



Ask the Experts

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Do you have a biosafety question and you're not sure whom to ask? Send your questions to the "Ask the Experts" column and I will get them answered for you. Drawing from my own experience or that of other experts in the field, I will try to compile a comprehensive answer to your questions. Please e-mail them to jkeene@biohaztec.com or to the Editor, Ira F. Salkin, at irasalkin@aol.com.

What precautions need to be taken when working with suspected Severe Acute Respiratory Syndrome (SARS) specimens in the clinical and research laboratory?

There is no doubt that SARS has claimed the attention of not only the general public, but the scientific and medical community as well. It is important that we handle specimens and cultures that might contain the SARS virus in an appropriate manner. There is also no question that biosafety professionals should critically review any guidelines that are promulgated to insure that work can be done safely and efficiently as we attempt to be in compliance with those guidelines. The Centers for Disease Control and Prevention (CDC) has published the following information for the handling of SARS specimens in the laboratory. Please read these guidelines critically and then note my request at the end of this article.

Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with (SARS)

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Background

The CDC and the World Health Organization (WHO) have received reports of patients with SARS from various international and domestic sources. The cause of these illnesses is unknown and is being investigated, but current findings strongly suggest a viral etiology with a coronavirus as the leading candidate. The primary way that SARS appears to spread is by close person-to-person contact. Most cases of SARS have involved people who cared for or lived with someone with SARS, or had direct contact with infectious material (for example, respiratory secretions) from a person who has SARS. Potential ways in which SARS can be spread include touching the skin of other persons or objects that are contaminated with infectious droplets and then touching your eye, nose, or mouth. This can happen when someone who is sick with SARS coughs or sneezes droplets onto themselves, other persons, or nearby surfaces. It is also possible that SARS can be spread more broadly through the air or by other ways that are currently not known.

It is estimated that several thousand diagnostic specimens from patients with SARS have been processed in routine clinical laboratories throughout the world and to date there have been no reported clusters of SARS illness among laboratory workers. Nonetheless, until more information about the transmission of the SARS agent in the laboratory setting is known, reasonable precautions should be taken in handling these specimens. Effective and timely communication between clinical and laboratory staff is essential in minimizing the risk incurred in handling specimens from patients in whom SARS is suspected. Specimens from patients with suspected

SARS should be labeled accordingly and the laboratory should be alerted to insure proper specimen handling. Listed below are interim biosafety guidelines for handling these specimens. A detailed description of recommended facilities, practices, and protective equipment for the various laboratory biosafety levels (BSLs) given below may be found in the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL)*.

A. Blood and Urine Specimens

1. These specimens may be handled using Standard Precautions (previously Universal Precautions) in BSL-2 laboratories. Laboratory workers should wear protective equipment, including disposable gloves, laboratory coats, eye protection and a surgical mask, or face shield to provide a barrier to mucosal surface exposure. Careful attention should be given to hand hygiene after removal of gloves and especially before touching the eyes or mucosal surfaces.
2. Any procedure with the potential to generate fine particulate aerosols (e.g., vortexing or sonication of specimens in an open tube) should be performed in a biological safety cabinet (BSC). The use of sealed centrifuge rotors or sample cups, if available, should be employed for centrifugation. Ideally, these rotors or cups should be unloaded in a BSC.
3. Procedures performed outside of a BSC should be performed in a manner that minimizes the risk of exposure to an inadvertent sample release.
4. Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents that are effective against lipid-enveloped viruses should be sufficient.
5. If the safety equipment described above is not available, administrative measures and/or additional personal protective equipment may be employed to reduce risk. This should be done in the context of a careful risk assessment by the laboratory safety officer. For example, the workflow of the laboratory may be adjusted so that a minimum number of workers are present during centrifugation.
6. Consideration may be given to implementing respiratory protection for workers for use during centrifugation or other procedures with increased potential for inadvertent sample release. Acceptable methods of respiratory protection include a properly

fit tested NIOSH approved filter respirator (N-95 or higher); or powered air-purifying respirators (PAPRs) equipped with high efficiency particulate air (HEPA) filters. Accurate fit testing is a key component of effective respirator use. Personnel who cannot wear fitted respirators because of facial hair or other fit-limitations should wear loose fitting hooded or helmeted PAPRs.

7. Consideration may also be given to referral of specimens to a suitably equipped reference laboratory.

B. Other Specimens

1. The following activities may be performed in BSL-2 facilities with standard BSL-2 work practices:
 - a. Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues.
 - b. Molecular analysis of extracted nucleic acid preparations.
 - c. Electron microscopic studies with glutaraldehyde-fixed grids.
 - d. Routine examination of bacterial and mycotic cultures.
 - e. Routine staining and microscopic analysis of fixed smears.
 - f. Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.
2. Activities involving manipulation of untreated specimens may be performed in BSL-2 facilities, but with more stringent BSL-3 work practices. Laboratory workers should wear protective equipment, including disposable gloves, solid front gowns with cuffed sleeves, and full face protection. Specimen manipulations should be carried out in a certified biological safety cabinet. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) must be used. Acceptable methods of respiratory protection include a properly fit tested NIOSH approved filter respirator (N-95 or higher); or powered air-purifying respirators (PAPRs) equipped with high efficiency particulate air

(HEPA) filters. Accurate fit testing is a key component of effective respirator use. Personnel who cannot wear fitted respirators because of facial hair or other fit-limitations should wear loose fitting hooded or helmeted PAPRs. Centrifugation should be carried out using sealed centrifuge cups or rotors that are unloaded in a biological safety cabinet.

Examples of these activities include:

- a. Aliquoting and/or diluting specimens.
 - b. Inoculation of bacterial or mycological culture media.
 - c. Performing diagnostic tests that don't involve propagation of viral agents in vitro or in vivo.
 - d. Nucleic acid extraction procedures involving untreated specimens.
 - e. Preparation and chemical- or heat-fixing of smears for microscopic analysis.
3. The following activities require BSL-3 facilities and BSL-3 work practices. When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of

personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) *must* be used.

- a. Viral cell culture
 - b. Initial characterization of viral agents recovered in cultures of SARS specimens.
4. The following activities require Animal BSL-3 facilities and Animal BSL-3 work practices:
- a. Inoculation of animals for potential recovery of the agent from SARS samples.
 - b. Protocols involving animal inoculation for characterization of putative SARS agents.

Author's Note

I have decided to take a slightly different bent in this issue of the journal and would like to challenge you to look at these guidelines critically. Please contact me via e-mail at jkeene@biohaztec.com with any comments regarding the published guidelines. I will compile the responses and provide a report in a future issue of the journal.