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.

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 1380m^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 P3laboratory, 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.

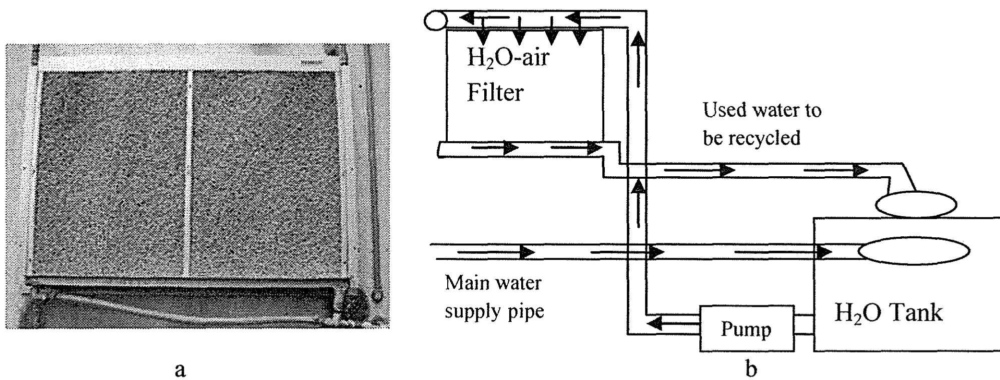


Figure 2: a) The water-air filtration system, and b) its water supply schema

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.

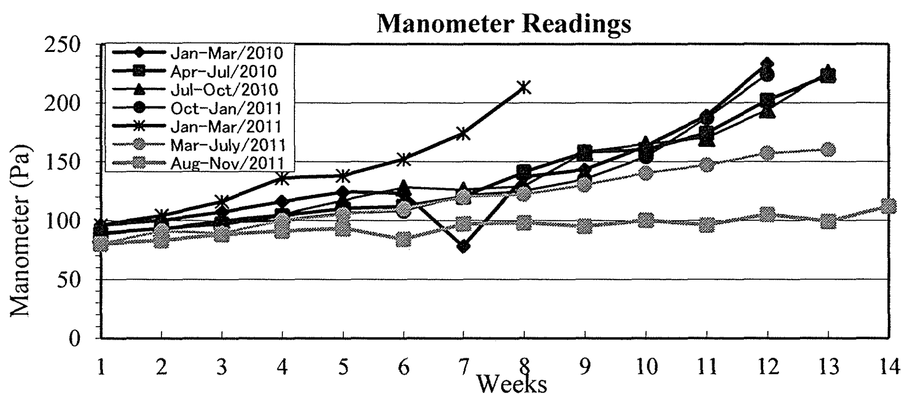


Figure 3: A graph showing manometer readings before and after installation of water-air filtration system

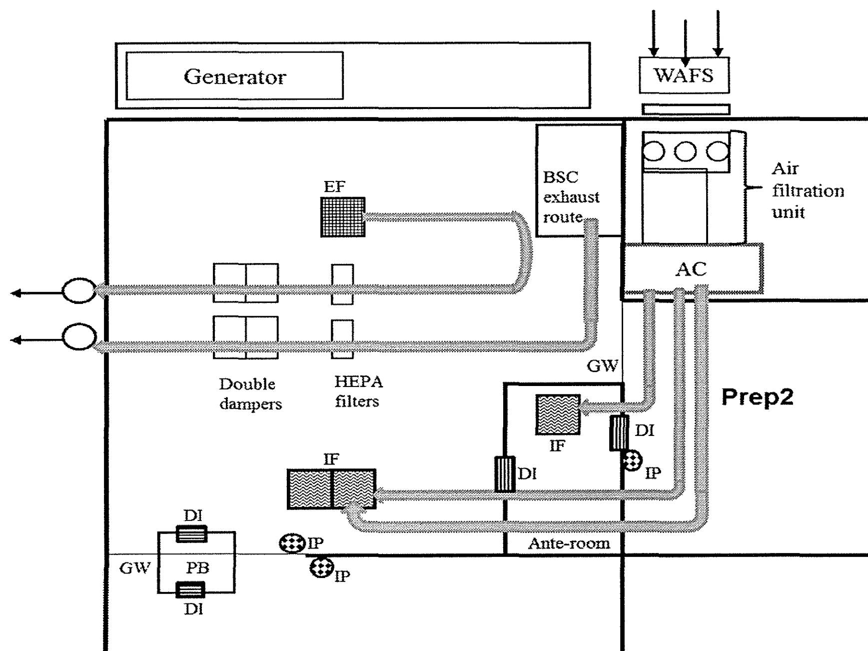


Figure 4: Physical features of a P3 laboratory; DI-door interlock, AC-air conditioner, IF-inlet fan, EF-exhaust fan, HEPA filters-high efficiency particulate air filters, GW-glass window, IP-interphones, PB-pass-box, WAFS-water-air filtration system

3.2 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 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 30^0 Celsius, hence eliminating the need for air conditioning, which saves power consumption in the facility by at least 30%.

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.

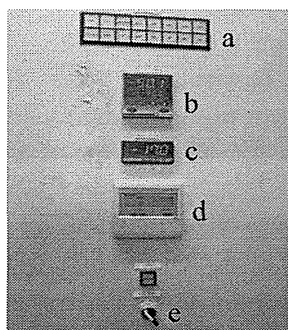


Figure 5

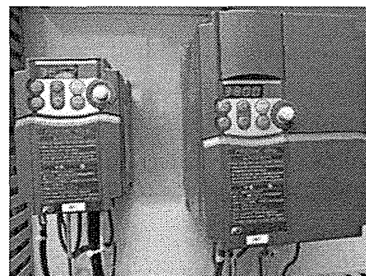


Figure 6

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

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.

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 IIA1, 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). 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).

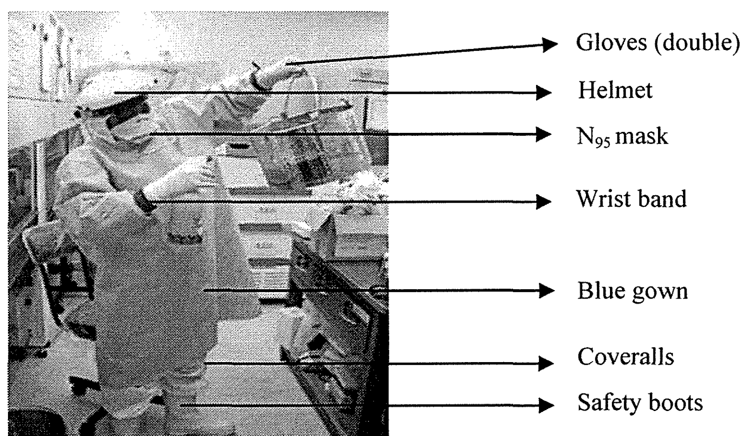


Figure 7: Personal protective equipments

4.2 Emergency response equipments

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 equipments.

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.

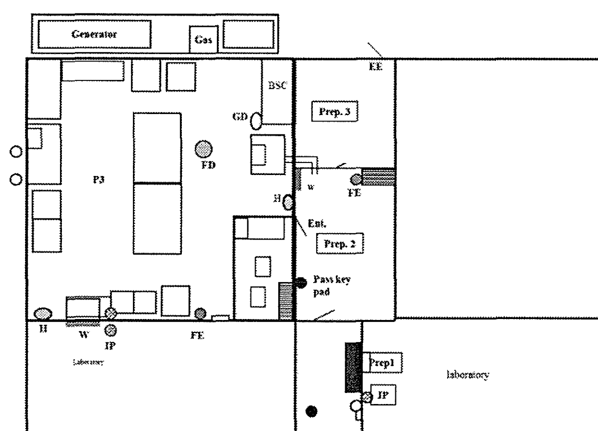


Figure 8: Aerial view of location of emergency response equipments within a P3 laboratory; GD-Gas detector, FD-fire detector, EE-Emergency exit, H-Hammer, IP-Inter-phone, FE-Fire extinguisher

Lack of lighting during power outages can be quite disastrous and a generator back-up 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 set-ups and evacuate.

5 Maintenance

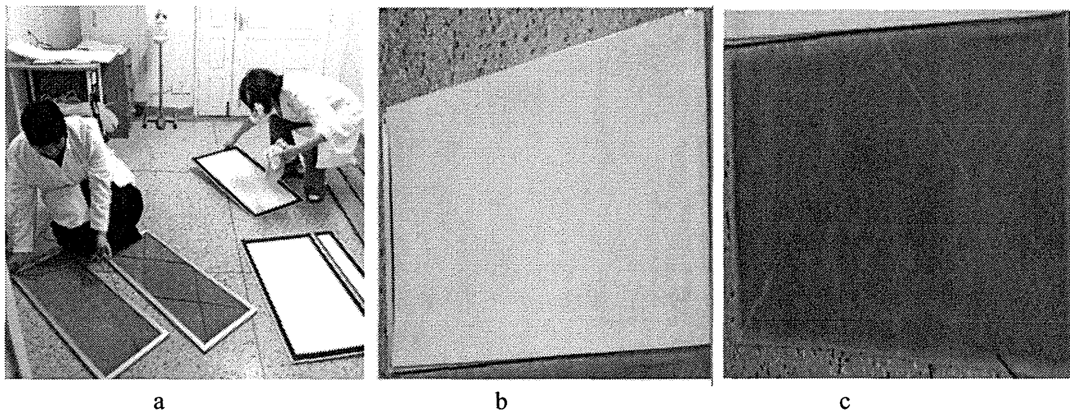
Maintenance of P3 facilities is a key aspect of biosafety management systems because use of faulty and unconditioned equipments can cause contamination. A P3 laboratory can increase the risk of transmission of biohazards due to the nature of pathogens handled in it hence regular maintenance is of utmost importance. For easy maintenance and optimum functioning of the laboratory, daily, weekly, monthly and yearly maintenance routines are carried out, overlapping 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.

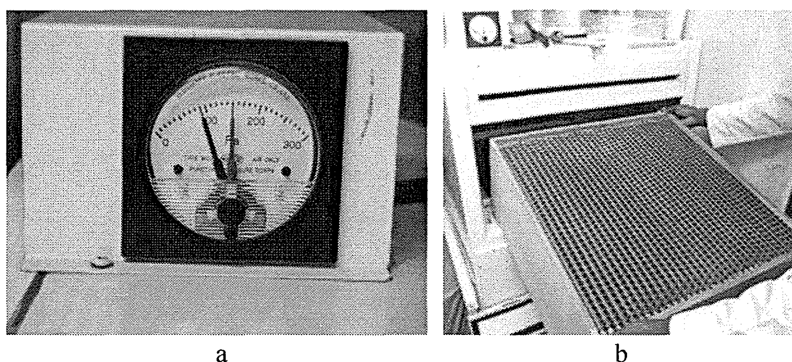


a b c

Figure 9: a) Changing of pre-filters, b) A clean pre-filter, c) A soiled pre-filter

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) below 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.



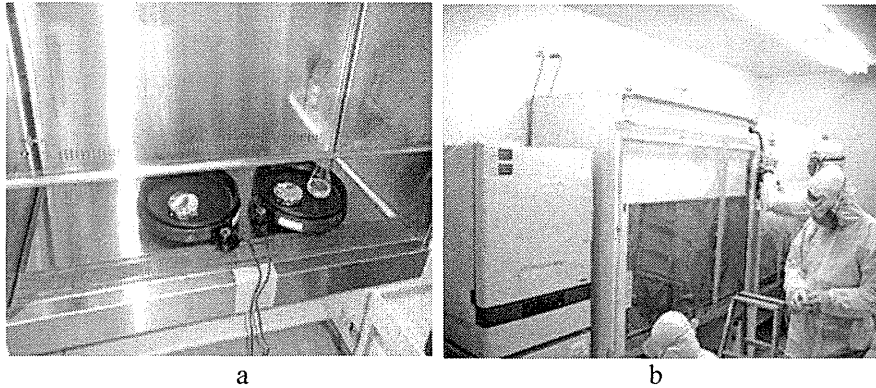
a b

Figure 10: a) The manometer, b) A used-up intermediate filter being removed from the filter chamber for changing

5.4 Yearly maintenance

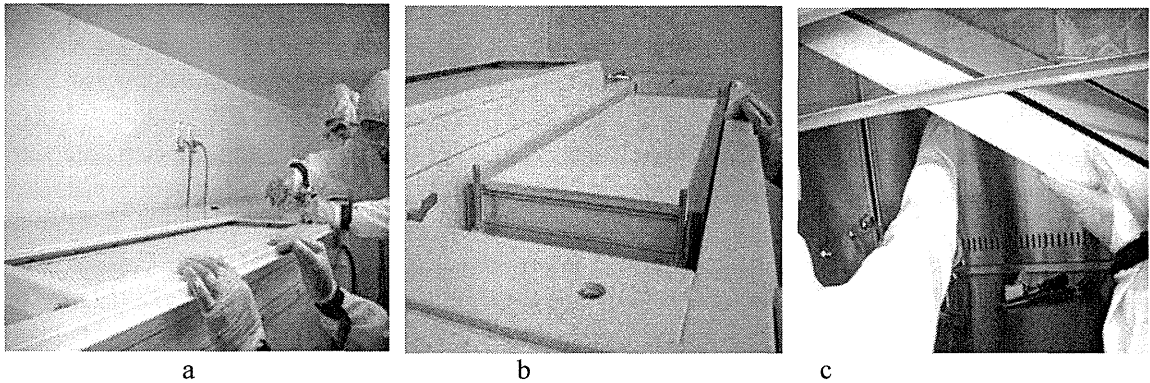
Every year, core P3 facilities are serviced, preferably by trained experts or by suppliers. Yearly maintenance mainly focuses on fumigation of biosafety cabinets and changing of HEPA filters.

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 11 (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 0.4m/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.



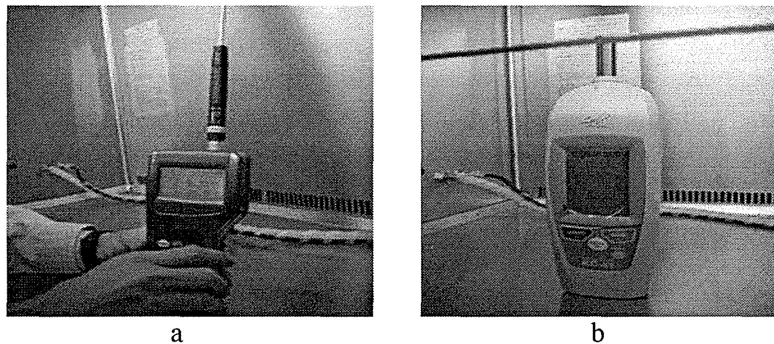
a b

Figure 11: Positioning hotplates (a) and sealing biosafety cabinets (b) in preparation for fumigation



a b c

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



a b

Figure 13: Confirmation of efficiency of fitted HEPA filters using air velocity testing (a) and air particle counting (b)

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 safety provided by safety equipments, additional safety and security features can be incorporated 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 measure 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 persons 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 hazardous 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 affixed 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 pathogen 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.

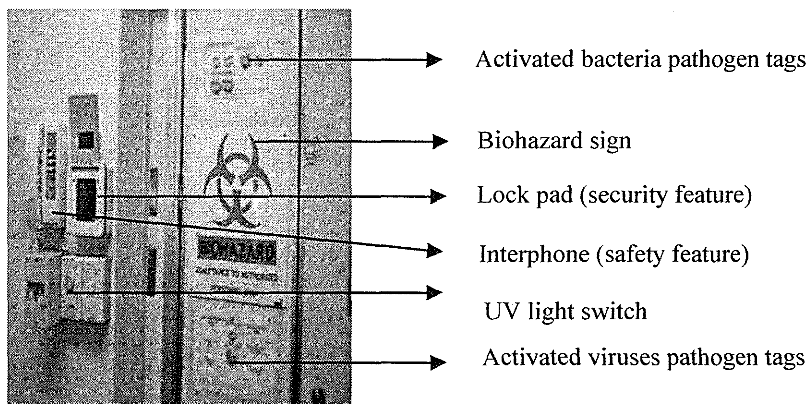


Figure 14: Safety and security features

Given the level of hazard associated with P3 laboratories, decontamination by ultraviolet light after each work session is necessary. The UV light switch bulb enables easy decontamination 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 communication 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 individual hazard that users are

likely to encounter. All signage features follow universal specifications 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.

Date / **Item**							
Inverter I							
Inverter II							
P3 Pressure							
Ante Pressure							
Room Temperature							
Remarks							

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.

8 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.

9 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.

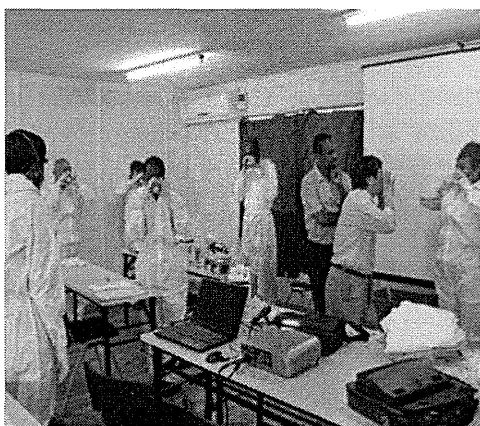


Figure 14: A training workshop's practical session

10 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 activities. 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.

11 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.

12 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.

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Acyclovir Reduces the Duration of Fever in Patients with Infectious Mononucleosis-like Illness

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Acyclovir is known for its antiviral activity against some pathogenic viruses such as the Epstein-Barr virus (EBV) that causes infectious mononucleosis (IM) and IM-like illness. Therefore, we empirically administered acyclovir to patients with suspected EBV-IM and IM like-illness, upon their admission to our hospital. We admitted 25 patients, who were hospitalized for fever and lymphadenopathy, to the Tohoku University Hospital Infectious Disease Ward. As part of treatment, 8 of these patients were given acyclovir (750 mg/day) with their consent and were assigned to the acyclovir group; the remaining 17 patients were assigned to the control group. The mean age of acyclovir patients (all men) was 42 ± 5.2 years, and that of control patients (13 men and 4 women) was 31 ± 3.0 years. The cause of illness was confirmed as EBV-IM in 6 patients (1, acyclovir; 5, control), and remained unknown for the other 19 IM-like illness patients (7, acyclovir; 12, control). A shorter duration of hospitalization and fever was observed in the acyclovir compared to that in the control patients (hospitalization duration: 16 ± 3.7 vs. 27 ± 7.7 days, $P = 0.36$; fever duration: 4.5 ± 1.8 vs. 18 ± 6.5 days, $P = 0.04$). Additionally, serum amyloid A (SAA) levels were lower in acyclovir than that in control patients (98 ± 37 vs. 505 ± 204 μg/mL, $P = 0.02$). Therefore, we propose that acyclovir is a potential therapeutic agent for both EBV-IM and IM like-illnesses. Future studies should further examine its mechanism of action.

Keywords: acyclovir; Epstein-Barr virus; fever; infectious mononucleosis like illness; lymphadenopathy
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Infectious mononucleosis (IM) is specifically caused by the Epstein-Barr virus (EBV), and is characterized by lymphadenopathy, prolonged fever, cervical lymph node swelling, and increased inflammatory reaction (Luzuriaga and Sullivan 2010). IM-like illness, which also has non-EBV etiologies, exhibits similar characteristic symptoms as IM. In the US, adult IM and IM-like illness are caused by EBV in 50-90% of the cases, human herpes virus type 6 (HHV6) in 9%, cytomegalovirus (CMV/HHV5) in 5-7%, herpes simplex virus (HSV)-1 in 6%, human immunodeficiency virus (HIV) in < 2%, and adenovirus in < 1% of the cases (Hurt and Tammaro 2007). However, IM-like illness due to CMV infection appears to be more common in Japan than in the US, and has been reportedly associated with 27.5% of IM-like illness cases in Japan (Naito et al. 2006). An evaluation and treatment algorithm involving patient history, physical examination, symptoms, and size of neck mass has been proposed (Schwetschenau and Kelley 2002).

The outcome of antiviral therapy largely depends on the pathogen. However, it would be impractical to test for all possible viruses due to time and cost constraints. Consequently, EBV-IM cases have been the ones most extensively studied with the aim of improving diagnosis and management, which resulted in the identification of RT-PCR as a useful tool for early diagnosis (Vouloumanou et al. 2012). Unfortunately, no anti-viral drug has been approved yet for the treatment of EBV-IM, and clinical trials involving anti-viral drugs have also yielded controversial results. On the other hand, administration of acyclovir to EBV patients reportedly gave beneficial results in immunocompetent patients (Torre and Tambini 1999; Rafailidis et al. 2010). One study showed that acyclovir pharmacokinetic parameters did not correlate with the virologic or clinical response in young adults with EBV-IM (Vezina et al. 2010). However, another study reported that valacyclovir therapy led to reduced EBV excretion and was clinically beneficial (Balfour et al. 2007). Other studies also showed that high-dose acyclovir and valacyclovir prophylaxis reduced the risk of CMV infection and disease following bone marrow transplantation, although it did not improve overall survival (Prentice et al. 1994); a more recent report

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revealed that antiviral agents such as ganciclovir or foscarnet should be used to treat CMV disease (Boeckh 2011). Neonatal HSV infection, caused by exposure to HSV in the genital tract during delivery, often results in severe fever and has a survival rate of 50% only. Antiviral therapy with high-dose acyclovir reportedly led to a reduction in the mortality of infants with disseminated disease (Corey and Wald 2009). The number of genital HSV infections among adults has increased in the last 30 years (Corey 2002). Varicella zoster virus (VZV), which belongs to the subfamily Alphaherpesvirinae, is commonly treated by nucleosides such as acyclovir, famciclovir, and valacyclovir (Rajan and Rivers 2001). These antiviral drugs have been approved for the treatment of herpes zoster and have shown beneficial results in reducing the duration of established postherpetic neuralgia. Although acyclovir anti-viral activity against herpes virus HHV8 could not be detected in vitro, some anti-herpes virus 6 activity was observed (De Clercq et al. 2001). One case report also showed that ganciclovir was effective in the treatment of adenovirus-associated hemorrhagic cystitis (Chen et al. 1997).

As described above, acyclovir seems to be potentially beneficial in the treatment of IM and IM-like illness. Therefore, we conducted this retrospective study involving IM and IM-like illness patients whose treatment empirically included or excluded acyclovir. We especially investigated the cases where patients were given acyclovir for the treatment of suspected viral infection upon admission to our hospital, and we examined the effects of this drug on patient condition.

Methods

Patients

In this retrospective cohort study, we collected data involving patients who were hospitalized for fever (> 37.5°C) and lymphadenopathy, from 2008 to 2010, at the Tohoku University Hospital Infectious Disease Ward. Since "conventional" IM diagnostic criteria only apply to EBV-caused IM, we enrolled 25 patients in this study, including patients with IM-like illness caused by other viruses, with the following inclusion criteria: duration of fever was defined as > 37.5°C for at least 1 day; sputum examination, including tuberculosis smear and PCR, did not indicate any bacterial infection; blood bacterial cultures were also negative for aerobes, anaerobes, and fungi; no antibodies against toxoplasma, chlamydia pneumonia, and mycoplasma were detected; antinuclear antibodies, indicating collagen diseases, did not exceed normal levels; sarcoidosis was excluded; HIV antibody and HIV RT-RNA levels were below the detection limit; lymphoma was not detected in any of the patients who underwent bone-marrow puncture and 18F-2-deoxy-fluoro-D-glucose positron emission tomography.

SRL laboratories (Tokyo, Japan) were used to perform HHV 1-8 viral PCR, assess HIV RNA quantity, conduct the CMV antigenemia (pp65 antigenemia, a component of the shell surrounding the virus nucleoprotein core) assay, and measure anti-EBV VCA IgG, IgM, and IgA as well as anti-EBV EA IgG and IgA and Epstein-Barr virus nuclear antigen (EBNA) antibody levels. EBV antibodies were measured using the fluorescent antibody technique, and antibodies against other viruses were measured using enzyme immunoassay (EIA). HIV antibody titrations were assessed at a Tohoku University clinical laboratory using the EIA method.

Statistical analysis

We expressed analyzed data as mean ± standard deviation. Comparisons between pre-treatment and post-treatment of the acyclovir and control groups were calculated by subtracting the post-treatment value from the pre-treatment value. The Mann-Whitney's U test was used to calculate the P values of the 2 groups. The Kruskal-Wallis test was used to evaluate clinical improvement. A P value < 0.05 was considered statistically significant. Analysis of data was performed using EXCEL software.

Results

Eight of the 25 patients were given acyclovir with their consent, as part of conventional therapy. Patient characteristics are presented in Table 1. Mean age, physical examination results, and laboratory results were compared between control patients (CP) and acyclovir patients (AP). Whereas all 8 APs were men, 13 of the CPs were men and 4 were women ($P = 0.13$). In general, clinical presentations including palpable surface lymph nodes (lymphadenopathy), severe throat pain (pharyngeal pain), continuous headache (headache), major multiple polyarthralgia (arthralgia), rash covering more than 1% of the body surface area (rash), and multiple mucosal bleeding (mucosal bleeding) were not significantly different between CPs and APs ($P > 0.05$). The largest liver and spleen diameter measurements observed also were not significantly different between CPs and APs ($P = 0.90$ and $P = 0.37$, respectively). Only the neck flexion test results were significantly different between the 2 groups ($P = 0.01$), and the 3 APs with positive neck flexion test results were clinically suspected of viral meningitis based on collected spinal fluid. Based on blood samples collected upon admission, laboratory test results showed that hemoglobin concentration, white blood cell count (WBC), white blood cell differentiation, platelet cell count, as well as C-reactive protein (CRP), serum amyloid A (SAA) proteins, ferritin, soluble IL-2 receptor (sIL-2R), lactate dehydrogenase (LDH), D-dimer, glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels were not significantly different between the 2 groups ($P > 0.05$).

Antibody titer tests were performed, and viral DNA was detected using PCR in order to identify the causes of fever; results are shown in Table 2. Patients with acute EBV infection were defined as those with anti-EBV VCA IgM positive (> 10) and EBNA negative (< 10) results. Accordingly, 5 CPs and 1 AP met the clinical criteria identifying acute EBV infection as the cause of IM. Since CMV antigenemia test results were negative for all patients, IM-like illness was unlikely caused by CMV. PCR results were positive for EBV DNA (> 10 copies/500,000 cells) in peripheral blood mononuclear cell (PBMC) samples of 2 CPs and 3 APs. Since PCR results were negative for HSV,

Table 1. Patient characteristics.

	Control Mean ± S.D.	Acyclovir Mean ± S.D.	P
n	17	8	
Male	13	8	0.13
Female	4	0	
Age (years)	31 ± 3	42 ± 5.2	0.06
Lymphadenopathy	14	7	0.74
Pharyngeal pain	11	7	0.24
Headache	13	6	0.94
Neck flexion test	0	3	0.01
Arthralgia	4	5	0.06
Rash	8	7	0.054
Mucosal bleeding	10	7	0.15
Liver (cm)	18 ± 0.5	17 ± 0.70	0.90
Spleen (cm)	10 ± 0.7	10 ± 1.2	0.37
Hemoglobin (g/dL)	13.7 ± 0.39	14.2 ± 0.39	0.49
White blood cell count ($10^3/\mu L$)	3,747 ± 909	8,675 ± 1,741	0.45
Neutrophil ($10^3/\mu L$)	4,203 ± 695	6,648 ± 1,608	0.11
Lymphocyte ($10^3/\mu L$)	2,058 ± 413	1,145 ± 244	0.16
Platelet cells count ($10^3/\mu L$)	215 ± 14	219 ± 32	0.89
CRP (mg/dL)	5.2 ± 1.2	8.7 ± 2.9	0.20
SAA (μg/mL)	349 ± 80	1,027 ± 519	0.07
Feritin (ng/mL)	581 ± 145	2,088 ± 1,632	0.20
sIL-2R (U/mL)	1,349 ± 160	1,044 ± 248	0.30
LDH (IU/L)	422 ± 60	409 ± 91	0.90
D-dimer (μg/mL)	3.4 ± 0.8	2 ± 0.6	0.24
GOT (IU/L)	64 ± 9.2	116 ± 63	0.29
GPT (IU/L)	84 ± 16	112 ± 71	0.61

CRP, c-reactive protein; SAA, serum amyloid A; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; GOT, glutamicoxaloacetic transaminase; GPT, glutamic-pyruvic transaminase.

Table 2. Antibody titer and viral DNA PCR.

		Control			Acyclovir		
		+	−	Unknown	+	−	Unknown
Antibody	EBV VCA IgG	11	5	1	7	1	0
	EBV VCA IgM	5	11	1	1	7	0
	EBV VCA IgA	2	12	3	0	8	0
	EBV EA IgG	1	14	2	0	8	0
	EBV EA IgA	0	15	2	0	8	0
	EBNA	7	9	1	4	4	0
Virus PCR	EBV	2	4	11	3	3	2

PCR of viral DNA was conducted on peripheral blood mononuclear cells.
VCA, viral capsid antigen; EA, early antigen; EBNA, epstein barr virus nuclear antigen.

CMV, VZV, or HHV DNA (< 10 copies/500,000 cells) in all patients, the pathogen was only identified in the EBV patients.

Acyclovir (750 mg/day) was daily administered intravenously with normal saline, as part of the treatment. The duration of treatment was similar between CPs and APs. Adverse effects of acyclovir such as skin eruption, liver injury, and shock were not observed in any patients. Antibiotic therapy was administered to 4 CPs and 4 APs, and gamma globulin was administered to 2 APs. The anti-

Table 3. Comparison between pre-treatment and post-treatment laboratory examination results.

	Control (n = 17)	Acyclovir (n = 8)	P
	Mean ± S.D.	Mean ± S.D.	
Hemoglobin (g/dL)	0.45 ± 0.19	1.1 ± 0.38	0.17
White blood cell count ($10^3/\mu$L)	888 ± 848	2,625 ± 1,186	0.25
Neutrophil ($10^3/\mu$L)	1,158 ± 591	2,940 ± 1,006	0.09
Lymphocyte ($10^3/\mu$L)	−460 ± 187	−163 ± 276	0.94
Platelet cell count ($10^3/\mu$L)	−61 ± 13	−50 ± 17	0.33
CRP (mg/dL)	3.2 ± 0.99	3.2 ± 0.86	0.40
SAA (μg/mL)	98 ± 37	505 ± 204	0.02
Ferritin (ng/mL)	25 ± 161	21 ± 49	0.75
sIL-2R (U/mL)	341 ± 123	−51 ± 84	0.55
LDH (IU/L)	−33 ± 29	42 ± 21	0.09

Table 4. Duration of hospitalization and of fever.

	Control (n = 17)	Acyclovir (n = 8)	P
	Days ± S.D.	Days ± S.D.	
Hospitalizaion (days)	27 ± 7.7	16 ± 3.7	0.36
Fever (days)	18 ± 6.5	4.5 ± 1.8	0.04

Table 5. Clinical presentation following treatment with acyclovir.

		No change	Improved	Worsened	P
Lymphadenopathy	Control	1	3	13	0.01
	Acyclovir	0	6	2	
Pharyngeal pain	Control	2	8	7	0.16
	Acyclovir	1	6	1	
Headache	Control	1	4	12	<0.01
	Acyclovir	1	6	1	
Arthralgia	Control	5	5	7	<0.01
	Acyclovir	1	6	1	
Rash	Control	6	4	7	0.17
	Acyclovir	1	5	2	
Mucosal bleeding	Control	3	5	9	<0.01
	Acyclovir	1	6	1	

P value is calculated by Kruskal-Wallis test.

biotic and gamma globulin treatments did not affect the patients' laboratory results.

Blood was collected from CPs and APs over a period of 2-3 days following acyclovir treatment. When we subtracted the laboratory examination post-treatment values from the pre-treatment values, the decline in WBC count was less prominent in the CP group (888 ± 848 × $10^3/\mu$L) compared to the AP group (2,625 ± 1,186 × $10^3/\mu$L; P = 0.25), as shown in Table 3, suggesting a beneficial effect of acyclovir on the condition of APs. This difference was mainly due to the much more prominent decrease in neutrophil count in APs (2,940 ± 1,006 × $10^3/\mu$L) compared to CPs (1,158 ± 591 × $10^3/\mu$L; P = 0.09). Although the reduction in CRP and ferritin levels was similar in APs and CPs (CRP, P = 0.40; ferritin, P = 0.75), SAA levels were significantly lower in APs (505 ± 204 μg/mL) than in CPs (98 ± 37 μg/mL; P = 0.02). Acyclovir administration also caused an increase in sIL-2R levels in APs (−51 ± 84 U/mL) and a reduction in CPs (341 ± 123 U/mL), although the change was not statistically significant (P = 0.55).

The duration of hospitalization was more prominently reduced in APs (16 ± 3.7 days) than in CPs (27 ± 7.7 days; P = 0.36), as was the duration of fever in APs (4.5 ± 1.8 days) compared to that in CPs (18 ± 6.5 days; P = 0.04), as shown in Table 4. The mean hospitalization period among EBV-IM patients was 18 days for the 1 AP and 12 ± 15.3