What you don’t know really can hurt you

By LTC Wade Aldous, PhD

Since the events of 9/11, including the anthrax mailings, how many of you ended up receiving all kinds of “specimens” for possible anthrax cultures, ranging from shoe powder to flour to small bits of styrofoam? That day our world forever changed, as did our laboratories. Now, we worry about recognizing agents of bioterrorism among all of those various culture specimens, and microbiologists have become more in demand because of their expertise.

We rarely get advance notice from physicians suspecting a select agent such as a Brucella species [see “Anatomy of an exposure: A hospital lab’s recovery of Brucella melitensis” by Colleen K. Gannon, MT(AMT), HEW, September 2003, page 22]. When that specimen comes, we seem to treat it differently — with greater respect — even though we should treat all specimens equally, with standard precautions. The more common scenario is that we receive a specimen asking for multiple cultures (aerobic, anaerobic, acid-fast bacillus, fungal). The specimen is plated and initial Gram-stain results show something like Gram-negative diplococci or very tiny Gram-negative cocccobacilli. When starting your work-up the next day, there are a couple of choices: a) start down a series of biochemical tests, performing all operations on the bench, or b) take that specimen directly to the biosafety cabinet for work-up. [Actually, I have seen both scenarios occur with very experienced and knowledgeable technologists working on the bench.]

When the identification comes back as Neisseria meningitidis or a Brucella species, all of a sudden, the anxiety level within the laboratory increases. The original bench tech and surrounding lab workers end up getting antibiotic prophylaxis and put under medical surveillance for a short period of time. This is, of course, a much better alternative than that reported in the Morbidity & Mortality Weekly Report (MMWR) where two independent clinical microbiologists working with patient specimens of Neisseria meningitidis both developed symptoms of meningococcal disease within 10 days of working with these isolates on the bench and expired shortly afterward.1

In another incident, 17 laboratory workers manipulated cultures of Burkholderia pseudomallei outside of a biosafety cabinet. Some of these individuals actually reported sniffing the culture plate because of the distinctive “earthy” odor.2 Fortunately, all workers were provided antibiotic prophylaxis within 48 hours of exposure with no patients developing melioidosis. (What were they thinking here? Olfactory excitement? Was this risky behavior and carelessness born out of on-the-job stress?)

LAIs: coming soon to a lab near you?
No laboratory professional wants to think he will ever become a patient by working with pathogenic organisms. But such situations do occur, and the result is recognized as a laboratory acquired infection (LAI). While anyone in the laboratory can become infected, microbiologists have greater chances of developing disease because they work with isolated cultures with greater concentrations of organism. No one really knows the prevalence rate, but the statistics are thought to be vastly underreported, as only obvious infections are reported. Fear of reprisal, loss of employment, or the appearance of incompetence cause this lack of disclosure.

There are, however, multiple incidents recorded in the scientific literature. Baron and Miller1 recently identified Shigella, Brucella, Salmonella, Staphylococcus aureus, and N meningitidis as some of the top bacterial agents.3 Some recent articles from Entrez PubMed list: vaccinia,4 methicillin-resistant Staphylococcus aureus or MRSA,5 leptospirosis,6 anthrax,7 human immunodeficiency virus or HIV,8 severe acute respiratory syndrome or SARS, virus,9 scrub typhus,10 and various parasites.11

Biohazard agencies and guidelines
Since we know laboratory-acquired infections occur from working with biohazards in the laboratory, how do we prevent these infections? Luckily, we have numerous resources and agencies to assist us in these matters including:
- American Society for Microbiology (ASM);
- Centers for Disease Control and Prevention (CDC);
- Clinical Laboratory Standards Institute (CLSI);
- College of American Pathologists (CAP);
- Occupational Safety and Health Administration (OSHA);
- Code of Federal Regulations (CFR);
- American Biological Safety Association (ABSA);
- Department of Health and Human Services (DHHS); and
- the United States Department of Agriculture (USDA).

A number of pertinent guidelines are also available for laboratorians to follow. The most recognized are:
- Biosafety in Microbiological and Biomedical Laboratories (BMBL);
- CAP accreditation checklists and proficiency surveys; and
- CLSI documents.

Biosafety in Microbiological and Biomedical Laboratories: A new 5th-edition BMBL was published in February 2007 and is vastly improved with new, pertinent information [Download BMBL PDF of 3.4 MB at www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm]. Printed booklets, HTML, and Spanish versions are not yet available, according to this website.] New topics were added including occupational medicine and immunization, decontamination and sterilization, and laboratory biosecurity and risk assessment. Additionally, all agent summary-statements and appendices were revised to include the most current guidance and regulations from federal agencies. New agents were added to this document including 1918 influenza virus, SARS virus, and several arboviruses and toxin agents. Throughout this document, laboratory directors are encouraged to “evaluate and ensure the effectiveness of their biosafety programs, the proficiency of their workers, as well as the capability...
of equipment, facilities, and management practices, to provide containment and security of microbiological agents.\textsuperscript{12}

Lab directors are to conduct risk assessments of their facilities, and develop tailored procedures and practices according to the existing equipment and engineering controls within their facilities. This should then be agreed upon with input from the institution’s biosafety committee, safety officers, public-health officials, and other experts. Several of the infectious agents summarized in the \textit{BMBL} are now classified as “Select Agents” (e.g., \textit{Bacillus anthracis}, \textit{Yersinia pestis}, or \textit{Francisella tularensis}). If any select agent is suspect, it needs to be sent off to a reference laboratory within the CDC’s Laboratory Response Network (LRN) for testing/confirmation [see “Straight talk on bioterror from the Army’s LRN Gatekeeper” by William F. Nauschuetz, PhD, June 2005, page 10]. If confirmed, the CDC is notified, and any culture materials kept at the original laboratory must be destroyed within seven days.

Because of the select-agent classification, biosecurity is a new feature added to protect biological pathogens or toxins from theft, loss, or misuse. Some facilities may not think that biosecurity applies to them, but regulatory agencies are cracking down on safety and security violations for fear of bioterrorism. In 2003, a distinguished and internationally renowned plague researcher from Texas Tech University reported missing vials of organism. The Federal Bureau of Investigation delved into the matter, and this researcher eventually was convicted, given a two-year prison term, and fined, as well as losing his medical license.

In a separate incident in 2007, Texas A&M University failed to report incidents of lab-worker exposure to Brucella and Coxiella species. The school was cited by the CDC for lab-safety failures, and high-containment research was suspended. Financial and criminal charges/penalties are still pending. Although these examples deal with research facilities, any laboratory can be cited for improper conduct. It will be very important to ensure that all regulations are correctly followed.

Overall, the \textit{BMBL} introduction encourages a “careful review” of the manual in order to be better prepared to work safely with infectious agents. It is important to emphasize that this is considered an advisory document and will continue to be a dynamic document as new agents are identified or technologies advance. Again, the guidance therein should be used by individual lab directors to develop and implement procedures and processes to ensure safety and security within their facilities.

\textbf{College of American Pathologists:} The CAP provides a plethora of information and training for the laboratorian including many proficiency surveys in which to participate, based upon the individual laboratory’s capabilities. One of the newer surveys specific to microbiology is the “Laboratory Preparedness Exercise” or LPX (previously known as the Laboratory Preparedness Survey or LPS), which tests the lab’s ability to handle potential public-health emergencies related to bioterrorism agents.

Live organisms with characteristics of bioterrorism agents are sent for identification including specific attenuated strains of \textit{B anthracis}, \textit{Y pestis}, \textit{F tularensis}, and \textit{Brucella abortus}. These agents — not on the CDC’s select-agent list — are modified and are safe for testing, but still represent potential pathogens and are meant to be tested under appropriate conditions. The CAP recommends this survey for those facilities participating in its full D-Bacteriology survey. Additionally from the CAP microbiology checklist, several questions under the biosafety header concern the need for: a) policies and procedures for recognition of isolates that may be used as agents of bioterrorism (ASM provides sentinel-level guidelines); b) participation in the institution’s bioterrorism response plan, and also c) policies and procedures developed to minimize the occupational risk of exposure to infectious agents.

\textbf{Clinical Laboratory Standards Institute:} CLSI provides materials dealing with laboratory safety. All of these materials provide greater knowledge concerning safety methods and are encouraged for all facilities:

- Clinical Laboratory Safety (GP17-A2) provides general guidelines for implementing a laboratory-safety program.
- Clinical Laboratory Waste Management (GP5-A2) provides information for proper disposal of hazardous wastes.
- Protection of Laboratory Workers From Occupationally Acquired Infections (M29-A3) gives guidance on transmission risk of infection from aerosols,

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Implementation challenge

Although lab personnel are generally very careful when working with patient specimens, lab-acquired infections occur anyway. This means that there is still room for improvement. Through careful study of the resources above, lab directors can take stock of their personnel and their facilities to develop and implement plans and processes to reduce the potential for LAI. The following might be used as a starting point.

**Determine where your hazards might be.** Typically, the hazards will be at the collection point with exposure to the patient or during processing of specimens within the laboratory.

**Recognize how exposures can occur.** The most common exposure routes include percutaneous, mucocutaneous, inhalation, and ingestion. Proper usage of personal protective equipment (PPE) and adherence to standard laboratory-safety procedures help eliminate these.

**Determine methods of prevention.** All personnel should be trained in specific laboratory methods and be competency-assessed before performing testing. Laboratory equipment and engineering controls will allow for better workflow practices. Personal protection is a must; use both barrier protection (PPE and biosafety cabinets) and vaccination against potential pathogens (HBV, Meningococcus, and others). A post-exposure plan for dealing with such incidents will ensure proper care and future treatment.

Finally, be very thorough in your studies of these listed organizations and resources in order to provide a safe and healthy environment within your laboratory.

**References**