

Regional report

The NUITM-KEMRI P3 Laboratory in Kenya: Establishment, Features, Operation and Maintenance

Shingo Inoue¹, Ernest Wandera², Gabriel Miringu², Martin Bundi², Chika Narita³, Salame Ashur¹, Allan Kwallah¹, Amina Galata², Mwajuma Abubakar², Sora Suka², Mohamed Shah², Mohamed Karama⁴, Masahiro Horio⁵, Masaaki Shimada⁶ and Yoshio Ichinose^{2*}

Received 11 January, 2013 Accepted 15 January, 2013 Published online 21 February, 2013

Abstract: A biocontainment facility is a core component in any research setting due to the services it renders towards comprehensive biosafety observance. The NUITM-KEMRI P3 facility was set up in 2007 and has been actively in use since 2010 by researchers from this and other institutions. A number of hazardous agents have been handled in the laboratory among them MDR-TB and yellow fever viruses. The laboratory has the general physical and operational features of a P3 laboratory in addition to a number of unique features, among them the water-air filtration system, the eco-mode operation feature and automation of the pressure system that make the facility more efficient. It is equipped with biosafety and emergency response equipments alongside common laboratory equipments, maintained regularly using daily, monthly and yearly routines. Security and safety is strictly observed within the facility, enhanced by restricted entry, strict documentation and use of safety symbols. Training is also engrained within the operation of the laboratory and is undertaken and evaluated annually. Though the laboratory is in the process of obtaining accreditation, it is fully certified courtesy of the manufactures' and constructed within specified standards.

Key words: P3 Laboratory, Kenya

INTRODUCTION

A P3 laboratory is a containment facility that enables the isolation and manipulation of dangerous biological materials for various research purposes. P3 laboratories are subjected to BSL-3 standards, which permit the handling of biological agents that can be transmitted through aerosols. The establishment and usage of P3 laboratory and facilities can be traced back to the mid-20th century, with the emergence of laboratory-acquired infections attributed to unsecured laboratory operations [1–3]. These findings stimulated the World Health Organization (WHO) to prepare designs and compile biosafety rules and strategies into a biosafety manual for purposes of providing practical guidance on biosafety techniques and procedures.

In the manual, microorganisms are classified into risk

groups in ascending order of pathogenicity and stipulate four main levels of biological safety that classifies laboratories into P1, P2, and P3 and P4 depending on their level of their biosafety systems. Countries were also required to develop codes of practice for the safe handling of pathogenic agents and institute basic concepts of biological safety relevant for their geographical borders, a mandate that several countries have complied with today. While P4 laboratories are not as popular, the number of P3 facilities around the world have steadily increased, especially in developing countries owing to the increasing complexity of public health problems and the need to manage and contain incidences of laboratory acquired infections. In Kenya, there are five P3 facilities; four at KEMRI's collaborative centers and one at the International Livestock Research Institute (ILRI). Among the five facilities, our research unit hosts

¹ Institute of Tropical Medicine, Nagasaki University, Nairobi, Kenya (Virology group)

² Institute of Tropical Medicine, Nagasaki University, Nairobi, Kenya (Bacteriology group)

³ Senior volunteer, Japan International Cooperation Agency, Nairobi, Kenya

⁴ Center for Public Health Research, Kenya Medical Research Institute, Nairobi Kenya

⁵ Tropical Medicine Museum, Institute of Tropical Medicine, Nagasaki University, Japan

⁶ Institute of Tropical Medicine, Nagasaki University, Nairobi, Kenya (parasitology group)

*Corresponding author:

Institute of Tropical Medicine, Nagasaki University, NUITM-KEMRI Project, c/o CMR. KEMRI P.O.Box 19993-00202 Nairobi, Kenya

Tel: +254-20-272-5120

Fax: +254-20-272-5144

E-mail: ichinose@nagasaki-u.ac.jp

one of the most active P3 laboratories in Kenya alongside existing BSL-2 facilities.

ESTABLISHMENT OF THE NUITM-KEMRI P3 LABORATORY

Nagasaki University, Institute of Tropical Medicine Kenya research station was established in 2005 in collaboration with KEMRI (NUITM-KEMRI) as a center for research in the area of tropical medicine with a core objective of undertaking research towards the prevention and management of tropical and emerging infectious diseases alongside building biomedical research capacity in Kenya. Currently, the station has diversified its research activities into more specialized areas including entomology, virology, TB, the eradication of Malaria and research in diarrheal diseases. In addition to these, the station is preparing to embark on a number of new research projects featuring fishery and engineering science research.

The P3 laboratory was set up in 2007 within the building that houses the Center for Microbiology Research (CMR), and it intended to support clinical, teaching and research activities involving dangerous biological agents (Fig. 1). The facility was imported from the NK system in Japan with approval from the NUITM committee but not without obvious difficulties, especially obtaining permits from relevant authorities. This was the first time a university attempted to export such a facility and, as might be expected, difficulties were encountered in obtaining an export permit from Japan's Ministry of economy, trade and industry and from Kenyan end, an import request from Ministry of finance for tax and duty exemptions. Certification was provided on the condition that the equipment was intended to use in enhancing biosafety and not as a biosecurity threat.

Since its establishment, the lab has played a significant role in improving research experiences and outcomes by en-



Fig. 1. Researchers in NUITM P3 lab

abling research activities involving highly contagious biological materials. A number of studies have been successfully completed or are on-going in our facility by researchers from this and other institutions. Among these is the Remox project, a collaborative clinical trial that sought to establish the efficiency of a moxifloxacin-containing treatment regimen in the management of drug-sensitive tuberculosis. The JICA-JSPS Arbovirus project and JICA-JST SATREPS project were also carried out in the P3 lab due to apparent pathogenicity of arboviruses. In addition to these, our staff regularly uses the lab to process BSL-3 agents for on-going projects, especially septicemia, TB and virological studies.

Our P3 laboratory is located at the furthest end of the CMR building for it to be physically separated from areas of unrestricted traffic. It is an airtight facility, complete with its own water and dust proof wall. The flooring material is seamless and resistant to damage by laboratory reagents and cleaning detergents. The walls and the ceiling are finished with nonflammable materials (polyurethane) that is easily cleaned, resistant to corrosion and devoid of cracks and disjointed junctions. In addition to normal physical features and structures, our facility is fully equipped to handle a variety of biological assays. Major functional areas of the laboratory are fully computerized, a feature that makes the laboratory more efficient and easy to maintain.

FEATURES OF THE P3 LABORATORY

A) Physical features- General features

a) Door interlock- this is a double door system that functions in maintaining negative pressure and the air conditioning system. The Main door (that opens into the ante-room) and the P3 room door cannot be opened at the same time. The doors are self-closing and lockable. The pass-box door also has a similar mechanism.

b) Air conditioning system- the P3 lab operates at a particular temperature range as extreme temperatures can be a source of discomfort for users and interfere with the normal operation of equipment or experiments. The air conditioning system therefore helps in maintaining optimum temperatures.

c) Ventilation system- an aeration system that oversees steady supply of fresh air into the facility and the removal of circulated air. It is structured in a manner that maintains the directional flow of air and the negative pressure system necessary for the functioning of a P3 lab. The exhaust system is supported by dampers that pump air out while regulating its flow while inlets have inverters that control the rate of air inflow. A unique feature of our system is the automated double damper system at each exhaust route, which is able

to automatically switch to a reserve damper in case of failure of the default system.

d) Glass windows- These are large screens, located on two separate locations around the lab. They allow people outside the P3 lab to view the lab, 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 an hammer from within the lab that can be used to break the window, creating an evacuation route in the event of an accident.

e) Interphones- these are communication gadgets located in the P3 and ante-room that are also connected to the rest of the telephone network within the institution. Interphones allow communication between the two rooms and to or from other offices and labs within the institution.

f) Pass-box- This is a large window-like structure that opens to the cell culture room. It acts as a link between the two rooms, providing an entry and exit route for samples, small equipment, and waste materials. It has double interlocking glass doors with UV lights.

g) Generator- This is a Denyo (DCA-13ESKT) generator of 10.5 KVA in power capacity backup system specific for the P3 laboratory that switches on automatically in case of failure of the main generator. It covers the air conditioning system, freezers, incubators, ventilation system and the negative pressure system. The generator is connected to the current stabilizer that also serves other sources for power stabilization in order to protect equipment from damage by fluctuating electric currents.

B) Physical features-Special features

a) Water-air filtration system (Fig. 2-A and 2-B)- this is one of the unique features of our facility. Normally, air for a P3 facility is drawn from the outside environment and passes through a normal air filtration system. However,

since the environment at our location is quite dusty, such a system would require weekly pre-filter cleaning and regular changing of the intermediate filter which would significantly increase maintenance costs. Our institution therefore improvised a water-air filtration system which capitalizes on the use of water to trap fine particles.

Water runs down a filter, made of a special fibrous material, wetting its filaments while providing a medium for the initial purification of air before it is drawn into the main air filtration system. The structure is connected to a water supply system, basically a water storage tank, arranged in a manner that enables the water to be recycled. This system has significantly reduced maintenance costs since intermediate filters can now be used for a longer duration. As shown in Fig. 3, before the installation of the filtration system, the manometer reading reached to 150 Pa within 6–9 weeks, at which point the intermediate filter would need to be changed. Following the installation of the system in March, 2011, it has been extended to more than 13 weeks, hence less frequent intermediate-filter change-over is needed, translating into reduced maintenance costs.

C) Operation features- General features

a) Run mode- run mode is the normal operation mode activated during the day, when the facility is in use or when 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.

b) Directional air flow- this refers to the 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 through ceiling ducts. Circulated air on the other hand is exhausted through an exhaust ceiling duct and the biosafety cabinet Class II B2 exhaust route, all of which are fitted with

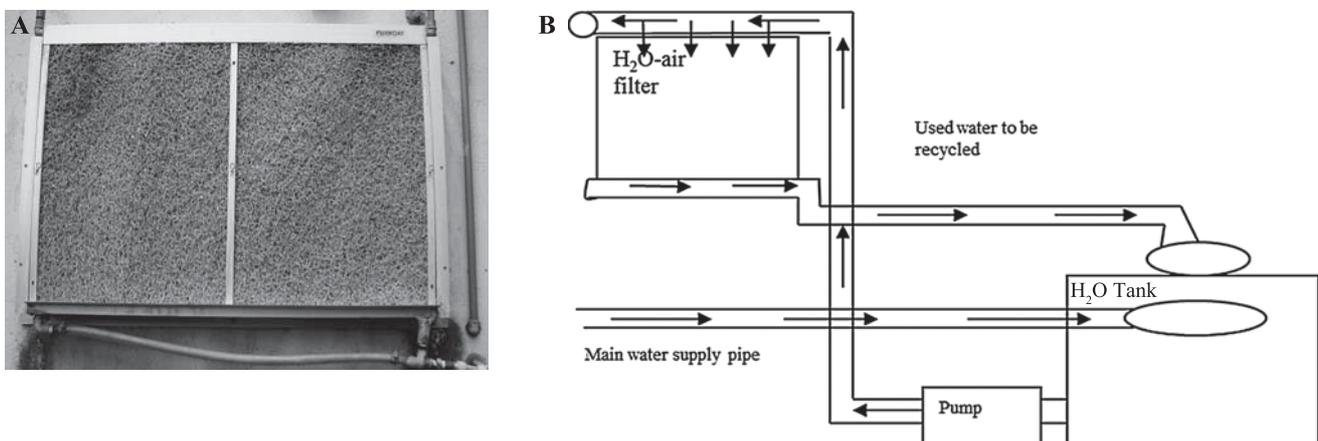


Fig. 2. A: Water-air filtration system, B: Filter's water supply scheme

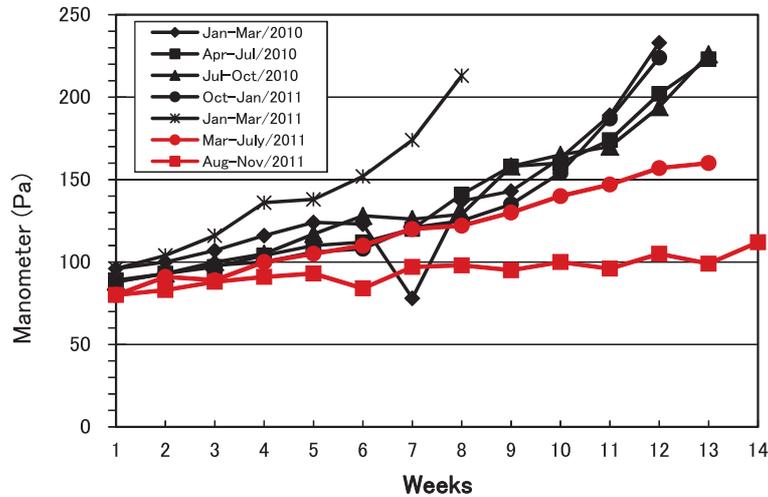


Fig. 3. Manometer Reading of Intermediate filter for P3 lab. Manometer readings before (Black) and after (Red) the installation of water-air filtration system.

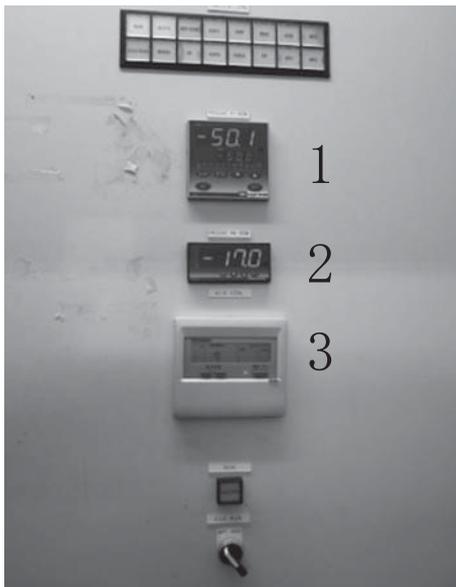


Fig. 4. Operation mode display. 1: P3 room pressure, 2: Ante-room pressure, 3: Temperature display



Fig. 5. Inverters I and II

HEPA-filters. Directed flow is further enhanced by the air-tight nature of the facility that limits the flow of air to designated routes.

D) Operation features-Special features

a) Eco-run mode- this is a special operation feature that causes the entire facility to go into a power saving mode. It is fully automated and turned on manually when the laboratory is not in use such as night time and weekends. The eco-run mode maintains normal power supply to vital equip-

ment and a minimal supply to those that require power to function but can operate on the minimal power supply when not in active use. It stops the supply of clean air into the ante-room and maintains the temperature in the P3 room at 30°C, hence eliminating the need for air conditioning while saving power consumption in the facility by at least 30%.

b) Computerized Pressure System (Fig. 4)- this is an automatic system that functions in maintaining a negative pressure. It is achieved by maintaining a rate difference between the exhaust and inlet air flow, whereby the exhaust speed is higher than the inlet speed. The efficiency of our system arises from use of two inverter control gadgets in-built within the control panel that automatically run inverters at the air inlets, maintaining them at a lower speed relative to exhaust speed (Fig. 5). The gadgets have a display screen and a number of control buttons that allow resetting and calibration of the system without having to modify the

actual inverters. The above features are controlled by a control panel that hosts all automated components in our facility. Operation mode systems are inbuilt within the panel from where they can be operated through a switch on the panel's display. Also built within the panel are air conditioning and negative pressure systems that automatically control the temperature and pressure. The control panel therefore runs the major operation components of our lab while providing a means for continuously monitoring functioning of the facility.

E) Equipment

a) Biosafety equipment

In addition to the usual laboratory equipment, our P3 lab is well equipped with sufficient biosafety equipment. There are three biosafety cabinets (BSCs); two of Class II AB and one of Class II B2. The Class II B2 is an exhaust type that allows manipulation of highly infectious agents. Class II AB biosafety cabinets offer primary containment and are used when manipulating less hazardous agents. Two autoclaves are provided to aid in waste disposal and infection prevention. They have different operating programs to allow for the decontamination of a range of wastes with varying levels and the nature of contaminants. Finally, the lab is sufficiently supplied with all the necessary Personal Protective Equipment (PPE). People using the lab are required to put on a full set of PPE upon entering the anteroom and are required to remain dressed until exit the facility (Fig. 6).

b) Emergency response equipment

These are gadgets that are meant to enable users and the rest of laboratory workers to respond to accidents mainly fires, power failures and gas leakage. The facility has two fire extinguishers; a carbon dioxide type in prep room 2 and a powder type in the P3 lab. These are regularly inspected and refilled, strategically stationed and have a user instruction manual attached. A gas detector and fire alarm are also provided. The gas detector is fixed on top of BSC Class II B2 and has an alert system that goes off in case of any gas leakage. The fire alarm is inbuilt within the lab from where it activates fire alerts at the onset of a fire, signaling the laboratory staff to respond appropriately. Our institution is not ignorant of the possibility of a complete power failure, by both generators and commercial power lines. A fluorescent lamp supported by a rechargeable battery is therefore built within our lab to provide emergency lighting. The bulb automatically switches on for 1h, following a total power failure to allow users to finish-up their experiments or undo set-ups and evacuate.



Fig. 6. P3 Lab user in full PPEs. 1: Gloves (double), 2: Face Shield, 3: N95 mask, 4: Wrist band, 5: Front covered gown, 6: Coverall, 7: Safety boots

MAINTENANCE

Maintenance is a key aspect of biosafety management systems because use of faulty and unconditioned machines can cause contamination. The P3 laboratory is quite sensitive due to the nature of the biohazards it handles hence regular maintenance is of the utmost importance. Maintenance schedules range from daily to yearly. Regular maintenance involves;

a) Daily- This includes floor cleaning, door and door handle disinfection, recording of various vital parameters, documentation of observed off-readings and waste management. Additionally, members of the staff are required to turn on the BSC UV light to decontaminate the cabinet. The P3 room is also decontaminated daily after work for at least an hour using preset UV lights.

b) Weekly- mainly involves the cleaning and changing of pre-filters (Fig. 7). Being part of the initial air filtration stages, the fibrous pre-filters trap the bulk of fine air contaminants (Fig. 8). It is therefore necessary to change them every week to eliminate chances of contaminating or damaging the entire system. The generator is also maintained at weekly intervals. It is supported by a control panel that is automatically set to turn on the generator on Monday morning between 9:30 am and 9:40 am to allow for routine maintenance, checking and recording of vital operating parameters.



Fig. 7. Lab staff changing pre-filters

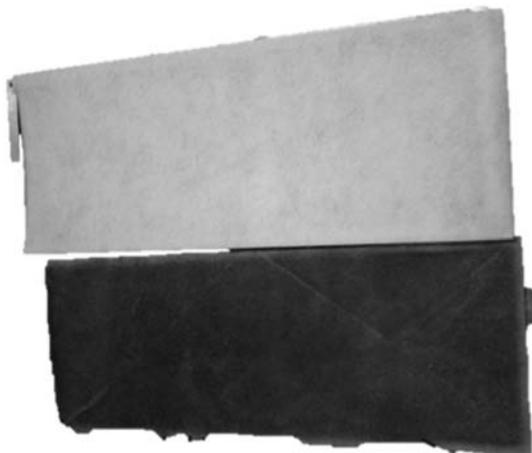


Fig. 8. Clean and soiled pre-filters

ters and battery recharge.

c) Monthly- maintenance is routinely performed mainly on the air filtration system depending on manometer readings. It involves replacement of intermediate filters which get soiled in the course of its usage. The manometer monitors the condition of these filters and indicates when they are due for replacement (Fig. 9). 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 time the intermediate filter should be changed (Fig. 10, 11).

d) Yearly- servicing is performed by experts from Japan during which the overall working condition of the facility is assessed and necessary corrective measures undertaken. BSCs are fumigated and HEPA filters are changed along-



Fig. 9. Manometer of intermediate filter



Fig. 10. Lab staff removing used-up intermediate filters

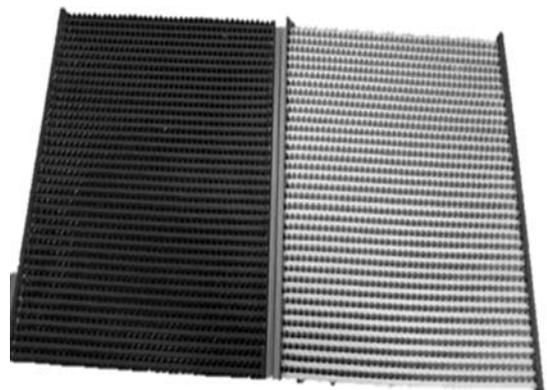


Fig. 11. Used-up and clean intermediate filters

side minor repairs and improvement of other components of the facility. BSCs are fumigated using paraformaldehyde powder and 100 ml of water for 1 h in a beaker and neutralized using ammonium hydrogen carbonate (Fig. 12). Train-



Fig. 12. Positioning of hotplates and sealing biosafety cabinets in preparation for fumigation

ing of NUITM laboratory staff in some core maintenance procedures is ongoing to permit our facility to perform more regularly with minimal maintenance costs (Fig. 13-A, 13-B, 13-C, 14-A, 14-B).

SECURITY FEATURES

Operations in the P3 lab call for adherence to strict security measures. In the first place, the lab is located in the rear end of the CMR lab where movement by other laboratory workers is minimal. Further, only authorized persons can access the laboratory, a measure that is further enforced by using secret opening passwords that are regularly changed (Fig. 15). New facility users must be trained or accompanied by our trained staff. There is a laboratory registration record that summarizes their activities in the laboratory, which can also be used for follow-up in case of any irregularity.

DOCUMENTATION

Documentation is a critical feature for the maintenance of safety standards. Our institution has variety of records for users and activities within the P3 facility. Generally, these records track activities in the P3 lab, movement in and out of the laboratory and the usage of laboratory equipment.

a) P3 lab in/out record- This is a record of persons who are working in the P3 facility. It captures daily activities in the P3 lab, the number of people using it and the usage of BSC.

b) BSC usage record- This record is used to monitor the usage of BSC which are core components of a P3 lab. It captures information on person, time and pathogens being handled in the facility. Apart from tracking usage of BSC, it can also be used to draw up a roster for smooth sharing of the facility among a number of research teams. The record can

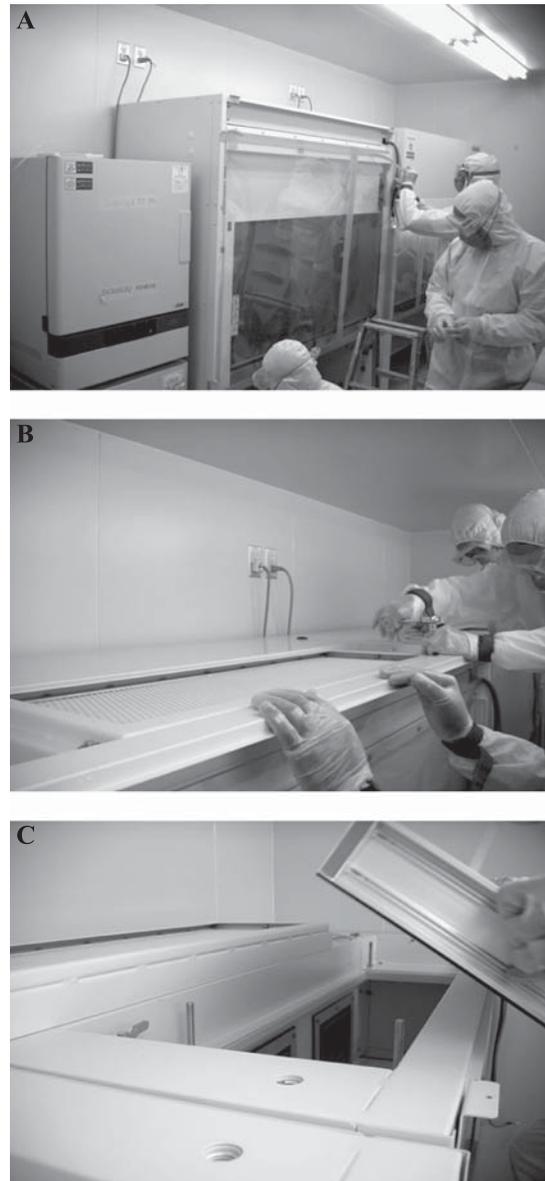


Fig. 13. A: Sealing of BSC for Formalin Fumigation, B: Removing of old HEPA filter from BSC, C: Replacing of new HEPA filter into BSC

also be used to schedule maintenance activities.

c) Daily check-points in ante-room- In the ante-room the daily check-points on observing the monitoring of the overall working conditions of the P3 lab. The parameters are shown on the control panel such as room temperature, negative pressure etc.

d) Standard operating procedures (SOPs)- Failure to adhere to good laboratory practices, laboratory worker errors and the misuse of equipment accounts for the majority of laboratory injuries and laboratory-acquired infections. Consequently, SOPs and standard laboratory practices must be

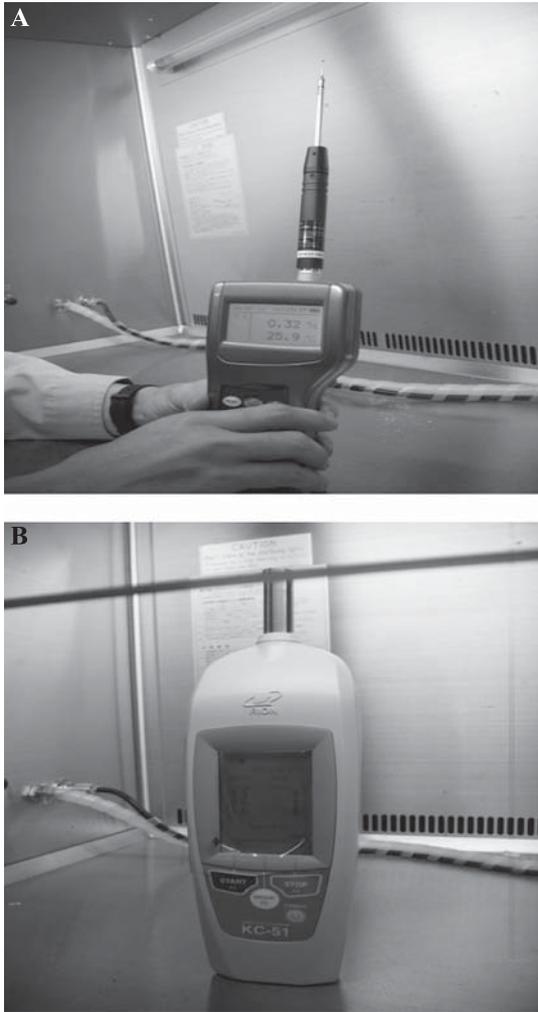


Fig. 14. A: Velocity testing in BSC, B: Particle counting in BSC

in place to prevent laboratory-acquired infections, minimize laboratory accidents and elevate biosafety and security measures. Our facility has adopted and enforced a biosafety and operation manual with reference to the WHO manual for safety applications which is used to inform and guide all operations within the P3 facility. Our facility maintains an SOP for each equipment within the P3 lab, for use within the lab and also for training purposes. SOPs provide a brief description of the equipment, how to operate it and calibration routines where applicable. Apart from internal SOPs, researchers from other institutions are required to develop and submit their project's SOPs well before they are permitted to commence any research activity in order to facilitate risk assessment procedures.

BIOSAFETY SYMBOLS

Our facility has a number of signage features that are located strategically in adherence to biosafety standards and specifications. First, there is an emergency exit indicator signaling a safe exit route through the back door in the case of an accident (Fig. 16). Biohazard signs, which are also located in relevant areas, indicate the need for extra caution. There is also an activated pathogen signage feature that alerts users to the possible presence of active pathogens at any given time (Fig. 15). This helps in preventing would-be infection and contamination of research materials. Further, there are access signage for areas of limited entry and those with special access conditions. For example, the P3 area has a signage that limits unnecessary traffic while the P3 lab main entrance has a signage indicating limited entry, indi-



Fig. 15. Multiple signage. 1: Activated pathogens symbol, 2: Biohazard sign, 3: Lock pad (a security feature)



Fig. 16. Fire exit sign

cating that only authorized persons are permitted to enter the laboratory. The facility's signage is openly displayed on all relevant areas. Most importantly, areas of multiple hazards are clearly indicated using multiple signs. All signage features follow universal specifications in terms of color scheme and images.

WASTE MANAGEMENT

Because the laboratory generates a variety of infectious wastes, the need exist for a proper waste disposal system in order to minimize the potential for exposure of laboratory workers who must handle these materials. Our institution therefore follows waste disposal procedures as provided in the KEMRI waste disposal guidelines and procedures protocol. The protocol classifies wastes into various categories, provides a unique color of waste containers and packaging and provides a set of handling and disposal policies for each category. A set of Policies are provided for each category, instructing users on how to handle and dispose of various types of wastes. Appropriate labeling is emphasized as a means of communicating to staff on the type of waste being handled while informing waste disposal activities. Our P3 lab is well equipped with all the necessary disposal equipment. Persons generating infectious wastes are responsible for preparing the wastes for eventual disposal through standard procedures. Following initial treatment, wastes materials are disposed of as per protocol by designated staff.

TRAINING

Usefulness, efficiency and safety at our facility depends on the level of awareness and expertise among researchers using P3 facility, hence the need for training. The training component in our P3 lab is of utmost importance not only because of the capacity development but also because of the relevance of P3 facilities in transforming research, since this is a relatively new concept in Kenya (Fig. 17-A, 17-B). Since 2007, the facility has trained about 70 researchers, equipping them with necessary knowledge and skills to independently work in a P3 lab. The effectiveness of the training sessions is evaluated through a standard examination in which the outcome informs the institution on existing gaps of knowledge and areas of weakness. The institution is therefore able to continuously improve the curriculum while improving training outcomes. P3 training is offered in the form of a continuing education course for fresh scientists who might need to use the facility in the future, NUITM staff and other users. Training is carried out through an annual two-day workshop with the help of a



Fig. 17. A: P3 Lab Training seminar (Lecture), B: P3 Lab Training seminar (Practicals)

short curriculum presented in the form of lectures, demonstrations and practicals. Participants are first introduced to the concept of biosafety and P3 facilities before being taught about the components of the laboratory and how to use them. Most specifically, training focuses on informing participants on the hazards associated with the facility and possible ways of minimizing personal harm while minimizing the risk of exposing other people to danger. Our staff also conducts refresher training for post-trained researchers before using the facility. This is basically a remedial on laboratory rules and regulations, good practices and safety procedures.

BIOSAFETY MEETINGS

These are meetings, which are held on the last Friday of every month brings together all researchers who have used the P3 lab within that particular month and other trained users (Fig. 18). The objective of meetings are to provide a forum for attendees to share their experiences with regards to using the P3 lab, identify and discuss areas of difficulty, update each other on upgrading or new installations and to provide information concerning ongoing activities.

Occurrences of alarms or system breakdowns are informed and discussed to identify their causes and generate preven-

tive procedures for these and other similar 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 is mandatory for all users and trained staff to attend and participate in the meeting.



Fig. 18. Monthly Biosafety Meeting

USAGE OF THE P3 LABORATORY

The P3 lab has been in use since 2007 although active use began in 2010. A wide variety of studies have been carried out in the lab and some are still going on. Users of the laboratory are KEMRI-NUITM staff and researchers from other centers of KEMRI. Research activities carried out in the lab can be large scale studies or short-term projects by individual researchers or students. Our staff also uses the

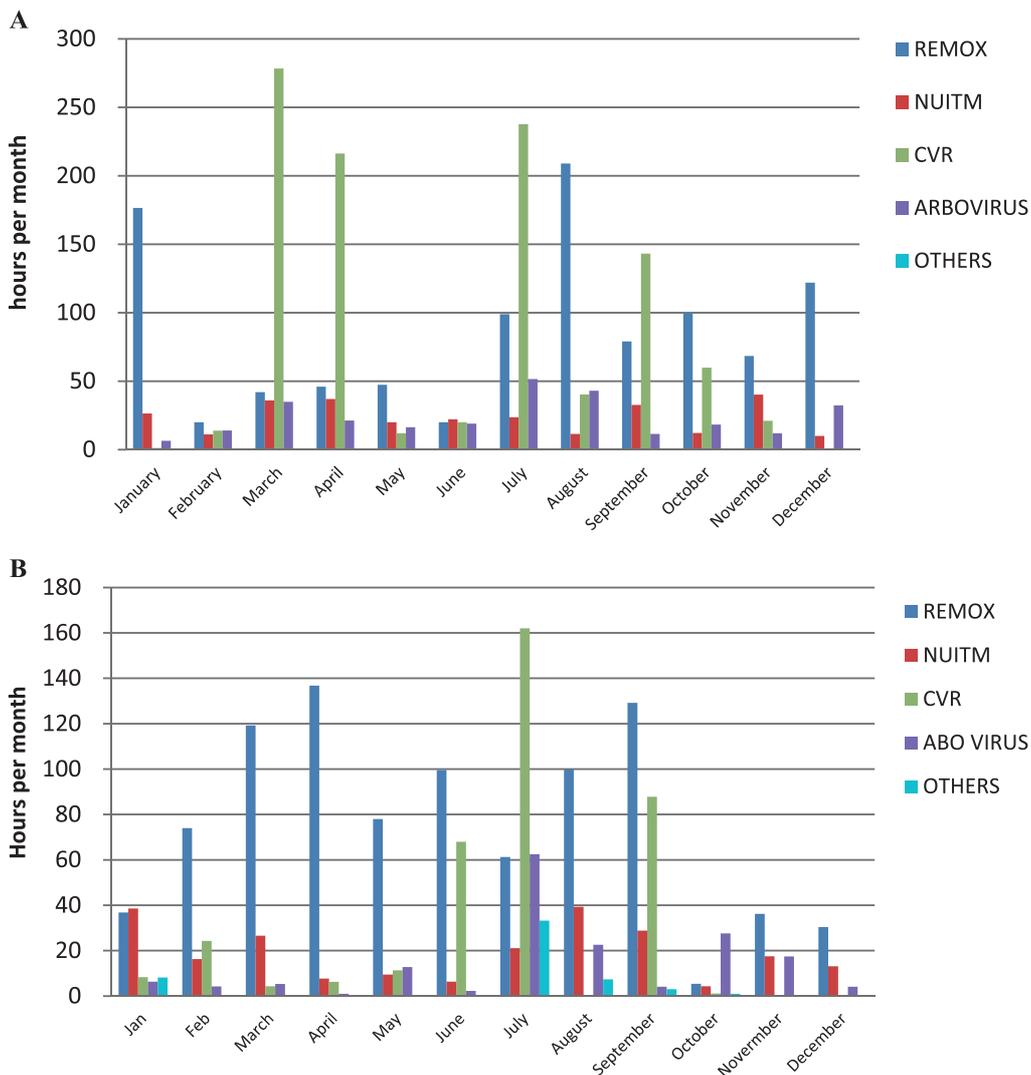


Fig. 19. A: Monthly working hours in P3 Lab in 2010. B: Monthly working hours in P3 Lab in 2011.

laboratory to process BSL-3 biological materials that are encountered in the course of routine research activities. To date, the majority of users of our facility have been research teams from other institutions, as shown in the graphs below, with the Remox project (CRDR-KEMRI) and CVR-KEMRI teams being the heaviest users (Fig. 19-A, 19-B). Through the entire course of its usage, a large number of pathogens have been isolated and/or propagated in the P3 lab. These include *Salmonella typhi*, *Bacillus anthracis*, *Vibrio cholerae*, yellow fever viruses and some strains of MDR-TB, among others. Bio-containment facilities in our laboratory have been of great use in enabling research activities involving such hazardous agents.

ACCREDITATION

Our facility is firmly established as per standard safety and security specifications [4] and has been in use for quite some time now. Operations within the P3 lab are in accordance with biosafety procedures, complete with standard operating procedures and necessary documentation as a prerequisite of accreditation of the facility. Currently, our institution is fully certified through the manufactures and is in the process of acquiring accreditation from local and international bodies.

CONCLUSION

In conclusion, our P3 facility is a comprehensive research unit that has continued to project our institution into a center of excellence in various fields of research. Most significantly, it has fully solved the problem of laboratory-acquired infections and threats posed by the manipulation of

hazardous biological materials in an unsecured system. The facility will continue to positively influence research experiences and outcomes while developing research capacity for the prevention and management of tropical diseases.

ACKNOWLEDGMENT

We thank Dr. Solomon Mpoke (Director, Kenya Medical Research Institute), Dr. Samuel Kariuki (Director, KEMRI-Centre for Microbiology Research) and Dr. Njeri Wamae (Former Director, KEMRI-Centre for Microbiology Research) for their collaborative support in setting up the P3 facility at the institute. We also acknowledge technical support offered by NK Systems company team, headed by Mr. Tetsuo Santoh in the establishment and the maintenance of P3 facility. We also thank all the lecturers for training P3 users on the occasion of annual P3 training seminar. This work was supported by JSPS KAKENHI Grant Number 19406014.

REFERENCES

1. Pike RM. Laboratory-associated infections: incidence, fatalities, causes, and prevention. *Annu Rev Microbiol* 1979; 33: 41–66.
2. Vesley D, Hartmann HM. Laboratory-acquired infections and injuries in clinical laboratories: a 1986 survey. *Am J Public Health* 1988; 78: 1213–1215.
3. Pedrosa PB, Cardoso TA. Viral infections in workers in hospital and research laboratory settings: a comparative review of infection modes and respective biosafety aspects. *Int J Infect Dis* 2011; 15(6): 366–376.
4. WHO. *Laboratory Biosafety Manual*, 3rd ed. Malta: World Health Organization; 2004.