

Laboratory Safety Aspects of SARS at Biosafety Level 2[†]

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Abstract

The severe acute respiratory syndrome (SARS)-associated coronavirus causes severe disease, is transmissible to the community and there is no effective prophylaxis or treatment – perhaps fulfilling the criteria for biohazard group 3 or 4. The recommendation to use Biosafety Level (BSL)3 practices within a BSL2 environment appears to have been a practical decision based on available resources; most diagnostic laboratories operate at BSL2. Safety is achieved with controls in administration, engineering and personal protective equipment/behaviour. At the heart of every safety policy is a risk assessment based on the exact manipulations employed. Excessive administrative and engineering controls are less important than the training and personal attitudes, abilities and understanding of the staff. The SARS outbreak focused our attention on the safety aspects of common mundane tasks, such as decapping blood tubes. Laboratories often claim they follow certain practices but casual observation does not always support these claims. Guidelines differed and created uncertainty. This was stressful for laboratory staff held accountable for their implementation. Attempts to categorise risks and their management into neatly wrapped parcels are attractive, but closer inspection reveals a subjective element that allows doubt to creep in with varying interpretations of the literature. Staff most at risk were those handling respiratory samples. Staff receiving samples via pneumatic tubes had least control over their exposure and were potentially exposed to aerosols from leaking samples. Risk assessment remains a balance between cost and benefit.

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Introduction

In Singapore, the majority of patients suffering from the severe acute respiratory syndrome (SARS) were cared for at Tan Tock Seng Hospital. The dramatic and serious nature of this outbreak in 2003 focussed unprecedented attention on laboratory safety practices. Our diagnostic laboratory operates at Biosafety Level 2 (BSL2), with viral cultures performed elsewhere. This paper is an edited transcript of a lecture describing our experiences that was delivered at the 14th Annual Scientific Meeting of the Chapter of Pathologists, Academy of Medicine Singapore on 20 September 2003.

Risk Assessment

The choice of safety measures depends on the perceived risk. The risk analysis is based on the hazard group of the organism and the detailed manipulations being carried out in the specific laboratory: different techniques require different measures.

Hazard Groups

The “Hazard Groups” (Table 1) are based on 4 questions (Table 2) although the normal route of transmission and infectious dose are important. Compromising factors need to be considered for specific staff members, such as pre-existing disease, compromised immunity, effects of medication and pregnancy. One glove does not fit all.

Biosafety Levels and Biological Safety Cabinets

In general, the biosafety level of a laboratory reflects the hazard group of the agents it handles.¹⁻³ For example, *Staphylococcus aureus* is handled at BSL2 and *Mycobacterium tuberculosis* at Biosafety Level 3 (BSL3). The use of a high-risk laboratory procedure may prompt staff to handle a particular organism at a higher containment level. Similarly, multi-resistant organisms, such as multi-resistant *M. tuberculosis*, deserve higher containment than fully susceptible strains.

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Safety is achieved by controls in 3 areas: administration, engineering and personal protective equipment (PPE). Biosafety Level 1 (BSL1) laboratories require the least stringent levels of control and Biosafety Level 4 (BSL4) the most stringent. Administrative controls include records of proper training, supervision, work practices and security. Engineering controls include the design of all equipment but especially airflow and air filtering. Air handling systems are expensive to install and to maintain. The biological safety cabinet (BSC) is the primary containment device. Airflow systems and personal respirators are back-up measures in case of BSC failure. Containment failure is normally an operational issue and is not due to mechanical failure. Sudden arm movements, the opening and closing of a door or simply walking past a BSC can all disturb the balance of airflow and allow air, momentarily, to escape. The BSC comes in many varieties depending on the scope of protection required. A simplified classification is shown in Table 3. Note that the correlation of laboratory biosafety levels and organism hazard groups does not extend to the BSC class levels. Hazard group 3 organisms can be manipulated in a Class I BSC in a BSL3 laboratory. PPE includes disposable gloves, masks and gowns. The word “masks” covers a range of equipment from simple surgical masks through N95 masks and full face shields to powered air-purifying respirators (PAPRs). Personal behaviour, knowledge and attitude are the best predictors of safety at work, irrespective of expensive hardware.

Despite being categorised at a certain biohazard level,

Table 1. Hazard Groups 1-4

Group	Characteristics of biological agent
1	Unlikely to cause human disease.
2	Can cause human disease and may be a hazard to employees. Unlikely to spread to the community. Effective prophylaxis or treatment is usually available.
3	Can cause severe human disease. Presents a serious hazard to employees. May present a risk of spreading to the community. Effective prophylaxis or treatment is usually available.
4	Causes severe human disease. Is a serious hazard to employees. Is likely to spread to the community. There is usually no effective prophylaxis or treatment available.

Table 2. Basic Risk Analysis

<ul style="list-style-type: none"> • Is the agent pathogenic for humans? • Is it a hazard to employees? • Is it transmissible to the community? • Is effective prophylaxis or treatment available?
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guidelines allow some agents to be handled at lower containment levels. This reflects the flexible nature of risk assessment. Examples of some hazard group 3 agents routinely handled at BSL2 are given in Table 4. This derogation anticipates “safe practices” and does not allow the culture of, for example, the human immunodeficiency virus; this would still require BSL3. Derogation requires risk analysis of the particular manipulation being performed in that laboratory. Important considerations include whether the work will amplify (by culture, centrifugation or filtration) the agent or generate aerosols.

The SARS Episode

In the absence of reliable information, we held a laboratory meeting to allow all levels of staff to ask questions and to reassure them that our normal safety practices were sound. We stressed compliance. A week later, new guidelines advised BSL3 practices in BSL2 laboratories. Initially, there was no distinction between different sample types. With an evolving outbreak affecting many ward staff and palpable concern and stress amongst our own staff, we accepted these guidelines and applied them indiscriminately to all sample types such as urine, blood and sputum. These

Table 3. Classes of Biosafety Cabinets

Class	Use
I	Protects the user. For hazard group 2 and 3 organisms.
II	Protects the user and the sample. For hazard group 2 and 3 organisms.
III	As for Class II but it is totally enclosed – access is via airlocks and sealed gloves: air must be exhausted to the outside. For hazard group 4 organisms.

Table 4. Common Hazard Group 3 Organisms

<ul style="list-style-type: none"> • HBV, HCV, HIV • <i>Burkholderia pseudomallei</i> • <i>Salmonella typhi</i> and paratyphi • <i>Shigella dysenteriae</i> • <i>Plasmodium falciparum</i> • <i>Brucella</i> species
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HBV: hepatitis B virus, HCV: hepatitis C virus, HIV: human immunodeficiency virus

Table 5. BSL3 Practices in BSL2 Labs

<ul style="list-style-type: none"> • Centrifugation – unload buckets in a BSC. • Decapping and opening samples only in a BSC. • Extra PPE, possibly N95 respirators. • No “open” work on the bench.

BSC: biological safety cabinet; BSL: biosafety level; PPE: personal protective equipment

BSL3 practices required extra measures (Table 5) that severely affected the workflow. Our laboratory performed thousands of tests during the epidemic. While this was much less than our normal workload, we were unable to work at the normal rate due to the “BSL3 practices”. Specific SARS-related tests, such as the polymerase chain reaction, were not the main worry. Our concern was about the “unknown”. We expected our safety measures to be reliable, if adhered to, but we could not apply these measures to samples from patients whose status was not known. Request forms did not reliably include any clinical information, let alone the SARS status. Hence, we initially decided to treat samples from wards with any SARS patients as if they were all from SARS patients. We updated notices daily so that the staff knew which wards were affected. As the outbreak progressed and the hospital became the “fever hospital”, with “non-fever” patients admitted elsewhere, we replaced our strategy with one where we regarded all samples from the hospital patients as SARS and those from community clinics as non-SARS. This was important as it allowed the staff to perform the community clinic work without implementing the BSL3 requirements.

In microbiology, almost all bacteriology samples are normally handled in a BSC and the staff are comfortable with the concepts and are trained in the use of the BSC and PPE: they had been fitted with N95 masks in 2000. Thus, the implementation of the BSL3 practices was relatively smooth, although the most dangerous samples, respiratory samples, were dealt with in this section. Other sections were less well-acquainted with, for example, the BSC. We held training sessions with particular emphasis on the dangers of the ultraviolet lights used for decontamination.

We conducted frequent “bench rounds” to assess compliance, look for risk areas and to talk to the staff. Specimen reception was identified as a particular risk area as the pneumatic pods are propelled by air pressure. In the event of a leaking sample, there might be a degree of aerosolisation. Our staff have to receive these pods without knowing whether they contain SARS samples or other dangerous organisms. The staff that normally deal with these pods are the least trained staff in the laboratory and perhaps the least likely to comply with PPE requirements/instruction.

We identified any wet microscopy or test that brought the sample within close proximity of the staff’s face and that could not easily be performed in a BSC as a special risk procedure. We informed the ward staff that we would not perform these procedures. They included wet microscopy, HbA1c and osmometry. Blood gases, however, were still measured on the open bench.

Our haematology analysers are “closed” systems. This

means that the equipment samples blood tubes by piercing the rubber bung. The blood tubes for chemistry, blood bank and other serologies are manually decapped as we use “open” analytical equipment. Decapping creates aerosols and droplets as it involves the equalisation of the air pressure within the vacutainer tube with that in the surrounding environment. Implementation of the “BSL3 practices in a BSL2 laboratory” rule meant we had to unload all the centrifuge buckets in a BSC. The tubes were then decapped to allow pressure equalisation and partially recapped before being taken to the analysers. The analyser hoods are usually kept open for workflow reasons as staff are continually adding or removing samples. We insisted that they be kept closed to offer some protection from any aerosols produced by the high-speed automatic pipettes. In microbiology, the small enzyme immunoassay readers and plate washers were moved into a BSC. The demand for BSCs was high.

All laboratory staff were mask-fitted. We discussed the separation of work areas. The current open design does not lend itself to the physical separation of clean from dirty areas. The clerical/administration staff were in the same area as sample reception and centrifugation. We discussed whether request forms should be considered “dirty”. We cancelled student attachments. We also considered sending non-essential staff home to reduce their exposure and whether we should split staff into cohorts, so that if someone in one cohort were to develop SARS, we could use the other cohort while the first was in quarantine. These considerations did not all bear fruit but continue to deserve attention. Theoretically, our staff were protected relative to the general public as the laboratory is a “controlled area”. Despite the dangers of handling samples, our controls may mean the laboratory is one of the safer places to be – if staff comply with protocols.

Changes and recommendations had to be evaluated and the benefits weighed against the costs. At the same time, we had to take into account the perceptions of not only the laboratory staff, but also that of the senior management who wanted an absolute assurance that laboratory-acquired infections would not occur. This was clearly unrealistic but served to elevate our discomfort as we tried to maintain an even balance. The laboratory staff took responsibility and placed their trust in long-established safety protocols. We had the reassurance of knowing that there had not been any laboratory-acquired SARS cases in Hong Kong, where many thousands of samples had already been processed.

Problems

Communication could have been better. Request forms were not labelled with the patients’ SARS status and, initially, when the hospital was still full of “non-SARS”

patients, it was not practical to treat all samples with BSL3 precautions. Laboratory staff felt unhappy as stratification was difficult without proper labelling.

The proper transport of samples is important for the validity of the test results and the safety of the staff. Leaking samples may contaminate the pod's exterior and may be aerosolised. We considered whether to insist that all samples be transported by hand to avoid this risk. However, the whole hospital was under immense pressure and the manpower required to do this would have been considerable. It would also have slowed down the service. We opted for requiring that all samples be wrapped in absorbent material, in the hope that any leaks would be contained. I suspect this had an impact on the nursing staff. It is worth noting that most leaks are due to lids being cross-threaded or not tightened. Cracked and broken tubes are usually due to staff forcing too many samples into a pod. The plastic bags in which samples are usually transported have a safety function as they will contain leaks if properly closed. The habit of stapling request forms to these bags breaches this "secondary containment".

Staff who transported samples to other institutions were not our staff. We did not know how much safety training they had had and whether they knew what to do in the event of a spill. Imagine the outcry should a road traffic accident result in body fluids from SARS patients being splashed over the road. These safety concerns resulted in the normal transport being withdrawn without notice on a Saturday morning. This action caused a lot of anxiety amongst our staff, who did not understand why and wondered if there were additional risks that they had not been alerted to. Thankfully, our hospital's ambulances stepped in to help. We, in turn, provided education and packed the samples in rigid containers lined with an absorbent material.

This whole experience was an opportunity to re-examine our safety practices for normal BSL2 work. Centrifugation is an aerosol-producing procedure and safety caps are now required for all clinical samples. However, many centrifuges do not have them and they may be unavailable for particular brands/models. The laboratories must decide whether to discard perfectly good equipment that falls short in this respect. Decapping blood tubes is a perennial problem. It produces droplets, if not aerosols. Decapping should be performed in a manner that protects the staff. Solutions such as using a "see through" screen are not ergonomically attractive. Decapping in a BSC has implications for workflow and space requirements. One option is to request staff to wear eye and face protection but this is not popular. Laboratories may satisfy requirements by specifying practices in their manuals but they may not actually enforce them. We currently ask our staff to cover the tubes with gauze and to twist the tops off gently, whilst pointing the

tube away from themselves. SARS samples were decapped in a BSC. Another option is to use closed systems, as mentioned earlier, but staff still have to decap tubes for tests not performed on these systems. Closed systems cost hundreds of thousands of dollars and may bring their own problems.

Although the laboratory is responsible for the design of request forms, we found ward staff designing and distributing new forms. This confused both ward and laboratory staff.

Storing samples was also an issue. Different sections of the laboratory keep samples for various lengths of time, depending on the likely need for retesting, for extra test requests ("add ons") by ward staff, the stability of the sample and the time to disposal. Whether a sample is merely awaiting disposal or is being refrigerated for a number of days before disposal, it is a potential source of exposure should it be knocked over/spilt. At any one time, there are hundreds of samples in the laboratory.

In Singapore, the regulations on disposal do not require samples to be decontaminated before they leave the laboratory, as they are all taken off for incineration by a licensed contractor. We choose to routinely autoclave cultures of a few particular pathogens, such as *Burkholderia pseudomallei*. During the SARS episode, we also autoclaved all SARS samples from all parts of the laboratory. Autoclave capacity became a problem when our main one broke down.

The enhanced PPE brought a few problems. It is difficult to wear a respiratory mask for a whole shift. We were concerned that staff who did were probably not wearing them properly. Simple issues such as what to do with the mask when on a break can be difficult to solve. The exterior of a mask is theoretically contaminated; recommendations other than disposal raise concerns. Initially, many staff wore disposable gowns or masks when it was unnecessary. This made it hard to immediately know if someone was missing some aspect of PPE, requiring correction, or whether they were simply being overcautious with one aspect of PPE. Goggles were provided but staff did not like sharing them. Guidelines did not clearly explain the need for goggles or a face shield. Similarly, it was not clear whether we should wear an N95 mask or a surgical mask. Faced with possible supply problems, we did not change PPE as frequently as we could have: should it be each session, day or week?

Guidelines

Guidelines can be very helpful but they can also cause difficulties – especially when they differ.⁴⁻⁶ Considering the passion in the air while dealing with this unknown agent, it was difficult not to adhere to every word of the

guidelines – but which ones? While we did not agree with all the recommendations, we felt uncomfortable not complying. They were issued by various authorities: the Public Health Laboratory Service (PHLS) in the United Kingdom (UK), the Communicable Disease Centre (CDC) in the United States (USA) and the World Health Organisation (WHO).

The PHLS stated that the Advisory Committee on Dangerous Pathogens (a UK committee) advised “level 3”, but a few pages later said that “non-micro samples be handled as advised by the Health and Safety Executive”. It then added that “for non-micro samples, samples from probable or suspect SARS cases be treated as normal for HBV/HIV”; this means normal practice in a normal BSL2. As they did not specify sample type, the question arose as to why a particular sample type in microbiology should be treated differently from the same sample type in chemistry.

Apart from this, they were wonderfully simple in relying on existing standards. This is presumably because many/most laboratories in the UK have a BSL3 facility and the safety culture is well-developed.

The WHO stated in their “Interim Guidelines for national SARS preparedness” document, published in May 2003, “The global consensus....to handle this previously unknown pathogen....is at BSL3” without specifying the sample type. Although laboratory staff interpret the word “handle” to refer to “culture”, it is not so easy to convince non-laboratory/administrative staff. If these guidelines were to be interpreted literally, then all the chemistry would have to be processed at BSL3! There were some other differences between guidelines and statements that were ambiguous.

The CDC document “interim Laboratory Biosafety Guidelines” published in April 2003 became a little confusing as it gave overlapping options, anticipating the lack of BSL3 in many parts of the world, and introduced the concept of “BSL2 with BSL3 practices”. The CDC and WHO, but not the PHLS, required the unloading of blood tubes from centrifuge buckets in a BSC, which was a considerable burden. The WHO left much up to local risk assessment, while the CDC introduced the need for “consideration of N95 masks or higher” for blood and urine. In times of stress, this amounts to an instruction! The CDC asked for “full face protection” while working at a BSC, but the WHO specifically stated that it was not necessary – perhaps reflecting an international tussle between experts!

In an article in the *New England Journal of Medicine* from the SARS working group, there was a clear message that sera should be inactivated before processing outside BSL3. This was far in excess of the requirements stipulated by the PHLS and WHO documents. If this message were extrapolated to its logical conclusion, then all chemistry and haematology samples should also be inactivated before processing at BSL2.

These differences had a large impact on workflow, efficiency and staff morale. Decisions that did not comply with the most exacting of these documents provided opportunities for staff and senior management to question them.

Conclusion

Safety regulation is difficult as many find it unattractive and undeserving until something goes wrong. Others use safety concerns to bully the administration into approving expensive equipment. We try to strike a balance and note that there have been no reported cases from diagnostic laboratories. While we can apply administrative, engineering and PPE controls, it is the safety culture and personal attitude of each staff member that really count.

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