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# **Biosafety and SARS Incident in Singapore September 2003**

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Report of the Review Panel on New SARS Case and Biosafety

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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 3<sup>rd</sup> 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 recirculating system.

The BSL-3 laboratory consists of an anteroom with a small autoclave and a laboratory; total size is about 30 m<sup>2</sup>. 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 CO<sub>2</sub> 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 1,000m<sup>3</sup>/h and exhaust is 1,200m<sup>3</sup>/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 H<sub>2</sub>O and laboratory against anteroom also minus 2-3mm H<sub>2</sub>O. 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 <sup>0</sup>-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.