



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 any comments or suggestions to karen_byers@dfci.harvard.edu or to the Chief Editor, Barbara Johnson, at barbara_johnson@verizon.net.

Sonication Revisited

The laboratory-acquired infections described in the Biosafety Tips column (Volume 7, Number 3, 2002) would have been prevented if Biosafety Level 2 or Biosafety Level 3 procedures had been followed.

- In one case, a researcher carefully followed a published procedure for purifying the proteins of *Orientia (Rickettsia) tsutsugamushi*. No biosafety precautions were listed in the publication. A biosafety cabinet was present in the laboratory; however, this researcher disrupted infected cells with a tissue grinder and broke up the rickettsial membranes with a sonicator—all on the open bench. This resulted in the first documented case of scrub typhus transmitted by the aerosol route (Oh, 2001).
- After two out of five laboratory staff assigned to work with *Chlamydia trachomatis* in the BSL-3 facility were hospitalized, a thorough audit of the facility was conducted. No problems were detected except that the sonicator used by the group was located, and used, in the hallway outside the BSL-3 facility (Peterson, 1982).

- A laboratory technician routinely sonicated gram-negative clinical isolates provided by a local hospital in order to extract aminoglycoside-inactivating enzymes. Unfortunately, one isolate was incorrectly identified as *Pseudomonas cepacia*; it was actually *Pseudomonas pseudomallei* (now *Burkholderia mallei*). The technician was hospitalized with acute melioidosis. (Schlech, 1981)

- A laboratory worker sonicated *C. trachomatis* after UV inactivation for 2 years without incident. Unfortunately, the UV inactivation step was eliminated and the worker continued to perform the procedure as before until his hospitalization 2 months later (Bernstein, 1984).

- An infectious disease physician was hospitalized 3 days after sonicating *C. trachomatis* out on the open bench (Bernstein, 1984).

If consulted, biosafety professionals would have prohibited these procedures, due to the risk of infection from uncontained aerosols of BSL-2 or BSL-3 organisms. But discouraging sonication on the open bench using BSL-1 organisms is a greater challenge for a biosafety professional. You can explain that:

- Open probe sonication does not meet the standards associated with good microbiological practices.
- A cup horn attachment to the sonicator allows sonication of cells in a closed tube placed in the ultrasonic bath.
- The sonicator could be moved to the biosafety cabinet.
- Allergic reactions to proteins aerosolized during sonication are a potential problem.

Enforcement activity with BSL-1 procedures is a real challenge. However, I reason that if sonicators are present, there is a potential for their inappropriate use (as in the examples above). So in my institu-

tion we worked on an educational campaign for the scientific staff. In our laboratories sonication is primarily used to lyse *E. coli* for extraction of expressed recombinant proteins. There are commercial reagents available now that are advertised as “an alternative to sonication” in the product literature. If you put “sonication” into the search engine Google, several detergent-based commercial reagents for lysing *E. coli* will appear on your screen. The product descriptions describe high, reproducible yields of proteins that are not denatured by the heat of sonication. To get the investigator’s perspective, I showed the product literature to the members of our Institutional Biosafety Committee and they agreed that a mailing of product literature to Principal Investigators would be appropriate. Next, I put the following note in the EH&S newsletter along with a few pieces of cut and pasted product literature:

What’s that WHINE?

Trying to reduce the amount of time you listen to that annoying whine? We mean the sonicator, of course. Check out BugBuster[®] Reagent and BugBuster plus Benzonase[®] simple extraction of soluble protein from *E. coli* without sonication at www.novagen.com/. We got this from a quick Google search... colleagues and a literature search might yield more alternatives!

I added a slide on sonicator use to the 2003 EH&S annual retraining session, and completed the educational campaign by inviting a regional sales representative for a cell lysis product (“Bugbuster”)

to speak at a laboratory safety officer’s meeting. The Novagen sales representative provided an informative talk comparing yields of protein from various methods of cell lysis. In addition to full-color brochures, the sales rep offered to send free samples of products to those who requested them. This approach was well received and several groups now use detergent-based reagents for protein extraction procedures instead of sonication. It’s a start!

References

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