container from which they escaped. Infected arthropods must not be killed with bare hands, and must be transferred using filtered mechanical or vacuum aspirators.

Accidental Release Reporting: A release procedure is developed and posted. This includes contacts and immediate mitigating actions. Accidents that result in release of infected arthropods from primary containment vessels, or that result in overt exposure to infectious material must be reported immediately to the insectary director who is responsible for ensuring that appropriate and documented action is taken to mitigate the release. Location, number, and type of material are prominently posted until the source is eliminated. Follow-up medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.

Movement of Equipment: All equipment must be appropriately decontaminated and disinfested before transfer between rooms within the insectary, and before removal from the insectary.

ACL-2 Safety Equipment (Primary Barriers)

Eye and Face Protection: Appropriate face/eye and respiratory protection are worn by all personnel entering the insectary.

Gloves: Gloves are worn when handling potentially infected arthropods, blood, and associated equipment and when contact with potentially infectious material is unavoidable.

Torso Apparel: White laboratory coats, gowns, and/or uniforms are worn at all times in the insectary when handling blood, vertebrate animals, and infected materials.

Protective Equipment: In addition to ACL-1 measures, personal protection equipment is used for all activities involving manipulations of infected or potentially infected arthropods.

ACL-2 Facilities (Secondary Barriers)

Location of Insectary: The insectary is separated from areas that are open to unrestricted personnel traffic within the building. It is recommended that this be accomplished by at least two self-closing doors that prevent passage of the arthropods. Increased levels of physical isolation are recommended, e.g., separate buildings, wings, suites.

Insectary Doors: Recommended entrance to the insectary is via a double-door vestibule that prevents flying and crawling arthropod escape. 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. Additional barriers (e.g., screened partitions, hanging curtains) are highly recommended.

Insectary Windows: 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).

Vacuum Systems: If a central vacuum system is installed, each service outlet is fitted with suitable barriers/filters to prevent arthropod escape. Filters are installed to permit decontamination and servicing. Other vacuum devices are appropriately filtered to prevent transfer and exhausting of arthropods.

Interior Surfaces: The insectary is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior walls are light-colored so that a loose arthropod can be easily located, recaptured, or killed. Gloss finishes, ideally resistant to chemical disinfectants and fumigants, are recommended. Floors are light colored, smooth and uncovered. Ceilings are as low as possible to simplify detection and capture of flying insects.

Floor Drains: Floor drains are modified to prevent accidental release of arthropods and agents. If present, traps must be filled with an appropriate chemical treatment to prevent survival of all arthropod stages (e.g., mosquito larvae).

Plumbing and Electrical Fixtures: Internal facility appurtenances (e.g., light fixtures, pipes, ducting) are minimal since these provide hiding places for loose arthropods. Penetrations of walls, floors, and ceilings are minimal and sealed/caulked. Ideally, light fixtures are flush with the ceiling, sealed, and accessed from above.

HVAC: Ventilation is appropriate for arthropod maintenance, but does not compromise containment of the agent or arthropod. Examples include: exhaust air is discharged to the outside without being recirculated to other rooms; appropriate filter/barriers are installed to prevent escape of arthropods; the direction of airflow in the insectary is inward; a progressively negative pressure gradient is maintained as distance from the main entrance increases; fans located in the vestibule and internal corridor can be used to help prevent escape of flying arthropods; air curtains are located in vestibules and doorways.

Sterilization Equipment: An autoclave is available conveniently located to rooms containing arthropods within the insectary building.

Sink and Shower: The facility has a hand-washing sink with hot water and with suitable plumbing to prevent arthropod escape.

Illumination: Illumination is appropriate for arthropod maintenance but does not compromise arthropod containment, impede vision, or adversely influence the safety of procedures within the insectary. Lighted (or dark) openings that attract escaped arthropods are avoided.

Facility Compliance Monitoring: The facility is evaluated annually for compliance to the ACL-2 level. The principle investigator or insectary director inspects the facility annually to ensure that alterations and maintenance have not compromised the containment characteristics. Adequacy of the practices and facility in view of changes in research protocols, agents, or arthropods are considered.

APPENDIX 4.16: ARTHROPOD CONTAINMENT LEVEL 3 (ACL-3)

ACL-3) involves practices suitable for work with potential or known vectors that are, or may be infected with, RG3 agents or BSL-3 pathogens associated with human disease. Arthropods that are infected or potentially infected with RG3 agents may pose an additional hazard if the insectary is located in an area where the species is indigenous, or if alternative suitable vectors are present, as an escaped arthropod may introduce the pathogen into the local population. ACL-3 builds upon the practices, procedures, containment equipment, and facility requirements of ACL-2. It differs in that access is more restricted, and the microbiological containment takes a more prominent role in determining the practices and facilities [31].

ACL-3 Standard Practices

- Location of Arthropods: Furniture and incubators containing arthropods are located in such a way that accidental contact and release by laboratorians, custodians, and service persons does not occur. This is usually achieved by locating arthropods in dedicated rooms, wings or suites in incubators located out of the traffic flow in areas of the building dedicated to BSL-3 activities.
- Supply Storage: Equipment and supplies not absolutely required for ongoing ACL-3 work are removed from the insectary after appropriate decontamination. Those present are 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 open only during access.
- General Arthropod Elimination: In addition to measures for general arthropod elimination within the insectary, materials used to wipe or mop are autoclaved before disposal. Only persons trained and equipped to work with arthropods and BSL-3 agents clean up spills.
- Primary Container Cleaning and Disinfection: Care is taken to disinfect primary containers in a manner that does not create aerosols. All primary containers are autoclaved or incinerated.
- Primary Container Construction: Cages used to hold arthropods are non-breakable and screened with mesh of a size to prevent escape. Containers are autoclavable or disposable. Openings are designed to prevent escape during removal and introduction of arthropods. Disposable containers are recommended.
- Disposal of Arthropods: In addition to ACL-2 disposal practices, the outer surfaces of containers are decontaminated before moving the material. All arthropod waste materials are autoclaved or incinerated.
- Isolation of Uninfected Arthropods: Where possible, only arthropods requiring ACL-3 procedures are housed in the ACL-3 insectary. If it is necessary to house ACL-2 or lower arthropods in the ACL-3 insectary, all procedures and practices must meet the ACL-3 standards.
- Primary Container Identification and labeling. As in ACL-1
- Prevention of Accidental Dispersal on Persons or via Sewer: Before leaving the insectary and after handling cultures and arthropods, personnel wash their hands, taking care not to disperse viable life stages into the drainage system. No material is disposed of through the sewer. Non-infected material may be destroyed by heat or freezing if followed by autoclaving or incineration.
- Pest Exclusion Program: As in ACL-1
- Escaped Arthropod Monitoring: Additional measures are taken to measure the effectiveness of the arthropod trapping program and these are documented. As part of the IBC review and commissioning process of a new facility, the physical integrity and security practices might be tested by a simple release-recapture study. A known number of non-infected arthropods would be released and then these would be recaptured to assess the physical integrity of security barriers. Exterior and within-building monitoring is considered. Records of exterior captures are maintained.
- Source and Harborage Reduction: As in ACL-2

- Microbiological and Medical Sharps: Sharps are stringently limited and use is justified only when alternatives are not available.
- Arthropod Sharps: In addition to minimizing arthropod handling sharps, these are restricted for use in the insectary regardless of infection status of material handled.
- Routine Decontamination: As in ACL-2
- Notification and Signage: ACL-2 measures are implemented with BSL-3 signage.
- Procedure Design: All procedures are carefully performed to prevent arthropod escape and the creation of aerosols or splatters. Protocols are practiced with non-infected arthropods / animals and modified before implementation.
- Safety Manual: As in ACL-2
- Training: The training required for laboratory personnel under ACL-3 is more detailed and extensive, and BSL-3 certification is required if infected materials are handled.
- Medical Surveillance: In addition to the measures required for medical surveillance under ACL-2, assessment is made by the occupational health physician for persons who may be at unusual risk.
- Access Restrictions: The insectary director limits access to the insectary to the fewest number of persons possible. Personnel who must enter the insectary for program or service purposes when work is in progress are accompanied by trained laboratorians and are advised of the potential hazard to themselves, co-workers, and the potential consequences of arthropod release. Because of the increased risk to non-trained personnel, laboratory staff should perform general cleaning activities that would otherwise be performed by custodial staff.
- Special Arthropod Handling Containers and Areas: All work is done within a primary barrier. Appropriate biological safety cabinets, other physical containment devices, and/or personal protective equipment are used whenever conducting procedures to infect arthropods with BSL-3 agents, or when handling arthropods. Appropriate designs will consider the life history and behavior of the arthropod and may differ from that required by the agent alone. Such modifications should be made in consultation with biosafety experts. Manipulation of arthropods and, for example, rearing of transovarially infected immature stages, are performed in a designated area. It is suggested to make a separate room or double screened area that is separated from the main insectary by rooms having two screened or solid doors that open inward and closing automatically."
- Safe Transport in the Laboratory. As in ACL-2

ACL-3 Special practices

- IACUC and IBC Approval: As in ACL-2
- Housing of Non-arthropod Animals: As in ACL-2
- Containment During Blood-Feeding: Recommendations for ACL-1 containment of arthropods during blood-feeding are strictly assured by special practices and container designs that prevent escape of arthropods.
- Blood Source: As in ACL-1
- Escaped Arthropod Handling: Loose arthropods must be killed and disposed of, or recaptured and returned to the container from which they escaped. Infected arthropods are not killed with hands, and must be transferred using filtered mechanical or vacuum aspirators. Only personnel properly trained and equipped to work with designated arthropods and BSL-3 infectious agents are to recover and/or kill escaped arthropods.
- Accidental Release Reporting: As in ACL-2
- Movement of Equipment: ACL-2

- Inventory of Arthropods: In addition to appropriate primary containment cages, when possible, the number of arthropods must be included on the label, and records are maintained to account for all arthropods from the time of transfer to the ACL-3 insectary to the time of termination. Vessels containing low mobility stages (e.g., eggs, pupae, hibernating adults) should not be stored within the ACL-3 insectary unless they meet the ACL-3 criteria.

ACL-3 Safety Equipment (Primary Barriers)

- Eye and Face Protection: As in ACL-2
- Gloves: Personnel wear gloves when handling infected arthropods or host animals and associated equipment. Gloves are removed aseptically.
- Torso Apparel: White laboratory coats, gowns, and/or uniforms in the insectary are worn at all times by all personnel entering the insectary. Wrap-around or solid-front gowns are worn over this clothing. Front-button laboratory coats alone are unsuitable. The gowns are removed and left in the insectary. Before leaving the insectary, scrub suits and uniforms are removed and appropriately contained and decontaminated before laundering or disposal.
- Foot Apparel: Boot, shoe covers, or other protective footwear, and disinfectant foot baths (with appropriate anti-arthropod measures) are available and used where indicated.
- Personal Clothing: As in ACL-2
- Arthropod-Specific Personal Protective Equipment: As in ACL-2
- Pesticide: Pesticide for emergency use is available in areas in which escape of arthropods is likely.

ACL-3 Facilities (Secondary Barriers)

- Location of Insectary: The insectary is strictly separated from areas that are open to unauthorized, untrained personnel within the building by locked doors. These are opened, for example, by key lock, proximity reader, or card key.
- Insectary Doors: Access to the facility is limited to trained, approved personnel by a self-closing and self-locking door. The external insectary entry doors are controlled by a key lock, card key, or proximity reader. Entry into the insectary is via a double-door entry that includes a change room and shower(s). Showers are plumbed to prevent arthropod escape. An additional double-door access (air lock) or double-door autoclave may be provided for movement of supplies and wastes into and out of the facility respectively. The two contiguous doors must never be opened simultaneously. Internal doors may open outwards or be sliding, but are self-closing, and are kept closed when arthropods are present. Additional barriers (e.g., hanging curtains) are recommended.
- Insectary Windows: Windows are not recommended. Any windows present are resistant to breakage (e.g., double paned or wire-reinforced) and well sealed. If present, fixed light windows are recommended.
- Vacuum Systems: As in ACL-2
- Interior Surfaces: In addition to the recommendations for ACL-2, spaces around doors are sealed to facilitate decontamination or troughs surrounding door frames can be installed and filled with sticky or greasy material that will trap crawling arthropods.
- Floor Drains: Floor drains are not recommended. If present, traps must be filled with an appropriate treatment to prevent survival of any arthropod stage (e.g., mosquito larvae). Ideally, all drains are plumbed to a holding tank to facilitate heat or chemical treatment to kill all stages of arthropod prior to disposal into the waste system.
- Plumbing and Electrical Fixtures: As in ACL-2
- HVAC: Ventilation is appropriate for arthropod maintenance, but does not compromise containment. Exhaust air is discharged to the outside without being re-circulated to other rooms. Exhaust must be

dispersed away from occupied areas and air intakes, or the exhaust must be HEPA-filtered. Appropriate filter/barriers are installed to prevent escape of arthropods. The direction of airflow in the insectary is inward. A progressively negative pressure gradient is maintained as distance from the main entrance increases. Personnel must verify that the direction of the airflow is proper (a visual monitoring device/meter is recommended to confirm directional inward airflow). Audible alarms alert personnel to system failure.

- Sterilization Equipment: An autoclave is available within the suite of rooms containing arthropods.
- Sink and Shower: In addition to the ACL-2 recommendation, an appropriately plumbed shower is available within the insectary suite.
- Illumination: As in ACL-2
- Biosafety Cabinets: HEPA-fitted exhaust air from Class II biological safety cabinets can be re-circulated into the insectary provided that it is certified annually. If exhausting to the outside, the cabinet must be installed appropriately. If Class III cabinets are used, they must be installed appropriately.
- Facility Compliance Monitoring: The completed ACL-3 insectary design and operational procedures must be documented by the PI and reviewed by the IBC. The insectary must be tested for verification that the design and operational parameters have been met prior to operation. ACL-3 insectaries are re-verified at least annually against these procedures as modified by operational experience.

APPENDIX 4.17: ARTHROPOD CONTAINMENT LEVEL 4 (ACL-4)

ACL-4 safety guidelines are for the most dangerous pathogen-infected arthropods. No compromise is acceptable at this level of work. BSL-4 agents are associated with a high risk of infection from aerosol exposure, and cause life-threatening disease. Certain other pathogens such as those listed as "restricted animal pathogens" may also necessitate BSL-4 containment if used in vectors. For vector work, production of aerosols is a potential risk when preparing infectious meals or inocula, and can also result from analytical practices involved in virus isolation. If work with vectors must be performed in a BSL-4 facility, then BSL-4 requirements must be strictly followed. As described below, vectors must be safely contained at all times possibly by use of specially designed apparatus that is tested and approved prior to use. Of the twelve viruses requiring BSL-4 containment in the USA, five are transmitted by arthropods [31]:

- Central European encephalitis
- Congo-Crimean hemorrhagic fever
- Kyasanur Forest disease
- Omsk hemorrhagic fever, and
- Russian Spring-Summer encephalitis

Only ticks have been implicated in their natural transmission cycles, although other arthropods have been experimentally infected with BSL-4 agents (e.g., *Aedes aegypti* with Marburg, and *Mesostigmata* mites with Junin). With this information one might at present only consider measures and protocols that safely contain species of ticks as relevant to BSL-4 research with arthropods. However, with the recent emergence of new diseases, it is perhaps necessary to consider other arthropods as potential vectors, particularly flying insects. Furthermore, research on newly discovered pathogens often requires experimental attempts to infect arthropods in an attempt to determine the life cycle. Species of arthropods - principally ticks that have been collected from areas in which infections with a BSL-4 agent are actively being or suspected of being transmitted - are processed as though they were infected with a BSL-4 agent.

As the number of BSL-4 laboratories is quite limited, the readers are referred to the appropriate sections of the BMBL [8] for arthropod work for a simple and minimalist approach to adopted. There are two types of BSL-4 laboratories: A) the cabinet laboratory where the agent is handled in a Class III Biological safety cabinet, and B) the suit laboratory. Personnel working in a BSL-4 suit facility shower in and then don one-piece positive pressure personnel suits ventilated by a life support system. Arthropods requiring ACL-4 would typically be adults for use in pathogen transmission studies. However, there may be circumstances in which immature stages such as nymphal ticks might be maintained to be able to stimulate pathogen reactivation to facilitate isolation. Construction of a BSL-4 facility, and required operating procedures, are sufficient to guarantee that no early life stage could survive, since, for example, all liquid waste is decontaminated.

When used in a BSL-4 facility, an arthropod must never be handled outside of a primary containment barrier e.g., cages are opened only in an arthropod secure glove box. As required for ACL-3, every arthropod is counted and accounted for throughout the experiment. No one enters or leaves the room until all arthropods are accounted for and secured in double taped cages and placed in secondary sealed holding trays. If one is missing and cannot be found, the facility is shut down and treated with a pesticide.

The nature of this research and the protective equipment required dictates that staff must be trained to the very highest level. Since working with arthropods often requires the use of small instruments and hence considerable dexterity, it is recommended that a specific person be designated for this work and be trained extensively using a space suit so that they are well rehearsed before actual ACL-4 work. Equipment that is used for ACL-3 work will be specially adapted for ACL-4 research, and such work would require extensive practice.

APPENDIX 4.18: PROCEDURES FOR EXPERIMENTS INVOLVING FISH AND OTHER AQUATIC ORGANISMS

Genetic manipulation work involving the use of fish and other aquatic organisms includes:

• Production or use of transgenic aquatic organisms;

• Use of genetically modified microorganisms to infect aquatic organisms.

The primary objective in designing containment facilities and procedures for genetic manipulation work involving aquatic organisms is to prevent escape of transgenic aquatic organisms or recombinant infectious agents into natural waterways.

Proposals for such work will be examined by IBC on a case-by-case basis. Investigators submitting proposals for research involving transgenic aquatic organisms shall describe in the proposal the containment facilities to be used and procedures proposed for treatment of wastewater from the facility.

Adherence to the biosafety guidelines does not exempt researchers from practicing responsible laboratory animal care and maintain basic welfare standards (useful references: Guide for the Care and Use of Laboratory Animals, National Research Council, 1996 and Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care). Researchers should also be aware that they are subject to laws that legislate against cruelty to animals.

The following general guidelines are provided regarding the basic requirements for genetic manipulation work involving aquatic organisms.

General

• The rearing area shall be confined in a secured building and be restricted to authorized personnel only.

• All water shall leave the plant through a common drain.

• All effluent water shall be passed through at least two screens. The size of the screening shall be set to retain the smallest life history stage of the organisms in use. The screens shall be cleared regularly to prevent blockage and overflow.

• Effluent water shall not discharge into a major system containing related fish species.

• The building shall be structurally sound and of sufficient elevation to preclude flooding or unintentional escape of these transgenic organisms.

Work with Transgenic Aquatic Organisms

• To prevent escape of adult aquatic organisms into the sewerage system, a grill or mesh, with an appropriate grid size to prevent passage of adults of the species being used, shall be fitted to outlets used for disposal of tank water.

• Water from tanks containing only adult transgenic aquatic organisms, and not involving use of recombinant infectious agents, may be discarded untreated down the sink, provided that a filtering mechanism to retain adult aquatic organisms is in place.

• Tank water that has any potential to contain embryos, sperm, eggs or larvae of transgenic aquatic organisms shall be treated to ensure inactivation of viable transgenic material before disposal. The proposed treatment procedures and evidence for their efficacy shall be provided to AVA for case-by-case assessment.

• Since many fish species have sticky eggs which attach firmly to substrates, nets and other equipment used in the tank during spawning shall be sterilized after use. If fish are induced to spawn on the sides of tanks, the tanks shall be decontaminated after use by a procedure of demonstrable efficacy in sterilizing eggs and sperm for the species used.

Work with Infectious Recombinant Microorganisms in Aquatic Organisms

• General practices shall be as required for BSL2 or BSL3 laboratory work depending on the microorganism.

• To prevent escape of adult aquatic organisms into the sewerage system, a grill or mesh, with an appropriate grid size to prevent passage of adults of the species being used, shall be fitted to outlets used for disposal of tank water.

• Tank water used for work with infectious recombinant microorganisms, or tank water that has any potential to contain embryos, sperm, eggs or larvae of infected aquatic organisms, shall be treated to ensure inactivation of viable material before disposal.

APPENDIX 4.19: REFERENCES, GUIDELINES AND LINKS

1. Gurley E. S., Montgomery J. M., Hossain M. J., Bell M., Azad A. K., Islam M. R., Molla M. A. R., Carroll D. S., Ksiazek T. G., Rota P. A., Lowe L., Comer J. A., Rollin P., Czub M., Grolla A., Feldmann H., Luby S. P., Woodward J. L., & Breiman R. F. (2007). Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerging Infectious Diseases, 13*(7), 1031-1037.
2. de Wit E., Prescott J., Falzarano D., Bushmaker T., Scott D., Feldmann H, Munster V. J. (2014) Foodborne Transmission of Nipah Virus in Syrian Hamsters. PLoS Pathog, 10 (3)
3. Newell-McGloughlin M. (2008) Nutritionally Improved Agricultural Crops. Plant Physiology, July 2008, Vol. 147, pp. 939–953
4. Qaim M. (2010) Benefits of genetically modified crops for the poor: household income, nutrition, and health. New Biotechnology, 27(5), 552-557
5. Bangladesh Biosafety Rules 2012 https://bangladeshbiosafety.org/wp-content/uploads/2017/04/Bangladesh_Biosafety_Rules_2012.pdf
6. Biosafety Guidelines of Bangladesh https://bangladeshbiosafety.org/wp-content/uploads/2017/09/BiosafetyGuidelineBD.pdf
7. Laboratory Biosafety Manual - Third Edition http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/
8. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
9. The Laboratory Biosafety Guidelines, 3rd Edition, Canada http://www.phac-aspc.gc.ca/publicat/lbg-ldmbl-04/pdf/lbg_2004_e.pdf
10. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories https://www.cdc.gov/mmwr/pdf/other/su6101.pdf
11. ASM Guidelines for Biosafety in Teaching Laboratories https://www.asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laborator
12. Biosafety Cabinetry: Design, Construction, Performance, and Field Certification https://www.nsf.org/newsroom_pdf/nsf_49-11_annex-e.pdf
13. Guideline for Hand Hygiene in Health-Care Settings https://www.cdc.gov/mmwr/PDF/rr/rr5116.pdf
14. WHO best practices for injections and related procedures toolkit. https://apps.who.int/iris/bitstream/handle/10665/44298/9789241599252_eng.pdf;sequence=1
15. WHO Guidance on regulations for Transport of Infectious Substances 2009-2010 https://www.who.int/csr/resources/publications/biosafety/WHO_HSE_EPR_2008_10.pdf?ua=1
16. The International Air Transportation Association (IATA), Dangerous Goods Regulations and Live Animals Transport Regulations IATA online store –http://www.iata.org
17. NIAID Emerging Infectious Diseases/ Pathogens. https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens
18. The Environment Conservation Rules, 1997 http://extwprlegs1.fao.org/docs/pdf/bgd19918.pdf
19. Guidance for non-poliovirus facilities to minimize risk of sample collections potentially infectious for polioviruses. http://polioeradication.org/wp-content/uploads/2018/04/polio-containment-guidance-for-non-poliovirus-facilities-20180410-en.pdf
20. WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use. http://polioeradication.org/wp-content/uploads/2016/12/GAPIII_2014.pdf
21. A Community Guide to Environmental Health, Chapter 19: Health Care Waste, https://en.hesperian.org/hhg/A_Community_Guide_to_Environmental_Health
22. Medical waste processing and management rules 2008 http://www.doe.gov.bd/site/view/policies/
23. Requirements for Decontamination by Autoclaving http://ehs.virginia.edu/biosafety/bio.documents/Autoclaving_Guidelines.pdf
24. NIH guidelines for research involving recombinant DNA molecules. https://blink.ucsd.edu/safety/research-lab/biosafety/nih/index.html
25. Laboratory biosecurity guidance. https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf

26. Singapore Biosafety Guidelines for GMO Research http://www.gmac.sg/pdf/Research/Singapore%20Biosafety%20Guidelines%20for%20GMO%20Research_Jan%202013.pdf
27. The National Institute of Health, USA, Design Requirements Manual (DRM) https://www.orf.od.nih.gov/TechnicalResources/Documents/DRM/DRM1.4042419.pdf
28. NationalInstitutesofHealthBiosafetyLevel3LaboratoryCertificationRequirements https://www.orf.od.nih.gov/TechnicalResources/Bioenvironmental/Documents/BSL3CertificationGuidelinesFINAL_508.pdf
29. National Guidelines on Medical Biotechnology 2010 http://www.dghs.gov.bd/index.php/en/mis-docs/important-documents/item/national-guidelines-on-medical-biotechnology-2010?category_id=115
30. 2017 User's Guide to Biosafety Regulatory Process for Genetically Engineered Plants in Bangladesh.https://bangladeshbiosafety.org/wp-content/uploads/2017/11/Users_Guide_to_Biosafety_Regulatory_Process_Bangladesh_2017.pdf
31. Arthropod Containment Guidelines (Version 3.1). http://www.ehs.wisc.edu/bio/ArthropodContainment.pdf