

Supplementary Guideline on Handling of Clinical Specimens in the Laboratory (Revised July 2006)

The following guidelines are supplementary to existing standard laboratory safety guidelines to ensure safety in the handling specimens that may possibly contain SARS-Coronavirus, avian influenza virus or other highly pathogenic micro-organisms. Please also refer to 'Guideline on Transport of Clinical Specimens' for guidance on transport of clinical specimens within hospitals and between laboratories/hospitals.

IT IS THE OBLIGATION AND RESPONSIBILITY OF EVERY LABORATORY WORKER TO FAMILARISE WITH AND TO STRICTLY OBSERVE LABORATORY SAFETY PRACTICES.

1. All laboratory workers shall practice **“STANDARD PRECAUTIONS” and GOOD LABORATORY PRACTICE in the course of their duties. Hands must be frequently washed and especially after removing gloves and on leaving the laboratory area.**
2. Disposable gloves should be worn when handling contaminated items or sorting of specimens.
3. The laboratory workers should wear **personal protective equipment, PPE (specified in the respective section below)** according to the risk of aerosol generation and exposure when performing specific manipulations.
4. All **personal protective equipment (PPE)** must be removed on leaving the laboratory area.
5. The following activities may be performed in biosafety level 2 (BSL-2) laboratories with appropriate BSL-2 work practices, and PPE (including disposable gloves, solid front or wrap around gowns with cuffed sleeves and a surgical mask): e.g.
 - Routine diagnostic testing of serum, blood and urine specimens;
 - Routine staining and microscopic analysis of fixed smears;
 - Routine examination of mycotic and bacterial cultures;
 - Pathological examination and processing of formalin-fixed /inactivated tissues;
 - Electron microscopic studies with glutaraldehyde-fixed grids;
 - Molecular analysis of extracted nucleic acid preparations.
6. Biosafety level **(BSL) -2 facilities and BSL-3 practices**, i.e. conducting the procedures within biosafety cabinet (BSC) and PPE (including disposable gloves, solid front or wrap around gowns with cuffed sleeves, and a surgical mask or full-face shield) should be adopted for procedures including the followings:
 - Any procedure that may generate aerosols or splashing (e.g. sonication, vortexing, grinding or blending);
 - Aliquoting and / or diluting specimens;

- Activities involving manipulation of untreated specimens;
 - Inoculation of bacterial or mycological culture media;
 - Preparation of chemical- or heat-fixing of smears for microscopic analysis;
 - Performing diagnostic tests on untreated specimens, but do not involve propagation of viral agents in vitro or in vivo;
 - Nucleic acid extraction procedures involving untreated specimens.
7. Under exceptional situation when such a procedure or process cannot be conducted within a biological safety cabinet (BSC), then **enhanced PPE** (including disposable gloves, solid front or wrap around gowns with cuffed sleeves, a N95 respirator, full face shield and where appropriate head covering and shoe cover) and other physical containment devices must be used.
8. Pneumatic tube should NOT be used for the transport of specimens from patients suspected of avian influenza/SARS or during **emergency response level**.
9. Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be unloaded inside a biological safety cabinet.
10. Smear Preparation: Fresh respiratory specimens submitted for cytological examination: smears should be prepared inside the biological safety cabinet and first fixed in 95% ethanol for 30 minutes and post-fixed in 70% ethanol or isopropyl alcohol for 15 minutes, the latter for disinfection before taking out of the biological safety cabinet for staining and other procedures. Alternatively, specimen can be fixed in equal volume of 10% formalin for 2 hours before smear preparation and staining.
- Peripheral blood: smears can be incubated on a hot plate heated up to 56°C for 15-30 minutes before methanol fixation and Romanowsky staining.
11. **Splashes and aerosols formation should be minimized by good laboratory technique and good practices. Blood and serum should be pipetted carefully, not poured. Mouth pipetting must be forbidden.**
12. The following activities require BSL-3 facilities and BSL-3 work practices e.g.,
- Cell culture for virus isolation;
 - Initial characterization of viral agents recovered in cultures of SARS specimens
13. Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents that are effective against lipid-enveloped viruses should be sufficient. Careful attention should be given to hand hygiene after removal of gloves and especially before touching the eyes and mucosal surfaces.
14. In case of spillage of infectious material in the laboratory, existing contingency measures in respective

laboratories should be activated as appropriate, depending on nature of specimens involved and procedures performed. In general, for spillage of blood or serum specimens, pour freshly prepared solution of 10,000ppm of sodium hypochlorite i.e., 1 in 5 dilution of domestic bleach (e.g., Clorox) over the spilt material and cover the site of spillage with thick cloth for 15-30 minutes and cleanse before resume work. Staff in the vicinity should be alerted of the hazardous nature of the spillage and to evacuate temporarily from the affected site.

15. All laboratory incidents must be reported and fully investigated by the laboratory management. Where appropriate, such as unprotected exposure to infectious materials, the hospital Infection Control Officer should be notified for post-exposure assessment.
16. All laboratory staff should report any sickness promptly by the current arrangement.
17. When a laboratory staff is suspected / diagnosed to have SARS, the following actions should be taken:
 - The laboratory in charge should notify the laboratory i/c / DM and the COS / Consultant in-charge and the latter to report to the HCE / CCE and the Hospital Infection Control Team for attention and subsequent follow up actions.
 - Workplace contact of the index staff should be identified and informed to seek medical advice as soon as possible, if any suspected symptoms occur. (Refer HA guidelines).
 - Laboratory safety procedures should be reviewed for any lapse and rectified.
 - Work area where the index staff is stationed should be appropriately disinfected if indicated before resuming work.
18. Concerning the maintenance of SARS-CoV containing or SARS-CoV related materials, a global advice to discard unnecessary clinical specimens collected during the period **01 Nov 2002 to 31 July 2003 inclusive** is recommended. Otherwise, cultures and specimens collected from patients as part of clinical investigations from **1st November 2002 to 31st July 2003 inclusive** should be stored and maintained according to the policy as set in Appendix 1.

Ref:

1. A Practical Guide for SARS Laboratories: From sample collection to shipment World Health Organization Regional Office for the Western Pacific, Manila, Philippines. 29 December 2003
2. Laboratory Biosafety Manual, 2nd ed. WHO 2003
<http://www.who.int/csr/resources/publications/biosafety/labbiosafety.pdf>
3. <http://www.cdc.gov/ncidod/sars/sarslabguide.htm>
4. WHO biosafety guidelines for handling of SARS specimens
http://www.who.int/csr/sars/biosafety2003_04_25/en/
5. WHO laboratory biosafety guidelines for handling specimens suspected of containing avian influenza A virus(revised 12 January 2005)
6. HA Safety Manual, chapter 25

Table 1: Summary

	BSL-2 standard + BSL-2 Practice	BSL-2 standard + BSL-3 Practice	BSL-3 standard + BSL-3 Practice
Requirement	Open Bench	BSC	BSC
PPE	Gloves, Gown, Surgical Mask	Gloves, Gown, Surgical Mask	Following individual laboratory's guideline
Enhanced PPE For exceptional BSL-3 practice	N/A	Gloves, Gown, Goggles, N95 respirator, Head covering and Dedicated shoes/Shoes cover as appropriate	N/A
Procedures	◆ Routine diagnostic testing of serum, blood and urine specimens ◆ Routine staining and microscopic analysis of fixed smears ◆ Routine examination of mycotic and bacterial cultures; ◆ Pathological examination and processing of formalin-fixed /inactivated tissues ◆ Electron microscopic studies with glutaraldehyde-fixed grids; ◆ Molecular analysis of extracted nucleic acid preparations.	◆ Any procedure that may generate aerosols or splashing (e.g. sonication, vortexing, grinding, blending) ◆ Aliquoting and / or diluting specimens; ◆ Activities involving manipulation of untreated specimens ◆ Inoculation of bacterial or mycological culture media ◆ Preparation of chemical- or heat-fixing of smears for microscopic analysis ◆ Performing diagnostic tests on untreated specimens ◆ Nucleic acid extraction procedures involving untreated specimens.	◆ Cell culture for virus isolation ◆ Initial characterization of viral agents recovered in cultures of SARS specimens

Appendix 1

SARS Co-V Specimens and Isolates Storage Policy

Category of specimens / isolates:

- A 、 Cell culture-grown SARS-CoV isolates
- B 、 Specimens that have been tested positive for SARS-CoV by molecular method or isolation.
- C 、 Clinical specimens collected from SARS patients (defined as patients under the Government SARS list). These refer to blood, respiratory samples, stool or rectal swabs, urine, body fluid, lesion swabs, tissues and all other types of specimens collected from 1st November 2002 to 31st July 2003, inclusively.
- D 、 Stool and respiratory specimens collected from non-SARS patients (defined as patients NOT under the Government SARS list) as part of clinical investigations from 1st November 2002 to 31st July 2003, inclusively.
- E 、 Specimens other than stool /respiratory specimens collected from non-SARS patients (defined as patients NOT under the Government SARS list) as part of clinical investigations from 1st November 2002 to 31st July 2003, inclusively.

Category of storage and access control:

1. Stored and locked in a freezer / liquid nitrogen tank, inside a Biosafety Level 3 laboratory. Storage inside a securely locked room with restricted access can be considered as a temporary measure while waiting for Biosafety Level 3 facility. Entrance and handling of the stored samples requires prior approval of the principal person-in-charge or his/her deputy. An access record should be maintained.
2. Stored and locked in a freezer / liquid nitrogen tank, inside a Biosafety Level 2 laboratory in a securely locked room with restricted access. Entrance and handling of the stored samples requires prior approval of the principal person-in-charge or his/her deputy. An access record should be maintained.
3. Stored in a freezer / liquid nitrogen tank with controlled access.

Category of inventory:

- I. Full inventory:
 - This includes patient name, patient identification number, specimen type, specimen laboratory number, specimen collection date, number of aliquots, subsequent movement and numbers of aliquots remained, SARS CoV RT-PCR result, SARS CoV isolation result and storage location. This inventory is to be maintained and updated by the principle person-in-charge of SARS specimen / isolate storage.
- II. Summarized inventory (clinical specimens):
 - This includes specimen type, corresponding total number of specimens, subsequent movement and number of aliquots remained, and storage location. This inventory is to be maintained and updated quarterly by the principle person-in-charge of SARS specimen / isolate storage.

Storage and access policy

Specimen category	Storage and access control	Inventory requirement
A	1	I
B	2	I
C	2	I
D	2	I
E	3	II