

BioSide Lines

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The Newsletter of the Office of Biological Safety, UW-Madison Safety Department

www.fpm.wisc.edu/biosafety

Recommendations Revisited

Recent BioSide Lines articles have presented recommendations on handling animals that have been treated with human cells and appropriate response to laboratory incidents. These risks should not be ignored, yet they seem remote and are easily dismissed. An illustrative example was presented recently at the American Biological Safety Association meeting.

"In early 1998 an animal technician employed at a Harvard affiliated institution developed a persistent high fever of unknown origin. He went on extended sick leave and over the next few months had substantial weight loss and other worrisome symptoms. Despite extensive testing his personal physician was unable to determine the causal agent. It is likely the physician did not realize the technician's occupation. Several months later 3 of 3 sentinel animals in a mouse room served by the technician tested positive for lymphocytic choriomeningitis virus (LCMV). Subsequently the recovering technician was tested and found to be LCMV seropositive. No other technicians, veterinarians or investigators using the room were LCMV seropositive. The technician regained his health within six months."

"How did LCMV enter the animal facility? An intensive investigation revealed a human tumor cell line used in the animal room served by the victim was contaminated with the virus. The cells were obtained from another institution where they had been passaged in nude mice over a period of 10 to 12 years. It is likely the cells became contaminated during this period. The original cell line has been in widespread use for over a decade without reports of LCMV contamination. A lesson learned from this experience is the necessity that supervisors, animal care staff and occupational health providers consider the possibility any staff illnesses may be occupationally acquired. When consulting a health care provider about an illness the staff member should inform his/her physician of their occupation."

Some additional relevant details were provided by the presenter:

- The technician was experienced.
- Open cages were used.
- There was no record available to the technician showing that the animals had been inoculated with anything. The experiment was done without biosafety and animal care committee approvals.
- The cell lines had been known in the past to be infected with LCMV but the original facility had tried to clear it.

The take home messages: Use biosafety level 2 precautions when working with human or non-human primate cell lines and animals treated with these potentially infectious materials. Use appropriate hazard communication. And, don't dismiss the possibility that an illness could be occupationally acquired; always inform the attending physician about your occupation and possible exposures to hazardous materials in the work place.

Source: Braun, A. and H. Warren. 2004. Case Report: Transmission of Lymphocytic Choriomeningitis Virus from Laboratory Mice to an Animal Technician. Conference Proceedings, 47th Annual Biological Safety Conference.

Options for Verifying Autoclave Efficacy

Autoclaves are a critical tool for disinfecting biological waste. It is important to test the efficacy of the autoclave periodically to ensure that it is functioning properly. In addition to reaching the desired temperature and pressure for a certain period of time, efficacy is affected by the nature of the material in the load and how the

load is prepared. The frequency with which efficacy testing should be done depends on the nature of the materials. It should be done every 40 hours when routinely disinfecting biological hazards such as human or non-human primate blood, tissues, clinical samples, or pathogens. Some recent advances have made efficacy testing with biological indicators easier.

Autoclave tape changes color after exposure to 121°C and will tell you only if the temperature was reached. The tape lacks indication of important parameters (how long and how hot) for disinfection. Tape should be used in conjunction with periodic use of biological indicators.

The gold standard for autoclave efficacy testing utilizes biological indicators. Spore strips or vials of *Geobacillus stearothermophilus*, a thermophilic bacterium that grows best at 55°C, are inserted into the center of a load and run in the autoclave, then mixed with culture medium, and incubated at 55°C for 48 hours. There will be no growth if sterilization was successful. If, however, sterilization was ineffective, the spores will germinate into vegetative cells that will grow. Growth is detected visually by checking for turbidity or a color change if the growth media contains a pH indicator. A different biological indicator, *Bacillus atrophaeus* (formerly *B. subtilis* var. *niger*), is used to test efficacy of ethylene oxide sterilizers. Some commercial products combine these two biological indicators and the incubation temperature will favor one indicator microbe over the other. Suppliers of biological indicators also provide small dry heat blocks so that expensive incubators are not needed.

A serious drawback to biological indicators is the slowness of the test. A rapid method for determining the efficacy of a sterilization cycle employs a chemical indicator that is indicative of the exposure temperature and time. 3M Comply™ Steam Chemical Integrators (SteriGage)¹ provide immediate visual results without incubation. This product contains a steam and temperature sensitive pellet of self-contained, non-toxic chemicals. Interaction with steam causes the pellet to melt and migrate along a paper wick to an acceptable point. The strip can be mounted on the daily autoclave log to record the results.

Another rapid method provides results in just 3 hours and is based on an enzymatic assay that produces fluorescence. An example is 3M Attest™ 1292 Rapid Readout Biological Indicators. A spore strip of *G. stearothermophilus* is inserted into the load to be autoclaved. It is then crushed to release the microorganism into growth medium that contains an indicator which changes from rose to brown when incubated. This process is easy for non-scientists to use.

A comparison² of a various types of indicators for steam sterilization concluded that the sensitivity of the rapid readout biological indicator was equivalent to the standard 48-hour biological indicator. This study also found that Comply SteriGage performed satisfactorily, but certain other chemical indicators failed to indicate adequate sterilization and could cause unnecessary recall of adequately sterilized items.

¹Mention of a specific product is not intended to be an endorsement.

²Rutala et al. 1996. Infect. Control Hosp. Epidemiol. 17:773.

Significant Changes in Shipping Regulations Require Retraining

Major changes in the air transport regulations (IATA) for Infectious Substances and Diagnostic Specimens become effective 1 January 2005. The changes include:

- The classification of Infectious Substances and Diagnostic Specimens has been revised. Infectious Substances are now divided into 2 categories (A & B) with category A being a higher hazard. Diagnostic Specimens are now classified as category B infectious substances, yet still are exempt from some requirements.
- The packing instructions for Infectious Substance and Diagnostic Specimens have been modified. A notable change is that packages of Diagnostic Specimens must display a UN3373 diamond label and be marked "Diagnostic Specimens" or "Clinical Specimens marked adjacent to the diamond. OBS will have a limited supply of these labels to distribute.

- The format for the dangerous good declaration has changed. An air transport certification statement must be added to the Shipper's declaration; it replaces the 1.3.3.1 statement.
- The technical name need not be used with the proper shipping name for Infectious Substances on the package marking. It still is required on the shipper's declaration.
- A minor error on the dangerous goods declaration, such as omitting a comma, will no longer be a violation.

Personnel who currently are certified will need to participate in additional training to be apprised of these changes; a web-based option is available to complete this requirement. Those who are not currently certified will need to attend a class and this option is always available to those who need to update their certification.

Shipping Infectious Substance and Other Biological Materials

The Office of Biological Safety will provide training and certification for shipping Infectious Substance and other biological materials, with a focus on safety and regulatory compliance for research laboratories. The Department of Transportation requires that persons involved in shipping hazardous materials in commerce be trained and certified in proper handling of these materials.

Wednesday, January 19, 2005
 Union South 1:30 – 3:30 p.m.
 Refreshments will be served.

Registration is required. Contact OBS at 263-2037 or biosafety@fpm.wisc.edu.

All staff are welcome to attend this class for initial training or re-certification. Staff approaching their two-year expiration for certification will receive a notice in advance of that date. Computer-based training is available only for those who attended the class for their initial certification.

Basic Biosafety Class Offered

This class will give an overview of basic biological safety. Topics include: biosafety levels and biohazard containment, good microbiological techniques, waste disposal, risk assessment, and emergency preparedness. It is intended primarily for students and staff who are new to this institution and/or new to working with biological materials in a laboratory. Everyone is welcome to attend.

Wednesday, February 9, 2005
 Union South 1:30 – 3:30 p.m.

Registration is required. Contact OBS at 263-2037 or biosafety@fpm.wisc.edu.

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