Operations at Biosafety Level III: 
The P3 Laboratory

Yoshio Ichinose, Shingo Inoue, Masaaki Shimada, Gabriel Miring’u, Betty Muriithi, Angela Makumi, Ernest Wandera, Martin Bundi, Chika Narita, Salame Ashur, Allan Kwalla, Amina Galata, Erick Odoyo, Sora Huqa, Mohammed Shah, Mohammed Karama, Masahiro Horio

1 Introduction

Biomedical research on pathogenic agents has steadily grown within the past decade, with increasing disease burden necessitating intensive research on highly infectious agents as well as organisms of unknown pathogenicity. Coupled with the need to ensure public health, protection of laboratory staff and the environment at large are of utmost importance, hence formulation of biosafety guidelines and subsequent development of containment laboratories.

Institution of biosafety strategies can be traced back to the mid-20th Century, when the first instances of laboratory–acquired infections occurred due to unsecured laboratory operations (Pike, 1979; Vesley & Hartman, 1988; Pedrosa & Cardoso, 2011). These stimulated the World Health Organization (WHO) to design biosafety guidelines that would guide development of codes of practice for safe handling of pathogenic microorganisms (WHO, 2004). Given varying pathogenicity of different microorganisms, differentiation of laboratory facilities into cumulative containment levels and Risk Group (RG) classification of microorganisms were developed. There are therefore four Biosafety Levels (BSL), or Protection levels; BSL-1, BSL-2, BSL-3 and BSL-4, each having specific design features, containment facilities, practices and operational procedures.

A BSL3 laboratory (or P3) laboratory is a medium containment facility that enables isolation and manipulation of pathogens that can be transmitted through aerosol (WHO, 2004). P3 laboratories apply BSL-3 principles and utilize various biocontainment strategies to provide total physical separation between a laboratory worker and a possible source of contamination. They have unique design and engineering features that facilitate containment in addition to biosafety equipments, all strategies aimed at ensuring maximum containment of infectious materials.

P3 laboratories can be found in hospitals, research institutions and food industries, and their importance ranges from securing laboratory procedures to protecting the public and the environment. Generally, disease burden is steadily rising, necessitating accurate diagnosis especially for diseases related to level three organisms, or even specimens suspected to be having microorganisms of unknown pathogenicity. There is also need to carry out intensive research on level three microorganisms alongside emerging and re-emerging infectious diseases,
procedures which need comprehensive analytical processes and user protection strategies that can only be found in P3 laboratories. Moreover, occupational and environmental safety has to be observed when dealing with extremely dangerous microorganisms. These are coupled with the fact that health sectors have now boldly embraced evidence-based practices, broadly informed by research. A P3 laboratory is therefore of utmost importance since it guarantees user and environmental safety, as well as safety of research procedures. In addition to these, a P3 facility generally creates a good working atmosphere by assuring safety of laboratory workers. Since the core purpose of a P3 laboratory is to contain contaminants, people working within it are guaranteed of optimum safety, even in case of an operational error. Further, P3 laboratories enhances adherence to biosafety rules and guidelines while strengthening vigilance against laboratory acquired infections.

A P3 laboratory is mainly used for manipulation of RG3 microorganisms such as *Rickettsia typhi*, *Bacillus anthracis*, *Brucella abortus*, Yellow FeverViruses and other arboviruses, and MDR-TB strains, among others. Generally, microorganisms are categorized into four risk groups (RG 1, RG 2, RG 3 and RG 4) based on their relative hazard (WHO, 2004). RG 1 comprises of microorganisms that are unlikely to cause any human or animal disease (WHO, 2004). RG 2 includes low risk microorganisms that can cause diseases in humans through percutaneous injury, ingestion or mucous membrane exposure, but which pose minimal risk to laboratory staff or the environment. (Schaechter, 2009; CDC, 2009). Laboratory exposure to RG 2 biological agents does not cause serious disease, risk of spread is minimal and therapeutic treatment is available (CDC, 2009). These organisms are normally manipulated on open benches though biosafety cabinets can be used for the more contagious ones. RG 3 microorganisms are agents that can be spread through aerosol. They cause serious but treatable human diseases and present a high individual risk but low community risk (Schaechter, 2009). Biosafety cabinets and other primary protective devices are required for safe manipulation of RG 3 microorganisms. Lastly, RG 4 organisms pose high individual and community risk, with directly and indirectly transmissible diseases, which have no effective treatment or preventive measures (WHO, 2004; Schaechter, 2009). They are best manipulated in Class III biosafety cabinets or in Class II biosafety cabinets combined with positive pressure suits.

In addition to RG3 microorganisms, WHO (2004) also recommends that large quantities or high concentrations of RG-2 organisms should be manipulated at BSL-3 due to increased risk of aerosol spread. Further, unknown specimens for either research or diagnostic purposes should be processed in a P3 laboratory.

2 Establishment of a P3 Laboratory

The process of establishing a P3 laboratory can be quite lengthy, due to high costs and biosecurity concerns that surround such facilities. Any institution setting up a containment facility is therefore required to adhere to recommended construction and operation guidelines, since the laboratory can pose a biosecurity threat in case of a technical or operational error. A P3 facility is purchased from certified medical and chemical equipments suppliers, certified by local and international bodies upon meeting set safety and quality standards. Initial staff training and regular technical maintenance are carried out by the supplier.
P3 laboratories are purchased as a pre-finished casework ready for installation. The case is made and finished using a non-corrosive water tight material, complete with a ceiling, a floor and provisions for creating necessary openings, using measurements of the actual room into which it will be installed. Interior surfaces of walls, floor and ceiling are therefore easy to clean and resistant to corrosion by laboratory reagents and cleaning detergents. Upon installation, power and air conditioning lines and systems are drilled into the walls or ceiling and any cracks or gaps sealed airtight.

As shown in Figure 1, biosafety cabinets are the most dominant features of a P3 laboratory due to their role in containing infectious material during sample processing. They are best installed in areas with limited movement and away from the door in order to enhance directional flow of air necessary for the cabinet to function optimally (WHO, 2004). At the middle is a work surface located in close proximity to other equipments, especially the biosafety cabinets to ease movement and reduce the risk of breach of containment. An autoclave is also an important component in the P3 laboratory, as a tool of managing contaminations. It is best located at the furthest edge of the laboratory, away from work benches to reduce chances of contamination. Beside the autoclave is a pass box used to move samples and small equipments into and out of the laboratory. Other equipments should be arranged in a manner that will assure sufficient working space and ease of access.

![Figure 1: Basic layout of a P3 laboratory.](image)

Basically, a P3 facility should be installed in a segregated location to allow for physical separation from areas of unrestricted traffic. Upon installation, necessary utilities and equipments are fitted, including analytical and biosafety equipment to allow the laboratory to function as an independent unit, and maintain containment by minimizing movement of specimens and equipments into and out of the laboratory. Major functional areas of the laboratory can be computerized in order to make it more efficient and easy to maintain.

### 3 Features of the P3 Laboratory

#### 3.1 Physical Features
3.1.1 Door Interlock

The door interlock is a double door structure that functions in maintaining negative pressure and optimum temperatures. It also locks in potentially contaminated air, preventing it from spreading to the environment. The interlock system includes the outer door that opens into the ante-room and P3 room door. The doors are controlled by an interlocking sensor, are self-closing, and cannot be opened at the same time. The P3 room door has to be well closed for the ante-room door to open.

3.1.2 Air Conditioning System

P3 laboratories operate at a particular temperature range since extreme temperatures can yield discomfort for users, interfere with normal operation of equipments, or impede analytical processes. The air conditioning system therefore functions to maintain optimum temperatures.

3.1.3 Ventilation System

This is an aeration system that maintains a steady supply of fresh air into the facility alongside removing circulated, potentially contaminated air. Generally, air is drawn from the environment into the water-air purification system, where particulate matter is removed before being channeled into the pre-filters, and finally through intermediate filters; the last stage of air purification. Purified air is then passed through the air conditioner where its temperature is adjusted to room temperature then channeled into the prep room and the P3 room through cellar fans. Recirculated air on the other hand is drawn out through cellar and Class IIB2 exhaust routes. The cellar exhaust route draws out air from within the laboratory at about 720m$^3$/hour. Biosafety cabinet, class IIB2 exhaust route also utilizes a negative pressure system, drawing out air at about 1380 m$^3$/hour. Both routes are supported by dampers that regulate rate of flow of air. The two also have a sterilization bulb that decontaminates air before it can be removed into the atmosphere and are fitted with High Efficiency Particulate Absorption filters (HEPA filters). For increased efficiency, the exhaust ventilation system can be fitted with an automated double damper system capable of automatically switching to a reserve damper in case of failure of the default one.

The role of the ventilation system is to maintain steady supply of clean air, hence preventing contamination of laboratory procedures by contaminants of environmental origin as well as enhancing biocontainment by maintaining directional flow of air and negative pressure.

3.1.4 Glass Windows

These are large screens, located on separate locations around the laboratory. They allow people outside the P3 laboratory to view the inside, communicate or observe its general condition without necessarily having to get in. The screens also serve as an emergency exit as they are provided with a hammer from within the laboratory that can be used to break the window, creating an exit route in the event of an emergency.

3.1.5 Interphones

These are communication gadgets located in the P3 and ante-rooms that are also connected to the rest of the telephone network within the institution. Interphones allow communication
between the laboratory rooms, and to or from other offices and laboratories within an institution. Further, they can serve as a security measure since they can be used to alert staff in case of an accident.

3.1.6 Pass-box

A pass-box is a big window-like structure that opens to adjacent laboratory units such as the cell culture room. It acts as a link between the P3 room and an adjacent laboratory, providing an entry and exit route for samples, small equipments, and waste materials. By providing a passage route, it eliminates the need to constantly open the main door which would otherwise interfere with directional airflow and negative pressure hence compromising containment. The pass-box also has interlocking glass doors.

3.1.7 Generator

A generator provides power back-up for a P3 laboratory, ensuring uninterrupted functioning of the laboratory while upholding containment. The generator should have sufficient capacity to cover essential components; mainly the air conditioning system, freezers, incubators, ventilation system and the negative pressure system. Like all power supply systems, it should be connected to a current stabilizer in order to protect equipments from damage by fluctuating electric currents.

3.1.8 Water-air Filtration System

Normally, air for a P3 facility is drawn from the environment then passed through the normal air filtration system. However, in some instances, the environment could be heavily laden with dust particles and particulate matter that rapidly clog the air inlets, necessitating regular changing of the intermediate filter, which significantly increases maintenance costs. A water-air filtration system (NIHON IKA Chemical Company), an improvisation which capitalizes on the ability of water to trap fine pollutants and particles can however be installed on the air inlet to filter off excess particulate matter before it enters into the main filtration channel. This improves efficiency of the air filtration system while reducing frequency of intermediate filter change-over.

As shown in Figure 2(b), water runs down a filter, made of special fibrous material, wetting its filaments while providing a medium for initial cleansing of air before it is drawn into the main air filtration system. The structure consists of a water storage tank, arranged in a manner that enables recycling of water. Air drawn through this system has a lower amount of particulate matter, therefore the rate at which the intermediate filters get clogged is lower hence they can be used for a longer duration.

Figure 3 shows intermediate filter consumption of a system before and after installation of a water-air filtration system. In the absence of the filtration system, the intermediate filter is consumed within 6-9 weeks. After installation of the system, it can take more than 12 weeks for the manometer to reach the 150Pa mark, hence less frequent intermediate-filter change over, translating to reduced maintenance costs. Though quantity of particulate matter in the environment is the main determinant of the rate of consumption of the intermediate filters, use of water-air filter significantly lengthens duration of usage of intermediate filters.
3.2 Operation features- General features

3.2.1 Run mode

Run mode is the normal operation mode activated during the day, when the facility is in use or when it is being prepared for use. It is activated using a manual switch button on the display of the control panel. At run mode, the laboratory operates optimally with maximum power consumption.

Eco mode, an alternative operation mode can also be installed to achieve energy efficiency. It is a modified operation feature that subjects the entire facility into a power saving

Manometer Readings

![Manometer Readings Graph]

**Figure 3:** A graph showing manometer readings before and after installation of water-air filtration system.
mode, and is turned on manually when the laboratory is not in use. Eco mode maintains normal power supply to vital equipments and minimal supply to those that require power to function but can operate on minimal power supply when not in active use. It also stops supply of clean air into the ante-room and maintains temperature in the P3 room at 30°C Celsius, hence eliminating the need for air conditioning, which saves power consumption in the facility by at least 30%.

**Figure 4:** Physical features of a P3 laboratory; DI-door interlock, AC-air conditioner, IF-inlet fan, EF-exhaust fan, HEPA filters-high efficiency particulate air filters, GW-glass window, IP-interphones, PB-pass-box, WAFS-water-air filtration system.

### 3.2.2 Directional Air Flow

This refers to directed flow of air in and out of the P3 facility. Purified air flows in through the inlet system into the ante-room and the P3 room through ceiling ducts. Circulated air on the other hand is exhausted through an exhaust ceiling duct and the BSC Class II B2 exhaust route, all fitted with HEPA-filters. Directed flow is further enhanced by the air-tight nature of the facility that limits flow of air to designated routes.

P3 facilities operate under negative pressure, achieved by maintaining a rate difference between exhaust and inlet air flow, which generates a higher exhaust speed relative to inlet speed. Both directional air flow and negative pressure facilitate biocontainment. Efficiency of such a pressure system can be increased through computerizing operation of inverters, by connecting these to control gadgets, which automatically run inverters at the air inlets. The gadgets have a display screen and control buttons that allow resetting and calibration of the system without having to modify the actual inverters.
These and other automated features of a P3 laboratory are controlled from the control panel whose display panel is shown in Figure 5. The operation mode system is inbuilt within the panel from where it can be operated through a switch on the panel’s display. Temperature and pressure control functions are also controlled from the control panel. The control panel therefore runs operation components of a P3 laboratory, with its display panel facilitating easy monitoring of functioning of the facility by displaying all vital readings.

![Control panel](image)

**Figure 5:** Control panel; a) Operation mode display, b) P3 room pressure, c) Ante-room pressure, d) Temperature display, e) Eco/run mode switch.

**Figure 6:** Inverter control gadgets.

### 4 Equipments

A P3 laboratory can be equipped with a range of equipments depending on intended usage, with biosafety equipments being the main basic necessities. Among these, biosafety cabinets and autoclaves are the most important. General laboratory equipments such as incubators, ELISA machines, PCR machines among others can be installed based on the laboratory’s operation protocol. A freezer is also considered a basic equipment since some specimens must be maintained under BSL-3 conditions. P3 equipments maintain physical containment or minimize chances of transmission of contaminants.

#### 4.1 Biosafety Equipments

Safety equipments form the core of biosafety, and biosecurity and containment cannot be achieved without a full set of these. They provide a physical primary barrier between the user and the source of contaminants, since aerosols are bound to be produced even after biosafety procedures have been followed. Major safety equipments include biosafety cabinets, autoclaves and protective clothing, though other safety enhancing equipments can be provided based on a laboratory’s function or research protocols.

Biological safety cabinets are a major component of a P3 laboratory due to their ability to contain aerosols, conferring protection to both the user and specimens being processed. There are three classes of biosafety cabinets; class I, II and III. Class I cabinets are open-front safety cabinets, fitted with exhaust HEPA filters only, and exhausts all air to the outside or
into the laboratory room (CDC, 2009). These cabinets protect the environment but offer minimal protection to specimens within the hood. Class II cabinets have considerable negative pressure, have HEPA filters at the exhaust route only and provide protection for both the environment and the specimens being manipulated (Maier, Pepper, & Gerba, 2009). Class II A1 recirculates 70% into the cabinet and exhausts 30% into the room or outside while class II B1 recirculates 30% and exhausts 70%. Class II B2 exhausts all air to the outside while Class II A2 are similar to A1, but with a higher face velocity (100 lfm) (CDC, 2009). Moderately risky pathogens such as Clostridium spp., Shigella spp., Microsporum spp., Entamoeba spp., adenoviruses and influenza viruses among others can be manipulated in Class II cabinets (Maier, Pepper, & Gerba, 2009). Class III cabinets are total containment cabinets that enable safe manipulation of high risk pathogens. They are fitted with one inlet filter and two outlet filters and have attached rubber gloves through which all work within the cabinet is performed (Maier, Pepper, & Gerba, 2009). Microorganisms such as Brucella spp., Rickettsia spp., Mycobacterium tuberculosis, Coccidioides immitis and Dengue virus among other high risk pathogens can be safely manipulated in class III cabinets. Class II A1, A2 and B1, B2 and class III safety cabinets are the most recommended for a P3 laboratory, though class IIB2 cabinets are more popularly used in place of class III.

Autoclaves on the other hand function in sterilization of infectious or contaminated materials for disposal or reuse. Preferably, an autoclave should have different inbuilt operating programs to allow for decontamination of a range of materials with varying levels and types of contaminants. Further, the laboratory should be sufficiently supplied with all the necessary Personal Protective Equipments (PPE) (Figure 7). PPE is normally stored in the anteroom, from where it can be worn before entering the P3 room and removed upon exit. Other safety equipments may include centrifuge cups, pipette aids and leak proof collection containers among others, which serve in containing hazardous materials (WHO, 2004).

![Figure 7: Personal protective equipment.](image)

### 4.2 Emergency Response Equipment

These are gadgets that enable laboratory workers to manage accidents or incidents, mainly fires, power failures and leakage of laboratory gases. Fire detectors, fire extinguishers, gas detectors and emergency lighting are the most essential emergency response equipment.
Fire extinguishers are preferably carbon dioxide type or powder type, located within the P3 room and in the ante-room. These should be conditioned regularly, strategically stationed and have a user instruction manual attached. The gas detector is normally fixed on top of a biosafety cabinet and has an alert component that goes off in case of any gas leakage. The fire alarm can be inbuilt within the laboratory from where it activates fire alerts at the onset of a fire. A hammer is provided on each glass window to be used to break the glass and provide a safe exit route, in addition to conventional emergency exits.

Lack of lighting during power outages can be quite disastrous and a generator backup power can fail. It is therefore necessary to provide emergency lighting by installing a fluorescent lamp supported by a rechargeable battery. The bulb automatically switches on for one hour, following a total power failure to allow users to finish-up their experiments or undo setups and evacuate.

![Figure 8: Aerial view of location of emergency response equipments within a P3 laboratory; GD-Gas detector, FD-fire detector, EE-Emergency exit, H-Hammer, IP-Interphone, FE-Fire extinguisher.](image)

## 5 Maintenance

Maintenance of P3 facilities is a key aspect of biosafety management systems because use of faulty and unconditioned equipments can cause contamination. A P3 laboratory can increase the risk of transmission of biohazards due to the nature of pathogens handled in it hence regular maintenance is of utmost importance. For easy maintenance and optimum functioning of
the laboratory, daily, weekly, monthly and yearly maintenance routines are carried out, over-lapping maintenance with daily usage rather than having to repair systems only when they breakdown.

5.1 Daily Maintenance

Daily maintenance mainly involves floor cleaning, disinfection of handles, waste removal, and monitoring of vital parameters at the control panel display as well as documentation of observed off-readings. Additionally, biosafety cabinets are decontaminated after each use by Ultraviolet (UV) light. The entire P3 room must also be decontaminated daily after work for at least an hour using UV light.

5.2 Weekly Maintenance

Changing of pre-filters is carried out weekly. Being part of the initial air filtration stages, the fibrous pre-filters trap large quantities of dust and fine particles. It is therefore necessary to change them every week in order to minimize chances contaminating or damaging the entire system. The pre-filters are washable hence their maintenance basically involves removal of dirty filters from the panels as shown in figure 9 (a) and replacement with a clean filter. The generator is also maintained weekly. Its control panel is programmed to turn on the generator once every week for ten minutes or so, to allow for routine maintenance, mainly checking and recording of vital operating parameters and battery recharge.

![Figure 9: (a) Changing of pre-filters, (b) A clean pre-filter, (c) A soiled pre-filter](image)

5.3 Monthly Maintenance

Monthly maintenance is applied on the air filtration system, depending on manometer readings. It involves replacement of intermediate filters since they get clogged with dust particles in the course of usage. The manometer in Figure 10 (a) monitors the condition of these filters and indicates when they are due for replacement. The black arm of the manometer shows meter readings, indicating how much of the intermediate filter has been consumed while the red arm shows maximum consumption, at which the intermediate filter should be changed.
5.4 Yearly Maintenance

Every year, core P3 facilities are serviced, preferably by trained experts or by suppliers. Yearly maintenance mainly focuses on fumigation of biosafety cabinets and changing of HEPA filters. Fumigation uses a bactericidal principle to get rid of aerosol contaminants that accumulate within the inner spaces of a biosafety cabinet in the course of its usage. It is carried out using paraformaldehyde and water, followed by neutralization using ammonium hydrogen carbonate. Hotplates holding the fumigants are placed within the hood as shown in Figure 10 (a). The front panel of the cabinet is then removed and the cabinet sealed using a thick film. Humidification with the fumigants is initiated within the sealed cabinet by switching on the hotplate containing paraformaldehyde and water for a while, while switching the biosafety cabinet on and off for at least one minute to allow the gas to circulate within the plenum. Following humidification, fumigation continues for at least twelve hours after which neutralization is carried out for about one hour using ammonium hydrogen carbonate. HEPA filters are then changed as shown in Figure 12 and their functionality confirmed using air velocity and air particle count tests. Figure 13 (a) shows the air velocity test that examines the rate of flow of air into and within the cabinet. Final velocity reading is the average of several readings taken at different points within the cabinet. Inside air velocity should be between 0.3 to 0.4 m/s while that at the entrance ranges between 0.7 to 0.8m/s. Air particle count shown in Figure 13 (b) confirms efficiency of fitted HEPA filters, by measuring penetration of the filter. Caution should be observed when changing HEPA filters because these could still be bearing contaminants, hence personal protective equipments should be used.

Yearly routines also cover major technical functions of the laboratory. Pressure, ventilation and air conditioning systems are checked alongside electrical systems, and repairs and maintenance undertaken where necessary. Breaks are also checked for within the P3 structure to ensure that there are no air spaces that could compromise its air tight function.

6 Safety and Security Features

Operations in a P3 laboratory call for adherence to strict safety and security measures. While physical features of the laboratory provide necessary secondary barrier in addition to primary
Figure 11: Positioning hotplates (a) and sealing biosafety cabinets (b) in preparation for fumigation.

Figure 12: (a) Removal of exhaust covers during replacement of HEPA filters, (b) Aerial view of old exhaust filter, (c) Fitting of new inlet filter.

Figure 13: Confirmation of efficiency of fitted HEPA filters using (a) air velocity testing, and (b) air particle counting.
safety provided by safety equipments, additional safety and security features can be incorpo-
rated to enhance biosafety. For physical separation, the laboratory should be located in an area
with minimal movements. Further, only authorized persons can access the laboratory, a meas-
ure that can be reinforced by use of an automatic locking pad operated using a password that
can be changed regularly. Only trained laboratory staff can use the P3. In case untrained per-
sons need to use the facility, they should be accompanied by trained personnel. A register is
provided and maintained to track activities in the P3 laboratory. Such a register can also be
used for follow-up in case of any irregularity.

Other safety features include safety signs and labels that aid in identification of hazard-
ous areas or materials or remind users of recommended safety procedures. A biohazard label
is the most dominant safety signage in P3 laboratories. It bears a biohazard symbol and is af-
fixed on the P3 room door, waste containers, refrigerators and freezers containing hazardous
materials and on any equipment that may be contaminated. An exit sign is yet another safety
label that directs users to a safe emergency exit route in case of any accident. Activated patho-
gen tags on a pre-printed chart can also be affixed on the door to inform users on particular
pathogens being handled in the laboratory at any given time. Some P3 laboratories also have
custom made biosafety symbols to fit its safety policies. For example, a limited entry symbol
can be used to limit entry of unauthorized users into the P3 area, alongside other more specific
symbols on P3 laboratory etiquette or even PPE usage.

Given the level of hazard associated with P3 laboratories, decontamination by ultravio-
let light after each work session is necessary. The UV light switch bulb enables easy decontam-
ination of the laboratory since it is preset to run for a pre-determined duration, sufficient to
fully sterilize the laboratory. Interphones are also provided to enhance safety by easing com-
munication in the event of an accident.

Labels and signs are openly affixed on any hazardous or potentially hazardous area
within the laboratory, or where extra caution needs to be observed. Most importantly, areas of
multiple hazards are clearly indicated using multiple signs as on Figure 14, each signaling in-
dividual hazard that users are likely to encounter. All signage features follow universal speci-
fications in terms of color scheme and images, for easy recognition.
7 Documentation

Documentation is a critical feature of safety management procedures. Biosafety management systems recommend design and utilization of records that capture information on users and activities intended to be carried out in the laboratory in a particular session. Generally, these records track activities in the P3 laboratory, movement in and out of the laboratory and usage of laboratory equipments. Such records include:

7.1 P3 In/Out Record

This is a general record on utilization of P3 laboratories. It records information on daily activities in P3, the number of people using it and usage of P3 facilities. It captures the name of laboratory staff, time in and out; number of biosafety cabinets used per session, purpose of using the laboratory and pathogens intended to be manipulated.

7.2 Biosafety Cabinet Usage Record

This is a record specific for usage of biosafety cabinets. It captures information on duration of usage, pathogens being handled in the facility and specific biosafety cabinets intended to be used. The record can be used to schedule maintenance activities, especially in cases where the biosafety cabinets are frequently being used.

7.3 Daily Check-point for Ante-room

This is the ante-room record in which daily observations made on the overall working condition of the P3 laboratory as shown on the control panel are entered. It documents pressure reading on inverters I and II, P3 pressure, ante-room pressure and temperature readings.

<table>
<thead>
<tr>
<th>Item</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inverter I</td>
<td></td>
</tr>
<tr>
<td>Inverter II</td>
<td></td>
</tr>
<tr>
<td>P3 Pressure</td>
<td></td>
</tr>
<tr>
<td>Ante Pressure</td>
<td></td>
</tr>
<tr>
<td>Room Temperature</td>
<td></td>
</tr>
<tr>
<td>Remarks</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: An ante-room checklist.

8 Biosafety Rules and Standard Operating Procedures (SOPs)

Failure to adhere to good laboratory practices, laboratory worker error and misuse of equipments accounts for majority of laboratory injuries and laboratory-acquired infections. Consequently, good laboratory practices and SOPs must be in place to prevent laboratory-acquired
infections, minimize laboratory accidents and maintain biosafety and biocontainment. A biosafety manual provides a code of conduct in compliance with good laboratory practices, whose proper implementation reduces occurrence of hazardous incidences. WHO (2004) provides a basic code of practice that can be adopted and fine-tuned to fit institutional needs. A good manual should define access requirements; restricting entry to only authorized persons, biosafety practices in daily utilization of laboratory facilities, personal protection and laboratory etiquette (WHO, 2004). For example, one should not eat or store food in the laboratory, personal protection equipments should be worn and used appropriately, biosafety protocols must be followed, and biosafety symbols must be used as necessary and should follow universal specification.

SOPs on the other hand should document analytical and maintenance procedures as well as safety procedures that can be applied in case of an accident or in order to avert a would-be incident. Biosafety management SOPs include accident and incident reporting procedure, disinfection and decontamination of work surfaces, entry and exit procedures and waste disposal among others (Zaki, 2010). Further, each equipment in P3 should have an SOP, for use within the laboratory and for facilitating training of new staff. SOPs provide a brief description of the equipment, how to operate it and guidelines on its maintenance and calibration routines where applicable. Each research project utilizing a P3 laboratory should also have an SOP.

9 Waste Management

P3 laboratories generate a variety of infectious wastes necessitating development of proper waste disposal system in order to minimize potential for exposure of laboratory workers who must handle the material. In most cases, institutions usually develop their own waste disposal protocol that fit their needs. A protocol defines wastes into various categories, provides a unique color of waste container and liner, and provides a set of handling and disposal policies for each category. A set of policies are also provided for each category, instructing laboratory workers on how to handle and dispose various types of wastes.

To ease waste disposal, P3 laboratory users initiate the disposal process, by autoclaving wastes where necessary and segregating wastes into respective waste containers. Following initial treatment, wastes are disposed as per the institution’s waste disposal protocol by designated staff.

10 Training

Usefulness, efficiency and safety of a P3 facility depends on the level of awareness and expertise among researchers, hence the need for training. The training component of a P3 laboratory is of utmost importance not only because of the need to develop manpower but also because of the relevance of P3 facilities in securing laboratory procedures.

An ideal P3 training curriculum covers biosafety and personal safety, and is delivered through lectures and practical demonstrations. Participants are first introduced to the concept of biosafety and the P3 laboratory in general before being instructed on operations at biosafety
level three. Most specifically, training focuses on informing participants on the hazards associated with the facility and possible ways of eliminating personal harm while minimizing the risk of exposing other people to danger. Maintenance and management of a P3 laboratory is also covered. Effectiveness of the training session is evaluated through an assessment test whose outcome can inform the institution on gaps of knowledge and areas of weakness, for which refresher courses can be scheduled.

Figure 15: A training workshop’s practical session.

11 Biosafety Committees and Biosafety Meetings

For a P3 laboratory, need to develop, implement and adhere to biosafety policies cannot be overemphasized, functions that are executed through biosafety committees and meetings. A biosafety committee can be a group of laboratory staff who have undergone intensive training on biosafety and biosecurity systems to acquire sufficient capacity to manage P3 laboratory security systems. It is in charge of ensuring that biosafety guidelines are adhered to, carrying out risk assessments on new projects utilizing P3 facilities, supervising laboratory maintenance routines, responding to alarms and emergencies, chairing biosafety meetings and training new users (Zaki, 2010). Led by a biosecurity officer, it is also the responsibility of the biosecurity committee to manage P3 facilitates and to ensure sufficient supply of laboratory consumables. Apart from training laboratory users, the committee creates awareness on matters of biosecurity within the institution to assure occupational safety of other workers.

Biosafety meetings are an output of biosafety committees. These are usually monthly meetings that bring together researchers who have used the P3 laboratory within a particular month and other trained users. The meetings provide a forum for attendees to share their experiences with regards to using the P3 laboratory, identify and discuss areas of difficulty, update each other on recent occurrences or new installations and get informed on ongoing activi-
ties. Occurrences, especially alarms or system breakdowns can be communicated and discussed to identify their causes and generate preventive measures against these and other would-be occurrences.

Attendees are also reminded of basic safety rules and practices, emphasizing on the need to observe personal safety and safety of other people around the institution. It should be mandatory for all users and trained staff to attend and participate in the meeting.

12 Accreditation

Given the complexity of operations in a P3 laboratory coupled with increasing public health threats, a P3 laboratory has to always be in optimum working condition and in a position to manage emerging challenges. Laboratory accreditation is therefore necessary since it assures that all recommended physical and operational features of a P3 laboratory are in place and well maintained and that biosafety guidelines are being adhered to. Moreover, an accredited laboratory instills confidence in users and assures them of personal safety and safety of their research procedures, as well as quality of their research outcomes. A P3 laboratory is first accredited by default by the supplier, since a containment facility manufacturer has to be certified before it can be allowed to supply. However, the laboratory must obtain more authentic accreditation by in-country and international bodies, though this can be quite a lengthy process especially in countries lacking proper accreditation systems.

13 Conclusion

Several uncertainties surround laboratory acquired infections. It is not only difficult to determine the actual risk for infection after exposure but it is also difficult to determine the actual source or mode of infection (CDC, 2012). Further, laboratory workers are at a greater risk of exposure to pathogens than the general public. Biosafety facilities therefore aim at minimizing the risk of exposure to infectious agents, in order to enable proper disease diagnosis or specimen processing the in case of research laboratories. Proper construction, utilization and maintenance of a P3 laboratory provides users with optimum safety necessary when handling risk group three organisms. However, equipments only cannot guarantee biosafety. Capacity to utilize the facility has to be developed continuously, while entrenching good laboratory practices among laboratory workers, to responsibly undertake biosafety observance.

Acknowledgements

Establishment of our P3 facility would not have been possible without the support of many partners. We wish to acknowledge the Director of Kenya Medical Research Institute (KEMRI) for the continued support and in particular, the Center for Microbiology Research who are hosting our laboratories. Special thanks to Nagasaki University (Japan) for supporting our research activities technically and financially. Finally, we thank Nippon Medical &Chemical Instruments Company (Japan), the suppliers of our P3 facility for providing labor for continuous maintenance of the laboratory.
Authors

Yoshio Ichinose, Shingo Inoue, Masaaki Shimada, Gabriel Miring’u, Betty Muriithi, Angela Makumi, Ernest Wandera, Martin Bundi, Chika Narita, Salame Ashur, Allan Kwalla, Amina Galata, Erick Odoyo, Sora Huqa, Mohammed Shah, Mohammed Karama, Masahiro Horio

Institute of Tropical Medicine, Nagasaki University, Nairobi, Kenya, Japan

References


