



Biosafety Tips

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Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I will share some biosafety insights for managing a variety of workplace situations. I welcome feedback or suggestions for future topics. Please e-mail them to karen_byers@dfci.harvard.edu or to the Editor, Ira F. Salkin, at irasalkin@aol.com.

Misplaced Sonicators

Researchers trying to save time by not moving the sonicator from the bench top to the biosafety cabinet before use may rationalize this lapse in appropriate procedure. Their rationalizations are often based on misconceptions that can and do lead to laboratory-acquired infections (LAIs). The following are accounts of laboratory-acquired infections that resulted from sonicating open tubes of infectious material on the bench.

Misconception #1

If a procedure is published in the scientific literature without safety advice, then safety precautions are not required.

Misconception #2

This organism is not routinely transmitted by aerosol, so aerosolization of this culture is not a problem.

A researcher followed a published procedure for purifying the proteins of *Orientia (Rickettsia) tsutsugamushi*. No biosafety precautions were mentioned in the published procedure. Twenty flasks with a surface area of 150 cm² were used to culture mouse L-929 cells; the

cells were then infected with *O. tsutsugamushi*. Although a biosafety cabinet was present in the laboratory, this researcher worked on the open bench while disrupting cells with a Dounce tissue grinder (Wheaton, Inc. NJ, USA) and then breaking up the rickettsial membranes with a sonicator (Oh et al., 2001).

Orientia tsutsugamushi, or scrub typhus, is usually transmitted by bite of an infected chigger. In the laboratory setting, infections have been acquired through needlestick, glassware injuries, rat bites, and possibly mite bites (Oh et al., 2001).

Twelve days after the protein purification procedure, the researcher became ill. Twenty-one days after the procedure, the researcher was hospitalized and the diagnosis was scrub typhus. *Orientia tsutsugamushi* isolated from the patient's blood was identified as the Gilliam serotype—the same serotype with which the patient was working. In addition, the pattern of lymph node response and the early onset of chest symptoms indicated the respiratory tract was the primary infection site. Apparently, during sonication of a concentrated preparation, this rickettsial agent was transmitted by aerosol. The researcher responded to doxycycline treatment and hopefully incorporated containment measures into his experimental procedures.

Misconception #3

The details of specific procedures do not have to be reviewed when research staff has been provided access to a Biosafety Level 3 facility.

A biosafety professional met with a laboratory director to discuss an upcoming project involving cloning the cell surface proteins of a number of infectious agents. After the discussion the director requested space to conduct this research at Biosafety Level 3.

When the Biosafety Level 3 space assignment was approved, the research project went forward without a detailed analysis of procedures.

Four to six weeks later, two members of the research group reported illness that required hospitalization. This prompted a major review of the Biosafety Level 3 facility, but no problems with the ventilation were discovered. The facility had been fully air-balanced, and the biosafety cabinets certified, only 4 months previously. The laboratory staff had reported no spills or accidents. Immunoassays were performed with pre- and postexposure serum samples from five of the research staff, including the two who were ill. Four of the five staff had a "striking" seroconversion to chlamydia polypeptides. *Chlamydia trachomatis* was one of the organisms under study (Peterson, 1982), so how had the infection been acquired?

During interviews with laboratory staff, the biosafety professional discovered that "sonications were repeatedly carried out by personnel using a Heat Systems Ultrasonics probe type cell disruptor in an open test tube in the corridor outside the biocontainment suite rooms. Undoubtedly, inhalation of aerosols generated by the sonication process led to illness and seroconversion to chlamydia polypeptides (Peterson, 1982).

Misconception #4

The material to be sonicated does not contain a pathogen.

The patient, ultimately diagnosed with acute melioidosis, was a lab technician who worked for a large pharmaceutical firm. He was hospitalized with fever, pleuritic chest pain, and swelling and tenderness in his upper calf. Since the patient did not improve following initial intravenous antibiotic therapy, the possibility of the presence of a calf abscess was explored surgically. The CDC identified a culture of the purulent material from the calf as *Burkholderia mallei*. A wide range of symptoms such as pulmonary cavitation, empyema, chronic abscesses, and osteomyelitis can result from infection. The patient recovered fully with 14 days of intravenous chloramphenicol treatment followed by 3 months of oral tetracycline.

The patient denied the possibility of occupational exposure and had never traveled to Asia or South America. A diagnosis of acute or chronic melioidosis is very unusual in the Western Hemisphere. Upon ques-

tioning it was revealed that the technician's specific laboratory responsibilities involved growing "large batches of resistant gram-negative organisms and preparing them for extraction of aminoglycoside-inactivating enzymes. This process, which involved disruption of the cell wall by sonication, was performed with a Raytheon sonicator in an open-flask system. Sonication was not done in a biologic safety hood. So how could the lab technician have been exposed to *Burkholderia mallei*? The two bacterial strains of *Pseudomonas*, which were sonicated by the technician on the open bench prior to his illness, were isolated by a local hospital clinical laboratory and identified as *Pseudomonas cepacia*. Unfortunately, one of the isolates was not *Pseudomonas cepacia*, but instead *Pseudomonas pseudomallei* (now *Burkholderia mallei*; Schlech et al., 1981). Most likely, the dissemination of this bacterium subsequent to an initial pulmonary infection resulted in the development of the abscess in the patient's calf.

Misconception #5

A change in experimental procedure does not require a change in biosafety precautions.

The patient, who worked in a laboratory, was admitted to the hospital after a 14-day history of general malaise, anorexia, intermittent fever, night sweats, shortness of breath, right pleuritic chest pains, and a dry cough. Antibiotic treatment was initiated after the patient was tentatively diagnosed with a *Mycoplasma pneumoniae* infection; the diagnosis was based solely on the symptoms. This treatment resulted in a gradual resolution of symptoms over a 2-month period, at which point the patient returned to work.

This patient's serum was routinely used in his laboratory as a normal control reagent in the antigenic analysis of *Chlamydia trachomatis* by gel immunoradioassay. However, when he returned to work it was observed that his serum could no longer be used as a negative control in the assay. This accidental finding provided the correct diagnosis; no antibody to *M. pneumoniae* was found. For 2 years prior to his illness, the patient had sonicated ultraviolet (UV) inactivated *C. trachomatis*; however, the UV inactivation step was eliminated from the procedure about 2 months before the onset of his illness (Bernstein et al., 1984).

Misconception #6

Physicians always know, and follow, appropriate laboratory precautions.

Three days after sonicating *Chlamydia trachomatis*, an infectious disease physician became ill. One week after the sonication, he was hospitalized with symptoms suggestive of lymphoma. Seroconversion was again the key for diagnosis of *C. trachomatis* infection (Bernstein et al., 1984).

Summary

Other LAIs related to sonication undoubtedly exist. These reports specifically point out not only aerosol exposures, but also sonicators as the point source of aerosols that transmitted the infections. Biosafety professionals cannot possibly anticipate every unsafe practice a goal-oriented researcher might adopt. We can, however, continue to make auditing of actual research procedures a priority, and advise containment when open probe sonication is proposed in an experimental procedure involving infectious agents.

References

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