

Laboratory accidents and breaches in biosafety – they do occur!



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In 2003 and 2004 the world became aware of three high profile microbiological incidents in Asia associated with infections with the SARS coronavirus (SARS CoV). Early in 2004 SARS was causing major disruption to many Asian countries, costing a considerable amount in lost trade and in handling the medical emergency. However, through strict quarantine measures, the disease was controlled and eradicated the virus as a cause of disease in humans (although it continues to exist in wildlife).

So when a PhD student in Singapore developed SARS in 2003¹, a senior researcher in Taiwan almost died of SARS in 2003², and 11 people in China became infected with SARS CoV in 2004³ (including one death of a contact person), WHO became involved in the investigation of these cases of wide community concern. All the findings indicated major problems with the management of biosafety in the institutes where these cases occurred and senior management were held responsible for failing to have proper safety management systems in place.

However, over the past 5 decades there have been thousands of accidental infections of staff in microbiology laboratories around the world⁴, and the problems are not just limited to laboratories in developing countries. The most common laboratory infections are with aerosol transmitted agents that have a low number of organisms required to produce an infection, such as *Brucella* spp. and *Mycobacterium tuberculosis*⁵.

The SARS CoV incidents 2003-2004 Singapore 2003

In 2003 a PhD student from the National University of Singapore (NUS) was studying the replication of flaviviruses in cells using electron microscopy. He had investigated the replication of

a low virulence isolate of West Nile virus at NUS but wished to determine whether there was a difference with the high virulence West Nile-New York isolate. The NUS laboratory was a Biosafety Level 2 (BSL-2) laboratory but the New York isolate had to be handled in a BSL-3 laboratory. The Environmental Health Institute (EHI) in Singapore had a BSL-3 laboratory where they worked on arboviruses, including West Nile virus, and the student's supervisor sought agreement that the student work there to handle the New York isolate.

During the SARS outbreak earlier in 2003, the EHI was one of three laboratories in Singapore able to work with the diagnosis of SARS CoV. After the SARS outbreak was over and the virus was no longer in Singapore, EHI still handled an isolate of the SARS CoV. The PhD student was supervised on the first two occasions he visited the laboratory. On the third occasion, on a Saturday morning, the virology technician had grown up a stock of the New York isolate and centrifuged the supernatant from infected cells. She had placed the centrifuge tubes in the Class II biological safety cabinet for the student to ampoule as a seed stock for his research. Because Saturday was the day when staff from EHI met, the technician left the student unsupervised in the laboratory.

Three and a half days later the student developed respiratory problems similar to a mild case of SARS. This was subsequently confirmed by PCR and seroconversion, and the SARS isolate was sequenced by the Singapore Genome Institute and found to be similar to the isolate being handled at the EHI. Subsequent investigation by a joint WHO-CDC team for the Singapore Ministry of Health concluded that the infection probably occurred at the EHI on that Saturday morning. Other findings were that neither NUS nor EHI had adequately trained the student to work at BSL-3 and that there was a failure to properly supervise the student. Analysis of the ampoules of seed West Nile virus that the student stored on that Saturday showed that the vials contained SARS-CoV as well as West Nile virus. Further, there were problems with the work practices within EHI and with the containment of the BSL-3 laboratory.

Within Singapore there were no adequate standards, regulations or guidelines on biosafety. There was inadequate training and practice at NUS, which is the main institute training future microbiologists. In addition, at the Biopolis, a number of new BSL-3 laboratories were about to come on line. Since 2003, Singapore has introduced legislation and regulations to cover the

handling of high risk infectious agents. They have also developed a laboratory accreditation system. Further, the Asian Pacific Biosafety Association (APBSA) has been established which trains and mentors biosafety specialists. At the NUS a system of rewards has been developed for teams with outstanding safety practices and cash rewards are made to these teams – a system of positive incentives to produce a change in safety culture and practices.

Taiwan 2003

In December 2003 a senior scientist at the Institute of Preventative Medicine, National Defense University in Taipei became infected with SARS and developed a critical illness. The facility was a BSL-4 laboratory utilising type III glove ported isolators. The researcher was working alone and had to travel to Singapore to address a meeting about his SARS research. He had a spill in an isolator cabinet and wished to quickly decontaminate the spill and clean it up before leaving the laboratory. The recommended way of decontaminating isolators was to use vaporised hydrogen peroxide, but this takes some hours. Instead the researcher used 70% ethanol, which he sprayed on the spill. He left the ethanol in contact with the spill for 10 minutes, then opened the isolator and cleaned up the spill without adequate respiratory protection. On returning from the meeting in Singapore, he reported to the Defense University clinic that he had developed influenza. Six days later he was critically ill and, on going to hospital, they readily diagnosed a case of SARS.

The WHO investigation team found many management problems associated with the activities in this facility, including working alone at a BSL-4, inadequate staff training, a lack of standard operating procedures (SOPs) and a failure to have a medical monitoring programme in place. Senior management could not explain why, after he had reported to the clinic with a respiratory disease, he was not followed up for the next 6 days when he was absent from work.

Beijing 2004

In April 2004 Chinese authorities reported that two staff at the China Centre for Disease Control and Prevention had developed SARS and that the infection had been transmitted to seven external people, one of whom died. On investigation and serological monitoring of staff, it was discovered that two other staff had clinical SARS probably in early February 2004.

None of the staff actually worked in the SARS laboratory. The SARS laboratory had inactivated SARS CoV antigen using a technique that inadequately inactivated the virus and the live virus was subsequently used in the open laboratory where the four staff were exposed. If the inactivation process had been adequately controlled and the material safety tested, then it is unlikely that this incident would have occurred. If a health

monitoring programme had been in place, then it is likely that the two earlier infections would have been detected and the subsequent incident avoided.

The factors involved in the incidents

The following factors⁵ are critical to infections that occur with different infectious agents:

- The route of infection – through the lungs (inhalation), through the mouth (ingestion), by contact with apparently unbroken skin and with mucous membranes (by injection, with hollow-bore needles and other sharps), through the conjunctiva, through the genitourinary tract and from animals' (including arthropods) bites and scratches.
- The infectivity of the agent.
- The relative concentration of the agent.
- The survival of the agent in the environment.

Figure 1 illustrates the type of laboratory accidents that are recorded to proceed laboratory acquired infections. As these represent only about 20% of the known causes, it has been concluded that aerosol transmission from unknown sources is probably the major cause. The use of biological safety cabinets to contain aerosols, the use of directional air flows away from laboratory doors to contain aerosols generated by spills and the use of high air exchange rates, usually between 8 and 15 per hour, to remove aerosols from the environment, are engineering controls for this situation.

The level of infectivity and the route of transmission of microorganisms are critical to whether an exposed person will become infected. Table 1 shows the infectivity of some agents and their routes of infection. Agents that are aerosol transmitted and have a relatively low infectious dose, such as brucella, tuberculosis, the rickettsial diseases such as Q fever, are high in the number of laboratory infections.

GAO report 2007

Following a report from an audit team from the CDC into the lack of compliance of Texas A&M University to the Select Agent Rule, the USA Congress asked the Government Accountability Office (GAO) to look at high containment laboratories in the United States⁷.

Texas A&M failed to report a brucella infection of a staff member to the CDC in a timely manner; further, the staff member infected was not authorised to work with brucella. Further examination of the staff indicated that a number may have been exposed to Q fever (*Coxiella burnetii*). There were also some other discrepancies with Texas A&M's compliance with the Rule.

The GAO report identified six lessons that could be learned from the Texas A&M incident, a power failure at CDC Atlanta and the foot-and-mouth virus escape at Pirbright in the United Kingdom.

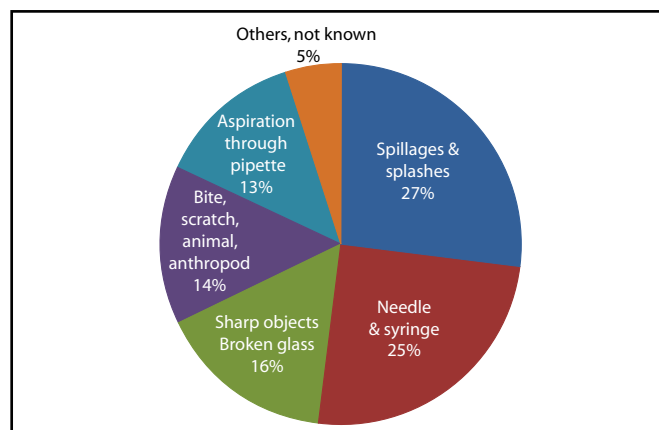
These were:

1. "Identifying and overcoming barriers to reporting in order to enhance biosafety through shared learning from mistakes and to assure the public that accidents are examined and contained.
2. Training lab staff in general biosafety, as well as in specific agents used in the lab, to ensure maximum protection.
3. Developing mechanisms for informing medical providers about all the agents that lab staff work with to ensure quick diagnosis and effective treatment.
4. Addressing confusion over the definition of exposure to aid in consistency of reporting.
5. Ensuring that all BSL-4 labs' safety and security measures are commensurate with the level of risk these labs present.
6. Maintenance of high-containment labs to ensure integrity of physical infrastructure over time."

Other incidents

A centrifuge accident occurred with Sabia virus⁸, an arenavirus, at Yale University in a visiting scientist who was working alone. It would appear that there were inadequate management guidelines and practices for visiting scientists. Also, a risk assessment should have indicated the Sabia virus was potentially a high risk agent, belonging to Risk Group 4 and should have been worked at a BSL-4 lab. Subsequently it was reclassified to Risk Group 4.

Figure 1. Types of accidents preceding infections in laboratories⁵.



There have been numerous laboratory infections with brucella. I know of a number that have occurred in recent years in Australia. In the USA two laboratory infections occurred in different laboratories with the same isolate⁹, probably because the agent is rarely seen in the laboratory and the staff were not familiar with its recognition. In the first case the agent was handled on the bench and in the second case the biochemical tests were done on the bench. In neither case was the agent recognised to be brucella at the time, and the small number of organisms required to infect by the aerosol route led to these laboratory acquired infections.

Table 1. Infectious doses for some diseases^{5, 6}.

Disease or agent	Route	Dose*
Scrub typhus	Intradermal	3
Q fever	Inhalation	10
Tularaemia	Inhalation	10
Syphilis	Intradermal	57
<i>Shigella flexneri</i>	Ingestion	180
Anthrax	Inhalation	≥1300
Typhoid	Ingestion	10 ⁵
Cholera	Ingestion	10 ⁸
<i>E. coli</i>	Ingestion	10 ⁹
<i>E. coli</i> O157	Ingestion	10
Brucellosis	Inhalation	10
<i>Mycobacterium tuberculosis</i>	Inhalation	10
Poliovirus type 1	Ingestion	2

* Numbers of organisms or appropriate viral units.

Tularaemia has been the cause of a number of laboratory accidents. Recently a particular case at Boston University occurred where students were working with what they thought was an attenuated vaccine strain on the bench¹⁰. It wasn't until the third laboratory infection occurred that the University recognised that there was a problem and subsequently confirmed the three infections as tularaemia. It was subsequently shown that the attenuated tularaemia preparation contained virulent tularaemia. There was inadequate risk assessment of the project, checking of the purity of the 'attenuated' material, incorrect work procedures (under the US Guidelines for Select Agents the agent should have been handled at BSL-3) and obviously the health monitoring programme was inadequate.

In a food laboratory run by the US Department of Agriculture located near Washington, two staff became infected with a Verotoxigenic (VTEC) isolate of *E. coli* O157^{6,11}. Experiments on the effects of various disinfectants on O157 were being evaluated by dipping a piece of apple in the *E. coli*, then treating with the disinfectant and then spinning the apple to remove liquid, using a salad spinner on the open bench. The salad spinner was disinfected with 70% ethanol and washed in the only sink in the laboratory. There was an obvious lack of training related to biosafety and a totally inadequate assessment of the risks of this work.

The above are just a few of the examples of recent laboratory incidents. It must be emphasised that it doesn't matter if the laboratory is in a developing country or a developed country, these incidents continue to occur. Most could have been prevented by adequate biosafety management, assessment of the risks involved with the work and competency based training of the staff.

Conclusions

Laboratory acquired infections will continue to occur until proper biosafety management systems are put in place in microbiology laboratories. It is the responsibility of management to ensure proper policies, guidelines and training occur. The community no longer finds it acceptable for poor practices to continue and result in infection of staff, and possibly the community, as occurred in the SARS infections in Beijing. A useful book on

laboratory biosafety is published by ASM Press and edited by Fleming and Hunt¹² and I strongly recommend that it be in every microbiology library.

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