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CONTENTS

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FOREWORD

Accurate and reliable diagnosis of a malady are critical for the provision of targeted treatment, proper prevention and vaccine selection, or for complementing epidemiological study. The array and variety of samples that most national laboratories around the world receive expose operators to possible pathogens and makes the threat of contamination a daily possibility. It is, therefore, important to make safety an engrained characteristic of any laboratory to protect its staff and the environment in which a laboratory is to function.

Biosafety is one of the key components of the Food and Agriculture Organization of the United Nation's biosecurity framework. The framework promotes a strategic and integral approach to analysing and managing risks to people, livestock and crops.

FAO remains committed to assisting member countries in building the necessary technical, institutional and information-sharing capacities for biosafety. This publication is a testament to this commitment.

This primer provides reference and general guidance for biosafety managers. It is envisaged to be a source of information and an important reference for many biosafety novices and experts. It covers basic introduction to biosafety and biosafety levels including policy, administration and practical guidance in biosafety implementation and management. It is a unique publication as it aims to be an all-in-one biosafety reference for national laboratories, but others may find it beneficial as well, such as those in the private sector or academic institutions.

Our FAO laboratory specialists including partners have contributed to ensuring that information in this publication are most current and most practical. It contributes to FAO's strategic objective of increasing each country's resilience to threats and crises.

We continually commit to protecting people and animals from disease threats.

Juan Lubroth

Chief Veterinary Officer

ABBREVIATIONS

BMBL Biosafety in Microbiological and Biomedical Laboratories Manual (CDC)

BSC Biosafety Cabinet

BSL Biosafety Level; BSL1 – BSL4

CDC Center for Disease Control & Prevenetion,
United States Department of Health & Human Services

CEN Comite Europeen de Normalisation

COSHH Control of Substances Hazardous to Health

CSO Clinical Safety Officer

DSO Designated Safety Officer

IATA International Ait Transport Association

ISO International Organization for Standardization

GMO Genetically modified organism

GMP Good Manufacturing Practice; CGMP – Current Good Manugfacturing Practice

H&S Health and Safety

HEPA High Efficiency Particulate Arrestor

HVAC Heating Ventilation and Air Conditioning

NSF National Sanitation Foundation

OIE World Organization for Animal Health

PAPR Powered Air Purifying Respirator

PI Prinicple Investigator

PPE Personal Protective Equipment

RG Risk Group; RG1 – RG4

RMW Regulated Medical Waste

RPE Respiratory Protective Equipment

SOP Standard Operating Procedure

TSE Transmissible Spongiforme Encephalopathy

WHO World Health Organisation

BACKGROUND AND SCOPE

This manual was originally compiled by Assoc. Prof. Stuart Blacksell to provide reference and general guidance for biosafety managers in Southeast Asia and elsewhere during the Regional Laboratory Network Training Of the Trainers (ToT) Workshop on Biosafety Management, 9-20 December 2013, Bangkok, Thailand which was hosted by Mahidol-Oxford Tropical Medicine Research Unit (MORU) and organized by Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific (FAO-RAP) with funding from the United States Agency for International Development (USAID) and the European Union. Since that workshop, this manual has provided a source of information for many biosafety novices and experts within Asia.

The manual has borrowed heavily from national and international guidelines such as the *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) published by the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention National Institutes of Health and the CEN workshop agreement CWA15793 *Laboratory Biorisk Management*. Furthermore, practical advice from many years of experience working at the MORU has also been provided in this document.

INTRODUCTION TO BIOSAFETY AND BIOSAFETY LEVELS

WHAT IS BIOSAFETY?

Biosafety is about the intrinsic hazards of living organisms and how to handle them safely. Genetic material as such ('naked' DNA) can be dangerous as well. Before starting to work with pathogens or genetically modified organisms (GMOs) in a laboratory one should stop and think about the possible risks of working with these organisms and take proportionate measures to minimise any risks for human health and the environment.

What are hazards and risks?

A hazard is any biological, chemical, or physical agent with the potential to cause an adverse health effect in an animal or human. A biohazard is specifically a biological agent, typically a pathogen, with the potential to cause an adverse animal or human health effect.

Risk is defined as the likelihood of occurrence and the likely severity of any biological or economic consequences on animal or human health due to an adverse event related to a hazard.

The process of Laboratory Risk Analysis includes (1) identification of the hazards in a laboratory, (2) assessement of how likely and how severe the consequences of an adverse event associated with the specific hazard would be, (3) management steps to mitigate the risk(s), (4) a communication plan to keep all those impacted informed, and (5) a verification plan to insure that the risk management steps are working (OIE: Chapter 1.01.04 Biosafety Biosecurity)

What are pathogens?

Pathogen is usually an infectious organism such as a virus, bacteria, parasite or fungus that is the capability of causing disease in humans, animals or plants.

What is virulence or pathogenicity?

Infectivity is the ability of an organism to infect a specific tissue or tissues of a host; ***pathogenicity*** is defined as the ability of an organism to cause harm (disease) to the host; and ***virulence*** is defined as the degree of damage caused to the host. The virulence associated with a group or species of microorganisms is typically indicated by case morbidity and mortality rates.

Modes of transmission of pathogens

The mode of transmission of an infectious agent will have a large affect on the biosafety implications for an infectious agent. Horizontal transmission occurs between hosts of the same species, in contrast to vertical transmission which tends to evolve symbiosis (after a period of high morbidity and mortality in the population) by linking the pathogen's evolutionary success to the evolutionary success of the host organism. Infectious disease is transmitted from some source. Defining the means of transmission plays an important part in understanding the biology of an infectious agent, and in addressing the disease it causes. Transmission may occur through several different mechanisms.

Aerosol: Respiratory diseases and meningitis are commonly acquired by contact with aerosolized droplets, spread by sneezing, coughing, talking, kissing or even singing.

Ingestion: Gastrointestinal diseases are often acquired by ingesting contaminated food and water.

Sexual: Sexually transmitted diseases are acquired through contact with bodily fluids, generally as a result of sexual activity.

Direct contact: Some infectious agents may be spread as a result of contact with a contaminated, inanimate object such as a coin passed from one person to another, while other diseases penetrate the skin directly.

Vectors: Transmission of infectious diseases may also involve a vector. Vectors may be mechanical or biological. A mechanical vector picks up an infectious agent on the outside of its body and transmits it in a passive manner.

RISK GROUPS

The relationship between risk groups and containment levels

Risk (or Hazard) groups are a simple method of communicating the risk relating to a specific infectious agent. This risk group classification can be applied to laboratories and to field activities. Risk groups are designated from 1 (RG1- lowest risk) to 4 (RG4- highest risk). The risk group designation is dependent on factors such as route of transmission, infectious dose, and treatment/prophylaxis, etc.

Biosafety level designations are based on a composite of the design features, construction, containment facilities, equipment, practices and operational procedures required for working with agents from the various risk groups. Laboratory facilities are designated as basic containment – Biosafety Level 1, medium containment – Biosafety Level 2, containment – Biosafety Level 3, and maximum containment – Biosafety Level 4. The features and characteristics of risk groups and containment levels are contained in the sections below.

> *It is very important to understand that risk groups do not equate to containment levels. For example, a risk group 3 organism does not need to be necessarily handled at containment level 3. There are a number of factors that need to be considered as part of a full risk assessment before the control measures and biosafety containment level can be determined including quantity of concentration and the methods being performed.*

What are risk groups?

There are several thousand known and characterized microorganisms which cause disease in man and animals. They are classified as bacteria, fungi, viruses, prions, and parasites and can be categorized by their level of hazard into risk groups (or hazard). Risk groups vary from country to country and a summary of the different risk groups is presented at the following website https://my.absa.org/Riskgroups. It is important to be aware of the risk (or hazard) groups as classified in your own country. It is not recommended to use the risk group designations from other countries.

It is important that biorisk assessments are performed for all procedures with infectious agents so that an appropriate biosafety level can be assigned. It is also important to remember that the absence of information regarding risk does not mean the agent cannot infect humans or animals.Irrespective of the country, organisms are divided into four categories of risk. Organisms that are not able to cause disease belong to risk group 1. Pathogenic organisms belong to the risk groups 2, 3 or 4, depending on their degree of pathogenicity and the

availability of effective treatment. To distinguish between the classification of natural non-modified pathogens and genetically modified organisms (GMOs), the pathogen classification uses the term risk groups or sometimes also biological risk class, while for the GMO classification the term risk class is used. Below an overview is given of the definitions of the different risk groups.

Risk group 1 are organisms that are unlikely to cause human or animal disease and are disease-producing organisms in animals that are enzootic but not subject to official control. Examples of risk group 1 organisms using the US risk group designations are as follows: *E.Coli K12, Lactobacilli* used in food processing, many thermophile bacteria, *Saccharomyces cerevisiae,* Lambda Phages, Tabaccomosaic virus, Vaccine strains.

Risk group 2 human pathogens are organisms that can cause disease in humans and pose a hazard to persons that are directly exposed to it. Their spread to the community is unlikely. Prophylaxis or effective treatment is mostly available. Risk group 2 animal pathogens are organisms that can cause disease in animals and that possess in different extend one of thefollowing properties: limited geographical importance, transmission to other limited or non-existent species, absence of vectors or carriers. Limited economic and/or medical impact. Prophylaxis and/or effective treatment is mostly available. Examples of risk group 2 organisms using the US risk group designations are as follows:

Viruses: Influenza viruses types A, B, C other than notifiable avian influenza (NAI); Newcastle disease virus; Orf (parapox virus)

Bacteria: *Alcaligenes spp.; Arizona spp.; Campylobacter spp.; Chlamydophila psittaci (nonavian); Clostridium tetani; Clostridium botulinum; Corynebacterium spp.; Erysipelothrix rhusiopathiae; Escherichia coli; Haemophilus spp.; Leptospira spp.; Listeria monocytogenes; Moraxella spp.; Mycobacterium avium; Pasteurella spp.; Proteus spp.; Pseudomonas spp.; Salmonella spp.; Staphylococcus spp.; Yersinia enterocolitica; Yersinia pseudotuberculosis*

Fungi: *Aspergillus fumigatus; Microsporum spp.; Trichophyton spp.*

Risk group 3 human pathogens are organisms that can cause serious disease in humans and pose a hazard to persons that are directly exposed to it. There is a risk of spread to the community. Prophylaxis or effective treatment is mostly available. Risk group 3 animal pathogens are organisms that can cause serious disease or epizootic in animals. Spread to other species is more than possible. Some of these pathogenic agents require specific sanitary measures. Prophylaxis or effective treatment is mostly available. Examples of risk group 3 organisms using the US risk group designations are as follows:

Viruses: Rabies virus; Equine encephalomyelitis virus (Eastern, Western and Venezuelan); Japanese B encephalitis virus; Louping ill virus

Bacteria: *Bacillus anthracis; Burkholderia mallei (Pseudomonas mallei); Brucella* spp.; *Chlamydia psittaci* (avian strains only); *Coxiella burnetti; Mycobacterium bovis*

Risk group 4 human pathogens are organisms that can cause serious disease in humans and pose a severe hazard to persons that are directly exposed to it. There is a high risk of spread to the community. Prophylaxis or effective treatment is mostly not available.

Risk group 4 animal pathogens are organisms that can cause a very serious pandemic or epizootic in animals with high levels of mortality or dramatic economic consequences for the afflicted regions. Medical prophylaxis is not available or one exclusive sanitary prophylaxis is possible or obligatory. Examples of risk group 4 organisms using the US risk group designations are as follows: Nipah/Hendra virus, Lassa virus, Junin virus, Ebola virus and Marburg virus.

Summary points: Risk Groups

- Risk Group 1 (RG1) - Agents that are not associated with disease in healthy adult humans
- Risk Group 2 (RG2) - Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available
- Risk Group3 (RG3) - Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk)
- Risk Group 4 (RG4) - Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).
- It is very important to understand that risk groups do not equate to containment levels.

CONTAINMENT LEVELS (BIOSAFETY LEVELS)

The WHO and CDC describe four biosafety levels (BSL-1 to BSL-4) consisting of combinations of standard and special microbiological practices, safety equipment (primary barriers), and laboratory facilities (secondary barriers).

BSL1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

BSL2 facility design and work is more rigorous than BSL1. It is suitable for work involving agents of moderate potential hazard to personnel and the environment. Most clinical laboratories use BSL2 for initial diagnostic procedures of unknown samples. BSL2 is what is considered a normal working laboratory in most situations and is able to perform the core functions required of most situations. It differs from BSL1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists
- Access to the laboratory is limited
- Extreme precautions are taken with contaminated sharp items
- Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

BSL3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the

Table 1. Routes of Transmission and Protective Measures for Infectious Agents in the Laboratory

Route of Exposure	Protective Measures
Mucous Membranes: Exposure to eyes, nose, or mouth. splash/splatter.	**Achieve face protection by:** • Wearing safety glasses or a full face shield • Working in a biosafety cabinet or behind a protective shield • Following good microbiological practices
Inhalation: Exposure to respirable aerosols (particles <5.0µm) centrifuge leaks, spills, poor pipetting, practice, pouring liquids.	**Avoid exposure to aerosols by:** • Working in a biosafety cabinet • Using sealed rotors or centrifuge cups • Following good microbiological practices • Use of respiratory protective equipment
Ingestion: Exposure to mouth mouth pipetting; eating, drinking or smoking in the laboratory; or failure to wash hands prior to exiting the lab.	**Prevent exposure via ingestion by:** • No eating, drinking or smoking or storage of food in the laboratory • Using mechanical pipetters • Following good microbiological practices • Use of gloves and frequent hand washing
Percutaneous: Exposure through intact or non-intact skin needlestick, puncture with a contaminated sharp object, animal scratch or bite, contact with a wound.	**Prevent percutaneous injuries by:** • Substituting plastic for glass • Using extreme caution with sharps • Care in handling sharps and disposal in a rigid leak-proof sharps container • Wearing cut resistant gloves and sleeves • Covering non-intact skin with waterproof bandages and wearing double gloves
Contact: Indirect exposure. Touching mucous membranes, contact lenses, or food with hands that have been in contact with contaminated surfaces such as benches, phones, computers; or hands that were not washed after working.	**Prevent indirect exposure by:** • Decontaminating work surfaces • Washing hands when finished working, if gloves develop a hole, and prior to exiting the lab • Not touching face with gloves or non-gloved hands until hands are washed • Not using lip balm or touching contact lenses within the laboratory • No eating or drinking in the lab

manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. A BSL3 laboratory is highly specialised and requires significant maintenance budgets to ensure the reliable performance of support systems such as HVAC (to provide directionally airflow) and liquid decontamination treatment (if specified). In most situations, BSL3 is not required and is reserved for situations where large volumes of concentrated infectious materials are cultivated or infectious animal work is being performed.

BSL4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. This is considered to be a maximum containment laboratory. Such laboratories are extremely rare and are highly specialised facilities and are outside of the scope of this manual.

BIOSAFETY MANAGEMENT – PROCEDURES AND POLICIES

A SYSTEMS APPROACH TO BIOSAFETY MANAGEMENT

A successful laboratory biosafety management standard must be based on a management systems approach. This implies that identifying, understanding and managing a system of interrelated processes for a given objective, improves the organization's effectiveness and efficiency. Application of the management systems approach principle leads to the following actions:

Defining the system by identifying or developing the processes that affect a given objective;

- Structuring the system to achieve the objective in the most effective manner;
- Understanding the interdependencies among the processes of the system;
- Continually improving the system through measurement and evaluation, and;
- Establishing resource constraints prior to action.

The systems approach outlined above has been successfully adopted by the International Organization for Standardization (ISO). Organizations which have already implemented systems for quality, environmental and/or occupational health and safety management, will find significant synergy between these systems and the one for biosafety management. The management system approach enables an organization to effectively identify, monitor and control the laboratory biosafety and biosecurity aspects of its activities. An effective management system approach should be built on the concept of continual improvement through a cycle of planning, implementing, reviewing and improving the processes and actions that an organization undertakes to meet goals. This is known as the PDCA (Plan-Do-Check-Act) principle:

Plan: Planning, including identification of hazard and risk and establishing goals,

Do: Implementing, including training and operational issues,

Check: Checking, including monitoring and corrective action,

Act: Reviewing, including process innovation and acting to make needed changes to the management system.

In order to improve biosafety management the organization needs to focus on the causes of non-conformities and undesirable events. Systematic identification and correction of system deficiencies leads to improved performance and control of biosafety. (CEN Workshop Agreement CWA 15793, p4). The OIE Terrestrial Manual chapter (1.01.04) Laboratory Biosafety and Biosecurity provides a standard for managing biological risk in veterinary laboratory and animal facilities using biorisk analysis for identifying and managing laboratory biosafety and

biosecurity risks for individual veterinary laboratories. Risk mitigation is accomplished through use of administrative, operational, engineering, and PPE-based controls that are selected, implmented and monitoried using the biosafety management system approach.

Keys to a successful biosafety management system

Some of the key factors in establishing and implementing a successful biosafety management system include:

Commitment by top management:

- Providing adequate resources, prioritization and communication of biosafety and biosecurity policy;
- Integrating of biosafety management throughout the organization; o identifying opportunities for improvement and prevention, determining root causes and preventing recurrence.

Focus on continual improvement:

- Making continual improvement an objective for every individual in the organization;
- Using periodic assessment against established risk-criteria to identify areas for potential improvement;
- Continually improving the effectiveness and efficiency of processes; o promoting prevention activities;
- Providing personnel in the organization with appropriate education and training including the methods and tools of continual improvement; o Establishing measures and goals for improvement;
- Recognizing improvement (CEN Workshop Agreement CWA 15793, p4-5).

Biosafety management policy

The organization's top management shall develop, authorize and sign a policy concerning the management of laboratory biosafety (laboratory biosafety and laboratory biosecurity). It shall clearly state the overall biosafety management objectives and a commitment to improving biosafety management performance.

The policy shall be appropriate to the nature and scale of the risk associated with the facility and associated activities and commit to:

- Protecting staff, contractors, visitors, community and environment from biological agents and toxins that are stored or handled within the facility;
- Reducing the risk of unintentional release of, or exposure to biological agents and toxins;
- Reducing the risk to an acceptable level of unauthorized intentional release of hazardous biological materials, including the need to conduct risk assessments and implement the required control measures;
- Complying with all legal requirements applicable to the biological agents and toxins that will be handled or possessed, and with the requirements of this standard;
- Ensuring that the need for effective biosafety management shall take precedence over all non "health and safety" operational requirements;

- Effectively informing all employees and relevant third parties and communicating individual obligations with regard to biosafety to those groups;
- Continually improving biosafety management performance. (CEN Workshop Agreement CWA 15793, p15).

Laboratory biorisk management standard (CWA15793:2011)

The CEN Workshop Agreement was been drafted and approved by a Workshop of representatives of interested parties (76 participants from 24 countries) in 2008. The scope of the laboratory biosafety management system standard CWA15793 is to set requirements necessary to control risks associated with the handling or storage and disposal of biological agents and toxins in laboratories and facilities.

The standard enables organizations to:

- Establish and maintain a biosafety management system to control or minimize risk to acceptable levels in relation to employees, the community and others as well as the environment which could be directly or indirectly exposed to biological agents or toxins;
- Provide assurance that the requirements are in place and implemented effectively;
- Seek and achieve certification or verification of the biosafety management system by an independent third party;
- Provide a framework that can be used as the basis for training and raising awareness of laboratory biosafety and laboratory biosecurity guidelines and best practices within the scientific community.

The standard is performance-based and sets out requirements for and places responsibility on organizations to demonstrate that appropriate and validated risk reduction procedures have been established and implemented.

The Laboratory biorisk management standard (CWA15793:2011) can be accessed at ftp://ftp.cenorm.be/CEN/Sectors/TCandWorkshops/Workshops/CWA15793_September2011.pdf

Summary points: Biosafety management

- A successful laboratory biosafety management standard must be based on a management systems approach.
- CWA15793 is a laboratory biosafety management system standard used to control risks associated with the handling or storage and disposal of biological agents and toxins in laboratories and facilities.
- An effective management system approach should be built on the concept of continual improvement known as the PDCA (Plan-Do-Check-Act) principle
- A biosafety policy be appropriate to the nature and scale of the risk associated with the facility and associated activities and commit to:
- Protecting staff, contractors, visitors, community and environment from biological agents and toxins that are stored or handled within the facility;
- Reducing the risk of unintentional release of, or exposure to biological agents and toxins;

- Reducing the risk to an acceptable level of unauthorized intentional release of hazardous biological materials, including the need to conduct risk assessments and implement the required control measures;
- Complying with all legal requirements applicable to the biological agents and toxins that will be handled or possessed, and with the requirements of this standard;
- Ensuring that the need for effective biosafety management shall take precedence over all non "health and safety" operational requirements;
- Effectively informing all employees and relevant third parties and communicating individual obligations with regard to biosafety to those groups;
- Continually improving biosafety management performance.

BIOSAFETY MANAGEMENT – DOCUMENTATION

Biosafety manual

A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

The purpose of a Biosafety Manual is to describe how the organisation complies with institutional policies and government regulations and to provide an overview of arrangements for ensuring the safety of all staff and visitors to the organisation. This Biosafety Manual applies to all sites within the organisation, and any other sites at which staff will be undertaking work on behalf of the organisation. It applies to all staff and visitors who are working in the organisation. Where potential difficulties or conflicts between organisational safety arrangements are identified, these should be discussed with the Biosafety Officer in the first instance.

The Biosafety Manual should be developed to identify hazards and measures to prevent or mitigate these hazards. This manual describes the mechanical and engineering controls, operational procedures, administrative controls, emergency preparedness plans and procedures, training programs, and other elements of the safety and biosecurity program. The Biosafety Manual supplements and signposts detailed safety precautions outlined in individual SOPs and is not intended to replace or reproduce them all. Use of the word 'must' implies that this requirement is mandatory and use of the word 'should' means that all possible efforts should be made to comply, subject to local constraints. The Biosafety Manual will be annually reviewed and revised as necessary.

Summary points: Biosafety Manual

- A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- A biosafety manual describes how the organisation complies with institutional policies and government regulations and to provide an overview of arrangements for ensuring the safety of all staff and visitors to the organisation.
- A biosafety manual describes the mechanical and engineering controls, operational procedures, administrative controls, emergency preparedness plans and procedures, training programs, and other elements of the safety and biosecurity program.

- A biosafety manual supplements and signposts detailed safety precautions outlined in individual SOPs and is not intended to replace or reproduce them all.
- A biosafety manual will be annually reviewed and revised as necessary.

Risk assessments

All protocols and procedures used at sites must have an associated risk assessment. The Principle Investigator must ensure that all procedures including new experimental procedures are fully risk assessed and have been approved by the Biosafety Officer. This risk assessment must include emergency procedures plus protective equipment and containment level guidance.

For performing risk assessments with infectious materials the following guidance is recommended,

- The risk assessment process should identify features of microorganisms as well as host and environmental factors that influence the potential for workers to have a biohazard exposure or illness.
- The Principle Investigator should consult with the Biosafety and other relevant Safety Officers to ensure that the laboratory is in compliance with established guidelines and regulations and that the basis for the risk assessment is sound and comprehensive.
- When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available. Risk assessments must be presented to the Biosafety and other relevant Safety Officers for final approval before the work starts.

The following should trigger either a new risk assessment or review of an existing one:

- Commencement of new work or changes to the programme of work including the introduction of new hazards (e.g. new biological agents, new chemicals, alterations to work flow or volume, etc.);
- New construction / modifications to laboratories, plant and equipment or its operation;
- Introduction of altered and unplanned staffing arrangements (including contractors, visitors and other non-core personnel);
- Alterations to Standard Operating Procedures (SOPs) or working practices (e.g. disinfection/ waste management methodologies, PPE provision / usage entry / exit protocols, etc.);
- When unexpected events that may have relevance for the management of biosafety are observed;
- when actual or potential non-conformity with internal / external rules and regulations is identified (e.g. introduction of new legislation or major accident exposure);
- When considering emergency response and contingency planning requirements;
- As part of the existing management system review process (e.g. annually or at another appropriate and predetermined frequency) (CEN Workshop Agreement CWA 15793, p17).

Hazard identification A hazard may be a physical situation (e.g. a fire or explosion), an activity (e.g. pipetting) or a material (in this case the principal hazard is most likely to be a biological agent or toxin, but others will include chemicals and asphyxiating gases such as nitrogen). The essence of a hazard is that it has the potential for causing harm, regardless of

how likely or unlikely such an occurrence might be. Biological hazards should be identified and assessed in relation to their potential damage to humans, animals, and the environment. Where hazardous materials are classified into hazard or risk groups based on international and/or foreign country classification schemes local diverging needs and constraints should be considered.

A hazard identification exercise should use information including:

- Group experience and knowledge;
- External or specialized expertise not found in the facility;
- Results of previous assessments;
- Surveys of previous accidents/incidents;
- Hazardous materials data;
- Information on hazardous organisms;
- Guidelines and codes of practice;
- Facility drawings;
- SOPs, manuals, etc.;
- Process maps.

Defined methodologies and approaches are available for conducting hazard identification exercises. Unless hazards are identified effectively, it is not possible to assess the risk associated with the facility and associated activities. Hazard identification should be appropriate in nature, structure and recorded to a level whereby others can review the process (CEN Workshop Agreement CWA 15793, p18).

Risk assessments The risk assessment should categorize risks to identify those which need to be eliminated or controlled. Two examples of forms for risk assessment are provided in Form 1 (UK Control of Substances Hazardous to Health (COSSH) and Form 2 (US CDC). Descriptions of likelihood and consequence, together with the acceptability of risk levels should be defined and used in the assessment. Such a classification can be achieved for example through the use of a risk matrix identifying likelihood and consequence categories, ordered to illustrate those falling into high, moderate and low zones. However, other approaches may also be relevant and appropriate. Assessments can be qualitative, semi-quantitative or quantitative, and a method suitable to the situation should be identified and followed. In conducting the assessment due consideration should be made of the inherent risk from the biological agents and toxins (e.g. from risk grouping descriptions, material safety data sheets etc.). After definition and implementation of control measures the risks should be reviewed to decide if the remaining risk is acceptable or whether additional controls need to be identified and implemented (CEN Workshop Agreement CWA 15793, p18). Factors to consider when evaluating risk include the following:

Pathogenicity: The severity of the disease caused by an agent influences its assignment to a risk group. Those diseases that are generally self-limiting and typically do not result in illness, disability or death are assigned to lower risks groups than those associated with high severity.

Host factors: This includes host health status, training, experience, attention to safety, age gender and many other factors. Certain medical conditions can increase risk of

exposure and infection. For example, psoriasis results in skin wounds increasing the cutaneous exposure hazard and treatment with immunosuppressant drugs increases risk of infection due to down regulation of the immune system. Other host factors decrease the risk of exposure and infection to include the use of PPE, good microbiological practices, and immunization.

Route of transmission: Agents that can be transmitted by the aerosol route have been known to cause the majority of reported laboratory-acquired infections. Because many common procedures inadvertently create aerosols in the lab (opening vials, centrifugation, culture, pipetting,), work with agents spread via the aerosol route often pose the greatest hazard to personnel.

Agent stability: The greater the potential for an agent to survive in the environment, the higher the risk. Consider factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfections when looking at the stability of an agent.

Infectious dose: Consider the amount of an infectious agent needed to cause infection in a normal person. An infectious dose can vary from one to hundreds of thousands of organisms or infectious units.

Concentration: Whether the organisms are in solid tissue, viscous blood, sputum, the volume of the material and the laboratory work planned (amplification of the material, sonication, centrifugation, .) influences risk. In most instances, the risk increases as the concentration of microorganisms increases and the procedures used either generate aerosols or increase the risk of mucosal or percutaneous exposure.

Origin: This may refer to the geographic location (domestic or exotic), host (infected human or animal), or nature of the source (zoonotic or associated with an epidemic disease outbreak). Exotic agents may pose a higher risk than endemic agents as there may not be vaccines or specific treatment modalities available.

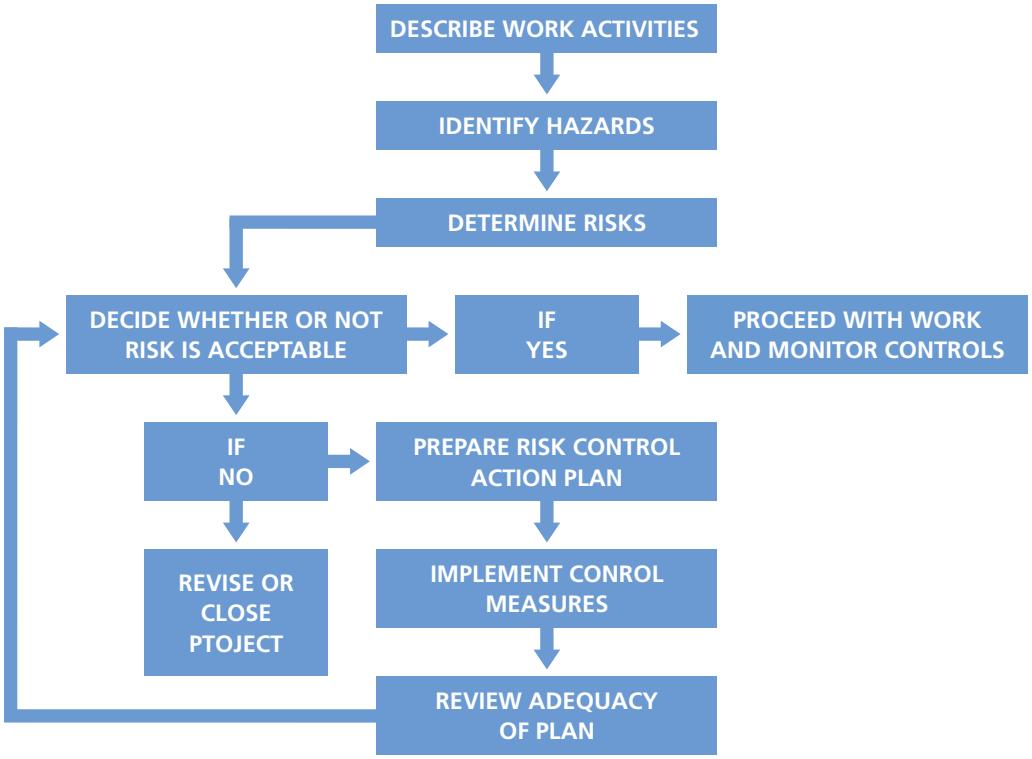
Availability of data from previous laboratory acquired infections, medical case study information, or animal studies: If human data is not available, information on the pathogenicity, infectivity, and route of exposure from animal studies may be valuable. Use caution when translating infectivity data from one species to another.

Availability of an effective prophylaxis or therapeutic intervention: Effective vaccines, if available, should be offered to laboratory personnel in advance of their of infectious material. However, immunization does not replace engineering controls, proper practices and procedures and the use of personal protective equipment. The availability of post-exposure prophylaxis must also be considered.

Medical surveillance: Medical surveillance programs may include serum banking, monitoring employee health status, participating in post-exposure management, employee counseling prior to offering vaccination, testing for respirator suitability, and annual physicals.

Experience and skill level of personnel: Laboratory workers must become proficient in specific tasks prior to working with microorganisms. Laboratory workers may have to work with non-infectious materials to ensure they have the appropriate skill level prior to working with biohazardous materials. Laboratory workers may have to go through additional training (e.g., Blood borne pathogen training, BSL-3 training, use of PPE) before they are allowed to work with materials or in a designated facility.

Figure 1. Risk assessment flowchart (CEN Workshop Agreement CWA 15793, p18).



Summary points: Risk assessments

- All protocols and procedures must have an associated risk assessment.
- The Principle Investigator must ensure that all procedures including new experimental procedures are fully risk assessed and have been approved by the Biosafety Officer/Biosafety Committee
- Risk assessment should identify features of microorganisms as well as host and environmental factors that influence the potential for workers to have a biohazard exposure or illness.
- Risk assessment must include emergency procedures plus protective equipment and containment level guidance (what to wear – where to work)
- Risk assessments must contain information regarding required vaccinations
- When performing a risk assessment, it is advisable to take a conservative approach if there is incomplete information available.
- Risk assessments must be approved before the work starts.

Form 1. Example COSHH risk assessment document

<table>
<tr><th colspan="2">COSHH risk assessment</th></tr>
<tr><td>Department
Microbiology
Location of work
Bangkok
Description of procedure
Immunofluorescence</td><td>Persons involved
Rickettsial staff
Substances used
Serum/Plasma</td></tr>
<tr><td>Quantities used
Small</td><td>Frequency of use
Daily</td></tr>
<tr><td>Hazards identified
Possible infection risk via puncture wound, mucous membranes and aerosol</td><td>Could a less hazardous substance be used instead? YES / NO
Justify not using it</td></tr>
<tr><td colspan="2">What measures have you taken to control risk?
Risk is minimal
Engineering controls:
i. Transport specimens using IATA 650 specifications
PPE:
i. Designated white gown
ii. Safety glasses
iii. Gloves</td></tr>
<tr><td colspan="2">Checks on control measures
Annual assessment</td></tr>
<tr><td>Is health surveillance required?
No
Emergency procedures:
1. Blood exposure
i. Contact CSO/DSO/Area supervisor
ii. Eyes – Eyewash procedure - Report
iii. Aerosol – Report
iv. Needstick- Make wound bleed by pressure. Wash exposed skin area thoroughly with alcohol, soap and water.
2. Spills
i. Clean up using 1% virkon</td><td>Training requirements:
i. Centrifuge
ii. Waste disposal
iii. Accident procedures</td></tr>
</table>

Form 2. CDC biological risk assessment document (source: http://www.cdc.gov/biosafety/publications/BiologicalRiskAssessmentWorksheet.pdf)

Save | Print | E-mail | Reset Form

Biological Risk Assessment Worksheet

Tracking # _____ **Building/Lab Room #** _____ **PI Name** _____

Laboratory protocols consist of one or more procedures. Each procedure in the protocol needs an agent-specific Biological Risk Assessment. Once an agent-specific Biological Risk Assessment has been completed for the procedure, it can be used for multiple protocols by referencing its tracking number. The procedure may be performed with additional precautions, if desired, but must be no less stringent than what is calculated below at Section II.

Keep a completed copy of this worksheet in your Biosafety Manual. The *Biosafety in Microbiological and Biological Laboratories* (BMBL) 5th Edition has additional guidance on facilities, work practices, PPE, and medical surveillance.

Section I: Complete All Data Entry in this Section

1. **Agent Used** _____
2. **Is a vaccine available?** Yes ○ No ○
3. **Risk Group of Agent** (check www.absa.org) 1 ○ 2 ○ 3 ○ 4 ○ {Inactivated agents = Risk Group 1}
4. **Procedure** _____
5. **For Risk Group 2-3, is there a splash potential?** Yes ○ No ○
6. **For Risk Group 2-3, does the procedure generate aerosol or large concentration?** Yes ○ No ○
   (e.g., cell culture, vortex, centrifuge, aerosol chamber, sonicate)

Section II: Data will be calculated in this Section according to the answers entered above in Section I

1. **Facility and Work Practices Biological Safety Levels (BSLs)**
   Facility BSL 1 ☐ 2 ☐ 3 ☐ 4 ☐ **Work Practices BSL** 1 ☐ 2 ☐ 3 ☐ 4 ☐
2. **Biological Safety Cabinet** Class I/II ☐ Class III ☐
3. **Personal Protective Equipment Needed for Procedure:** (left to right = increased protection)
   a. **Gloves** latex/nitrile required
   b. **Eye** safety glasses ☐ goggles + face shield ☐
   c. **Lab coat** white ☐ blue smock/coveralls ☐ space suit ☐
   d. **Respirator*** N-95/PAPR ☐ space suit ☐
4. **Medical Protection and Surveillance**
   a. **Medical Monitoring required** ☐ b. **Hearing Conservation Program** ☐
   c. **Vaccine recommended*** ☐ d. **Respiratory Protection Program** ☐
5. **Comments** _____

Note: *Vaccines and respirators require separate risk assessments.

Biosafety Officer's Signature

ACCIDENTS AND INCIDENT REPORTING – ROOT CAUSE ANALYSIS

Each laboratory must document all accidents, incidents and "near misses" so that remedial actions can be formulated and implemented. Accidents should be reported to the principal investigator and Biosafety and other relevant safety officers. Depending on the nature of the accident it will be investigated by the Safety officer and other senior management staff to ensure that there is reduced likelihood of a repeat of the same accident.

Details of accident and incident reporting requirements are contained in standard operating procedure. The findings of the accidents and incidents report including record analysis must be reviewed at local safety committees (see example Form 3).

Types of accidents and incidents and appropriate management

Personnel exposure to a hazardous substance such as infectious material or chemical: An "exposure incident" is specific contact (eye, mouth, other mucous membrane, respiratory tract via inhalation, non-intact skin, or parenteral) with potentially infectious materials that results from the performance of an employee's duties. An employee who sustains a known or potential exposure incident must immediately contact their line manager, or in their absence, Clinical Safety Officer the Biological Safety Officer, or Principal Investigator.

Near misses: Reporting of near misses (e.g. incidents that might have resulted in injury to a member of staff or visitor, or that could have led to the release of a biological agent, radiation or chemical hazard, but did not) are just as important to report as incidents which did lead to serious adverse consequences, as they allow root causes to be analysed and remedied and patterns to be observed.

Physical injury: Staff members who sustain any physical injury should be assessed by the Occupational Health Officer an accident report completed.

Infectious material spills: See "Emergency Procedures" section for details.

Form 3. Accident reporting document

Accident/Incident Report Form	No

Injured person's name	Age	Home address, postcode and telephone number
	Gender F ☐ M☐	

Job title	Department	Supervisor's name

Time and date of accident	Where did the accident happen?	How was the injured person treated ? (Tick the appropriate box)	Was the injured person absent from work or unable to perform their normal duties as a result of the accident? If yes, state the duration of their absence or incapacity (whole days only).
		First aid ☐ Hospital ☐ Other ☐	No ☐ Yes ☐ days If hospital treatment was needed, did the injured person remain there more than 24 hours? No ☐ Yes ☐

Accident or incident details – give a full description of what happened, including details of any injury or damage.

Describe the action to be taken to prevent a recurrence of this type of accident or incident.

Name and signature of person completing form	Name and signature of supervisor or administrator

This section is for Safety Office use

STANDARD OPERATING PROCEDURES

A Standard Operating Procedure (SOP) is a set of step by step instructions for carrying out a specific technique. They can be used for any technique, but this guide focuses on their use in documenting monitoring techniques, specifically for species and ecological communities. SOPs are used to stipulate how the monitoring will be undertaken, and provide quality assurance that the data collected will be consistent and therefore comparable. They should be clear and concise but with enough detail so that users with a basic understanding but limited experience can reproduce the procedure. The following is a list of key SOPs that relate to biosafety practices. However, all SOPs should include safety information and must have an associated risk assessment that details the precautions that must be observed to mitigate hazards and risks. A guide to writing SOPs is detailed in appendix 2.

- General laboratory rules
- Entry and Exit procedures
- Staff training
- Use of autoclave
- Verification of autoclave performance using Biological indicators
- Use of Biosafety cabinet class II
- Formaldehyde fumigation of the Laboratory
- Procedures for contractors working in BSL2/3
- Fumigation of biosafety cabinet laboratory
- Cleaning the small equipment in BSL3 for bring to outside BSL3
- Decontamination for the pipette
- Use and selection of Personal protective equipment
- Chemical/biological spill kit and spill clean up
- Infectious waste procedures
- Chemical waste procedures
- Non-infectious/non-chemical waste procedures
- Reporting Accidents and Incidents
- Sending and receiving sample
- Sterility testing reagents to remove from BSL3
- Packaging infectious materials
- Labelling of infectious materials
- Use of -80˚C Freezer
- Use of liquid nitrogen

BIOSAFETY MANAGEMENT – ADMINISTRATION

Biosafety Officer/ Biosafety Manager/ /Biosafety Advisor

A biosafety officer, also called a biosafety manager or biosafety adviser must be appointed at each institution. The function of the biosafety officer should normally be regarded as an advisory position and not directly responsible for managing biosafety, as this rests with those conducting and managing the work within the organization (e.g. scientific director, principal investigator, department head, laboratory manager, group leader). The role and knowledge of the biosafety advisor is important to develop, implement, maintain and continually improve a biosafety and biosecurity programme based on a management system. The advisor should be competent to perform the role, and allocated sufficient time and other resources to do the job effectively. In the execution of his/her biosafety management duties the advisor should be independent from those responsible for implementing the programme of work and have direct access to the top management representative when necessary.

Functions of the biosafety management advisor should include:

- Verifying, in conjunction with other relevant personnel, that all relevant biosafety considerations have been addressed;
- Advising or participating in the reporting, investigation and follow-up of accidents / incidents, and where appropriate referring these to management / biosafety management committee;
- Ensuring that relevant and up-to-date information and advice on biosafety management is made available to scientific and other personnel as necessary;
- Advising on biosafety management issues within the organization (e.g. management, biosafety management committee, occupational health department, security);
- Contributing to the development and / or delivery of biosafety training activities;
- Ensuring that all relevant activities are performed in compliance with biosafety regulations and that required biosafety authorizations for work are in place (CEN Workshop Agreement CWA 15793, p21).

Summary points: Biosafety manager

- The role of the Biosafety manager/advisor is:
- Verifying, in conjunction with other relevant personnel, that all relevant biosafety considerations have been addressed;
- Advising or participating in the reporting, investigation and follow-up of accidents / incidents, and where appropriate referring these to management / biosafety management committee;
- Ensuring that relevant and up-to-date information and advice on biosafety management is made available to scientific and other personnel as necessary;

- Advising on biosafety management issues within the organization (e.g. management, biosafety management committee, occupational health department, security);
- Contributing to the development and / or delivery of biosafety training activities;
- Ensuring that all relevant activities are performed in compliance with biosafety regulations and that required biosafety authorizations for work are in place.

Biosafety management committee

A biosafety management committee shall be constituted to act as an independent review group for biosafety issues. Reporting to senior management, the committee shall:

- Have documented terms of reference;
- Include a representative cross-section of expertise, appropriate to the nature and scale of the activities undertaken;
- Ensure issues addressed are formally recorded, actions allocated, tracked and closed out effectively;
- Be chaired by a senior individual (normally the biosafety officer);
- Meet at a defined and appropriate frequency, and when otherwise required (CEN Workshop Agreement CWA 15793, p21).

The functions of biosafety management committee include the following:

- To ensure all tasks performed in the workplace are carried out safely, efficiently and effectively.
- To oversee changes in the workplace which may affect the health, safety and welfare of employees.
- To regularly review the Programme occupational health policy and SOP's.
- To ensure regular risk assessment is performed.
- To keep track of statistics on accident records, ill health and sickness absence.
- To ensure all (laboratory) staff have health and safety training.
- To ensure there is regular inspection of the workplace by enforcing authorities or management staff, as appropriate.
- To regularly review and update the Programme biosafety manual.
- To approve the SOPs for the use of biological materials in laboratories annually or when changes are requested.
- To discuss and implement policies for the proper handling, disposal and safeguarding of biohazardous materials, chemical materials and radioactive materials.
- To review any incidents or accidents of a biological or chemical nature and recommend remedial actions to prevent reoccurrence.

- To review and update the Programme fire safety policy(ies), including working with flammable substances.
- In consultation with host organisations, develop fire evacuation plans and ensure necessary communication to staff.
- To review electrical safety in offices and laboratories.
- To review newly proposed protocols, revisions to projects that may increase risk, and projects involving work with new pathogens.

Summary points: Biosafety management committee

A biosafety management committee shall act as an independent review group for biosafety issues that reports to senior management. Major issues dealt with by the biosafety management committee include

- To ensure all tasks performed in the workplace are carried out safely, efficiently and effectively.
- To regularly review the Programme occupational health policy and SOP's.
- To ensure there is regular inspection of the workplace by enforcing authorities or management staff, as appropriate.
- To approve the SOPs for the use of biological materials in laboratories annually or when changes are requested.
- To discuss and implement policies for the proper handling, disposal and safeguarding of biohazardous materials, chemical materials and radioactive materials.
- To review any incidents or accidents of a biological or chemical nature and recommend remedial actions to prevent reoccurrence.
- To review newly proposed protocols, revisions to projects that may increase risk, and projects involving work with new pathogens

Induction/Training/Appraisal

Health and safety induction All staff and visitors must undergo an induction that includes consideration of health and safety (H&S) issues. It is the responsibility of the line manager or supervisor responsible for the staff member/visitor to contact the Biosafety administrator who will arrange a H&S induction as well as an appointment to see the clinical safety officer. The H&S induction must include:

- Documents that must be read and understood
- The identification of training needs
- Introduction to key staff members
- A site tour, with identification of fire escapes and procedures
- Staff health evaluation (vaccination requirements PPE, any specific underlying conditions requiring special consideration).

The Induction Form for staff to work in BSL2 and BSL laboratories must be signed off by the line manager or supervisor responsible for the staff member/visitor and the Area Safety Adviser (see example Form 4).

Health and safety training Prior to working in any laboratories, all staff must be trained in individual SOPs and protocols before undertaking them without supervision. The following should be considered and addressed:

- Definition of biosafety training needs;
- Provision of required biosafety training;
- Determination of effectiveness of biosafety training;
- Provision of refresher biosafety training;
- Restrictions on personnel to ensure they do not perform tasks for which they are not trained;
- Maintenance of adequate records (CEN Workshop Agreement CWA 15793, p25).

It is the responsibility of the individual's line manager or supervisor to provide this training. The following general guidance is provided.

Undergraduate and work-experience students must receive instructions and clear guidelines on all the techniques to be used. They must be under close supervision at all times and must not be allowed to undertake any techniques in which they have not been trained nor are they allowed to undertake any laboratory work alone.

Departments are expected to provide additional and more specific biosafety training for *inexperienced post-first qualification employees and students* (e.g. postgraduate students or research assistants, newly qualified technicians or scientific officers). Line managers and supervisors must also ensure specific training on the techniques to be used is provided. The importance of using safety equipment appropriately must be emphasized in such training.

Newly appointed postdoctoral workers or experienced research assistants or technicians may require only minimal biosafety training to work at BSL1 or 2 when sufficient instruction is provided. The supervisor must assess competence and apply judgement as to what levels of supervision are then required. All work at BSL3 requires competence to be demonstrated andapproved both in handling the organism(s) to be used and in the functioning of the BSL3 facility. Newly approved BSL3 workers must be supervised for a period of time appropriate to the hazardous nature of the organism and to the degree of competence gained by the worker. Where an organism is to be used for the first time, competence can be demonstrated by undertaking training in other laboratories and/or by the use of sentinel organisms in a lower hazard group at full BSL3.

Assessing competence Competence is defined in relation to appropriate education, training and / or experience, together with a demonstrable ability to perform the task in a safe / secure manner. Procedures should address:

- Definition of competency needs;
- Demonstration of successful completion of required training;
- Demonstration of ability to perform tasks under supervision and unsupervised;
- Restrictions on personnel who have not demonstrated competence to ensure they do not perform tasks for which they are not eligible;

- Maintenance of adequate records.

No worker should be exempt from demonstrating competence irrespective of rank, experience or background (CEN Workshop Agreement CWA 15793, p24).

Summary points: Inductions and training

- All staff and visitors must undergo an induction that includes consideration of health and safety (H&S) issues.
- Prior to working in any laboratories, all staff must be trained in individual SOPs and protocols before undertaking them without supervision. The following should be considered and addressed;
  - Definition of biosafety training needs;
  - Provision of required biosafety training;

    Determination of effectiveness of biosafety training;
  - Provision of refresher biosafety training;
  - Restrictions on personnel to ensure they do not perform tasks for which they are not trained;
- Competence is defined in relation to appropriate education, training and / or experience, together with a demonstrable ability to perform the task in a safe / secure manner.

Staff health

The organization shall ensure that risk to worker health, and that of other personnel whose health could be directly impacted by exposure to biological agents and toxins, is managed effectively including prevention and protection measures (CEN Workshop Agreement CWA 15793, p31-32). The requirements of the staff health/medical surveillance programme shall be determined by a defined health hazard identification and risk assessment process involving all relevant personnel including the following staff,

- the biosafety management advisor or biosafety officer;
- the occupational health professional (also known as the clinical safety officer);
- facility personnel and employee representatives;
- external experts, including emergency responders;
- biorisk/biosafety management committee members;
- veterinary and animal care facility staff;
- communicable disease specialist;
- scientific management (CEN Workshop Agreement CWA 15793, p31-32).

The programme should address the needs of all individuals who may be associated with the facility, including providing assurance that contractors (including maintenance staff) and visitors receive the required level of protection and advice in line with the activities they will perform,

Form 4. Staff induction document

Staff Induction Checklist

Name:		Date of employment:	
Buddy:		Status (please circle):	Employee/ Visitor
Area Safety Officer:		Project Supervisor:	

Notes for completion of this document

1. This document is to be completed by the staff member BEFORE starting work in the laboratory with the assistance of the Project supervisor and the Area Safety Officer (ASO)

2. This document is used to familiarise the new staff member with the physical and safety aspects of MORU.

3. This document is also used to identify training requirements.

4. A buddy must be appointed to the staff member to assist the new staff member for the first week of employment with familiarisation of the laboratory and surroundings.

5. If you are unclear about any aspect of this document or have any safety concerns please contact your buddy for clarification.

6. It is you responsibility to read and understand the safety policy. You must not start work in the laboratory unless you have read and understood this document.

1. Induction checklist

	Action	**Done?**
Introduction	Have you met the director?	Yes/No/NA
	Have you met the Office Manager	Yes/No/NA
Security	Do you require keys or swipe card?	Yes/No/NA
	Do you require email and network access (if necessary)?	Yes/No/NA
	Inform of after hours procedures, e.g. Exits available and times of locking?	Yes/No/NA
Safety	Have you made an appointment to meet the Biological Safety Officer	Yes/No/NA
	Have you made an appointment to meet the Clinical Safety Officer?	Yes/No/NA
	Have you been shown fire exits, fire equipment and fire assembly points?	Yes/No/NA
	Have you been provided with any necessary protective clothing. Lab coats, etc.	Yes/No/NA
	Have you read and understood the Institutional Safety Policy?	Yes/No/NA

2. Training requirements

This section must be completed in conjunction with the project supervisor.
The project supervisor is responsible for ensuring all training is completed.

	Training required	Training signed off by trainer
Centrifuge use & cleaning		
Microfuge use & cleaning		
Biological safety cabinet class ii		
Use & cleaning		
Autoclave		
Power paks		
Electrophoresis equipment		
Low temperature freezers		
Gel doc equipment		
BSL 3 Air handling equipment		
Flammable chemicals -Use and Storage		
Liquid nitrogen		
Disposal of infectious waste		
Disposal of non-infectious waste		
Disposal of radioactive waste		
Disposal of carcinogenic/mutagenic waste (especially ethidium bromide)		
Disposal of solvent waste		
Disposal of glass waste		
Category 3 lab restrictions		
Sharps		
Handling of biological materials		

3. Short Course Training / Practical

1.Subject Leptospirosis Melioidosis Scrub Typhus Malaria Other

2. Scope .....

3. Training / Co-ordinator .....

4. Date .....

Please read the next section carefully. I declare that I have,

1. Read and understood the institutional safety policy; and

2. Received all required training necessary to perform my work in a safe manner.

Signed (Staff member/Visitor) _____ **Date** _____

Signed (Project supervisor) _____ **Date** _____

Signed (Area Safety Officer) _____ **Date** _____

Signed (Departmental Safety Officer) _____ **Date** _____

as well as safeguarding workers' families. Personnel considered to have significant risk of exposure should be identified and their healthcare needs assessed. This should include the need for vaccination, PPE requirements and emergency measures that encompass isolation / testing in the event of exposure. The health including the immune status of the individual should be considered and periodic checks as appropriate to work conditions should be established (CWA 15793:2011, p31-32).

An overall staff health policy should also include first aid and the following are recommended,

- An immunisation policy that includes an assessment of immunization requirements prior to starting any laboratory or clinical procedures and any individual health issues. Based on risk, the need for vaccination shall be identified and shall cover groups identified as being potentially exposed to biological agents or toxins. Note that measures should be implemented to identify non-responders to vaccination for example, post-vaccination immunity testing. Areas requiring vaccinations to enter should be posted.
- Staff with personal health issues that requires any additional protection (eg, pregnancy, diabetes, epilepsy, heart disease) must report to the Clinical Safety Officer for counselling as soon as the issue arises. This counseling will remain confidential.
- Eye wash facilities are available in all laboratories. If these are located at a sink using a hose type arrangement then these must be flushed weekly. If eye washers are of the bottle variety then these must be checked for expiry at least annually.
- First aid kits must be available in all laboratory areas or in common room areas. First aid kits are checked monthly to ensure that stocks of all items are maintained and not out of date.
- Staff are strongly encouraged to attend first aid training when it is provided.
- It is strongly suggested that staff carry a MEDICAL ALERT CARD (see figure 2) so that in the case of a medical emergency, medical staff are aware that they may be dealing with a laboratory acquired infection (CEN Workshop Agreement CWA 15793, pp44).

Summary points

- All staff must be medically assessed prior to starting work
- Staff with personal health issues that require any additional protection (e.g. regnancy, diabetes, epilepsy, heart disease) must report this issue immediately
- An immunisation policy that includes an assessment of immunization and post-vaccination requirements prior to starting any laboratory or clinical procedures be implemented
- Areas requiring vaccinations to enter should be posted.
- First aid kits must not be out of date and must be available in all laboratory areas or in common room areas.
- Staff are strongly encouraged to attend first aid training when it is provided.
- Staff carry a MEDICAL ALERT CARD in case of medical emergency

Figure 2. Example of a MEDICAL ALERT CARD as suggested by CDC (accessed at http://www.cdc.gov/biosafety/publications/MedicalAlertTemplate.docx**)**

Company Logo

Medical Alert
Attending Physicians

This card holder works with at [insert: workplace] in areas where hazardous biological, chemical, or radioactive agents or materials may be used. In the event of an unexplained illness, please contact the supervisor(s) listed on reverse side for information on specific agents or materials to which this person may have been exposed.

Company: **Clinic:** **Hours Phone:**

Employee Name _____

Employee Emergency Contact _____

Telephone Number _____

Organizational Unit _____

1° Supervisor **Work Telephone** **Alternate Telephone**

2° Supervisor **Work Telephone** **Alternate Telephone**

Supervisor **Work Telephone** **Alternate Telephone**

Supervisor **Work Telephone** **Alternate Telephone**

Note:

The template above is meant to serve as text for two sides of a standard business-size card (a dashed line separates the two sides). Modify/ resize according to your institution's standards before printing as cards.

Site safety /biosafety inspections and audits

The organization shall ensure that a programme of inspection and audit is conducted which is appropriate to the risk associated with the facility. Inspections and audits shall be conducted at planned intervals (normally at least annually) to determine if the biosafety management system conforms to the documented plans, the physical and technical facilities are compliant and that it is effectively implemented and maintained using a standard template such as those described in the CDC BMBL (Appendix 2 & 3).

Audits should be performed by competent individuals who are independent of the activity being audited. Random, unannounced inspections and inventory audits can help ensure compliance at all times, not just in time for scheduled inspections. Inspections may be frequent checks on specific areas conducted to ensure sufficient standards are being maintained (e.g. disinfectant levels / concentrations and air exchange rates / maintenance of directional air flow), or more extensive but less frequent inspections of laboratories, facilities or other operations.

Management responsible for the area being inspected / audited shall ensure that any actions are taken without undue delay to eliminate detected non-conformities and their causes. Follow-up activities arising shall include the verification of the actions taken and the reporting of verification results. Staff responsible and deadlines for rectifying non-compliance must be identified. Records should be maintained of findings of inspections / audits, including action taken to close out any non-conformities or improvement opportunities (CWA 15793:2011; p44)

Summary points: Inspections and Audits

- Safety/biosafety audits be performed at least one time per year
- If possible, the auditor should be independent or not directly working in the area
- Use a standard template for inspections to identify areas of non-compliance
- Follow up on areas of non-compliance
- Identify staff responsible and time limits to rectify areas of non-compliance
- Random inspections may be useful.

Biosafety management – Work practises

Laboratory Code of Practice The Laboratory Code of Practice must be complied with at all times. Below is a suggested list of rules that must be followed when working in the laboratory.

- Access is restricted to authorized persons
- An appropriate risk assessment must be completed prior to any new work starting, in order to identify additional controls above those already outlined in this & other documentation.
- Eating, chewing, drinking, smoking, applying cosmetics, storing of food and outdoor clothing in the laboratory is banned.
- Mouth pipetting must not be used under any circumstances for any reason.
- All workers in the laboratory must cover cuts and abrasions with a waterproof dressing before entering the laboratory.

- All laboratory procedures with infected material must be conducted in a microbiological safety cabinet.
- Sharps and glassware should not be used in the laboratory unless there is no alternative and a risk assessment is in place. If sharps are used then they must be placed directly in a sharps bin for disposal.
- Standard glass bottles and containers should not be used in the BSL3. All groups should ensure that safe break or reinforced glassware is used where a plastic alternative is not available. Broken glass must be autoclaved before disposal. Never handle broken glass directly.
- Samples must be centrifuged only in sealed safety buckets.
- All waste materials must be made safe before leaving the laboratory
- Surfaces must be disinfected with 1% Virkon or another suitable disinfectant for 30 minutes then 70% ethanol following spillages. Safety cabinets must be disinfected after use.
- All cardboard should be removed from the Containment Level III suites. It must be made safe by autoclaving before leaving the laboratory.
- Accidents:
  - All accidents should be immediately reported to the Supervisor and the Safety Officer.
  - In the event of an accident resulting in a wound, immediately encourage it to bleed, wash thoroughly with soap and water but DO NOT SCRUB, cover with a waterproof dressing.
  - In the event of contamination of skin, conjunctivae or mucous membranes, immediately wash thoroughly
  - All accidents and incidents should be immediately recorded in the accident book.

PERSONAL PROTECTIVE EQUIPMENT (PPE) AND RESPIRATORY PROTECTIVE EQUIPMENT (RPE)

PPE POLICY

The organization shall ensure that PPE needs are identified and suitable equipment is specified, made available, used and maintained appropriately within the facility. Measures in place should include:

- Ensuring adequate information is used in selecting PPE (e.g. risk assessments, review and analysis of tasks, employee feedback, etc.);
- Ensuring all personnel who have to use PPE (including scientific staff, visitors and contractors) are identified and supplied with correct fitting equipment and clothing;
- Explicitly addressing selection and use of PPE in SOPs, training and competency assessments;
- Defining and conducting an appropriate programme to ensure that routine checks and maintenance of PPE are defined and carried out;
- Defining and addressing the need for and provision of replacement and spare PPE;
- Identifying and controlling the hazards associated with PPE itself (e.g. impaired dexterity or visibility);
- Providing adequate PPE for use during both normal and emergency working conditions;
- Ensuring procedures are in place for the cleaning and if appropriate the validated decontamination of used PPE including the safe storage prior to decontamination.

Personal protective equipment should be used in conjunction with, but never as a substitute for, reasonable and appropriate administrative and engineering controls. PPE should be used in accordance with established standards and manufacturers specifications. PPE should be made available by the employer at no cost to the staff (CEN Workshop Agreement CWA 15793, p31).

Examples of PPE application with differing Risk Group organisms

The following is general guidance for the application of PPE

RG2 organisms working at BSL2 The following PPE must be worn at when working at BSL2 with RG2 agents.

- Gloves Latex or Nitrile
- Cloth gown or lab coat
- Goggles or glasses
- Closed-toed shoes

RG3 organisms working at BSL3 with no aerosol generation The following PPE must be worn at when working at BSL3 with RG3 agents.

- Gloves Latex or Nitrile
- Cloth gown or lab coat
- Goggles or glasses
- Covered shoes

RG3 organisms working at BSL3 with aerosol generation The following PPE must be worn at when working at BSL2 with RG2 agents when there is the potential for aerosol generation such as sonication or vortexing as specified by the SOP.

- Fit tested N95 mask, full-face respirator or PAPR
- Gloves Latex or Nitrile
- Cloth gown or lab coat
- Goggles or glasses
- Covered shoes

The following guidance on PPE should be applied,

- Covered footwear must be worn (i.e. no open-toed shoes, sandals or flip-flops).
- Unique color laboratory coats should be assigned to the BSL3. They must be worn at all times whilst in the laboratory, removed before leaving and autoclaved before laundering.
- Double gloves must be worn at all times within the BSL 3 laboratories. These gloves should be pulled over the sleeves of the laboratory coat to limit any possible skin exposure during work. It is acceptable to change your gloves whilst in containment level III, so long as exposed hands do not touch any potentially infectious surface
- Disposable gloves must not be reused.
- In the event of gloves becoming damaged or grossly contaminated the gloves must be discarded, hands washed and new gloves put on.
- On completion of the work and before leaving the suite, hands must be washed.
- Appropriate eye protection (e.g. safety glasses) must be worn at all times.
- Where the risk from splashing is significant, then additional eye protection in the form of goggles or visor should also be worn.
- On completion of work, the workstation and all equipment must be disinfected.

Fit testing of respirators

A "fit test" tests the seal between the respirator's facepiece and the user's face. It takes about fifteen to twenty minutes to complete and is performed at least annually. After passing a fit test with a respirator, you must use the exact same make, model, style, and size respirator on the job. There are two types of fit tests: qualitative and quantitative.

- **Qualitative fit testing** is a pass/fail test method that uses your sense of taste or smell, or your reaction to an irritant in order to detect leakage into the respirator facepiece. Qualitative fit testing does not measure the actual amount of leakage. Whether the respirator passes or fails the test is based simply on you detecting leakage of the test substance into your face-piece. There are four qualitative fit test methods:
  - Isoamyl acetate, which smells like bananas;
  - Saccharin, which leaves a sweet taste in your mouth;
  - Bitrex, which leaves a bitter taste in your mouth; and
  - Irritant smoke, which can cause coughing.
- **Quantitative fit testing** uses a machine to measure the actual amount of leakage into the facepiece and does not rely upon your sense of taste, smell, or irritation in order to detect leakage. The respirators used during this type of fit testing will have a probe attached to the facepiece that will be connected to the machine by a hose. There are three quantitative fit test methods:
  - Generated aerosol;
  - Ambient aerosol; and
  - Controlled Negative Pressure.
  - Quantitative fit testing can be used for any type of tight-fitting respirator.

Many workers need to wear prescription glasses or personal protective equipment, such as safety goggles or earmuffs, while performing a job. If you fall into this category, then you must wear these items during the fit test to be sure they don't interfere with the respirator's fit.

Also, the fit of your respirator must be retested whenever you have a change in your physical condition that could affect the fit of you respirator. Such changes could include:

- large weight gain or loss;
- major dental work (such as new dentures);
- facial surgery that may have changed the shape of your face; or
- significant scarring in the area of the seal.

Any of these changes could affect the ability of your respirator to properly seal to your face, which could allow contaminated air to leak into your respirator facepiece.

Summary points: PPE policy

The organization shall ensure that PPE needs are identified and suitable equipment is specified, made available, used and maintained appropriately within the facility. Measures in place should include:

- Ensuring adequate information is used in selecting PPE (e.g. risk assessments, review and analysis of tasks, employee feedback, etc.);
- Ensuring all personnel who have to use PPE are identified and supplied with correct fitting equipment and clothing;

- Explicitly addressing selection and use of PPE in SOPs, training and competency assessments;
- Defining and conducting an appropriate programme to ensure that routine checks and maintenance of PPE are defined and carried out;
- Defining and addressing the need for and provision of replacement and spare PPE;
- Identifying and controlling the hazards associated with PPE itself
- Providing adequate PPE for use during both normal and emergency working conditions;
- Ensuring procedures are in place for the cleaning and if appropriate the validated decontamination of used PPE including the safe storage prior to decontamination

PPE DON AND DOFF GUIDANCE

The safe and effective use of PPE requires that the user be trained and competent in the proper way to put-on (don) and take off (doff) PPE in order to prevent exposures or contamination of themselves and the laboratory environment. Appendix 5 includes as an example the PPE Don and Doff section from a Biosafety Manual used by INIA – Spain. Appendix 5 demonstrates the useful approach of including Don and Doff guidance in SOP format, as well as demonstrates appropriate don and doff technique using clear step-wise photographs and checklists to verify compliance to the PPE requirements of the laboratory.

BIOLOGICAL SAFETY CABINETS

Class II biological safety cabinets (BSCs) are vital pieces of laboratory equipment in many life science applications requiring contamination control. In addition to providing a workstation with aseptic conditions for product protection, a BSC also helps protect laboratory personnel from exposure to aerosols of hazardous substances and prevents the release of such hazards into the environment. Field certification is the method by which proper BSC operation is verified over time, to be sure that product, personnel, and environmental protection are maintained. Requirements for Class II biological safety in the United States are established by the NSF (National Sanitary Foundation) and published under NSF/ANSI Standard 49. Class1 and class III BSC also exist and are generally used only for special applications. Class II cabinets are by far the most prevalent, and NSF 49 is specific to Class II BSCs. NSF 49 includes definitions of the types and function, acceptable materials, design and construction requirements, and performance requirements for Class II BSCs. NSF International manages a program of type testing and products of dictation for class to BSCs. To ensure that individuals performing certification are properly certified and qualified, NSF International also administers a program biosafety cabinet Field certified accreditation.

TYPES OF CLASS II BIOLOGICAL SAFETY CABINETS

All Class II biological safety cabinets offer product, personnel, and environmental protection from biological and other aerosolized contaminants. Product protection is offered by unidirectional (commonly called "laminar") downflow air in the work chamber, generated by the cabinet blower pushing air through the supply HEPA filter. Personnel protection comes from the intake air pulled into the front access opening of the cabinet. Environmental protection is provided by HEPA filters in the exhaust air stream of the cabinet. Different types of Class II BSCs are utilized when protection from chemical vapor hazards is also a concern, as HEPA filters do not capture gases or vapors. Type B1 and B2 cabinets must be directly connected to the building's exhaust system for venting to the outdoors via a hard connection. Type A1 and A2 cabinets usually return their filtered exhaust air to the room but may optionally be connected to the building exhaust system with a canopy, formerly called a "thimble," connection (see Fig. 3). Note that many existing installations of exhausted Type A cabinets may utilize a direct connection to the exhaust system. However, more recent revisions of the NSF 49 standard recommend use of the canopy exhaust, and in the future hard connections for Type A cabinets will most likely not be allowed at all. Note that many existing installations of exhausted Type A cabinets may utilize a direct connection to the exhaust system. However, more recent revisions of the NSF 49 standard recommend use of the canopy exhaust, and in the future hard connections for Type A cabinets will most likely not be allowed at all. Current NSF 49 Standards require that Class II Type A1 or A2 cabinets with canopy connections must be fitted with an audible alarm and visible exhaust alarm to indictate when air flow is being returned to the room rather than being exhausted out.

USE OF CLASS II BIOLOGICAL SAFETY CABINETS (BSC)

When properly located in a room, maintained, and used in conjunction with good laboratory techniques, BSCs provide effective primary containment for work with human and animal pathogens. The effectiveness containment while working in a BSC is contingent upon employees being trained in its correct use and following those procedures.

Class II BSC

Class II BSCs are designed for personnel, product and environmental protection. They are designed for work involving microorganisms in Biosafety levels (BSL) 2, 3 and in BSL-4 suit laboratories. Class II BSC and are classified as type A2, or B1, B2 based of construction type, airflow velocities and patterns, and exhaust systems. Regardless of the type, they all employ HEPA filtration to purify incoming and exhaust air.

BSCs are designed to be operated 24 hours per day, and some investigators find that continuous operation helps to control the laboratory's level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, maintaining room air balance is an overriding consideration. In some instances, room exhaust is balanced to include air discharged through ducted BSCs. In cases where flexibility to allow for the of minute use of volatile chemical and radionuclides is desired, Class II A2 BSC may be preferred as they can accommodate this research requirement. They are more energy conservative than Class II B1 and B2 BSC.In the case of Class II BSC it is noteworthy that the air curtain at the front of the cabinet is fragile and can be disrupted by rapid movements of the user, people walking parallel to the BSC, by open windows, opening and closing the lab door, air supply registers or laboratory equipment that creates air movement nearby (e.g., vacuum pumps, centrifuges).

Table 2. Description of the differing types of Class II biosafety cabinets

Class II BSC Type	Minimum intake velocity (fpm)	Construction	Airflow pattern	Volatile toxic chemicals (gases or vapors) permitted
A1	75	May have biologically contaminated ducts and plenums under positive pressure to the room	Downflow and inflow air mix in a common plenum, approximately 70% recirculated as downflow, 30% exhausted	No
A2	100	Biologically contaminated ducts and plenums must be under negative pressure to the room, or surrounded by negative pressure ducts and plenums	Downflow and inflow air mix in a common plenum, approximately 70% recirculated as downflow, 30% exhausted	No – when exhaust air is vented back into the room Yes – minute quantities allowed when canopy connected and exhausted to the outdoors
B1	100	Biologically contaminated ducts and plenums must be under negative pressure to the room, or surrounded by negative-pressure ducts and plenums	Approximately 60% of downflow air exhausted through a dedicated duct; the remainder of downflow air (approximately 40%) mixes with intake air and is recirculated	Yes – minute quantities allowed
B2	100	Contaminated ducts and plenums must be under negative pressure to the room, or surrounded by negative- pressure ducts and plenums	Downflow air drawn from laboratory; inflow and downflow air exhausted with no recirculation in the cabinet or return to the laboratory (100% exhausted)	Yes – as an adjunct to micro-biological work

Start-up procedures for work in the Class II BSC

- Prepare a written checklist of materials necessary for a particular activity.
- Turn off UV lights if in use and ensure that the sash is in the appropriate position.
- Turn on fluorescent light and cabinet blower, if off.
- Ensure the drain valve under the work surface is closed prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.

- Check the air intake and exhaust grilles for obstructions and materials that may have become lodged beneath the grille.
- If the cabinet is equipped with an alarm, test the alarm and switch it to the "on" position.
- Confirm inward airflow by checking the cabinet magnahelic gauge or other indicator.
- Wash your hands prior to donning gloves. Laboratory coats should be worn buttoned (BSL-2) or closed front gowns (BSL-3) over street clothing; latex, nitrile or other similar gloves are worn to provide hand protection. A solid front, back-closing lab gown provides better protection of personal clothing than a traditional lab coat. Gloves should be pulled over the knitted wrists of the gown, rather than worn inside. Elasticized sleeves are an option to be worn to protect the investigator's wrists.
- To the extent you can reach without opening the sash, disinfect the work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:100 dilution of household bleach (i.e., 0.05% sodium hypochlorite), or other disinfectant as determined by the Investigator to meet the requirements of the particular activity. When bleach is used, a second wiping with sterile water or 70% EtOH is needed to remove the residual chlorine, which will eventually cause pitting or corrosion of stainless steel surfaces. Wiping with non-sterile water may re-contaminate cabinet surfaces, a critical issue when sterility is essential (i.e. performing work with tissue, organ and cell cultures).
- Wipe down materials required for work and place the materials required for the procedure in the cabinet.
- The working surface may be lined with absorbent paper with plastic backing to facilitate routine cleanup and reduce splatter and aerosol formation during an overt spill. It can be folded and placed in an autoclave biohazard bag when work is completed.
- Segregate "clean" items from "contaminated" items and plan to work consistently moving from clean to dirty.
- Horizontal pipet trays or aspiration flasks with decontaminant and small biohazard containers should be placed in the dirty area toward the back of the BSC.
- Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment. Extra supplies (e.g., additional gloves, culture plates or flasks, additional supplies of culture media) should be stored outside the cabinet. Do not overload the BSC, only the materials and equipment required for the immediate work should be placed in the BSC.
- Nothing should be placed on or obstruct the vents (grills), e.g. no papers, pipette tip boxes, pipetters, gloves, glassware, etc.
- Wait 5 minutes to purge airborne contaminants from the work area once the cabinet is prepared.

Procedures for working in the Class II BSC

- Before beginning work, the investigator should adjust the stool height so that his/her face is above the front opening and they are ergonomically comfortable. The front grill must not be blocked with research notes, discarded plastic wrappers, pipetting devices. The users arms should not rest on the front grill, rather they should be slightly elevated so air flows smoothly into the grille and across the work surface.

- Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants.
- Perform operations on the work surface at least four to six inches from the inside edge of the front grille and optimally toward the rear 1/3 of the work area when using aerosol-generating equipment (i.e., vortex mixers, microfuges, sonicators).
- Avoid movement of materials or excessive, sweeping movement of hands and arms through the front access opening during use; when you do enter or exit the cabinet, do so by moving arms in and out slowly and perpendicular to the face opening of the cabinet, to reduce disrupting the air curtain. Allow the cabinet to stabilize before resuming work.
- Keep discarded, contaminated material to the rear of the cabinet; do not discard materials in containers outside of the cabinet.
- Do not work with open flames inside the cabinet, rather, use disposables when possible, or pre-sterilize loops and supplies prior to introduction into the BSC. A small micro incinerator can be used for sterilizing loops.
- If using an aspiration flask, employ an overflow flask with decontaminating agent between it and the building vacuum system, or place a cartridge filter between the vacuum trap and the source valve in the cabinet.
- If there is a spill during use, surface decontaminate all objects in the cabinet; disinfect the working area of the cabinet while it is still in operation (do not turn the cabinet off).

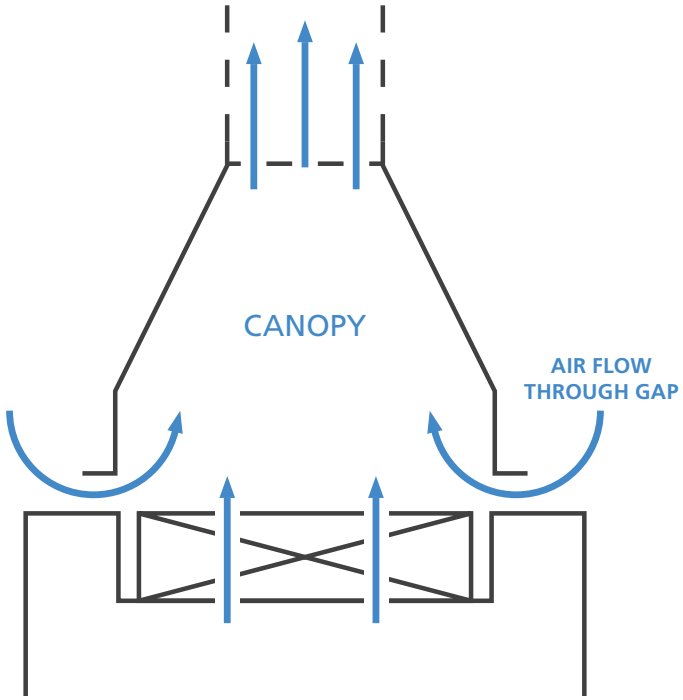
Procedures upon completion of work in Class II BSC

- Allow the cabinet to run for 5 minutes with no activity.
- Close or cover open containers before removing them from the cabinet.
- Surface decontaminate objects in contact with contaminated material before removal from the cabinet or place items to be decontaminated in a sealable container for transfer to an autoclave or other means of decontamination.
- Remove contaminated gloves and dispose of them as appropriate; wash hands.
- Don clean gloves, and ensure that all materials are placed into biohazard bags within the cabinet.
- Using a suitable decontaminant to clean the interior surfaces of cabinet. If the decontaminant is corrosive, followed this procedure by wiping down with a non-corrosive disinfectant (e.g., 70% ethanol).
- Turn off the fluorescent light and cabinet blower when appropriate (some cabinets must be left on at all times; if you are unsure, check with your cabinet certifier, safety officer or building maintenance personnel).
- Allow the BSC to run for 5 minutes to purge and clean the interior air.
- Remove gloves, and be sure to wash your hand before leaving the lab.

Summary points: Biological safety cabinets

- BSCs must be used for all infectious work at with RG2 organisms or above
- Staff must be training in the use of a BSC
- BSCs must meet NSF49 or equivalent standard
- BSCs must be field certified at least annually by certified testers
- BSCs must be field certified on installation and commissioning
- Practice emergency procedures for spills in a BSC
- Use an uninterruptable power supply (UPS) to ensure continuity of electricity supply

Figure 3: Class II A2 biological safety cabinet exhaust system with a canopy



DISINFECTION AND WASTE MANAGEMENT

INFECTIOUS WASTE MANAGEMENT AND POLICY

The organization shall establish and maintain an appropriate waste management policy for biological agents and toxins. The organization should have a validated procedure for the inactivation of biological agents and toxins waste products. The following elements should be considered for a waste management policy:

- Ensure programme is in place to minimize the waste production;
- Ensure effective waste audit trails are in place and documented;
- Provide adequate facilities and procedures for the storage of waste (including short term storage);
- Ensure methods are available for effective segregation and decontamination of mixed waste (e.g. infected animals that have received radioactive materials);
- Ensure appropriate packaging material is used to contain the waste and to maintain its integrity during storage and transportation.
- Clear workflow from infectious and waste materials is performed to determine ingress and regress of samples and waste to prevent accidental exposure of staff to infectious materials (CEN Workshop Agreement CWA 15793, p30).

The following is some general guidance on dealing with different types of waste.

Biological waste

All waste from the laboratory must be autoclaved (121oC for 30 minutes) prior to disposal, with the exception of aerosol or pressurized containers, radioactive waste and volatile substances.

- All waste must be autoclaved prior to outside disposal A biological indicator is also placed within every infectious waste load to verify the performance of the autoclave.
- After autoclaving the autoclave bag is placed inside a black plastic bag taken by the cleaning staff for outside disposal.

Blood waste

All laboratory waste heavily contaminated with human blood products must be kept separate, soaked in 3% Virkon for at least 30 minutes, autoclaved using the above-mentioned procedures and placed in a black bag for incineration. Do not use chlorine based disinfectants in autoclaves or other metal surfaces as it causes corrosion.

Glass Vacutainers

Glass Vacutainers should be soaked in a minimum of 3% Virkon for at least 30 minutes. The tubes should then be placed in a sharps box prior to disposal. Other glass must be kept separate and once autoclaved placed in an approved glass bins in the wash room. Clean broken glass must be kept separate from empty bottles. Glass should not be used in a BSL3 lab.

Sharps

Sharps should not be used in BSL3 laboratory unless there is no alternative. A risk assessment must be completed for work involving sharps in the BSL3 laboratory. At BSL2, sharps bust be disposed of in dedicated sharps disposal containers and disposed of when it reaches the FULL indicator line. The sharps container should be placed inside and autoclave bag and autoclaved prior to being sent for disposal.

Chemical waste

The person or group who generates or possesses the chemical is responsible for ensuring that the waste is disposed of correctly. Only work with chemicals in the fume cupboard. A risk assessment must be written for all chemical activities and waste disposal.

Space and Equipment Decontamination

The natural form of formaldehyde is a gas. However, formaldehyde is more readily available as a 40% aqueous solution called 'formalin'. Gaseous formaldehyde is used for the fumigation of buildings, rooms or equipment which can be sealed. Fumigation with formaldehyde is effective against most viruses and bacteria, including the acid-fast Mycobacteria. Formaldehyde gas is relatively unstable and can sometimes explode. It is difficult to achieve an even distribution and penetration of formaldehyde gas throughout buildings, which may lead to incomplete effect. For formaldehyde fumigation to be complete, the temperature must be above 55°F (13°C) and relative humidity must be above 70%. Spraying with hot water is sometimes necessary to achieve these conditions. For fumigation purposes, formaldehyde gas can be produced by oxidizing formalin with potassium permanganate, by heating paraformaldehyde, by mechanically generating a mist of formalin, or by applying complex mixtures from which formaldehyde is slowly released after application.

Formaldehyde fumigation is normally performed following and infectious organism spill or leakage or prior to testing or repair of equipment. The use of formaldehyde in disinfectant situations is declining, due to the strong, irritant odour, corrosiveness, fibrolytic properties and toxicity. The use of formaldehyde is illegal in some countries due to environmental concerns. Alternatives to formaldehyde include vaporised hydrogen peroxide (VHP) and chlorine dioxide (CD) however these decontamination methods must be validated prior to use. The following principles should be adhered to when performing gaseous decontamination procedures:

- Due to the toxic nature of these fumigants only trained personnel should be allowed to perform this procedure.
- Staff training for performing fumigations should include PPE, signage, validations, methodologies, gas monitoring, and emergency procedures.
- All staff (including ancillary staff such as cleaners) must be informed in advance of fumigations so that they can plan their work accordingly.
- Warning and Do Not Enter signs must be placed strategically to prevent access to the fumigation. Note – consider appropriate signage for staff who may be unable to read or where multiple languages are spoken.
- All fumigations must have their performance validated using biological indicators. Results must be recorded including failures.

Verification of disinfection and sterilisation processes.

Whatever the biological agents and toxins handled, it is likely that a number of effective inactivation methods will be available. The organization should ensure that there are data available to demonstrate that the methodology selected is capable of inactivating the biological agents and toxins under the specific conditions encountered in the facility.

Therefore, it is imperative that the three critical steps in testing the efficacy of the sterilizer process is followed which include: Physical monitoring, the use of external and internal CI (chemical indicators) and the frequent use of BI's (biological indicators).

- Physical Monitoring: Includes the recording of the sterilizer's temperature, time and pressure on digital printouts, recorders, displays and/or gauges. This provides real-time assessment of the sterilization cycle and a permanent record in the form of a chart or printed readout. Physical monitoring helps to detect malfunctions sooner allowing you to take corrective actions.

- Chemical Indicators: Chemical Indicators are a critical part of an effective quality assurance program and should be used in conjunction with physical monitoring and biological indicator testing. Best practices recommend that external and internal CI's should be used for all items being sterilized. Chemical indicators range from Class 1,2,3,4 and 5 as briefly described below:

  - Class 1 chemical indicator: A process indicator often referred to as an external indicator. They distinguish between processed or unprocessed items and indicate that they were exposed the sterilizer. These are seen as indicators on pouches, sterilizer tapes or labels.

  - Class 2 chemical indicator: Is designed for a specific test procedure (e.g. The Bowie-Dick test used to determine if air removal has been adequate in dynamic-air-removal sterilizers)

  - Class 3 chemical indicator: Is a single parameter indicator. It is designed to react to one of the critical parameters of sterilization.

  - Class 4 chemical indicator: Is a multi-parameter indicator. It is designed to react to two or more of the critical parameters of sterilization. An example is a Browne's tube which is a small glass tube containing a red heat-sensitive dye used as a chemical indicator for sterilization. The dye changes colour to green after a defined period of time at a certain temperature but is not proof of sterilization

  - Class 5 chemical indicator: Is an integrating indicator. This chemical indicator reacts to all three parameters of sterilization which include proper amount of time, temperature and pressure of the sterilizer. They have been correlated to the performance of a biological indicator when used according to the manufacturers conditions noted on the label.

- Biological Indicators: (Also known as spore tests or BI's) are paper filter strips inoculated with millions of spores. They provide the only way the sterility process can be measured Sterilizer manufacturers validate their sterilizers by using biological indicators, and therefore BI's should be used to check the sterilizer's effectiveness frequently and on a routine basis.

GUIDANCE ON THE SELECTION OF CHEMICAL DISINFECTANTS

Based on http://www.ucl.ac.uk/medicalschool/msa/safety/docs/laboratorydisinfectioncop.pdf

Properties and usage of recommended disinfectants

Considerations There must be adequate contact with the disinfectant to enable it to be effective, for example; objects should be fully immersed and air pockets should not be present. Deposits of organic matter should be removed prior to disinfection. Adequate contact time should be allowed for the disinfectant to perform its function. This time will vary according to the type of disinfectant, the presence of inactivating or interfering factors (such as excessive organic material or and the presence of chemicals) and the microbial load. Disinfectants do not necessarily kill all biological agents and do not usually destroy bacterial spores. Only freshly prepared in-use dilutions should be used since stored dilutions may lose activity. The HSE have recommended that autoclaving of dry discard should replace discard pots with disinfectant wherever possible.

Hypochlorites are highly effective against vegetative bacteria, viruses and fungi. They have limited activity against bacterial spores and are not very effective against mycobacterium spp. They are compatible with anionic and non-ionic detergents, but are inactivated by organic matter and may corrode metals and damage rubber. Hypochlorites are commonly available as solutions of sodium hypochlorite and as powdered or tableted sodium dichloroisocyanurate (NaDCC) which are also recommended for spillages. Sodium hypochlorite stock solutions will decay with time, light and temperature and should be stored in cool and dark conditions. Working solutions of any hypochlorite need to be changed frequently (at least daily) because of deterioration caused by the addition of organic matter. Commonly used dilutions are:

- 1000 ppm (0.1%) for general wiping of equipment and benches (not spillages)
- 2500 ppm (0.25%) for discard containers (if required)
- 10,000 ppm (1%) for spillages
- 20,000 ppm (2%) for work involving prions/TSE agents

Note: hypochlorites should not be mixed with acids as gaseous chlorine is released at low pH, nor with formalin or formaldehyde as a bis-chloromethyl 3 ether (a lung carcinogen) is released.

Peroxygen-based disinfectants Virkon is a multi-component peroxygen based oxidising agent. It is effective against bacteria, fungi, and viruses. Activity against bacterial spores and Mycobacterium spp. is variable for peroxygen-based disinfectants. It does cause some corrosion to metals (as hypochlorous acid is formed), but less than hypochlorite solution. Virkon in solution is stable for 7 days and has a pink colour which gradually fades with inactivation; however, good infection control practice indicates that in-use disinfectants should be replaced daily. Virkon (1%) may be suitable in laboratories performing low risk microbiological or Class 1 genetic modification work.

Alcohols are effective against many bacteria including Mycobacterium spp. and fungi. They have variable activity against viruses (less effective against nonenveloped viruses) and have no activity against bacterial spores. Alcohols poorly penetrate organic matter, particularly proteinaceous material therefore cleaning beforehand is essential. Due to their flammability they should not be used near flames or equipment likely to generate sparks. Alcohol sprays must not be use on electrical equipment whilst connected to the mains. When used to

decontaminate centrifuges, allow time for the alcohol to safely evaporate before turning the equipment on. A surface wipe is a convenient method of disinfection, but due to evaporation has a limited effect and therefore should be confined to surfaces with no visible contamination. Alcohols should not be used undiluted. The most effective strength for alcohol disinfection is a 70-80% (v/v) solution of isopropanol or ethanol in water.

Aldehydes There are two distinct types with differing uses.

1. **Formaldehyde gas** is used to fumigate microbiological safety cabinets and rooms. Safety cabinets should be fumigated at least 6 monthly and before issuing a permit to work. The procedure of fumigating a safety cabinet requires a written risk assessment.
2. **Glutaraldehyde** has long been recognized as a cause of ill health, with dermatitis and respiratory problems being the most significant effects. It is now also classified as an asthmagen and respiratory sensitiser and has been assigned a Maximum Exposure Limit (MEL) of 0.05 ppm. While glutaraldehyde is an effective disinfectant, its use in the open laboratory is not recommended. Small amounts may be used for disinfecting equipment inside safety cabinets, but these cabinets must vent to the exterior and not be of the recirculating type.

Chlorine dioxide (Tristel) (an aqueous solution of chlorine dioxide) has an activity that differs from hypochlorite as it does not produce free chlorine. It is active against bacteria, including Mycobacterium spp., viruses, fungi and spores.. At higher concentrations (280 ppm) it has rapid bactericidal activity against Mycobacterium spp. and is a more effective sporocide than hypochlorite. It may affect some metals.

Recommendations for specific procedures

- Routine disinfection of benches: sodium hypochlorite or Virkon
- Disinfection of safety cabinets: Virkon followed by 70% alcohol
- Disinfection of centrifuges: 70% alcohol
- Discard pots: sodium hypochlorite or Virkon

Summary points: Infectious waste management

The organization shall establish and maintain an appropriate waste management policy for biological agents and toxins. The organization should have a validated procedure for the inactivation of biological agents and toxins waste products. The following elements should be considered for a waste management policy:

- Ensure that the appropriate chemical sterilization/disinfection method is used and documented in SOPs
- Ensure programme is in place to minimize the waste production;
- Ensure effective waste audit trails are in place and documented;
- Provide adequate facilities and procedures for the storage of waste (including short term storage);
- Ensure methods are available for effective segregation and decontamination of mixed waste

- Ensure appropriate packaging material is used to contain the waste and to maintain its integrity during storage and transportation.
- Clear workflow from infectious and waste materials is performed to determine ingress and egress of samples and waste to prevent accidental exposure of staff to infectious materials.
- Validate sterilization/disinfection procedures and record the results.

Table 3. Provides a comparison of the effectiveness of disinfectant classes on differing microorganisms.

Disinfectant	Bacteria	Bacterial spores	Fungi	Enveloped viruses	Non-enveloped viruses	Myco-bacteria	TSE and prion agents
Phenolic	+	-	+	+	2	+	-
Hypochlorite	+	-	1	+	+	1	+
Alcohols	+	-	-	+	+	+	-
Aldehydes	+	+	+	+	+	+	-
Surface active agents	+	-	1	2	2	-	-
Peroxide compounds	+	+	+	+	+	+	-

+ = Generally effective

- = Generally ineffective

1 = Limited activity

2 = Depends on the virus

Table 4. Provides a comparison of the compatibility of disinfectant classes on differing surfaces and situations

Disinfectant	Hazard class	Organic matter	Hard water	Detergent	Corrosive	Flammable
Phenolic	**Toxic**	**-**	**+**	1	**-**	**-**
Hypochlorite	**Toxic, corrosive**	**+**	**-**	1	**+**	**-**
Alcohols	**Harmful, flammable**	**-**	**-**	**-**	**-**	**+**
Aldehydes	**Toxic, Irritant**	**-**	**-**	**-**	**-**	**-**
Surface active agents		**+**	**+**	2	**-**	**-**
Peroxide compounds	**Irritant (dust)**	**-**	**-**	**-**	3	**-**

+ = Compatible
- = Incompatible
1 = Inactivated by cationic detergents
2 = Inactivated by anionic detergents
3 = Virkon can "cloud" stainless steel

TRANSPORT OF INFECTIOUS MATERIALS

IATA REQUIREMENTS FOR PACKAGING, LABELING AND SHIPPING CATEGORY A AND CATEGORY B INFECTIOUS SUBSTANCES BY AIR (DGR 2007)

It is very important that the correct guidelines for transport of infectious materials are followed. The guidelines issued by the International Air Transport Association (IATA) and the purpose of the regulations is to protect the public, emergency responders, laboratory workers, and personnel in the transportation industry from accidental exposure to the infectious contents of the packages. All shipped goods must be classified to define dangerous goods that are shipped by commercial carriers. Classification allows the shipper to select the proper IATA packing instructions and directions to use, and provides information necessary to complete required documentation. The following information is based on the WHO Transport of infectious diseases guidelines and further details an can be found at:

http://www.who.int/csr/resources/publications/biosafety/WHO_HSE_EPR_2008_10/en/index.html

Category A Infectious Substance (UN 2814 and UN 2900)

A Category A material is an infectious substance that is transported in a form that is capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals when exposure to it occurs. An exposure occurs when an infectious substance is released outside of its protective packaging, resulting in physical contact with humans or animals. Category A infectious substances are assigned to identification number "UN 2814" for substances that cause disease in humans or in both humans and animals, or "UN 2900" for substances that cause disease in animals only.

Figure 4 shows an example of the UN standard triple packaging system for materials known or suspected of being a Category A infectious substance. The package consists of a watertight primary receptacle or receptacles; a watertight secondary packaging; for liquid materials, the secondary packaging must contain absorbent material in sufficient quantities to absorb the entire contents of all primary receptacles; and a rigid outer packaging of adequate strength for its capacity, mass, and intended use. Each surface of the external dimension of the packaging must be 100 mm or more. The completed package must pass specific performance tests, including a drop test and a water-spray test, and must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95 kPa. The completed package must also be capable of withstanding, without leakage, temperatures in the range of -40 °C to +55 °C. The completed package must be marked "Infectious substances, affecting humans, UN 2814" or "Infectious substances, affecting animals, UN 2900" and labeled with a Division 6.2 (infectious substance) label. In addition, the package must be accompanied by appropriate shipping documentation, including a shipping paper and emergency response information.

List of indicative Category A agents affecting humans and animals (this list may not contain all agents, confer with IATA)

- Bacillus anthracis (cultures only)
- Brucella abortus (cultures only)
- Brucella melitensis (cultures only)
- Brucella suis (cultures only)
- Burkholderia mallei - Glanders (cultures only)
- Burkholderia pseudomallei – Pseudomonas pseudomallei (cultures only)
- Chlamydia psittaci - avian strains (cultures only)
- Clostridium botulinum (cultures only)
- Coccidioides immitis (cultures only)
- Coxiella burnetii (cultures only)
- Crimean-Congo hemorrhagic fever virus
- Dengue virus (cultures only)
- Eastern equine encephalitis virus (cultures only)
- Escherichia coli, verotoxigenic (cultures only)
- Ebola virus

- Flexal virus
- Francisella tularensis (cultures only)
- Guanarito virus
- Hantaan virus
- Hantaviruses causing hemorrhagic fever with renal syndrome ‡
- Hendra virus
- Hepatitis B virus (cultures only)
- Herpes B virus (cultures only)
- Human immunodeficiency virus (cultures only)
- Highly pathogenic avian influenza virus (cultures only)
- Japanese Encephalitis virus (cultures only)
- Junin virus
- Kyasanur Forest disease virus
- Lassa virus
- Machupo virus
- Marburg virus
- Monkeypox virus
- Mycobacterium tuberculosis (cultures only)
- Nipah virus
- Omsk hemorrhagic fever virus
- Poliovirus (cultures only)
- Rabies virus (cultures only) ‡
- Rickettsia prowazekii (cultures only)
- Rickettsia rickettsii (cultures only)
- Rickettsia typhi (cultures only)
- Orientia tsutsugamushi (cultures only)
- Rift Valley fever virus (cultures only) ‡
- Russian spring-summer encephalitis virus (cultures only)
- Sabia virus
- Shigella dysenteriae type 1 (cultures only)
- Tick-borne encephalitis virus (cultures only)

- Variola virus
- Venezuelan equine encephalitis virus
- West Nile virus (cultures only)
- Yellow fever virus (cultures only)
- Yersinia pestis (cultures only)

Category B infectious substances (UN3373)

A Category B infectious substance is one that does not meet the criteria for inclusion in Category A. A Category B infectious substance does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs. The proper shipping name for a Category B infectious substance, "Biological specimen, Category B," is assigned to identification number "UN 3373." Following are examples of possible Category B substances:

Typical clinical, diagnostic, or patient specimens, e.g., blood, biopsies, swab specimens, excreta, secreta, body fluids, or tissues (a) being shipped for routine culturing or screening testing for non–Category A infectious microorganism(s), or (b) suspected of containing a non–Category A microorganism(s);

Typical clinical laboratory cultures (usually on solid or in liquid media) of non–Category A microorganisms routinely encountered and manipulated in clinical microbiology laboratories

Figure 5 shows an example of the triple packaging system for materials known or suspected of containing a Category B infectious substance. A Category B infectious substance must be placed in a packaging consisting of a leakproof primary receptacle, leakproof secondary packaging, and rigid outer packaging. At least one surface of the outer packaging must have a minimum dimension of 100 mm by 100 mm. The packaging must be of good quality and strong enough to withstand the shocks and loadings normally encountered during transportation. For liquid materials, the secondary packaging must contain absorbent material in sufficient quantities to absorb the entire contents of all primary receptacles. The primary or secondary packaging must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of 95 kPa. The package must be constructed and closed to prevent any loss of contents that might be caused under normal transportation conditions by vibration or changes in temperature, humidity, or pressure. The completed package must be capable of passing a 1.2-meter (3.9 feet) drop test. The package must be marked with a diamond shaped marking containing the identification number "UN 3373" and with the proper shipping name "Biological substance, Category B." In addition, the name, address, and telephone number of a person knowledgeable about the material must be provided on a written document, such as an air waybill, or on the package itself.

Exempt human (or animal) specimens

Exempt human or animal body site specimens are those for which there is "minimal likelihood there are pathogens present". Examples of such specimens include urine or serum to be tested for glucose, cholesterol, hormone levels, prostate-specific antigen, and analytes used to evaluate heart and kidney function.

Exempt substances

Many substances commonly encountered in clinical laboratories are exempt from strict infectious substance shipping requirements. Examples of such substances are

Figure 4: Example of the UN standard triple packaging system for materials known or suspected of being a Category A infectious substance

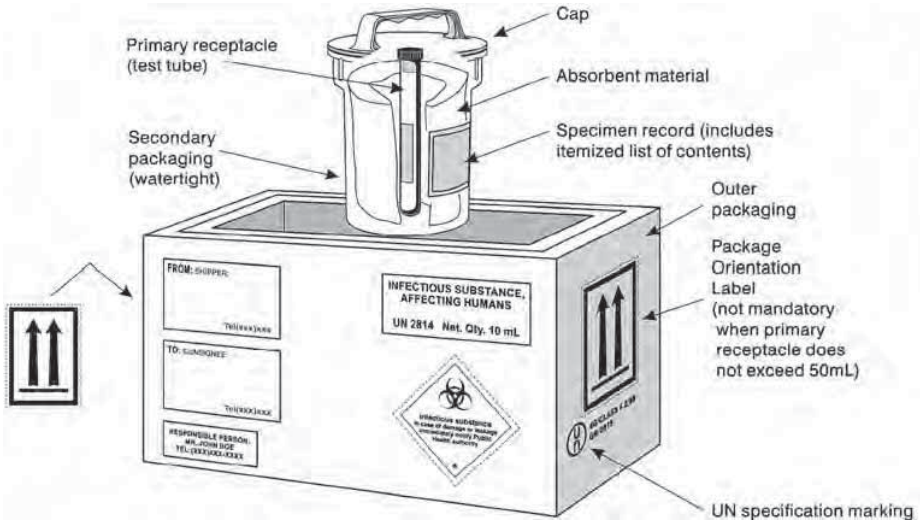
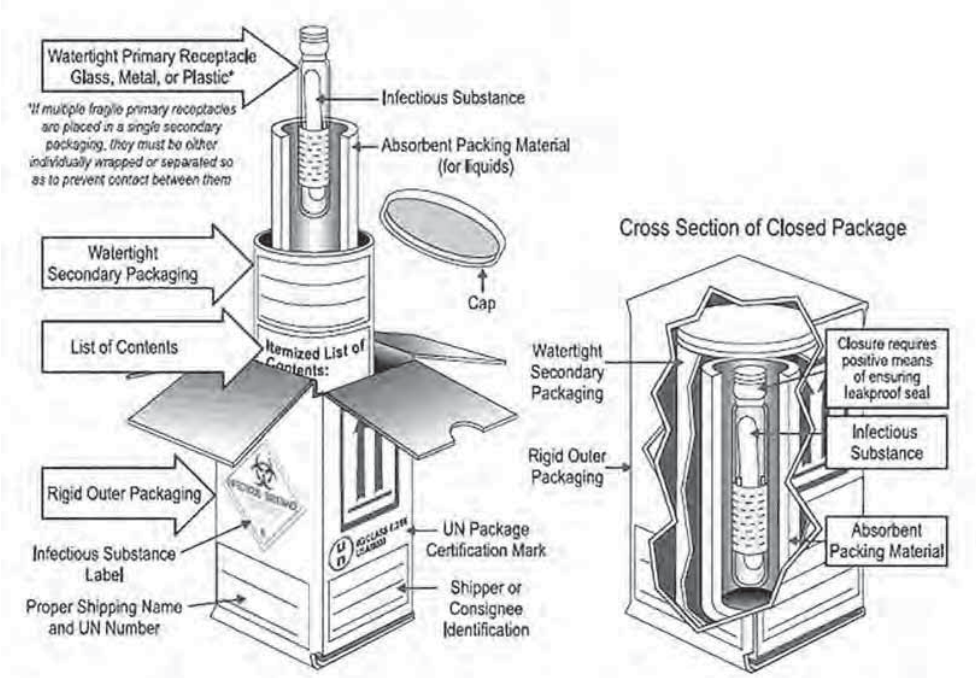
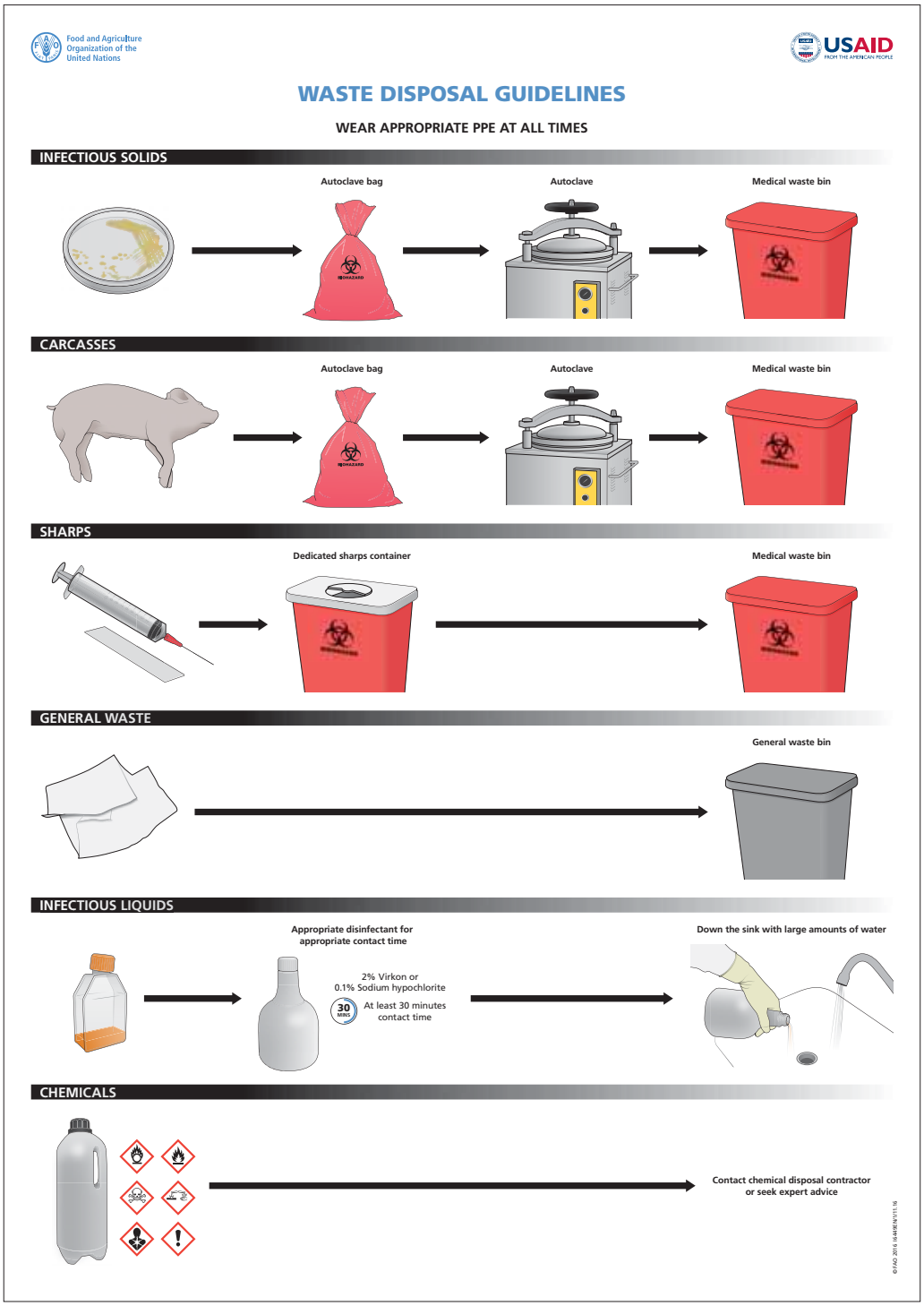


Figure 5: Example of the triple packaging system for materials known or suspected of containing a Category B infectious substance



Note 1: *The smallest external dimension of the outer packaging must not be less than 100 mm (3.9 inches)*
Note 2: *The primary receptacle or the secondary packaging must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa*
Note 3: *Follow package manufacturer's closure instructions*

Figure 6: Waste disposal guidelines



Substances that do not contain infectious substances or are unlikely to cause disease in humans and animals;

- Most environmental samples (e.g., food, soil);
- Substances that contain neutralized or inactivated microorganisms;
- Samples submitted for forensic analysis;
- Dried blood spots and fecal occult blood screen specimens;
- < 30 mL of 10% formalin per primary container when the formalin is used as a preservative.

Patient specimens

- A "patient specimen" is material collected directly from humans or animals for diagnostic, treatment, prevention, investigational, or research purposes.
- Patient specimens that meet Category A or Category B criteria must be classified as Category A or Category B substances.
- Patient specimens that meet neither Category A nor Category B criteria must be treated as exempt human or animal specimens.

Genetically modified organisms

Genetically modified organisms usually meet either Category A or Category B criteria. If this is not the case, the organism must be classified as a "genetically modified microorganism" (Class 9, Miscellaneous Dangerous Goods) and packed and shipped as such.

GENERAL SAFETY PRACTICES

The following is guidance on general safety practices. It is not exhaustive but should provide enough information to allow safe working practices in most cases.

Electrical safety

The following general principles should be applied to electrical safety

- All electrical outlets must be earthed
- Where possible individual items of electrical equipment should be earthed
- Electrical loads should be regularly checked
- All electrical equipment must be evaluated regularly.
- Regular "on load" testing of generators should be performed

If at any time there is any concern regarding electrical equipment for electrical safety please contact the Biosafety Officer or your line manager/supervisor. If you or anyone else has received an electric shock from any item of equipment, no matter how small, make sure that no one else touches it until it has been investigated further. ***Never try to repair any electrical equipment or tamper with electrical equipment if you suspect that there is a problem.***

Manual Handling/Working at height

- All staff should be aware of the dangers of working at heights, and training and awareness sessions will be held regularly or as required.
- All ladders must be checked for integrity before they are used, and must have non-slip feet.
- Anyone working on a ladder above head height must have a colleague who ensures that the ladder remains stable.
- Chairs or stools must never be used for working at height.
- Storage of items above head height should be kept to a minimum and only lighter items should be stored at high levels.
- All slips, trips and falls occurring must be reported as incidents so that the root causes may be investigated.
- Anyone who notices that a floor is slippery or that there are items that might cause a risk of tripping must bring these to the attention of the local manager.
- Appropriate signs must be used when floors are being wet-washed.
- All staff whose job will involve regular manual handling or lifting must have specific training in appropriate techniques.
- A trained staff member should be made responsible for manual handling issues, including the training of staff.
- Staff who have not been specifically trained should not lift items that they cannot lift with ease but should seek someone trained in manual handling techniques to do this.

Chemical and radiological safety

- Risk assessments must be performed using the a standardised format which conforms to the requirements of the Control of Substances Hazardous to Health (COSHH) regulations, prior to use of infectious agents, chemicals or radiation sources. Normally this would form part of the SOP or protocol that describes the work.
- Before working with any radiation sources you must contact the relevant safety officer so that appropriate risk assessments can be performed and \ regulatory practices put in place.
- Principle Investigators are responsible for storing and disposal of hazardous and toxic chemicals safely. Where the principal investigator is not normally located in a laboratory the principal investigator must delegate this responsibility to a senior technician and this responsibility must be performed in liaison with the area safety adviser. All hazardous chemicals (flammable, toxic, oxidising, strong acids and strong alkalis) should be stored in appropriately marked, lockable cupboards. Incompatible chemicals, such as strong acids and strong alkalis, should not be stored in the same cupboards. No new chemicals can be brought into the laboratories without a risk assessment in terms of use, storage, disposal and clean up.
- Disposal of obsolete hazardous chemical is performed by authorised Chemical disposal contractors only.

Liquid Nitrogen

It is extremely important that staff are aware of the risks involved with working with liquid nitrogen. These include asphyxiation, cryogenic burns from splashes and spills and explosion from stored pressure within cryovials. In summary, whenever working with liquid nitrogen be aware that there is this asphyxiation risk and that there was sufficient oxygen within the room, always wear cryogenic gloves as well as a face shield to protect oneself from splashes.

Lone working

Lone working (working in an isolated location or working outside of normal hours) is generally not encouraged. Is the responsibility of the supervisor of the staff and of the area to ensure that staff do not work alone for significant periods of time and all efforts are made to ensure that start for aware of the risks involved with lone working. Should out of hours work be required it is the responsibility of the staff and the supervisor to come to an arrangement where they are satisfied that all eventualities are considered and covered. Lone working in the BSL3 laboratories or other containment type facilities for low risk activities may be performed – however out of hours should be strictly forbidden and lone working is generally discouraged during normal work hours.

Therefore, best practice would dictate that in the majority of circumstances such as in the event that lone working is performed at BSL2 areas outside of normal working hours staff should contact their supervisor prior to starting work and contact their supervisor on completion of work.

Visitors and external contractors

The following is general guidance for visitors and external contractors to a BSL2 or BSL3 laboratory.

- All visitors must complete a safety induction.
- Short term visitors (less than one week) who will not be working in laboratories should have a named supervisor (buddy) who should inform them about the arrangements for fire safety and evacuation.
- It is normal for visitors to be assigned a "buddy" and that lone work by visitors in BSL3 laboratories is not acceptable.
- Anyone working in laboratories or who will be spending more than a week working in other facilities must have a standard induction procedure undertaken by their line manager or named supervisor.
- Line managers/supervisors must determine the experience and competence of a visitor and identify what procedures may be undertaken, what equipment can be used, and what hours may be worked.
- In all cases visitors must, in the first instance, be under close supervision at all times and must receive adequate instruction to work safely.
- Any contractor who must enter sites to perform repair work or to install new equipment must have prior authorisation to do so.
- Contractors must be provided with a visitors badge and registered at reception.
- A staff member must be assigned to the external contractor and must accompany all external contractors at all times. At no time should contractor be unsupervised for any reason.

Emergency procedures

Fire safety All sites must have a site-specific fire and evacuation policy that includes the following,

- There must be appropriate fire detection systems in place
- Fire safety equipment must be provided and maintained
- There must appropriate fire-related signage to provide guidance in times of emergency
- There must be regular fire and emergency evacuation drills.

The following general guidance is provided in case of fire.

Small fire

- Use the fire extinguisher or fire blanket to put out the fire
- Press emergency alarm and immediately report to the relevant Safety Officer, area safety officer or others senior staff member.
- If any staff have been injured, immediately contact the Occupational Health Officer.
- If fire cannot be extinguished after using fire extinguisher or Fire Blanket, please refer to large fire.
- Complete an incident report form

Large fire

- Inform other staff by pressing emergency alarm and call the Fire Department (know the telephone number).
- If possible, use the fire extinguisher or Fire Blanket to put out the fire
- Press emergency alarm and immediately report to the relevant Safety Officer, Area Safety Advisor or other senior staff member.
- Get out of the lab as quickly as possible if the fire is not containable.
- Staff must assemble at a predetermined location.
- If any staff have been injured, immediately contact the Occupational Health Officer.
- Complete an Accident/Incident report form.

Chemical spills In the event of a chemical spill or leakage contact your line manager or the relevant Safety Officer. Ensure that you know what chemical has been spilled or leaked and communicate this to the senior staff. ***Do not attempt to clean up the spill yourself without consulting senior staff member. In the majority of cases a staff member with specific training will clean up this spill***

The following guidance is recommended,

- For chemical spills, determine the nature of the chemical that has been spilled.
- Warn others and if necessary evacuate all other personnel in vicinity.
- Place signs around the area of the spill to warn others.
- Seal access to area, and inform supervisor and/or appropriate safety officer as soon as possible

- Obtain SOPs, COSHH assessments to determine, the nature of the risk, and how to perform the clean-up procedure.

Infectious material spills In the event of an infectious material spill of greater than 10 mL of liquid contact the Biosafety Officer or the principal investigator. Communicate to the senior staff what is the nature of the spill and the infectious agent in question. ***Do not attempt to clean up the spill yourself without consulting a senior staff member or safety officer.***

Spill Clean-up Materials or Biological Spill Kit

Laboratories using infectious materials, including recombinant microorganisms must develop spill response plans addressing foreseeable occurrences. The following materials should be assembled in one place in laboratories using infectious materials; all personnel must know of the location.

- Disinfectant solution*
- Forceps, tongs, broom, dust pan
- Personal protective equipment (PPE): safety glasses, goggles, or face shield, utility Gloves, wrap-around lab coat, shoe covers (optional)
- 'Biohazard' bag, sharps container
- Paper towels or other absorbent

**A 1/10 dilution of household bleach or 2% Virkon solution , prepared fresh daily is effective in most situations.*

Personal exposure takes priority over clean up

If you are exposed, immediately remove contaminated clothing and other protective equipment and leave it in the laboratory. Keep a spare tyvek suit in the laboratory for such cases. If medical follow-up is warranted it should be sought immediately.

Laboratory Spill Clean-up Procedures

- Spills involving RG2 microorganisms (low to moderate risk agents)
  - Clean-ups must proceed according to the guidance for the relevant biological safety level.
  - Alert people in immediate area and get out of the laboratory without causing panic.
  - Put on PPE.
  - Cover an area twice the size of the spill with disinfectant soaked-paper towels. Or, surround spill with disinfectant.
  - Allow a 30 minute contact period.
  - Wipe down any contaminated stationary equipment or furniture with disinfectant.
  - Use forceps, tongs, or broom to remove broken glass and other items; place in sharps container or red bag.
  - Remove towels and re-clean area with disinfectant solution.
  - Decontaminate (autoclave, chemical treatment) reusable clean-up items and other reusable equipment.

- Inform laboratory personnel when the clean-up is complete

- Spills involving RG3 microorganisms, including recombinant microorganisms (risk of serious disease via inhalation exposure)
  - Alert personnel in the area to hold their breath, leave the room, and close the door.
  - Notify your line manager or the Biosafety Officer.
  - Wait thirty minutes to allow airborne organisms to settle.
  - Collect all needed spill response supplies; don PPE for RG3 work, including a respirator or PAPR.
  - Return to lab and clean-up as per directions in RG2 section above.
  - Autoclave all spill-related materials and then dispose in appropriate RMW container.
- Spills inside a Biological Safety Cabinet
  - Keep the cabinet running. Clean-up as per directions above, making sure to wipe down back and side walls of cabinet.
  - If material has spilled into the catch basin beneath the work surface, add a volume of disinfectant equal to the quantity in the basin, wait 20 minutes, and absorb with paper towels.
  - After completion, allow cabinet to run for ten minutes before resuming work.
- Spills inside a centrifuge
  - Shut centrifuge off and do not open the lid for 20 minutes to allow aerosols to settle.
  - Put on PPE.
  - Use a squeeze bottle to apply disinfectant to all contaminated surfaces within the chamber, taking care to minimize splashing.
  - Allow 20 minute contact period and then complete clean-up of the chamber.
  - Remove buckets and rotors to nearest Biological Safety Cabinet; disinfect and clean as per manufacturer's instructions.
- Spills Outside the Laboratory
  - Viable organisms should only leave the laboratory in a well sealed primary (inner) and secondary (outer) container with a closable top. A test-tube rack inside a tray is not acceptable.
  - The exterior of the secondary container should be wiped down with disinfectant prior to leaving the laboratory so that it can be transported without wearing gloves.
  - Carry paper towels and if a spill occurs use the towels to cover the spill but do not attempt a clean-up without appropriate disinfectant and personal protective equipment.
  - Notify people in the immediate area and collect clean-up material and proceed with clean-up Facilities maintenance and commissioning

Figure 7a: Hazardous Spill Clean-up – Stage 1

Food and Agriculture Organization of the United Nations

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HAZARDOUS SPILL CLEAN UP PROCEDURE

STAGE 1:

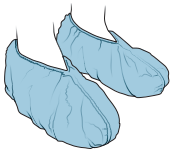
1

Inform other people to leave the contaminated area.

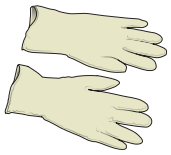
2

If any of your PPE or clothing is contaminated following the spill, please remove and discard it in the following order:

1. Shoe covers



2. Outer gloves

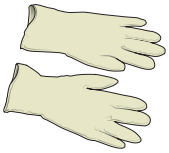


3. Lab coat

4. Goggles

5. Face mask

6. Inner gloves



As you remove each item, dispose of it in an autoclave bag.

3

Place a no entry sign outside the laboratory. Leave it there for at least 30 minutes.

4

Ask someone to assist you.

5

Bring a spill kit to the area of the spill.

Figure 7b: Hazardous Spill Clean-up – Stage 2

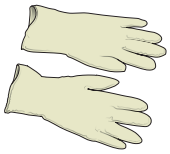
STAGE 2:

6

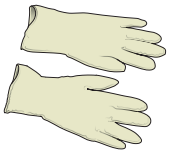
Put on fresh PPE.

1. Face mask

2. Inner gloves



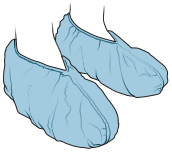
3. Outer gloves



4. Goggles

5. Lab coat

6. Shoe covers



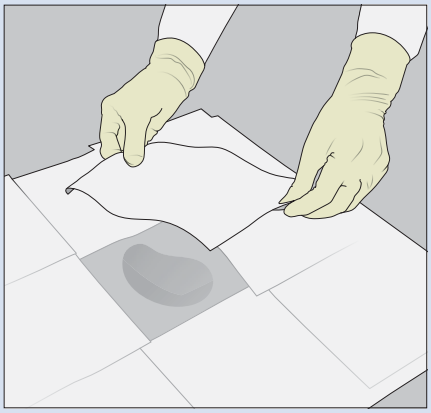
7

Prepare 1% Virkon solution disinfectant.

For example – 1% solution = 1 gram Virkon in 100 ml water.

8

Place paper towels over the area of the spill.



9

Pour the disinfectant on the area of the spill. Leave it for at least 20 minutes.

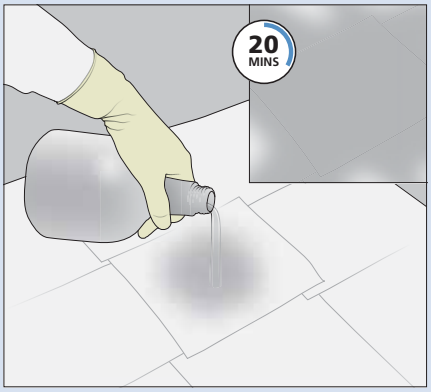


Figure 7c: Hazardous Spill Clean-up – Stage 3

STAGE 3:

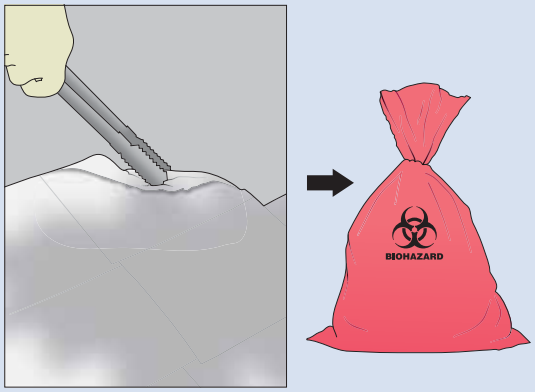
10

After 20 minutes, use tongs to put any contaminated sharps into a sharps bin.

Always use tongs to pick up sharps!

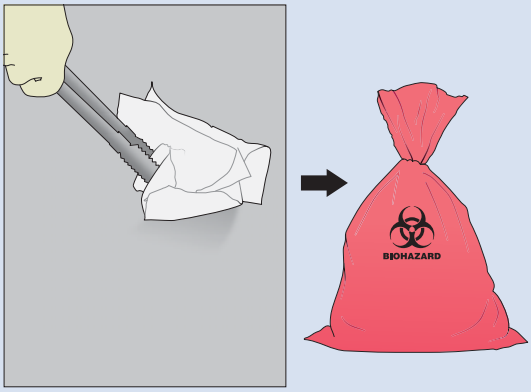
11

Put all the disinfectant soaked paper towels into an autoclave bag.



12

Absorb any residual contamination with fresh paper towels and dispose of in an autoclave bag.



13

Decontaminate all potentially contaminated re-useable tools with the 1% Virkon solution.

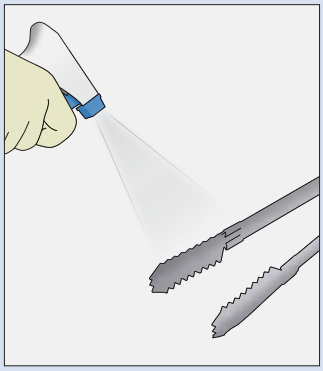


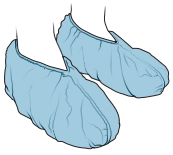
Figure 7d: Hazardous Spill Clean-up – Stage 4

STAGE 4:

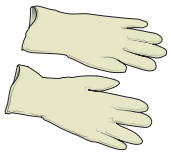
14

Carefully remove your PPE in the following order:

1. Shoe covers



2. Outer gloves

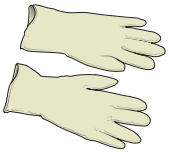


3. Lab coat

4. Goggles

5. Face mask

6. Inner gloves



As you remove each item dispose of it in an autoclave bag.

15

Wash your hands thoroughly.

16

Remove the no entry sign and invite people back into the laboratory.

17

Write an accident report.

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FACILITIES MAINTENANCE AND COMMISSIONING

Preparations of sustainable building and laboratory maintenance

Following tasks still need to be performed to guarantee a sustainable and effective preventive maintenance for building systems and the laboratory:

- Identify and interview/evaluate potential companies/individuals for the preventive maintenance services (this includes delivery of spare parts and emergency stand-by services).
- The Diesel Generator and Automatic Transfer Switch System (ATS) systems need parameter tuning to get them to work reliably and safe. This process needs time due to repeated test-runs at various situation that will be necessary during this testing and setting time the building will have several power failures – so this task is best done during a weekend.
- Create easy to understand systematic manuals (SOP's) and maintenance instructions for each essential machine (e.g. Generator, Incinerator, Air Handling Units) in local language and English. Those can then be laminated and attached to each of the machines together with a holder for a Logbook that will have to be filled in by the person that performs the check-ups or maintenance tasks.
- Train local technical staff for the daily / weekly and monthly maintenance of system components. Ideally during the first real maintenance.

Annual Preventive Maintenance (prioritized list)

Following is a list of tasks for annual preventative maintenance for BSL2 and BSL3 laboratories that should be prioritized as highest need to ensure sustainable building systems and the smooth running of the laboratory.

- Diesel Generator and ATS (Automatic transfer switch) systems: This includes preventive change of parts each year – such as the battery of the diesel generator, diesel and engine oil filters, air intake filters and also cooling water and engine lube
- Electrical distribution system (Generator house and each floor): Cleaning, check for hot-spots, check amps, check loose connections and eventual sparking and overloads
- Water booster pump – including checks for leaks and check valve testing
- Chiller system with Chiller, Pumps and chilled water piping system and armatures/fittings
- Biosafety cabinets yearly re-certification (according to NSF49 or other agreed standards)
- Plant room: Exhaust HEPA filter leak testing
- Plant room: Air handling units– this includes motor, inverter, bearings, shaft, belts, prefilter and filter change, sensor checkup and calibration, cleaning
- Plant room: Exhaust fan and plant room exhaust axial fan – this includes motor, inverter, bearings, shaft, sensor checkup and calibration, cleaning
- Calibration and signaling check of all field sensors (Temperature, velocity, pressure)

- Field wiring checks (this is time consuming but very essential) all terminals should be thermally checked and tightened once a year. This includes also breaker boxes for each floor
- Control system: Check all plugs and terminals, check internal battery, check heat buildup and clean the fins (follow instruction in manuals)
- Full Elevator safety check and preventive maintenance (at least twice a year or according to local building codes and laws) – this includes cleaning, check + replacement of batteries
- Duct work maintenance including actuators (pneumatic and electric)
- Air compressor (including draining of condense water) – according to manual. This includes belt change and tests, bearing, drain valve check, air tightness and pressure reducer check
- Diesel operated incinerator (incinerator house) including cleaning – see technical manual
- Yearly re-certification of BSL3 lab – this includes a failure scenario test
- Check and maintain building systems: Rain drainage, sanitary system including the septic tanks and treatment tank, fire extinguishers, emergency escape lamps and their batteries

Field tests for biological safety cabinets

BSC field tests should be performed by the certifier upon installation and relocation of cabinets, after major maintenance is conducted, changing of HEPA filters, and at regular intervals thereafter. NSF49 recommends a maximum interval of 12 months between certification, though many organizations re-certify more frequently. For example, pharmacies certify their BSCs every 6 months as required by USP Chapter on sterile compounding.

In order to verify proper operation of a biological safety cabinet, the following tests are performed. These tests are related to the containment and product protection provided by the cabinet, and results must correlate to the values obtained by NSF for type testing of that particular make, model, and size of cabinet.

- Downflow velocity profile test
- Inflow velocity test
- Airflow smoke pattern tests
- HEPA filter leak test
- Cabinet integrity test (for Type A1 cabinets with positive-pressure contaminated plenums only)
- Alarm function verification
- Blower interlock (for Type B1 and B2 cabinets)
- Exhaust system performance (for any cabinet connected to the building exhaust)

While not part of Class II BSC field certification to NSF Standard 49, for certain installations additional validation tests may be required. For example, most cGMP compliant facilities will verify air cleanliness with a particle counter to the required ISO class per IEST protocols. Any such validation tests should be performed in conjunction with field certification. The following tests, related to worker comfort and safety, may optionally be performed. These tests verify functions of the cabinet not directly related to containment or product protection.

- Lighting intensity test
- Vibration test
- Noise level test
- Electrical tests (leakage, ground circuit resistance, and polarity)

BSC certification and commissioning

The goal of commissioning is to verify and document that the facility and its systems meet defined objectives and criteria for performance. Commissioning is performed prior to occupying new laboratory facilities to ensure that systems are operating as specified. Many organizations also repeat commissioning, or at least some portion thereof, at regular intervals to ensure continued proper operation of building systems. This "re-commissioning" is a good practice to guarantee that all building systems continue to function as required. One of the most important systems considered in the commissioning process for laboratory facilities is the HVAC (heating, ventilation, and air conditioning) system. This is where commissioning and certification have a vital overlap and

interdependency. Proper HVAC design and operation is crucial to the proper operation of BSCs. While the majority of BSC installations simply return cabinet exhaust to the room, they may still impact, or be impacted by, the function of the HVAC system.

From a heating and cooling standpoint, a typical 4-ft Class II, Type A2 cabinet may generate 2,000 to 3,000 BTUs/hr. Air conditioning and heating systems should be verified to maintain desired environmental conditions when BSCs are in operation, as well as when cabinets are turned off.

More crucial to the safety of laboratory workers and the integrity of aseptic work processes is the possible impact of air currents in the room on the performance of the BSC itself. Location of room air supplies and returns is critical, as cross-drafts may negatively affect the performance of BSCs. This dependency should be taken into account when designing room air ventilation and should be verified during the commissioning process.

Another level of complexity is introduced when BSCs are vented to the outdoors via the building exhaust system. With such an installation, the BSC itself becomes the first piece of the system ductwork and needs to be designed for and tested as such. In addition to the possible impact of cross-drafts, the commissioning process for BSCs should also verify that the required exhaust and room supply air to operate the BSC are available and that HVAC controls are interfaced with the BSC controls.

Supply air Supply air requirements for BSCs are often overlooked. However, they can be just as critical as exhaust air requirements to proper cabinet function. Whatever volume of air is exhausted by the BSC must also be supplied to the room in order to avoid "starving" the cabinet of air. The supply air available to the BSC should be verified as well as the supply air to maintain desired room pressurization and air exchange rates. A BSC cannot be certified if a lack of supply air causes a low or inconsistent inflow or downflow velocity.

Exhaust airflow and static pressure Biological safety cabinets are constant volumetric airflow devices, and the fan energy to exhaust the cabinet must be provided by the building exhaust system. In addition to the exhaust airflow, the static pressure requirements for hard connected Type B cabinets are relatively high (up to –2.5 in. of water column for a 6-ft B2 cabinet). This is due to the resistance added to pull air through the cabinet and its exhaust HEPA filters. For canopy connected Type A cabinets, the cabinet blower overcomes the resistance of the exhaust filter; however, sufficient exhaust flow is still important. Biological safety cabinets will not

function properly and cannot be certified if the exhaust flow or static pressure is not sufficient (or potentially if either is too high).

HVAC and cabinet controls Low exhaust flow can lead to a loss of containment at the front access opening of the BSC and pose a risk to workers. As indicated by NSF 49, exhausted BSCs should have airflow monitors that alarm when the exhaust flow is too low. This feature is required for hard connected Type B cabinets and should be considered as good practice for canopy connected Type A cabinets. The alarm function should be verified during commissioning, as well as for certification. It is often desirable, or even required, to control the BSC and HVAC systems in con- cert, and verification of any such interlocks should be included in the commissioning process. Interlocks in the controls allow the room to maintain required pressurization, air changes, and exhaust flow when the cabinet is in operation as well as when the cabinet is turned off. A great majority of the problems encountered in field certification and proper operation of BSCs could be avoided if a thorough commissioning process were followed. The commissioning process should include documentation and verification of the requirements for BSCs as they relate to the building's HVAC systems. Field certification can besuccessfully completed only after the HVAC requirements for BSCs have been verified through commissioning.

Accreditation for field certifiers Because of the specialized knowledge required for accreditation, a field certifier must pass a written and a practical test administered by NSF. Additionally, continuing education and periodic re-examination are required in order to maintain accreditation. When selecting a service provider for certification, BSC owners should be sure that the vendor company's technicians are accredited field certifiers. To help locate a certifier, NSF maintains a searchable listing on its web site of accredited certifiers. Certification companies also provide service when problems with BSC function occur between regular certification intervals, or when maintenance tasks such as filter changes are necessary. If an organization performs certification using its own employees, such technicians should also obtain NSF accreditation to be sure that they possess the knowledge and experience required for field certification of BSCs.

Conclusion Biological safety cabinets are critical pieces of contamination control equipment in many settings. Field certification is essential to verify that BSCs continue to provide the product, personnel, and environmental protection that they were designed to offer. While commissioning and field certification technicians have distinct roles, it is vital to each that they collaborate to ensure that HVAC systems will support BSCs and that BSCs function as intended. Field certification of BSCs should occur at regular intervals and be conducted by field certifiers accredited by NSF International.

(Note: this section was adapted in part from http://forums.pharmacyonesource.com/phos/attachments/phos/pharmacy_ops/29/1/CertificationofClassIIBSC.pdf)

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APPENDIX 1.

CDC/NIH BMBL 5TH EDITION LABORATORY BIOSAFETY CONTAINMENT LEVEL CRITERIA

CDC/NIH BMBL 5th Edition Laboratory Biosafety containment level criteria

This section is adapted from "Biosafety in Microbiological and Biomedical Laboratories fifth edition" and can be accessed at the following web address https://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf

The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

The following standard practices, safety equipment, and facility requirements apply to BSL-1.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. 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.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. 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:

    5.1. 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.

5.2. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

5.3. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

5.4. Broken glassware must not be handled directly. Instead, it must be removed usingbrush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. 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.

8.1. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

8.2. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. 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.

10. An effective integrated pest management program is required.

11. 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. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged.

B. Special Practices

None required.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as BSCs, are not generally required.

2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

3. 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.

4. 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, BSL-1 workers should:

    4.1. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

    4.2. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

    4.3. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.
2. Laboratories must have a sink for hand washing.
3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

    4.1. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

    4.2. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratories windows that open to the exterior should be fitted with screens.

Biosafety Level 2

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. 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 Mouth pipetting is prohibited; mechanical pipetting devices must be used.

4. 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:

    4.1. 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.

    4.2. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

    4.3. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

    4.4. 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.

5. Perform all procedures to minimize the creation of splashes and/or aerosols.

6. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

7. 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:

    7.1. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

    7.2. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

8. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory.

9. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required. (See Appendix G.)

11. 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. Therefore, all laboratory personnel and particularly women of childbearing 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.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
2. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
3. 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 such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
4. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
5. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

D. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
    1.1. 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.
    1.2. 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.

2. 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, and 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.

3. 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

    3.1. 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.

4. 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, BSL-2 laboratory workers should:

    4.1. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

    4.2. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

    4.3. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

    4.1. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

    4.2. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not

interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines should be protected with liquid disinfectant traps.
8. An eyewash station must be readily available.
9. 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.
10. HEPA filtered exhaust air from a Class II BSC can be safely recirculated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. 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.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. 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.

5.1. Precautions, including those listed below, must always be taken with sharp items. These include:

5.1.1. 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.

5.1.2. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

5.1.3. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

5.1.4. 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.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

9. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured or transport.

10. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

11. A sign incorporating the universal biohazard symbol must be postedat the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

12. An effective integrated pest management program is required. (See Appendix G.)

12.1. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautionsto 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. Therefore, all laboratory personnel and particularly women of childbearing 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.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
    a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
    b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. 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 such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. 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.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated. Eye and face protection (goggles, mask, face shield or other splash 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.

3. 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, BSL-3 laboratory workers:

    a. Changes gloves when contaminated, glove integrity is compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

4. Eye, face, and respiratory protection must be used in rooms containing infected animals.

D. Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Laboratory access is restricted. Access to the laboratory is through two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.

3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

    a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

    b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

    c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

    Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. All windows in the laboratory must be sealed.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

8. An eyewash station must be readily available in the laboratory.

9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.

    a. Laboratory personnel must be able to verify directional airflow. A visual monitoring device, which confirms directional airflow, must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

    b. The laboratory exhaust air must not re-circulate to any other area of the building.

    c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

    HEPA filter housings should have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

10. 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. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, or other validated decontamination method).

12. Equipment that may produce infectious aerosols must be containe in primary barrier devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out

capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices, such as biometrics.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BSL-4 laboratories:

1. A Cabinet Laboratory—Manipulation of agents must be performed in a Class III BSC; and
2. A Suit Laboratory—Personnel must wear a positive pressure supplied air protective suit.

BSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

The following standard and special safety practices, equipment, and facilities apply to BSL-4.

A. Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. 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.
3. Mechanical pipetting devices must be used.
4. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.

    4.1. Precautions, including those listed below, must be taken with any sharp items. These include:

        4.1.1. 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.

        4.1.2. Use of needles and syringes or other sharp instruments should be restricted in the laboratory, except when there is no practical alternative.

4.1.3. Used needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal or decontamination. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal, located as close to the point of use as possible.

4.1.4. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

5. Perform all procedures to minimize the creation of splashes and/or aerosols.

6. Decontaminate work surfaces with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.

7. Decontaminate all wastes before removal from the laboratory by an effective and validated method.

8. A sign incorporating the universal biohazard symbol must be postedat the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

9. An effective integrated pest management program is required

10. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautionsto 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 nterventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should self identify for a full counseling and guidance.

B. Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements in accordance with institutional policies.

1.1. Only persons whose presence in the facility or individual laboratory rooms is required for scientific or support purposes are authorized to enter.

1.2. 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.

1.3. While the laboratory is operational, personnel must enter and exitthe laboratory through the clothing change and shower rooms except during emergencies. All personal clothing must be removed in the outer clothing change room. All persons entering the laboratory must use laboratory clothing, including undergarments, pants, shirts, jumpsuits, shoes, and gloves (as appropriate). All persons leaving the laboratory must take a personal body shower. Used laboratory clothing mustnot be removed from the inner change room through the personal shower. These items must be treated as contaminated materials and decontaminated before laundering.

1.4. After the laboratory has been completely decontaminated and all infectious agents are secured, necessary staff may enter and exit without following the clothing change and shower requirements described above.

2. Laboratory personnel and support staff must be provided appropriate occupational medical services 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.

3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared. The biosafety manual must be available, accessible, and followed.

5. The laboratory supervisor is responsible for ensuring that laboratory personnel:

   5.1. Demonstrate high proficiency in standard and special microbiological practices, and techniques for working with agents requiring BSL-4 containment. Receive appropriate training in the practices and operations specific to the policy changes occur.

6. 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 BSL-4 containment unless inactivated by a validated method.

7. Laboratory equipment musts be routinely decontaminated, as well as after spills, splashes, or other potential contamination.

   7.1. Spills involving infectious materials must be contained, decontaminated, and cleaned up by appropriate professional staff, or others properly trained and equipped towork with infectious material. A spill procedure must be developed and posted within the laboratory.

   7.2. 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.

   7.3. 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.

8. 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.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. Supplies and materials that are not brought into the BSL-4 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

    10.1. are brought into the facility. The doors of the autoclave or fumigation chamber are interlocked in a manner that prevents opening of the outer door unless the autoclave or fumigation chamber has been operated through a decontamination cycle.

    10.2. Only necessary equipment and supplies should be stored inside the BSL-4 laboratory. All equipment and supplies taken inside the laboratory must be decontaminated before removal from the laboratory.

11. 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.

12. 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.

C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

Cabinet Laboratory

1. All manipulations of infectious materials within the laboratory must be conducted in the Class III biological safety cabinet.

    1.1. 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.

    1.2. 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.

    1.3. 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.

    1.4. 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.

1.5. Class III cabinet gloves must be inspected for damage prior to use and changed if necessary. Gloves should be replaced annually during cabinet re-certification.

1.6. The cabinet should be designed to permit maintenance and repairs of cabinet mechanical systems (refrigeration, incubators, centrifuges, .) to be performed from the exterior of the cabinet whenever possible.

1.7. 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.

1.8. The Class III cabinet must be certified at least annually.

2. Workers in the laboratory must wear protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. 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 prior to removal from the laboratory for laundering.

3. 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.

4. Disposable gloves must be worn underneath cabinet gloves to protect the worker from exposure should a break or tear occur in a cabinet glove. 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.

Suit Laboratory

1. All procedures must be conducted by personnel wearing a one-piece positive pressure supplied air suit.

1.1. All manipulations of infectious agents must be performed within a BSC or other primary barrier system.

1.2. Equipment that may produce aerosols must be contained in primary barrier devices that exhaust air through HEPA filtration before being discharged into the laboratory. These HEPA filters should be tested annually and replaced as needed.

1.3. 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 specifications.

2. Workers must wear laboratory clothing, such as scrub suits, before entering the room used for donning positive pressure suits. All laboratory clothing must be removed in the dirty side change room before entering the personal shower.

3. Inner disposable 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 entering the personal shower. Dispose of used gloves with other contaminated waste.

4. Decontamination of outer suit gloves is performed during laboratory operations to remove gross contamination and minimize further contamination of the laboratory.

D. Laboratory Facilities (Secondary Barriers)

Cabinet Laboratory

1. TBSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

    1.1. Rooms in the facility must be arranged to ensure sequential passage through an inner (dirty) changing area, a personal shower and an outer (clean) change room upon exiting the room(s) containing the Class III BSC(s).

    1.2. An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on an uninterrupted power supply (UPS).

    1.3. A double-door autoclave, dunk tank, fumigation chamber, or ventilated airlock must be provided at the containment barrier for the passage of materials, supplies, or equipment.

2. A hands-free sink must be provided near the door of the cabinet room(s) and the inner change room. A sink must be provided in the outer change room. All sinks in the room(s) containing the Class III BSC must be connected to the wastewater decontamination system.

3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

    3.1. All penetrations in the internal shell of the laboratory and inner change room must be sealed.

    3.2. Openings around doors into the cabinet room and inner change room must be minimized and capable of being sealed to facilitate decontamination.

    3.3. Drains in the laboratory floor (if present) must be connected directly to the liquid waste decontamination system.

    3.4. Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

    3.5. Decontamination of the entire cabinet must be performed using a validated gaseous or vapor method when there have been significant changes in cabinet usage, before

major renovations or maintenance shut downs, and in other situations, as determined by risk assessment.

3.6. Selection of the appropriate materials and methods used for decontamination must be based on the risk assessment.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with 4.1. a non-porous material that can be easily decontaminated.
5. Windows must be break-resistant and sealed.

6. If Class II BSCs are needed in the cabinet laboratory, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. Class II cabinets should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the cabinet room. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory.

9. A dedicated non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3-Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

    9.1. The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory.

    9.2. Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

    9.3. The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

    9.4. Supply air to and exhaust air from the cabinet room, inner change room, and fumigation/decontamination chambers must pass through HEPA filter(s). The air exhaust discharge must be located away from occupied spaces and building air intakes.

    9.5. All HEPA filters should be located as near as practicable to the cabinet and laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

    9.6. The HEPA filter housings should be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. 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 the manufacturer's recommendations. If BSC exhaust is to be recirculated to the outside, BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a hard ducted, direct connection ensuring that cabinet exhaust air passes through two (2) HEPA filters—including the HEPA in the BSC—prior to release outside. Provisions to assure proper safety cabinet performance and air system operation must be verified.

    10.1. Class III BSCs must be directly and independently exhausted through two HEPA filters in series. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the cabinet room(s). Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.

12. Liquideffluentsfromcabinetroomsinks,floordrains,autoclavechambers, and other sources within the cabinet room must be decontaminated by a proven method, preferably heat treatment, before being dischargedto the sanitary sewer.

    12.1. Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

    12.2. Effluents from showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable and airtight and capable of expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended 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.

    13.1. Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that unfiltered air or steam exposed to infectious material cannot be released to the environment.

14. The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually. Verification criteria should be modified as necessary by operational experience.

15. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer). Provisions for emergency communication and emergency access or egress must be developed and implemented.

Suit Laboratory

1. The BSL-4 suit laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. Laboratory doors must have locks in accordance with the institutional policies.

    1.1. Rooms in the facility must be arranged to ensure exit by sequential passage through the chemical shower, inner (dirty) change room, personal shower, and outer (clean) changing area.

    1.2. Entry into the BSL-4 laboratory must be through an airlock fitted with airtight doors. Personnel who enter this area must wear a positive pressure suit supplied with HEPA filtered breathing air. The breathing air systems must have redundant compressors, failure alarms and emergency backup.

    1.3. A chemical shower must be provided to decontaminate the surface of the positive pressure suit before the worker leaves the laboratory. In the event of an emergency exit or failure of the chemical shower system, a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.

    1.4. An automatically activated emergency power source must be provided, at a minimum, for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets.

    1.5. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit controls, and security systems should be on a UPS.

    1.6. A double-door autoclave, dunk tank, or fumigation chamber must be provided at the containment barrier for the passage of materials, supplies, or equipment in or out of the laboratory.

2. Sinks inside the suit laboratory should be placed near procedure areas and be connected to the wastewater decontamination system.

3. Walls, floors, and ceilings of the laboratory must be constructed to form a sealed internal shell to facilitate fumigation and prohibit animal and insect intrusion. The internal surfaces of this shell must be resistant to chemicals used for cleaning and decontamination of the area. Floors must be monolithic, sealed and coved.

    3.1. All penetrations in the internal shell of the laboratory, suit storage room and the inner change room must be sealed.

    3.2. Drains, if present, in the laboratory floor must be connected directly to the liquid waste decontamination system. Sewer vents must have protection against insect and animal intrusion.

    3.3. Services and plumbing that penetrate the laboratory walls, floors, or ceiling must be installed to ensure that no backflow from the laboratory occurs. These penetrations must be fitted with two (in series) backflow prevention devices. Consideration should be given to locating these devices outside of containment. Atmospheric venting systems must be provided with two HEPA filters in series and be sealed up to the second filter.

4. Laboratory furniture must be of simple construction, capable of supporting anticipated loading and uses. Sharp edges and corners should be avoided. Spaces between benches,

cabinets, and equipment must be accessible for cleaning and decontamination. Chairs and other furniture must be covered with a non-porous material that can be easily decontaminated.

5. Windows must be break-resistant and sealed.

6. BSCs and other primary containment barrier systems must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Central vacuum systems are not recommended. If, however, there is a central vacuum system, it must not serve areas outside the BSL-4 laboratory. Two in-line HEPA filters must be placed near each use point. Filters must be installed to permit in-place decontamination and replacement.

8. An eyewash station must be readily available in the laboratory area for use during maintenance and repair activities.

9. A dedicated, non-recirculating ventilation system is provided. Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if gas-tight dampers and HEPA filters isolate each individual laboratory system.

    9.1. The supply and exhaust components of the ventilation system must be designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow as appropriate between adjacent areas within the laboratory.

    9.2. Redundant supply fans are recommended. Redundant exhaust fans are required. Supply and exhaust fans must be interlocked to prevent positive pressurization of the laboratory.

    9.3. The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. A visual monitoring device must be installed near the clean change room so proper differential pressures within the laboratory may be verified prior to entry.

    9.4. Supply air to the laboratory, including the decontamination shower, must pass through a HEPA filter. All exhaust air from the suit laboratory, decontamination shower and fumigation or decontamination chambers must pass through two HEPA filters, in series, before discharge to the outside. The exhaust air discharge must be located away from occupied spaces and air intakes.

    9.5. All HEPA filters must be located as near as practicable to the laboratory in order to minimize the length of potentially contaminated ductwork. All HEPA filters must be tested and certified annually.

    9.6. The HEPA filter housings must be designed to allow for in situ decontamination and validation of the filter prior to removal. The design of the HEPA filter housing must have gas-tight isolation dampers, decontamination ports, and ability to scan each filter assembly for leaks.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to the manufacturer's recommendations. Biological safety cabinets

can also be connected to the laboratory exhaust system by either a thimble (canopy connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. Pass through dunk tanks, fumigation chambers, or equivalent decontamination methods must be provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the BSL-4 laboratory. Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.

12. Liquid effluents from chemical showers, sinks, floor drains, autoclave chambers, and other sources within the laboratory must be decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.

    12.1. Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.

    12.2. Effluents from personal body showers and toilets may be discharged to the sanitary sewer without treatment.

13. A double-door, pass through autoclave(s) must be provided for decontaminating materials passing out of the cabinet laboratory. Autoclaves that open outside of the laboratory must be sealed to the interior wall. This bioseal must be durable, airtight, and capable of expansion and contraction. Positioning the bioseal so that the equipment can be accessed and maintained from outside the laboratory is strongly recommended. 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.

APPENDIX 2: GUIDE TO WRITING STANDARD OPERATING PROCEDURES (SOPS)

A Standard Operating Procedure (SOP) is a set of step by step instructions for carrying out a specific technique. They can be used for any technique, but this guide focuses on their use in documenting monitoring techniques, specifically for species and ecological communities.

SOPs are used to stipulate how the monitoring will be undertaken, and provide quality assurance that the data collected will be consistent and therefore comparable. They should be clear and concise but with enough detail so that users with a basic understanding but limited experience can reproduce the procedure.

The advantages of having a SOP is that they:

1. Provide personnel with numbered step by step instructions on a specific procedure (or procedure used to carry out a method) with minimum variability;

2. Ensure that the procedures are performed consistently and in compliance with government regulations;

3. Protect the health and safety of personnel by enabling jobs to be carried out in the safest possible way. They ensure that all of the safety, health, environmental and operational information is available to perform specific procedures with minimal impact;

4. Facilitate training in procedures, for both new personnel and for those that need re-training (ie. after extended absence from a position. Having step by step instructions aids trainers to ensure that nothing is missed;
5. Serve as a historical record for use when modifications are made to that procedure and when the SOP is revised
6. Promote quality though consistent collection of the data, even if there are changes in the people undertaking the monitoring; and
7. Encourage improvements and work evaluation by ensuring that the procedures are completed, and can be used in incident investigations to improve operations and safety practices

It is not possible, nor practical to provide all the appropriate headings that could be used in a template. The following guide has been developed for the general requirements of an SOP.

1. SOP Title

This is a description of the SOP. The title should be concise, but descriptive enough to indicate what technique the SOP provides instructions for.

2. SOP No

A SOP will be allocated a SOP number after it has been reviewed and approved. The numbering system involves placing SOPs in categories (e.g. Fauna Observation, Fauna Capture, Remote Observation etc.) with a sequential number applied in each category. The first digit in a SOP number therefore refers to the category and the second digit refers to the specific SOP.

3. Prepared by

Identifies the personnel who prepared the SOP. The first author listed will be the author to which correspondence/enquiries will be directed unless otherwise indicated.

4. Prepared for

Lists the organization or project for which the SOP has been prepared.

5. Version

The version number is allocated when the SOP is approved. Version numbers increase incrementally by hundredths (e.g. version 1.1, version 1.2, …etc) for minor changes. Major revisions should be designated with the next whole number (e.g. version 2.0, 3.0, 4.0 …).

6. Revision history log

This table records the version number, revision date, author and a brief summary of the changes that have been made to the SOP. The revision history log must be completed whenever an alteration to the version number is made (including approval for the first time).

Revision History Log			
Version	**#Revision Date**	**Author**	**Changes**

7. Approved by

Identity of the personnel approving the SOP for distribution and use.

8. Acknowledgements

Lists any other personnel (e.g. reviewers) or documents which aided in the preparation of the SOP.

9. Purpose

This is an introductory section to provide background information on the procedure. It explains why the SOP has been written and what the SOP is to be used for.

10. Scope

This section identifies the agencies and jurisdictions to which the SOP will apply. It specifies which situations the SOP will and will not apply to, and identifies those personnel who should be familiar with its content.

11. Definitions

This section should contain a list of any terms contained in the SOP that may be unfamiliar to, or misinterpreted by the reader. These must be in alphabetical order. References should be provided wherever possible. Approved methods

Briefly describe the methods that are approved and covered by the SOP. This section can be deleted if the SOP only covers one technique.

12. Procedure outline

In this section each specific task required to perform the procedure should be identified, listed and labeled. The easiest way to write this section is to perform the task, writing down each step as it is performed, in the same way that a recipe would be written for making a cake.

The procedure outline should describe when and how all aspects of the procedure are done, including troubleshooting tips. It can contain diagrams, photos, charts or tables. Users tend to have difficulty with long SOPs, so if the procedure is more than 10 steps long, break major tasks undertaken into subheadings. Other SOPs may be referred to if required.

When writing the SOP, you should consider:

1. What is needed before starting the task, the materials needed and how they are used;
2. Health and safety considerations, and specify them at the appropriate steps;
3. Whether there may be two methods which must be done at the same time. This needs to be clearly stated so that there is no confusion;
4. Providing personnel with alternative steps if there is a possibility that a step may not work (ie. under specific circumstances);
5. The time that the task may or should take, or how long it should be performed for this should be clearly stated; and
6. Using drawings or graphics, as well labeled drawings are sometimes better than text – a picture tells a thousand words! Level of Impact

The next two sections are for projects involving fauna only. The level of impact will determine the level of scrutiny the projects using the procedure will receive from the Animal Ethics Committee. You need to ask yourself if the procedure will have a potentially high, medium or low impact on animals and whether the procedure can be managed to reduce the level of impact.

13. Ethical Considerations

This section covers the issues that the Animal Ethics Committee will be reviewing, to ensure that the project proponents have adequately assessed them and put in place management strategies to minimise the impact on the animal.

14. Competencies and Approvals

In this section, details of all permits, licenses and experience which personnel must have prior to the procedure being carried out should be provided. This ensures personnel are trained and experienced in the procedure and identifies where training is required.

APPENDIX 3: INSPECTION CHECKLIST FOR BSL-2 LABORATORIES (BMBL 5TH EDITION; NIH GUIDELINES)

Inspection Checklist for BSL-2 Laboratories (BMBL 5th edition; *NIH Guidelines*)		
Lab PI/Contact Person:	Inspection Date:	Inspected By:
Lab Location (Bldg/Rm #)	Dept:	Phone #:
Inspection Type: ( ) Initial ( ) Annual		
List of Agents In Use ( ) Recombinant DNA: ( ) Bacteria ( ) Parasite: ( ) Toxin: ( ) Virus: ( ) Prion: ( ) Fungus: ( ) Human: ( ) Select Agent: ( ) Other:		

Reference	Statement	Response			Comments
		Yes	No	NA	
A Standard Microbiological Practices					
BMBL: A1 NIH: G-II-B-1-a NIH: G-II-B-2-b	The PI must enforce the institutional policies that control access to the laboratory. Access is limited/restricted by the PI when work is in progress.				
BMBL: A2 NIH: G-II-B-1-f	Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory				
BMBL: A3 NIH: G-II-B-1-e	Eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food for human consumption must not be permitted in laboratory areas.				
BMBL: A3	Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose				
BMBL: A4 NIH: G-II-B-1-d	Mouth pipetting is prohibited; mechanical pipetting devices must be used				
BMBL: A5	Policies for safe handling of sharps must be developed and implemented.				
BMBL: A5 NIH: G-II-B-2-j	Whenever practical, laboratory supervisors should adopt improved engineering/work practice controls that reduce risk of sharps injury.				
BMBL: A5	Precautions, listed below, must always be taken with sharps items.				
BMBL: A5-a	Needles must not be bent, sheared, broken, recapped, removed from disposable syringes or otherwise manipulated by hand before disposal.				
BMBL: A5-b	Place used disposable needles/syringes in puncture-resistant containers.				
BMBL: A5-c	Non-disposable needles are placed in a hard walled container for transport to decontamination area (autoclave).				
BMBL: A5-d	Broken glassware must be handled with a mechanical device (tongs, forceps, dustpan/brush) not with hands.				
BMBL: A5-d	Substitute plastic ware whenever possible.				
BMBL: A6 NIH: G-II-B-1-g	Perform all procedures to minimize the creation of splashes/aerosols.				
BMBL: A7 NIH: G-II-B-1-b	Decontaminate work surfaces after completion of work and after any spill or splash of biohazardous material. Use the appropriate disinfectant.				
BMBL: A8 NIH: G-II-B-1-c	Decontaminate all cultures, stocks, and other biohazardous material before disposal.				
BMBL: A8	Depending on where the decontamination will be performed, use the following methods prior to transport.				
BMBL: A8-a	Materials to be decontaminated outside of the immediate lab must be placed in a durable, leak-proof container and secured for transport.				
BMBL: A8-b	Materials to be removed from the facility for decontamination must be packed in accordance with applicable regulations.				
BMBL: A9	A sign incorporating the universal biohazard symbol must				

NIH: G-II-B-2-d	be posted at the entrance to the lab when biohazardous materials are present. Posted information must include: the biosafety level, the responsible person's name, telephone number, and entry/exit procedures.				
BMBL: A9	Agent information should be posted in accordance with institutional policy.				
BMBL: A10 NIH: G-II-B-2-e	An effective integrated pest management program is required. See appendix G.				
BMBL: A11	The lab supervisor must ensure that lab personnel receive appropriate training regarding their duties, precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedures or policy changes occur.				
BMBL: A11	Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, 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.				
NIH: G-II-B-1-h	Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same lab.				
BMBL: B Special Microbiological Practices					
BMBL: B1 NIH: G-II-B-2-c	All persons entering the lab must be advised of the potential hazards and meet specific entry requirements.				
BMBL: B2	Lab personnel must be provided medical surveillance and offered appropriate immunizations for agents in the lab.				
BMBL: B3 NIH: G-II-B-2-l	When appropriate, a baseline serum sample should be stored.				
BMBL: B4 NIH: G-II-B-2-m	A lab-specific biosafety manual must be prepared and adopted as policy.				
BMBL: B4	The manual must be available and accessible.				
BMBL: B5	The lab supervisor must ensure that lab personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.				
BMBL: B6 NIH: G-II-B-2-a	Potentially biohazardous material must be placed in a durable, leak-proof container during collection, handling, processing, storage, or transport within a facility.				
BMBL: B7	Lab equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.				
BMBL: B7-a	Spills involving biohazardous material must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with biohazardous material.				
BMBL: B7-b	Equipment must be decontaminated before repair, maintenance or removal from the lab.				
BMBL: B8 NIH: G-II-B-2-k	Incidents that may result in exposure to biohazardous material must be immediately evaluated and treated according to procedures described in the lab biosafety manual. All such incidents must be reported to the lab supervisor.				
BMBL: B9 NIH: G-II-B-2-g	Animals and plants not associated with the work must not be permitted in the lab.				
BMBL: B10	All procedures involving the manipulation of biohazardous material that may generate an aerosol should be conducted in a BSC or other physical containment device.				
BMBL: C Laboratory Facilities (Primary Barriers)					
BMBL: C1-a NIH: G-II-B-3-a NIH: G-II-B-3-a-(1)	Properly maintained BSCs (preferably Class II), other appropriate PPE, or other physical containment devices must be used whenever: Procedures with potential for creating infectious aerosols or splashes are conducted (examples: **pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of material, intranasal animal inoculation, harvesting infected tissues from animals or eggs**).				

BMBL: C1-b NIH: G-II-B-3-a-(2)	Properly maintained BSCs (preferably Class II), other appropriate PPE, or other physical containment devices must be used whenever: High concentrations or large volumes of material are used. Such materials may be centrifuged in the open lab using sealed rotor heads or centrifuge safety cups.				
BMBL: C2 NIH: G-II-B-2-f	Protective lab coats, gowns, smocks or uniforms designated for lab use must be worm while working with hazardous material.				
BMBL: C2 NIH: G-II-B-2-f	Remove PPE before leaving non-lab areas (e.g. cafeteria, library admin offices).				
BMBL: C2	Dispose of PPE appropriately or deposit it for laundering by the institution. It is recommended that lab clothing not be taken home.				
BMBL: C3	Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of material when handled outside of the BSC.				
BMBL: C3	Eye and face protection must be disposed of with other contaminated lab waste or decontaminated before reuse.				
BMBL: C3	Persons who wear contact lenses in labs should also wear eye protection.				
BMBL: C4 NIH: G-II-B-2-h	Gloves must be worn to protect hands from exposure to hazardous material.				
BMBL: C4	Glove selection should be based on an appropriate risk assessment.				
BMBL: C4	Alternatives to latex gloves should be available.				
BMBL: C4	Gloves must not be worn outside the lab.				
BMBL: C4-a	In addition, BSL-2 workers should: Change gloves when contaminated, integrity has been compromised or when otherwise necessary.				
BMBL: C4-a	In addition, BSL-2 workers should: Wear two pairs of gloves when appropriate.				
BMBL: C4-b	In addition, BSL-2 workers should: Remove gloves and wash hands when work with the material has been completed and before leaving the lab.				
BMBL: C4-c	In addition, BSL-2 workers should: Not wash or reuse disposable gloves.				
BMBL: C4-c	In addition, BSL-2 workers should: Dispose of used gloves with other contaminated lab waste.				
BMBL: C4-c	Hand washing protocols must be rigorously followed.				
BMBL: C5	Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.				
BMBL: D Laboratory Facilities (Secondary Barriers)					
BMBL: D1	Lab doors should be self-closing and have locks in accordance with institutional policies.				
BMBL: D2 NIH: G-II-B-4-d	Labs must have a sink for hand washing. The sink may be manual, hands-free or automatic. It should be located near the exit door.				
BMBL: D3 NIH: G-II-B-4-a	The lab should be designed so that it can be easily cleaned and decontaminated.				
BMBL: D3	Carpets and rugs are not permitted.				
BMBL: D4 NIH: G-II-B-4-c	Lab furniture must be capable of supporting anticipated loads and uses.				
BMBL: D4 NIH: G-II-B-4-c	Spaces between benches, cabinets and equipment should be accessible for cleaning.				
BMBL: D4-A NIH: G-II-B-4-b	Bench tops must be impervious to water, resistant to heat, organic solvents, acids, alkali and other chemicals.				
BMBL: D4-b	Chairs used in lab work must be covered with a non-porous material that can be easily cleaned and decontaminated.				
BMBL: D5 NIH: G-II-B-4-e	Lab windows to the exterior are not recommended. However, if a lab does have windows that open to the exterior, they must be fitted with fly screens.				
BMBL: D6	BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations.				
BMBL: D6	BSCs should be located away from doors, windows that can be opened, heavily traveled lab areas, and other possible lab flow disruptions.				

BMBL: D7	Vacuum lines should be protected with HEPA filters or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.				
BMBL: D8	An eyewash station must be readily available.				
BMBL: D9	There are no specific requirements on 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 lab.				
BMBL: D10	HEPA filtered air form a Class II BSC can be safely recirculated back into the lab if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the lab exhaust system by either a thimble canopy or by a hard connection.				
BMBL: D10	Provisions to assure proper BSC performance and air system operation must be verified.				
BMBL: D11 NIH: G-II-B-2-i NIH: G-II-B-4-	A method for decontaminating all lab wastes should be available in the facility (e.g. autoclave, chemical disinfection, incineration or other validated decontamination method).				

APPENDIX 4: INSPECTION CHECKLIST FOR BSL-3 LABORATORIES (BMBL 5TH EDITION; NIH GUIDELINES)

Inspection Checklist for BSL-3 Laboratories (BMBL 5th edition; *NIH Guidelines*)		
Lab PI/Contact Person:	Inspection Date:	Inspected By:
Lab Location (Bldg/Rm #)	Dept:	Phone #:
Inspection Type: ( ) Initial ( ) Annual		
List of Agents In Use ( ) Recombinant DNA: ( ) Bacteria ( ) Parasite: ( ) Toxin: ( ) Virus: ( ) Prion: ( ) Fungus: ( ) Human: ( ) Select Agent: ( ) Other:		

Reference	**Statement**	**Yes**	**No**	**NA**	**Comments**
CFR: Section 12(a)	An individual or entity required to register under this part must develop and implement a written biosafety plan that is commensurate with the risk of the agent or toxin, given its intended use.				
CFR: Section 12(a)	The biosafety plan must contain sufficient information and documentation to describe the biosafety and containment procedures.				
CFR: Section 12(b)	The biosafety and containment procedures must be sufficient to contain the select agent or toxin (e.g., physical structure and features of the entity, and operational and procedural safeguards).				
CFR: Section 12 (c)(1)	In developing a biosafety plan, an individual or entity should consider: The CDC/NIH publication, "Biosafety in Microbiological and Biomedical Laboratories, including all appendices. Copies may be obtained from the Superintendent of Documents, U.S. Government Printing Office, Post Office Box 371954, Pittsburgh, Pennsylvania, 75250-7954 or from the CDC website at http://www.cdc.gov/. Copies may be inspected at the Centers for Disease Control and Prevention, 1600 Clifton Road, Mail Stop E-79, Atlanta, Georgia.				
CFR: Section 12(d)	The plan must be reviewed annually and revised as necessary.				
CFR: Section 12(d)	Drills or exercises must be conducted at least annually to test and evaluate the effectiveness of the plan.				
CFR: Section 12(d)	The plan must be reviewed and revised, as necessary, after any drill or exercise and after any incident.				
BMBL A. Standard Microbiological Practices					
BMBL: A1	The laboratory supervisor must enforce the institutional policies that control access to the laboratory.				
BMBL: A2	Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.				
BMBL: A3	Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas.				
BMBL: A3	Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.				
BMBL: A4	Mouth pipetting is prohibited; mechanical pipetting devices must be used.				

BMBL: A5	Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented.				
BMBL: A5	Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.				
BMBL: A5	Precautions, including those listed below, must always be taken with sharp items. These include:				
BMBL: A5-a	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.				
BMBL: A5-b	Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.				
BMBL: A5-c	Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.				
BMBL: A5-d	Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.				
BMBL: A5-d	Plasticware should be substituted for glassware whenever possible.				
BMBL: A6	Perform all procedures to minimize the creation of splashes and/or aerosols.				
BMBL: A7	Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.				
BMBL: A8	Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.				
BMBL: A8	A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).				
BMBL: A8-a	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.				
BMBL: A8-b	Depending on where the decontamination will be performed, the following methods should be used prior to transport: Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.				
BMBL: A9	A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present.				
BMBL: A9	Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory.				
BMBL: A9	Agent information should be posted in accordance with the institutional policy.				
BMBL: A10	An effective integrated pest management program is required.				
BMBL: A11	The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.				
BMBL: A11	Personnel must receive annual updates or				

	additional training when procedural or policy changes occur.				
BMBL: A11	Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, 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.				
BMBL: A11	Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.				
BMBL B Special Microbiological Practices					
BMBL: B1	All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.				
BMBL: B2	Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.				
BMBL: B3	When appropriate and giving consideration to the agent handled based upon the facility's risk assessment, each institution must establish policies and procedures describing the collection and storage of baseline serum samples from at-risk personnel.				
BMBL: B4	A laboratory-specific biosafety manual must be prepared and adopted as policy.				
BMBL: B4	The biosafety manual must be available and accessible.				
BMBL: B5	The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.				
BMBL: B6	Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.				
BMBL: B7	Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.				
BMBL: B7-a	Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.				
BMBL: B7-b	Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.				
BMBL: B8	Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual.				
BMBL: B8	All such incidents must be reported to the laboratory supervisor.				
BMBL: B8	Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.				
BMBL: B9	Animals and plants not associated with the work being performed must not be permitted in the laboratory.				
BMBL: B10	All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices.				
BMBL: B10	No work with open vessels is conducted on the bench.				
BMBL: B10	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.				
BMBL: C Laboratory Facilities (Primary Barriers)					
BMBL: C1	All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.				
BMBL: C2	Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory.				
BMBL: C2	Protective clothing is not worn outside of the laboratory.				
BMBL: C2	Reusable clothing is decontaminated with appropriate disinfectant before being laundered.				
BMBL: C2	Clothing is changed when contaminated.				
BMBL: C3	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.				
BMBL: C3	Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse.				
BMBL: C3	Persons who wear contact lenses in laboratories must also wear eye protection.				
BMBL: C4	Gloves must be worn to protect hands from exposure to hazardous materials.				
BMBL: C4	Glove selection should be based on an appropriate risk assessment.				
BMBL: C4	Alternatives to latex gloves should be available.				
BMBL: C4	Gloves must not be worn outside the laboratory.				
BMBL: C4-a	In addition, BSL-3 laboratory workers should: Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.				
BMBL: C4-a	In addition, BSL-3 laboratory workers should: Wear two pairs of gloves when appropriate.				
BMBL: C4-b	In addition, BSL-3 laboratory workers should: Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.				
BMBL: C4-c	In addition, BSL-3 laboratory workers should: Do not wash or reuse disposable gloves.				
BMBL: C4-c	In addition, BSL-3 laboratory workers should: Dispose of used gloves with other contaminated laboratory waste.				
BMBL: C4-c	Hand washing protocols must be rigorously followed.				
BMBL: C5	Eye, face, and respiratory protection must be used in rooms containing infected animals.				
BMBL: D Laboratory Facilities (Secondary Barriers)					
BMBL: D1	Laboratory doors must be self closing and have locks in accordance with the institutional policies.				
BMBL: D1	The laboratory must be separated from areas that are open to unrestricted traffic flow within the building.				
BMBL: D1	Access to the laboratory is restricted to entry by a series of two self-closing doors.				
BMBL: D1	A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.				
BMBL: D2	Laboratories must have a sink for hand washing.				
BMBL: D2	The sink must be hands-free or automatically operated.				
BMBL: D2	The sink should be located near the exit door.				
BMBL: D2	If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone.				

BMBL: D3	The laboratory must be designed so that it can be easily cleaned and decontaminated.				
BMBL: D3	Carpets and rugs are not permitted.				
BMBL: D3	Seams, floors, walls, and ceiling surfaces should be sealed.				
BMBL: D3	Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.				
BMBL: D3-a	Floors must be slip resistant, impervious to liquids, and resistant to chemicals.				
BMBL: D3-a	Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.				
BMBL: D3-b	Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.				
BMBL: D3-c	Ceilings should be constructed, sealed, and finished in the same general manner as walls.				
BMBL: D3	Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs.				
BMBL: D3	Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.				
BMBL: D4	Laboratory furniture must be capable of supporting anticipated loads and uses.				
BMBL: D4	Spaces between benches, cabinets, and equipment must be accessible for cleaning.				
BMBL: D4-a	Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.				
BMBL: D4-b	Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.				
BMBL: D5	All windows in the laboratory must be sealed.				
BMBL: D6	BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations.				
BMBL: D6	BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.				
BMBL: D7	Vacuum lines must be protected with HEPA filters, or their equivalent.				
BMBL: D7	Filters must be replaced as needed.				
BMBL: D7	Liquid disinfectant traps may be required.				
BMBL: D8	An eyewash station must be readily available in the laboratory.				
BMBL: D9	A ducted air ventilation system is required.				
BMBL: D9	This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas.				
BMBL: D9	The laboratory shall be designed such that under failure conditions the airflow will not be reversed.				
BMBL: D9-a	Laboratory personnel must be able to verify directional air flow.				
BMBL: D9-a	A visual monitoring device which confirms directional air flow must be provided at the laboratory entry.				
BMBL: D9-a	Audible alarms should be considered to notify personnel of air flow disruption.				
BMBL: D9-b	The laboratory exhaust air must not re-circulate to any other area of the building.				
BMBL: D9-c	The laboratory building exhaust air should be dispersed away from occupied areas and from				

	building air intake locations or the exhaust air must be HEPA filtered.				
BMBL: D10	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. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection.				
BMBL: D10	Provisions to assure proper safety cabinet performance and air system operation must be verified.				
BMBL: D10	BSCs should be certified at least annually to assure correct performance.				
BMBL: D10	Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.				
BMBL: D11	A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).				
BMBL: D12	Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.				
BMBL: D13	Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.				
BMBL: D14	Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability.				
BMBL: D14	The HEPA filter housing should allow for leak testing of each filter and assembly.				
BMBL: D14	The filters and the housing should be certified at least annually.				
BMBL: D15	The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation.				
BMBL: D15	Facilities must be re-verified and documented at least annually.				

APPENDIX 5: PPE DON AND DOFF GUIDANCE

Procedure for taking off nitrile gloves and sleeves whilst working in biological safety cabinets. SSB/02/PROTECTION/NCB3

Aim

Establish a procedure for taking off protective nitrile gloves and sleeves when working in a biological safety cabinet.

Area of Application

For all those who work in the Centre's laboratories and biological safety.

Action

Gloves and sleeves must be decontaminated before being removed. To decontaminate, use the appropriate decontaminant for the area, zone and the biological agent being handled.

PHASES

1.- In the biological safety cabinet, decontaminate the gloves with an appropriate decontaminant solution.

2.- Once the gloves and the sleeves that have been used in the biological safety cabinet have been decontaminated, with one hand pinch the outside of the upper part (nearest the elbow) of the sleeve of the opposite arm and pull off towards the hand so that the sleeve ends up covering the hand.

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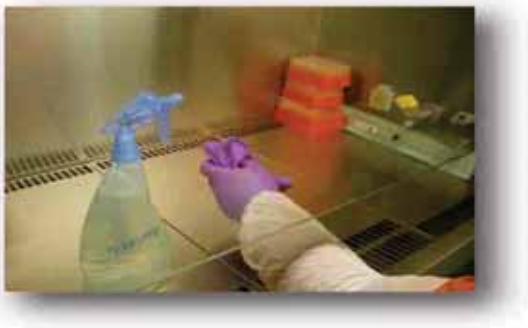
3.- Then, again with the other hand, pinch the upper outside part of the other glove and pull off towards the fingers to free the hand.

4.- Both the glove and sleeve can then be fully removed together by the (still-gloved) other hand.



5.- To remove the other sleeve, place the thumb or index finger of the free hand inside of the sleeve without touching the outside. Pull off towards the fingers so that the sleeve covers the gloved hand.

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6.- To remove the other glove, place the thumb or index finger of the free hand inside the second glove without touching the outside. Pull off towards the fingers so that the second glove covers the first glove and sleeve (which are being held inside the second hand). In this way, you ensure that the inside of the used gloves and sleeves is exposed on the outside of this 'ball' of used material.

7.- Remove the hands from the biological safety cabinet and deposit the gloves and sleeves in the container for plastic waste.

CHECK LIST

Question	YES	NO
Did you decontaminate the gloves?		
Did you do it inside the cabinet?		
Did you take off the gloves correctly inside the cabinet? (i.e. one glove and sleeve are rolled up in the other one, and without touching the outside of the gloves and sleeves with the free hands)		
Did you place the used gloves and sleeves in the correct waste container?		
Might you have followed incorrectly any of the steps in this procedure?		

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Procedure for removing nitrile gloves. SSB/03/PROTECCIÓN/EXTERIOR-NCB2-NCB3

Aim

Establish a procedure for taking off protective nitrile gloves.

Application

For all those who work in the Centre's laboratories, Animal Facilities, biological safety, maintenance, cleaning and general safety.

Action

Gloves must be decontaminated before being removed. Medical, cleaning and general safety staff should decontaminate gloves with a hydroalcoholic solution.

Staff from the Animal Facilities, laboratories, biological safety and maintenance should decontaminate with the appropriate decontaminant for the area or zone in which they are working, or for the biological agent they have handled or any other they may have come into contact with.

PHASES

1.- Decontaminate the gloves with the hydro-alcoholic solution or spray with a decontaminant solution.

2.- Once decontaminated, with one hand pinch the glove on the other hand on the outside of the part nearest the elbow and pull off towards the fingers to free the hand.

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3.- Keep hold of the removed glove in the other (still-gloved) hand.

4.- Place the index finger of the freed hand inside the second glove and, without touching the outside, pull off the glove towards the fingers.

5.- The second glove will cover the other glove (held in the hand) and the inside of both gloves will be exposed to the outside. Place in a container for waste plastic.

For more information, contact the Biological Safety Service.

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Procedure for putting on and taking off filtering half masks. SSB/04/PROTECTION/EXTERIOR-NCB2-NCB3

Aim

Establish a procedure for putting on and taking off FFP1, FFP2 and FFP3 filtering masks.

Application

For all those who work in the Centre's laboratories, Animal Facilities, biological safety, maintenance, cleaning and general safety.

Action

Gloves must be decontaminated before being removed. Medical, cleaning and general safety staff should decontaminate gloves with a hydro-alcoholic solution.

Staff from the Animal Facilities, laboratories, biological safety and maintenance should decontaminate with the appropriate decontaminant for the area or zone in which they are working, or for the biological agent they have handled or any other they may have come into contact with.

PUTTING ON FILTERING MASKS

1.- First, check to see that the mask is in good condition. In the case of adjustable masks, place the rubber straps in their slots.

2.- With one hand, place the mask over the nose and mouth with the elastic straps resting on top of (not under) the hands. Place the lower strap around the neck and then the upper strap over the crown of the head.

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3.- Once in place, for masks with adjustable straps, tighten the lower strap to fit the mask well to the chin and then the upper straps to fit the mask to the nose.

This operation is not applicable to masks without adjustable straps.

4.- Next, adjust the metal clip on the nose, readjusting the position of the mask by tightening or loosening the straps if necessary.

6.- Finally, place both hands over the filter and cover the exhalation valve (if there is one) as much as possible to carry out the positive or negative pressure test.

For the positive pressure test, with the hands covering the mask, breathe out normally to detect any leakage along the adjustable edge of the mask at the face, nose or chin.

For the negative pressure test, cover the mask and valve and breathe in to test for any leakage.

If in either case a small escape of air is detected, the elastic straps must be tightened and the test repeated.

TAKING OFF THE MASK

The procedure for taking off the mask is as follows:

1.- Hold the mask with one hand and loosen the elastic straps beginning with the lower straps and then continuing to the upper straps.

2.- Still holding the mask in one hand and once the straps are sufficiently loose, pass the straps over the hand holding the mask (first the lower strap and then the upper one).

3.- Finally, completely remove the mask from the face and place it in a container for plastic waste.

The gloves should be removed according to the procedure described above.

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Procedure for putting on and taking off coveralls TYCHEM/TYVEK with respirator. SSB/08/PROTECTION/NCB3

Aim

Establish the procedure for use of coveralls TYCHEM or TYVEK with respirator.

Application

This procedure must be followed by all those who have to use the protective coveralls described herein. Never enter a contaminated area alone. Always work in pairs. Both workers should be aware of what the other is doing, with special attention paid to the putting on and taking off of personal protective equipment (PPE).

Action

PUTTING ON PPE

1. In a clean area, put on and do up the zips.

2. Put on the footwear or boots, which should be taped to the outside of the leg of the coverall.

3. Put on the first pair of gloves and pass the coverall's thumb strap between the thumb and index finger. Tape up the glove leaving a non-adhesive tab exposed.

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4. Before placing the hood of the suit in its final position, put the mask on following the established procedure and conduct a positive or negative pressure test.
5. Next, put on the anti-splash goggles and then adjust the hood to its final position. Pay special attention to ensuring that the edge of the hood fits snugly with the mask and goggles.
6. Close the main zip completely and fix the horizontal and vertical adhesive tapes at the neck.

TAKING OFF PPE

1. Before leaving the contaminated area, each member of the pair should spray the other with decontaminating solution (all the coverall, gloves, goggles, soles of footwear, etc.).

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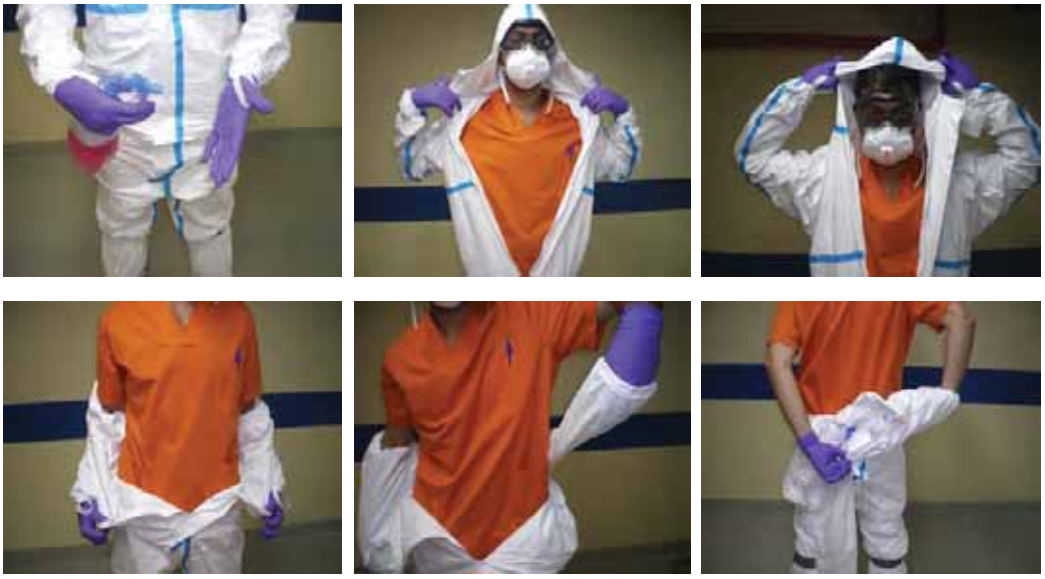
2. Wait for the decontaminant to act
3. Still inside the contaminated area, begin to remove the adhesive tape and second pair of gloves following the stipulated procedure. Place waste in the containers for plastic waste.
4. Decontaminate the second pair of gloves and leave the contaminated area.
5. Once in the non-contaminated area, take off the coverall.

6. Decontaminate again the gloves and unzip the coverall. Take the coverall off slowly, touching only the inside of the suit and ensuring that the inside part is then left exposed outwards.



7. Untape the footwear or boots. If boots have been worn, take them off and place them in an appropriate place.
8. Without yet taking off the footwear, remove the coverall, ensuring that its inside is exposed to the outside. Place suit in the container for plastic waste.

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9. Next, to remove eye protection, decontaminate the gloves again with a low-pressure spray. Remove the goggles with a backwards movement and take them off from the top of the head first. Place them in an appropriate place for further use.
   Once the goggles have been taken off, remove the filtering mask following the stipulated procedure and place it in the plastic waste container.



Check List

Question	YES	NO
Did you check the coverall, gloves, footwear and filtering mask before use?		
Did you zip up the zip and put on the first pair of gloves?		
Did you fix the coverall's elastic thumb strap by passing it between the thumb and index finger?		
Did you tape the second pair of gloves to the coverall and leave a tab of non-adhesive material for easy removal after use?		
Did you tape the footwear to the suit and leave a tab for easy removal after use?		
Did you put the FFP3 filtering mask on correctly?		
Did you test to see if the mask was correctly adjusted?		
Did you test the anti-splash goggles correctly?		
Did you check that the hood completely covered all your hair and face and left no exposed parts?		
Did you decontaminate the gloves before decontaminating your partner?		
When decontaminating your partner, did you pay special attention to the soles of his/her footwear, armpits, legs and back of the coverall?		
Did you decontaminate the outer gloves before removing them?		
Did you take off the hood with a backwards movement as stipulated?		
Did you take off the coverall ensuring that the inner part was left exposed on the outside?		
Did you decontaminate the gloves again?		
Did you take off the anti-splash goggles as stipulated?		
Did you take off the mask as stipulated?		
Did you take off the gloves as stipulated, leaving one pair in the other without touching the external surface of the glove with your free hands?		
Did you separate as stipulated the PPE in the container for plastic waste?		
Might you have followed incorrectly any of the steps in this procedure?		

For more information, contact the Biological Safety Service.

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Food and Agriculture Organization
of the United Nations
Regional Office for Asia and the Pacific
Maliwan Mansion
Phra Atit Road
Bangkok 10200, Thailand

FAO-RAP@fao.org

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