
Biosafety and SARS Incident in Singapore September 2003

Report of the Review Panel on New SARS
Case and Biosafety

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Terms of Reference

- 1) Review epidemiologic data on the SARS case.
- 2) Review biosafety requirements and practices at the laboratories in relation to the recent SARS case in Singapore, in the following areas:
 - Standard Microbiological Practices
 - Safety Equipment
 - Laboratory facilities
 - Training in biological safety
 - Other special practices
- 3) Recommend changes to address biosafety issues, improve practices and structural design of the laboratories.
- 4) Recommend the approaches to the adoption of National Standards, Audit and Accreditation for biological safety.

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Review Panel

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Executive Summary

The Singapore Ministry of Health asked an 11-member panel to review epidemiologic data on the recent SARS case and the biosafety requirements and practices at the Singapore BSL-3 laboratories in relation to this event.

From the results of the epidemiologic investigation surrounding the recent case of SARS, it appears that inappropriate laboratory standards and a cross contamination of West Nile virus samples with SARS coronavirus in the laboratory led to the infection of the doctoral student. No evidence could be found of any other source of the infection. West Nile virus and SARS coronavirus were detected in the virus samples handled in the laboratory. There is no evidence of secondary transmission and this is an isolated case of SARS.

Analysis of the Singapore BSL-3 laboratories showed a large range of biosafety structures and practices:

- The Environmental Health Institute inspection revealed several structural problems within the BSL-3 laboratory. The training of the laboratory workers is insufficient and depends on formal training in other institutions. A good record keeping policy should be instituted and implemented. It has been recommended that BSL-3 work cease until the laboratory deficiencies have been addressed and subjected to external audit.
- The Singapore General Hospital BSL-3 is correctly designed and the safety practices are well understood. Some minor structural problems should be addressed to facilitate laboratory work and improve safety. The renovation of the Department of Pathology BSL-2 laboratories lead to mixed BSL-2 / BSL-3 activities in the same working place that prejudice good safety practices.
- The National University laboratory is composed of several BSL-2 laboratories. Limited space and crowding are chronic problems. Overall, a practical culture of safety needs to be developed among the scientists and the students. The later will be tomorrow's scientists.
- The Defence Science Organization has a good laboratory structure and safety. No significant problems were identified. If the laboratory is to be more used in the future, minor structural changes are needed.

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Singapore has a real opportunity to address the adoption of Standards for Biological Safety and a range of approaches utilising international standards are recommended for adoption. These should be part of the National legislation, perhaps associated with the proposed Factories and other Workplaces Act. The control of importation of micro-organisms needs to be harmonised between Agri-Food and Veterinary Authorities and the Ministry of Health and there needs to be control on the transfer of imported agents. Competency based training in biological safety should be adopted and all groups involved in BSL-3 facilities should undergo retraining. Singapore needs to agree on a small list of restricted high risk organisms that should be tightly controlled and handled only in approved facilities. A regular audit process should be put in place for BSL-3 facilities.

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Recommendations

Epidemiological Investigation

Recommendation 2.1: To rule out subclinical secondary transmission, repeat serologic testing should be carried out for all contacts (work and household) of the patient at least 29 days after last contact with the case patient.

- a. Work contacts: On or after September 24th
- b. Household contact: On or after October 2nd

Recommendation 2.2: In order to complete the investigation, it would be desirable to sequence the SARS coronavirus from the cell supernatant that the patient was working on in the laboratory and compare it to the sequence obtained from his clinical specimens. (However, these findings are not likely to change the results of the investigation).

Environmental Health Institute

Recommendation 3.1: That the EHI BSL-3 laboratory only be allowed to reopen once it has been re-audited and issues related to the structure, the use of the BSL-3 laboratory, training of staff, and a risk assessment of work have been carried out to a level acceptable by a Safety Committee. The laboratory should be pressure tested before reopening. The stocks or virus passages done in the BSL-3 during the time when SARS coronavirus was present in the laboratory should be destroyed.

Recommendation 3.2: That, after a complete disinfection (fumigation) of the laboratory, structural changes should be done to the laboratory to reach the BSL-3 standards: the air supply unit for BSL-3 laboratory should be separated; Particulates Air filter (97% efficiency) filter should be installed; Pressure gauges indicating negative pressure level of change room and the Laboratory must be installed in a convenient place with adequate alarms; Doors should have cardkey access and biohazards signs must be displayed on them; Autoclave should be moved inside the laboratory or better a double-door autoclave should be installed; a low-temperature freezer should be moved inside the laboratory to keep the BSL-3 virus stocks; CO₂ bottles should be installed outside of the main lab. An eye wash station should be installed in the change room, and if possible, a personal shower.

Recommendation 3.3: That a daily checklist should be put in place to record date, time, name and laboratory pressure; That standardized and computerized inventory of the virus stock in the freezers must be implemented and enforced.

Recommendation 3.4: That the Institute develop, or offer, appropriate training (including refresher course) in biological safety and that this training be competency based. Training in the operation at BSL-3 could be mandatory if work is to be done under these conditions. Training records should be kept. Appropriate SOP should be available and enforced across the laboratories.

Recommendation 3.5: That the biosecurity aspects have to be considered at EHI. Access to the BSL-2 and BSL-3 laboratories should not be free and cardkeys system should be introduced

Singapore General Hospital

Recommendation 4.1: That the Hospital Safety Committee ensures that safety standards for BSL-2 and BSL-3 are not mixed. Each them require a separate set of standards and the current condition is very prejudicial for safety. An accurate risk assessment of the work carried out should be done. A BSL-2 laboratory should be made available as soon as possible or if impossible, the BSL-2 manipulation should be done following the BSL-3 standards.

Recommendation 4.2: That a few changes should be introduced to facilitate a good safety management of the laboratory: modify the location of the pressure gauges; creation of an external checklist; installation of biohazards signs on the doors; preparing a kit for disinfection in case of accidental infectious spill; installation of ports for decontamination and testing of the HEPA filters in the supply and exhaust air pipes; The positive pressure of the entrance room should be reverted to negative; the pressure differential between the rooms should be increased to facilitate the air balances.; provide extra CO₂ bottle for replacement; Creation of a procedure for systematic disinfection of the discarded liquids (sinks, shower) before release in the hospital sewage.

Recommendation 4.3: That the Department of Pathology responsible for the BSL-3 develop regular refresher safety courses for the people working in the BSL-3. The dress code in the laboratory should be standardized. The recent safety recommendations don't require wearing mask if the potentially infectious work is done under safety cabinets. A procedure for disinfection and laundering reusable surgical gowns should be developed.

National University Singapore

Recommendation 5.1: That the Microbiology laboratories be re-audited to ensure that they meet safety standards and that each laboratory provides a risk assessment of the work that they carry out. The local Safety Committee signs off on the adequacy of the audit and the risk assessment.

Recommendation 5.2: That front opening lab coats be banned from use in the microbiology laboratories and back fastening gowns be introduced. Gown hooks should be provided near the laboratory doors (inside) for gowns to be left inside and a procedure for disinfection and laundering the gowns developed. There are a number of makes of reusable surgical style gowns suitable for this purpose.

Recommendation 5.3: That the University develop, or offer, appropriate training in biological safety and that this training be competency based. Training in the operation at BSL-3 could be considered.

Recommendation 5.4: That the Microbiology Department as a team objective adopt the goal of creating a culture of safety for all staff and students within the Department.

Recommendation 5.5: That the virology laboratory be allowed to reopen once it has be re-audited and issues related to the used of the biological safety cabinet, excess paper and other materials within the laboratory, training of staff, use of gowns and a risk assessment of work has been carried out to a level acceptable by the Safety Committee and the Head of the Microbiology Department.

Defence Science Organization

Recommendation 6.1: That minor structural changes be made. DSO laboratory is more likely to be involved in the future during national emergencies requiring BSL-3 conditions. Several minor modifications should be done and the laboratory supervisors have already planned most of them. Pressure gauges need to be put in a better place for easy checking; Outside checklist should be implemented; a disinfection kit should be made available in case of accidental spill; the air conditioning should be removed from inside and conditioned the supply air; a double door autoclave will make manipulations safer and easier for the personnel; If a CO₂ incubator need to be installed, the supply bottles need to be outside of the laboratory; a computer connected to the outside should be easily installed.

Recommendation 6.2: That the group responsible group for the BSL-3 develop regular refresher safety course for the people working in the BSL-3.

Biological Standards

Recommendation 7.1: Need for a National Legislative basis for Standards in Biosafety Laboratories for Singapore. It is recommended that the detailed safety codes be attached to the legislation and be made mandatory. The codes should be reviewed by an expert panel, at least once every 5 years, and there should be a system in place to allow timely updates.

Recommendation 7.2: A structure should be created for laboratory certification covering both structure integrity and operating procedures. These certifications should be renewed on an annual basis. It would be more cost efficient if these audits were carried out under a quality control standard such as ISO17025 using experienced and accredited external auditors.

Recommendation 7.3: Creation of the tracking system for importation, exportation to and from Singapore. The tracking system should also cover the transfer of infectious agent among laboratories in Singapore. This system should be harmonised between the Ministries of Health and Agriculture

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Introduction

Singapore has continued surveillance for SARS since the last case was reported in May 2003. Early September 2003, the Singapore General Hospital (SGH) identified a new probable SARS case. The patient was isolated at the Communicable Disease Centre (CDC) and has since recovered. This single case of SARS occurred in a 27-year-old doctoral student who worked in virology laboratories in Singapore. Positive laboratory results obtained from the case, which was first identified by Singapore health authorities, have now been confirmed by the Centers for Disease Control and Prevention in Atlanta. The Singapore case is an isolated event and has not produced secondary. It is not regarded as an international public health concern.

The Singapore Ministry of Health asked an 11-member panel that included four international experts to review epidemiologic data on the SARS case and the biosafety requirements and practices at the Singapore BSL-3 laboratories in relation to this event. The panel will make recommendations on biosafety practices and standards to be developed in Singapore. With the large number of BSL-3 laboratories that are due to come on line soon in the BioPolis, and in view of the preliminary results of this investigation, there is an urgent need to develop a uniform and comprehensive biosecurity training program for the laboratory workers.

This report presents the results of the epidemiologic investigations surrounding the recent case of SARS in Singapore, the analysis of the biosafety structures and practices in the Singapore BSL-3 laboratories, and the recommendations for the development of safety standards in Singapore. These data were obtained by the four international experts during visits and interviews with the parties involved.

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Epidemiological Study

Overview

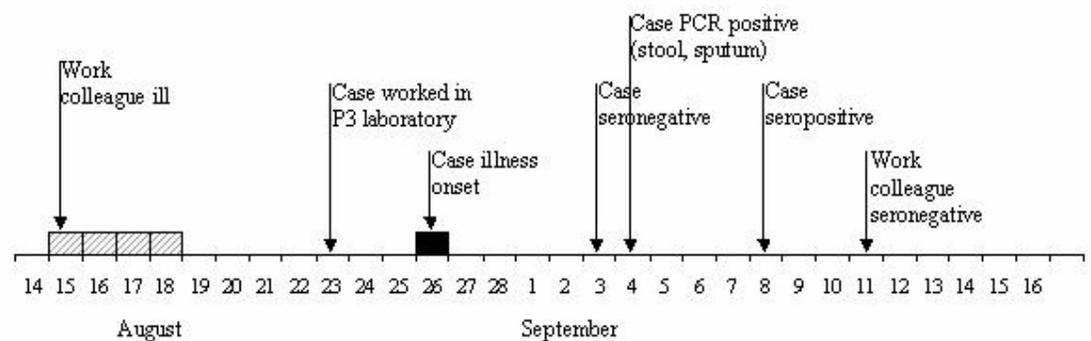
The patient is a 27-year-old man in his third year of a doctoral program in microbiology at the National University of Singapore (NUS). On the evening of August 26, he developed fever and muscle pain. Between August 27th and September 3rd, the patient visited his general practitioner, Singapore General Hospital (SGH) emergency room, and a Chinese physician. On September 3rd, the patient had persistent fever and returned to SGH; at this time he was admitted. Interviews with the patient revealed that the patient was in a laboratory where SARS coronavirus work was being conducted, and several specimens were taken for testing.

A review of the patient's clinical records suggests that he had an illness that was consistent with SARS. The patient had fever with a dry cough, and evidence of pneumonia on chest radiograph and CT. In addition, the laboratory results support this assertion. On September 8, stool and sputum specimens were tested for SARS coronavirus by reverse transcriptase polymerase chain reaction (RT-PCR) and were positive. Serial serum samples also documented seroconversion to the SARS coronavirus. Repeat testing in other laboratories confirmed these findings. Testing for a whole range of other pathogens was negative.

During the epidemiologic investigation, we interviewed the patient as well as his family and work colleagues. The timeline of events is depicted in the figure below. We developed three transmission hypotheses and the evidence for and against are given below. As a result of the investigation, we conclude that the patient most likely acquired the infection in the laboratory as a result of accidental contamination. This was an isolated case and there is no evidence of secondary transmission.

Figure. Timeline of events in Singapore, August and September 2003

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Transmission Hypotheses

1) Patient acquired infection a while ago and carried it latently

Evidence against:

a) Patient has recent documentation of seroconversion that coincides with clinical infection (Figure).

b) Patient denies contact with any known SARS case or travel to previously SARS affected areas.

2) Patient recently acquired infection from someone

Evidence against:

a) One work contact had recent illness, but clinical picture was not consistent with SARS (nausea and vomiting; no fever). This person did not work with live SARS coronavirus. Serum taken from this person 27 days after illness onset was negative for antibodies to the SARS coronavirus.

b) Patient denies contact with any other ill person. Interviews with family and work colleagues support this assertion.

3) Patient acquired infection through laboratory contamination

Evidence for:

a) Patient worked in BSL-3 laboratory 3.5 days before his illness onset. This is consistent with the expected incubation period for SARS. Although the patient reported only working on West Nile virus, the laboratory was doing live SARS work around the time.

b) Poor record keeping makes it difficult to ascertain if there was live virus in the BSL-3 laboratory on the day of his visit, but we do know it was there 2 days before.

c) Procedures for laboratory safety differed widely between laboratory personnel at EHI and were not always appropriate.

d) Testing of the frozen specimen that patient worked with on August 23 was positive by RT-PCR for the SARS coronavirus and West Nile virus, suggesting contamination.

e) The laboratory only works on one strain of the SARS coronavirus, so the laboratory strain and patient strain were sequenced for comparison. Approximately 91% of the genome was sequenced from patient and found to be most closely related to the sequence of the laboratory strain. Minor differences observed are likely the results of the natural mutation rate for the virus.

Recommendations

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Environmental Health Institute

Description of the facility

The Environmental Health Institute (EHI) is mainly a research institute for vector-borne microbiology. There are 27 employees, 24 of which work in the laboratories. The work is divided between mosquito taxonomy and vector-borne virology. EHI laboratories consist of insectarium, BSL-2 and BSL-3 laboratory.

Work with micro-organisms

The laboratory engages in work on Dengue virus (1-4), Japanese encephalitis, Yellow fever, Chikungunya, West Nile, Kunjin, and Hantavirus (only stock). Work includes growing stocks and virus isolation attempts from wild-caught mosquitoes.

The handling of SARS coronavirus started the 14 April 2003. Experiments on *Mycobacterium tuberculosis* (TB) also occurred in the BSL-3 laboratory beginning in June 2003. Specific SARS work includes virus stock preparation, plaque reduction neutralization test, RNA production, production of IFA slides for serodiagnostic, and infections of different cell lines. Only one strain of SARS coronavirus was used, but some human clinical materials were also handled. Virus was grown in T25 and T75 flasks and in 24 and 96-well plates.

Containment facility

EHI is located in the 3rd floor of the Building and entrance to the floor requires cardkey access. Laboratory access is not controlled. The office area is separated clearly from the laboratory area by a door that is not locked. The floor was constructed mainly for laboratory use, including BSL-3 laboratory work (by DE MAX Design PTE LTD). The BSL-3 laboratory was completed in April 2002 and opened in July 2002.

In the whole laboratory, there are 7 zones for air supply units. Pre-filtered air is supplied to all zones. There are 4 exhaust air units, and those of BSL-3 and Chemical and Mosquito laboratories are independent. The other unit has a re-circulating system.

The BSL-3 laboratory consists of an anteroom with a small autoclave and a laboratory; total size is about 30 m². There is no biohazard label on the door of BSL-3 laboratory, or on the freezers in the BSL-2 laboratory. There is a sink with 2.5 litre trap capacity. There is no secondary tank for disinfection. Wastewater is directly released to the building sewage. There are two class IIA biosafety cabinets (BSCs). Exhaust air from the BSCs is recirculated in the room. There are two CO₂ incubators (top dedicated to SARS virus, bottom for others viruses) supplied by a gas bottle situated in the main laboratory. Other equipment includes a bench-top centrifuge with safeguard buckets, a microscope, a water-bath, and a refrigerator. The autoclave for decontamination is

situated in the change room. The low temperature freezer for virus storage is situated outside in the BSL-2 area.

There is one air supply and one exhaust HEPA filter unit on the ceiling each in the laboratory and anteroom. Air supply to FCU-5 air conditioning unit supplies air to both BSL-3 and BSL-2 laboratories. Supply air volume to the BSL-3 laboratory is 1,000m³/h and exhaust is 1,200m³/h. Exhaust air volume is more than 10% larger than supply air volume. The setting of negative pressure level of anteroom against outside room is minus 2-3mm H₂O and laboratory against anteroom also minus 2-3mm H₂O. Volume control dampers (VCD) are present in the air supply in interstitial space and these are operating only manually. There is a maintenance service every month with air balance checks and adjustments if necessary. Values are reported to EHI. The BSL-3 laboratory is not running 24-hours a day. There is a small control panel on the outside wall. The automatic timer is set between 8 am and 7 pm.

Each air-condition unit has a pre-filter, but no medium efficiency PA filter. This pre-filter is re-usable after washing. Capture rate is 65% (HEPA, 99.999%). Although it seems to be insufficient for pre-filtration, the supply HEPA filter in the laboratory was replaced only once about two months ago. No fumigation of laboratory has been done and exhaust fan and supply fan were going while this replacement was performed. The filter was packaged and disposed under regulation of EHI. The laboratory had never been full decontaminated since it opened.

There are no pressure gauges indicating negative pressure level of BSL-3 laboratory, but when the main switch is off a red alarming device light is turned on. If the exhaust fan is down during operation, an alarm sound and a flashing light is turn on. There are two security personnel among the laboratory staffs. They will contact the maintenance service company if a problem is identified.

Staff procedures and SOP

There is a Standard Operating Procedure (SOP) manual entitled "Biosafety & Laboratory Practices in EHI", beta edited in April 2002 by the Ministry of the Environment, Singapore. There are only 3 pages for BSL-3 laboratory. One page is for the ground plan and the others are for laboratory practices. Although it refers to a daily record, there is no daily record of BSL-3 laboratory such as date, user name, time-in and out, remarks including negative pressure level and experiment except BSC usage record.

Personal equipment required consists of lab coat; cover shoes, masks (N95 or surgical) only during SARS coronavirus manipulations, no overhead, and gloves to be taped to lab coat. These norms are not always respected.

Because there are no low-temperature -80°C freezers inside the BSL-3 laboratory, virus ampoules are disinfected under the class II biosafety cabinet then transferred in the BSL-2 area for storage. There is no central database for virus stock and location in the freezers. There is a record for maintenance service for BSCs (sticker on the cabinet) but no written report.

There are two types of waste generated during manipulation. The solid waste (pipettes, plates and tips) are soaked in hypochlorite then sprayed with alcohol at the end of the session and followed by a 10MN UV irradiation, then left under the biosafety cabinet until the next morning. At that time, another 10mn UV irradiation occurs and then the trash is bagged and autoclaved. Sinks are not used to dispose of infectious material. Only disinfectant-treated liquids are discarded in the sink.

Formalin (plates fixation) and acetone (slides fixation) are done in the laboratory. Clorox (Sodium hypochlorite), 70% alcohol are used to inactivate contaminated materials, and Hibiscrub (4% chlorhexidine gluconate) for hand-washing. Every other Friday, a general cleaning of the BSL-3 laboratory is done.

People can work alone in the laboratory.

No radioactive material or animals are handled in the BSL-3 laboratory.

There are no mandatory health clinic visits or follow-up for laboratory workers. No vaccinations (Japanese encephalitis or Yellow fever) are required and there is no follow-up for those that have received vaccine.

Staff training

The personnel working in the BSL-3 had attended some lectures. The “Biosafety & Laboratory Practices in EHI” manual is presented to the newcomers. The most senior laboratory personnel then do orientation training in the BSL-3 laboratory. Some visitors (e.g., during the *M. tuberculosis* experiments) received a longer training session by university mentors. Training for newcomers and re-training for staffs seems to be insufficient.

Recommendations

Recommendation 3.1: That the EHI BSL-3 laboratory only be allowed to reopen once it has been re-audited and issues related to the structure, the use of the BSL-3 laboratory, training of staff, and a risk assessment of work have been carried out to a level acceptable by a Safety Committee. The laboratory should be pressure tested before reopening. The stocks or virus passages done in the BSL-3 during the time when SARS coronavirus was present in the laboratory should be destroyed.

Recommendation 3.2: That, after a complete disinfection (fumigation) of the laboratory, structural changes should be done to the laboratory to reach the BSL-3 standards: the air supply unit for BSL-3 laboratory should be separated; HEPA filter should be installed; Pressure gauges indicating negative pressure level of change room and the Laboratory must be installed in a convenient place with adequate alarms; Doors should have cardkey access and biohazards signs must be displayed on them; Autoclave should be moved inside the laboratory or better a double-door autoclave should be installed; a low-temperature freezer should be moved inside the laboratory to keep the BSL-3 virus stocks; CO₂ bottles should be installed outside of the main lab. An eye wash station should be installed in the change room, and if possible, a personal shower.

Recommendation 3.3: That a daily checklist should be put in place to record date, time, name and laboratory pressure. That standardized and computerized inventory of the virus stock in the freezers must be implemented and enforced. Recommendation 3.4: That the Institute develop, or offer, appropriate training (including refresher course) in biological safety and that this training be competency based. Training in the operation at BSL-3 could be mandatory if work is to be done under these conditions. Training records should be kept. Appropriate SOP should be available and enforced across the laboratories.

Recommendation 3.5: That the biosecurity aspects have to be considered at EHI. Access to the BSL-2 and BSL-3 laboratories should not be free and cardkeys system should be introduced

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Singapore General Hospital

Description of the facility

The department of Pathology of the Singapore General Hospital is responsible for any laboratory diagnosis in the hospital and is accredited by the American College of pathology.

Work with micro-organism

During the SARS outbreak, SGH Department of Pathology acted as the principal laboratory for the processing of human specimens and confirming the results. Several procedures were used: virus isolation on Vero cell culture, RNA extraction for RT-PCR, antibody detection by indirect immunofluorescence and ELISA assays.

No security clearance is required for working in the laboratory. Several virus strains and human clinical specimens are available. Most of the potentially infectious specimens are kept in the BSL-3 freezer with limited access.

Containment facilities

The BSL-3 laboratory was opened a year ago. It is situated on the top of the building and can only be accessed through a restricted corridor. Access to the main laboratory is done through 3 successive locked doors; each of them can only be open by punching a secret code. The access room is in positive pressure; this could be potential problem in case of autoclave malfunction. A clothes change room is included in the passageway and is equipped with a personal shower. Water from the shower, the change room and the laboratory sinks goes to a designated tank before being release in the hospital sewage.

Walls, floor, and bench surfaces are constructed for easy cleaning and disinfection. Penetrations in the laboratory of ducts and wires are sealed and should allow disinfection by fumigation (not done yet).

The supply and exhaust air ventilation system for the 3 rooms is pre-filtered and HEPA filtered. There is no recirculation of the exhaust air and the exhaust air is discharged away from the air intakes. The differential pressure/directional airflows between adjacent rooms is monitored and linked to a control room with a sound and light alarm in the lab. A visual pressure-monitoring device is available at the entrance of the change room and the main laboratory room. The pressure differentials between the different levels are around 30 Pascals.

Laboratory waste is evacuated through a double door autoclave. All the three HEPA-filtered class II cabinets are tested annually, two are connected to air exhaust system and one, after filtration, recirculated the air in the laboratory.

There is no ultra or high-speed centrifuge. Bench-top centrifuges equipped with aerosol safeguard buckets are available. Freezers are situated inside the laboratory and CO₂ bottle for the incubators are situated outside of the contained area.

The hospital generator backs up the laboratory electric system. The technical staff is on campus and has a good knowledge of the systems.

Staff procedure

Access to the laboratory is controlled by the laboratory director and is restricted to trained personnel. There is one designated laboratory manager. A biosafety manual specific to the laboratory is available.

People used disposable gowns, on the top of the lab coat, and gloves. Due to the department BSL-2 laboratory maintenance, some BSL-2 work is done in the laboratory and personnel used different types of respirators and masks in the same area.

All manipulations of infectious materials are conducted in the class II biological safety cabinets. All contaminated materials are disinfected using Virkon and hypochlorite before being autoclaved and disposed. Protective laboratory clothing and gloves are not worn outside of the laboratory and decontaminated by autoclaving before being disposed outside of the laboratory

No radioactivity material and no animals are handled in the laboratory.

Staff training

Two laboratory workers have attended a safety course in Boston in 2002. A long-term planned visit at the Centres for Disease Control and Prevention was cancelled several times and is supposed to happen next February.

During the last visit of Dr. Ksiazek (CDC) several safety lectures were organized. There is a laboratory manual and all new personnel are going through a training period and a checklist of items to be covered during the training is available.

Recommendations

Recommendation 4.1: That the Hospital Safety Committee ensures that safety standards for BSL-2 and BSL-3 are not mixed. Each them require a separate set of standards and the current condition is very prejudicial for safety. An accurate risk assessment of the work carried out should be done. A BSL-2 laboratory should be made available as soon as possible or if impossible, the BSL-2 manipulation should be done following the BSL-3 standards.

Recommendation 4.2: A few changes should be introduced to facilitate a good safety management of the laboratory: modify the location of the pressure gauges; creation of an external checklist; installation of biohazards signs on the doors; preparing a kit for disinfection in case of accidental infectious spill; installation of ports for decontamination and testing of the HEPA filters in the supply and exhaust air pipes; The positive pressure of the entrance room should be reverted to negative; the pressure differential between the rooms should be increased to facilitate the air balances.; provide extra CO₂ bottle for replacement; Creation of a procedure for systematic disinfection of the discarded liquids (sinks, shower) before release in the hospital sewage.

Recommendation 4.3: That the Department of Pathology responsible for the BSL-3 develop regular refresher safety courses for the people working in the BSL-3. The dress code in the laboratory should be standardized. The recent safety recommendations don't require wearing mask if the potentially infectious work is done under safety cabinets. A procedure for disinfection and laundering reusable surgical gowns should be developed.

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Department of Microbiology

Description of the facility

The Department of Microbiology teaches both graduate and post-graduate students in science and medicine. It covers a wide range of research areas including immunology, genetics, virology, bacteriology and applied and industrial microbiology. The Department appears only to work with Biosafety level 1 and 2 organisms and therefore it has not been assessed to the same level of the other laboratories that have been reviewed. There is no evidence that live SARS coronavirus has been handled in the Department.

Work with micro-organisms.

Wide ranges of micro-organisms are handled in the Department, including bacteria, viruses, fungi and parasites. Most of the organisms are ones that require procedures at Biosafety levels 1 and 2, although there are some organisms held in storage that require Biosafety level 3, and *Burkholderia pseudomalli* is considered to now require containment at Biosafety level 3, except when handling diagnostic specimens.

Containment facility

BSL-2 laboratories are along a corridor, which has cardkey control on the access. Like many universities, there is significant over crowding and also the laboratories are also being used as sub-stores. The biosafety procedures obviously vary between laboratories, but at least one of the laboratories was using Bunsen burners in class II biological safety cabinets. This is a very dangerous practice as it causes disruption of the protective laminar flow and can cause the air to flow out of the cabinet and thus contaminating the operator and the environment.

Offices for the supervisor are within the laboratory and there are write up areas for the students in the corridor. It is now recommended that all write up areas be outside the laboratory. It was also noted that a flask with a pump was outside the BSCII, and used for sucking off infected tissue culture fluid. There was no in line filter to protect the pump and the flask was outside the cabinet. This was another lapse in understanding in how to work at Biosafety level 2.

Otherwise, the structure of the laboratory and the access control was adequate for a Biosafety level 2 laboratory.

Staff procedures and SOP

The University Safety Manual was well presented but not very practical, and it is doubtful that students and staff used it. It lacked detailed procedures for development of procedures for biological safety and did not encourage teams to carry out risk assessment of their work and the laboratory environment. There was no evidence of local safety procedures developed by the research teams and approved by the safety committee.

A safety audit of the Microbiology Department was supplied to the expert panel and it was evident from the brief inspection we carried out that the audit was inadequate and that a proper checklist process was not in place. Although there was clear evidence that the University had a strong management commitment to OH&S, there was little evidence that there was a strong culture within the staff and students.

The practice of using front opening laboratory gowns needs to be discontinued. Laboratory gowns should only be worn within the laboratory area and not outside the laboratory. Students should be taught how to handle spills, with the contaminated clothing being left within the laboratory. An understanding on how organisms are transmitted and the need to contain the spread of organisms needs to be taught and understood.

Staff training

Training is carried out for safety, chemical safety, radiochemical safety and biological safety. It was unclear the extent to which staff and students had been trained in the Microbiology Department. What is clear is that not all students are being adequately trained to understand and act safely.

It is critical that safety be accepted as a line management responsibility, with staff and students each taking responsibility. Further, supervisors of students have a critical roll in making sure their students are adequately trained and that they work in a safe environment. We saw examples where this had been done well and others where an assumption that the host institution would make sure everything was okay. Much of this was based on trust because so many microbiologists in Singapore either trained or worked at NUS. This was not a good assumption for the student who worked at EHI and was a failure of the supervisor to adequately ensure that the student was properly trained and working in a safe environment.

With the large number of BSL-3 laboratories that are due to come on line soon in the BioPolis (we understand 16), there is an urgent need to ensure that there is adequate training for those who will potentially be working in these laboratories. The Microbiology Department could take a leadership role in this area. If the safety culture

of many of the students coming out of NUS does not improve, then there will be more microbiological incidents, some of which could have serious consequences.

There is also a need to provide an upgrade in the training on Biosafety of all Microbiologists in Singapore. The University could play a role in the provision of this training. Such training needs to be competency based and not only should there be an evaluation following the course, but a re-evaluation around 3 months post training. Shorter annual refresher courses should also be held. It is also clear that training needs to take into account the Microbiological Safety Standards that Singapore operates under and supervisors need to be fully trained in understanding of their responsibilities for their staff and students.

Recommendations

Recommendation 5.1: That the Microbiology laboratories be re-audited to ensure that they meet safety standards and that each laboratory provides a risk assessment of the work that they carry out. The local Safety Committee signs off on the adequacy of the audit and the risk assessment.

Recommendation 5.2: That front opening lab coats be banned from use in the microbiology laboratories and back fastening gowns be introduced. Gown hooks should be provided near the laboratory doors (inside) for gowns to be left inside and a procedure for disinfection and laundering the gowns developed. There are a number of makes of reusable surgical style gowns suitable for this purpose.

Recommendation 5.3: That the University develop, or offer, appropriate training in biological safety and that this training be competency based. Training in the operation at BSL-3 could be considered.

Recommendation 5.4: That the Microbiology Department as a team objective adopt the goal of creating a culture of safety for all staff and students within the Department.

Recommendation 5.5: That the virology laboratory be allowed to reopen once it has been re-audited and issues related to the use of the biological safety cabinet, excess paper and other materials within the laboratory, training of staff, use of gowns and a risk assessment of work has been carried out to a level acceptable by the Safety Committee and the Head of the Microbiology Department.

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Defence Science Organisation

Description of the facility

The Defence research institute is involved in chemical and bacteriological research, including several agents with potential bioterrorism implications.

Work with micro-organisms.

The laboratory agents regularly used are: *Bacillus anthracis*, *Francisella tularensis*, *Clostridium*, *Burkholderia mallei* and *B. pseudomallei*. During the SARS outbreak, DSO was involved in the national network by helping to do extraction of RNA for RT-PCR from clinical material. No isolation attempt or culture of SARS-coronavirus was done.

Security clearance is required to work in the laboratory. Code and biometric (fingerprint) are required to access the BSL-3. Two cameras (motion triggered) are installed in the laboratory. A freezer containing bacteria and virus stocks and potentially infectious clinical specimens is in the restricted access laboratory and locked.

Containment facility

The laboratory design was finished in 2001 and is situated in the basement of the building (underground). It was not supposed to be a BSL-3 laboratory but a BSL-2 with some class III cabinets where live agents were handled. The access doors are not tight. Access to the main laboratory is done through 2 successive locked doors; to open the first one, laboratory workers need to use a mechanical (code) and biometric devices. A clothes change room is included in the passageway and is equipped with a personal shower. Water from the shower, the change room and the laboratory sinks goes to a designated tank, and disinfectant is added before release in the building sewage.

Walls, floor, and bench surfaces are constructed for easy cleaning and disinfection. Penetrations in the laboratory of ducts and wires are sealed and allow disinfection by fumigation (was done several times, including one at the end of the SARS campaign). The class III cabinets are regularly fumigated before opening and at the end of the series of manipulations involving the same agent.

The supply and exhaust air ventilation system for the 2 rooms is pre-filtered and HEPA filtered. There is no recirculation of the exhaust air and the exhaust air is discharged

away from the air intakes. The differential pressure/directional airflows between adjacent rooms is monitored and linked to an alarm in the lab. A visual pressure-monitoring device is available inside the change room and the main laboratory room. Floating indicators on the both supply and exhaust air can be seen from outside through the doors. A device inside the laboratory does the conditioning of the laboratory air. There is also a dehumidifier device inside the laboratory.

Laboratory waste is evacuated after been autoclaved in the main lab. The two HEPA- and charcoal-filtered class III cabinets are connected to air exhaust system and are tested annually. The HEPA-filtered class II cabinet with recirculation in the room is also annually tested.

There is no ultra or high-speed centrifuge. Bench-top centrifuges (one on the bench and one in a class II cabinet) are not equipped with aerosol safeguard. The freezer is situated inside the laboratory and there is no CO₂ incubator. There is no computer with access to the LAN inside.

The Institute generator backs up the laboratory electric system. The scientists working in the laboratory are acting as technical staff and have a very good knowledge of the systems.

Staff procedures and SOP

Access to the laboratory is controlled by the laboratory director and is restricted to trained personnel. There are three designated laboratory managers. A biosafety manual specific to the laboratory is available.

Notification of one of the laboratory managers is required before going in the laboratory and when the work is completed. The buddy system is mandatory.

During SARS manipulations, people used disposable gowns, on the top of the lab coat, and gloves. Personal shoes are used in the laboratory (sticky paper in the change room). During the SARS outbreak, scientist wore full-face respirators and designated shoes in the laboratory.

A checklist of the material is done everyday, and recorded data are maintained inside the lab. The freezer is locked and controlled by motion-detector cameras.

All manipulations of potentially infectious materials are conducted in the class III biological safety cabinets. All contaminated materials are disinfected using Virkom, formalin, or hypochlorite before being autoclaved and disposed. Protective laboratory clothing and gloves are not worn outside of the laboratory and decontaminated by autoclaving before being disposed outside of the laboratory. Before opening, the class III cabinets are disinfected by fumigation. After being autoclaved, trash bags are carried in the change room and put in a clean bag, then discarded with other laboratory trash

The shower in the change room is used in case of spill accident, but every time when the laboratory was considered BSL-3 (SARS episode). Discarded liquids are disinfected before being release in the general sewage.

No radioactivity material and no animals are handled in the laboratory.

There is agent-specific vaccination available for the personnel beside influenza, tetanus and hepatitis B vaccines and no dedicated health clinic but a specific general practioner is a point of contact.

Staff training

The three laboratory managers visited the DSTL laboratory in United Kingdom and had training in the Swedish containment laboratory. These scientists are responsible for the training of new personnel. Standards procedures for the laboratory are available on the local LAN for all people working in the laboratory and safety books are on the shelves inside the laboratory.

Recommendations

Recommendation 6.1: DSO laboratory is more likely to be involved in the future during national emergencies requiring BSL-3 conditions. Several minor modifications should be done and the laboratory supervisors have already planned to address most of them. Pressure gauges need to be put in a better place for easy checking; Outside checklist should be implemented; a disinfection kit should be made available in case of accidental spill; the air conditioning should be removed from inside and conditioned the supply air; a double door autoclave will make manipulations safer and easier for the personnel; If a CO₂ incubator need to be installed, the supply bottles need to be outside of the laboratory; a computer connected to the outside should be easily installed.

Recommendation 6.2: That the group responsible group for the BSL-3 develop regular refresher safety course for the people working in the BSL-3.

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Biological Standards

Biological safety is a combination of facility criteria and staff operating procedures. These are usually set by the National Governments in legislation or by attachment to acts as a code of practice. Laboratories are designed to meet the standard and as part of their commissioning they are tested to ensure they met the containment requirements. This is particularly important at BSL-3 and above where the standards are set not only to protect the staff but also the external environment. Moving from BSL-2 to BSL-3 is a very significant step where the level of biological hazard greatly increases and the consequent to the individual and the community is significant if the agents gets out of the facility. Staff working at BSL-3 usually have significant experience working at BSL-2 before they are considered for the higher level responsibility. Training needs to be through, competency based (testing the knowledge of the trained person) and a significant period of direct supervision is required in order for the supervisor/trainer to be satisfied that the person is adequately trained. Training on the responses to emergency situations is critical, because failure to react correctly can result in an incident moving from manageable to an uncontrollable situation.

It is also important to encourage an atmosphere of trust and confidence that the team members are working safely and feel that they can openly communicate any of their concerns without discrimination. Staff need to be willing to immediately report any biological safety incidents and feel that they will be treated openly and without the threat of punishment. Otherwise, incidents may go unreported and then there may a very serious situation to resolve. Incidents must be reported immediately they occur, any correctively actions taken immediately (after appropriate consideration of the safety issues) and then thoroughly investigated. Steps or changes must be put in place and documented to ensure that future occurrences of incidents are prevented. The incidents should be used for group discussion and learning teams of staff, and are a very effective teaching tool.

Singapore has no accepted or legislated standards for biological safety (biosafety). Biosafety level criteria, vertebrate/invertebrate animal biosafety criteria, nor recommended biosafety levels for infectious agents and infected animals. Consequently, there is no organisation or institution for laboratory or investigator accreditations. Such criteria are well described by some foreign national (USA, Australia, UK, Canada, etc.) and international organizations. All are based on guidelines developed by the World Health Organisation and form the basis of separation of micro-organisms into 4 risk groups. These standards include safety equipments, facility design and construction, laboratory practice and techniques.

Singapore needs to develop such guidelines, probably by the adoption of Safety Standards, such as the USA or Australian Standards. These standards could be attached as mandatory "Codes Of Practice" under the relevant legislation, thus allowing them to be modified without having to pass a new act. The Standard needs to be translated into a local set of safety procedures and approved by the local institute safety committee. It is critical that staff have ownership of safety and a safety culture

is developed within the teams. In addition, there needs to be appropriate competency based safety training performed that verifies that the training has been comprehended.

International Standards for Biological Safety

There is a range of standards for biological safety. Some examples are listed below:

- WHO Guidelines
<http://www.who.int/csr/resources/publications/biosafety/Labbiosafety.pdf>
- CDC/NIH Guidelines <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>
- Canadian Standard
- Australian and New Zealand Standard (AS/NZS 4423.3:2002)
<http://www.research.deakin.edu.au/biosafety/labbiostandards.htm>
- United Kingdom Standard

Rather than Singapore carry out the process of developing a new standard from scratch, it is recommended that they pick one of the internationally accepted safety standards and modify them for the local Singapore requirements. Biological Safety should be one of range of safety areas, including laboratory design, chemical, biological, radiochemical and other safety standards. Also the specifications to which safety equipment is designed, tested and operated needs to be identified.

The Ministry of Manpower is in the process of developing guidelines for laboratories and production facilities in the biomedical sciences industry. These are useful guidelines but lack the detail to be specific standards and are not testable. It is desirable that a set of standards be adopted that fully specify the requirements, as detailed in Australian and USA Standards. Otherwise it will become impossible to certify that facilities are operating correctly and that staff have the correct practices to handle micro-organisms safely.

Accreditation and Certification of Biosafety Level 3 Laboratories

This will describe the twin role of laboratory certification for structure integrity and for operating procedures and an annual accreditation demonstrating that the facility and staff are operating at the correct level.

The roles of the Director of the Institute, of line management, and of the local safety committee are critical to creating the right environment for biological safety. The Director must take full responsibility for safety within the institute and should be fully accountable for safety. The Director may delegate roles to other staff, usually done in writing, but remains finally responsible, so he must hold those with delegated powers fully accountable.

The cost of external audit processes needs to be taken into consideration. Any system that is developed should seek to reduce administrative overheads and costs, and as far as possible harmonise activities between the various Singapore Ministries. It would be very useful to encourage the development of quality control standards and accreditation within medical and veterinary diagnostic facilities, under ISO17025. It would be desirable to incorporate evaluation for biological safety as part of the annual external audit for laboratories operating under this standard.

Transport of Infectious Substances

Standards should be developed to cover the transportations inside and outside the national boundaries. There are already several regulations available from the UN, ICAO and IATA in air transport. Standards Australia is in the process of developing a standard for transport of infectious substances by road and this may be of use for transport of infectious substances and specimens by pathology services and others, rather than utilise the more stringent IATA regulations that are designed for air transport.

Centers for Disease Control and Prevention Office of Health and Safety (CDC, OHS): Interstate transportation of etiologic agents — in revision
Select Agent Regulation, Importation of Etiologic Agents of Human Disease
<http://cdc.gov/od/ohs>

U.S. Department of Transportation: <http://hazmat.dot.gov>

U.S. Postal Service, Domestic Mail Manual (DMM), mail ability from Etiologic Agents: <http://pr.usps.gov>

International Air Transport Association (IATA), Dangerous Goods Regulations:
<http://www.iata.org> or <http://www.iata.org/dangerousgoods/index>

U.S. Department of Agriculture (USDA), Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases <http://www.usda.gov>
<http://aphisweb.aphis.usda.gov>

U. S. Department of Interior, U.S. Fish and Wildlife Service: Importation and Exportation of Endangered Species of Animals and Plants:
<http://www.fws.gov>

Movement of samples between laboratories should be regulated and it is important that a process of approval to move imported agents be put in place in order for the Government to know where agents are held.

Importation of Micro-organisms and their control

Concern has increased regarding the national and international transfer of infectious agent. A list, specific for Singapore, of humans, animal, plants pathogens prohibited or restricted for importation, use or transfer should be established. A structure, involving several ministries, should be created to follow and regulate the movements of a designated list of infectious agents.

Biological Controls have already been put in place, such as The Australia Group export controls (<http://www.australiagroup.net/>) and the USA's Select Agent List (<http://cdc.gov/od/ohs>). Most of the norms are aimed at agents with real potentials for use by bioterrorists, the working group should also be aware of the burden of such regulations. The right equilibrium should be found. Only those agents with real and serious potential for use by terrorists should be registered and controlled. If the list is too wide and contains endemic disease agents, then Singapore's public health system will find it difficult to work and almost impossible to respond to a major disease outbreak.

Recommendations

Recommendation 7.1: Need for a National Legislative basis for Standards in Biosafety Laboratories for Singapore. It is recommended that the detailed safety codes be attached to the legislation and be made mandatory. The codes should be reviewed by an expert panel, at least once every 5 years, and there should be a system in place to allow timely updates.

Recommendation 7.2: A structure should be created for laboratory certification covering both structure integrity and operating procedures. These certifications should be renewed on an annual basis. It would be more cost efficient if these audits were carried out under a quality control standard such as ISO17025 using experienced and accredited external auditors.

Recommendation 7.3: Creation of the tracking system for importation, exportation to and from Singapore. The tracking system should also cover the transfer of infectious agent among laboratories in Singapore. This system should be harmonised between the Ministries of Health and Agriculture

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