



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 Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

***Neisseria meningitidis* Exposures in Clinical Laboratories**

In the United States, approximately 3,000 times each year, clinical microbiology laboratories isolate *Neisseria meningitidis* and another diagnosis of meningococcal meningitis is confirmed. The reported fatality rate for community-acquired meningococcal disease due to *N. meningitidis* is 10-14%; 11-19% of those who recover have permanent hearing loss, mental retardation, loss of limbs, or other serious sequelae (Bilukha, 2005). The fatality rate of laboratory-acquired infections (LAI) with *N. meningitidis* is estimated to be as high as 50% (Sejvar, 2005). To prevent this disease, the Advisory Committee for Immunization Practices (ACIP) recommends the tetravalent meningococcal polysaccharide-protein conjugate vaccine for the following risk groups: “young adolescents, college freshmen living in dormitories and other populations at increased risk (i.e., military recruits, travelers to areas in which meningococcal disease is hyperendemic or epidemic, **microbiologists who are routinely exposed to isolates of *Neisseria meningitidis***, patients with anatomic or functional asplenia, patients with terminal complement deficiency, and persons exposed to active or passive tobacco smoke)” (Bilukha, 2005). The vaccine reduces the risk of infection from serogroups A, C, Y, and W-135. Unfortunately, it does not protect against serotype B. The importance of various serotypes in causing clinical infection varies according to geographic regions. In sub-Saharan Africa, there is a “meningitis belt” with large seasonal epidemics caused by serotype A, C, and W-135. In Asia, most infections are caused by serotype A; New Zealand reports a large number of cases from serotype B.

The majority of the cases in the Americas and Europe are caused by both serotypes B and C (World Health Organization, 2003).

To prevent occupational infections from *N. meningitidis*, CDC recommends the use of the biosafety cabinet, an important Biosafety Level 2 engineering control, for handling isolates from sterile sites such as blood, cerebrospinal fluid, or inner ear fluid. The bacteria can be found in the pharyngeal exudates of some healthy carriers, but pharyngeal isolates are generally considered less invasive and have not caused any reported laboratory-acquired infections (Sejvar, 2005). Occupational infections are linked to manipulation of the invasive isolates of *N. meningitidis* from sterile sites such as blood, cerebrospinal fluid, or inner ear fluid. Clinical microbiologists may find it inconvenient to use a biological safety cabinet to manipulate blood and cerebrospinal fluid cultures, since these procedures were previously carried out on the open bench, but consistent use of the biosafety cabinet is critical to prevent rare, but serious, cases of occupational transmission. For example, one fatal laboratory-acquired infection occurred from the first *N. meningitidis* isolation, which had occurred in that laboratory in four years (CDC, 2002). Experienced microbiologists who routinely handle *N. meningitidis* have also been infected. In one case, an experienced scientist worked with suspension cultures on the open bench without incident. After this individual was hospitalized, a spinal fluid isolate was determined by restriction fragment length polymorphism to be identical to one of the three Brazilian strains studied in the lab the previous week (Knudsen, 1992). A fatal infection also occurred in a state laboratory; the infected microbiologist handled an average of four *N. meningitidis* isolates a month (CDC, 2002).

In a recent review of laboratory-associated infections, Harding and Byers (2006) cited 31 cases of *N. meningitidis* infections in clinical microbiologists that had occurred between 1979-2004; 11 were fatal. Five cases were described in a retrospective study in England and Wales during the 15-year period between 1 January 1985 and 31 December 1999 (Boutet, 2001). Boutet reported that all of the infected microbiologists had prepared suspensions on the bench, outside of biosafety cabinet, within seven days before the onset of meningococcal disease. The review data available on laboratory-acquired infections with *N. meningitidis* can be summarized with the following state-

ment. In 30 out of 31 cases, the infected microbiologist had handled *N. meningitidis* isolates on the open bench, usually within 3-4 days of becoming ill, and the isolates from the original patient and the laboratory-acquired case were identical in serotype and other markers.

Procedures Associated with LAIs

Identification procedures for *N. meningitidis* begin with “preparing a heavy suspension.” Specific procedures performed by the clinical microbiologists who became infected were:

- Slide agglutination testing and observing colony morphology (CDC, 2002);
- Aspirating samples from blood culture bottles, performing Gram stains, handling plates, and subculturing cerebrospinal fluid (CDC, 2002);
- Culturing blood and subculturing isolates (CDC, 1991);
- Performing agar diffusion antibiogram with serogroup C strain isolated from cerebrospinal fluid (Guibordenche, 1994);
- Determining antibiotic susceptibility using a Steers-type replicating device with 10 isolates (Guibordenche, 1994);
- Preparing a suspension, vortexing, seeding agar plates, and pipetting the suspension into the wells of a plate to perform a commercial identification procedure on the workbench (Paradis, 1994);
- Making a heavy suspension of *N. meningitidis* to inoculate an identification test strip using a pipette, by collecting a colony with a swab, and suspending it in saline (CDR, 1992); and
- Preparing negative stains for electron microscopy by adding 0.1 ml bacterial suspension to 0.9 ml 3.5% formalin solution (Bhatti, 1982).

In 2002, CDC reported two fatalities from meningococcal laboratory-acquired infections in *Morbidity and Mortality Weekly Report* and they requested information on additional case reports in the article, as well as on websites and email lists of various infection control and infectious disease organizations. Responders provided case reports from the United States, Canada, Germany, Switzerland, and Australia that had occurred between 1985 and 2000. Dr. Sejvar kindly compared the CDC case reports with the literature reports, eliminated overlapping reports, and confirmed that, in the period between 1979 and 2004, information was available on 31 laboratory-acquired infections with *N. meningitidis* (Sejvar, personal communication). In 2006, Sejvar et al. published an analysis of the occupational case reports of *N. meningitidis* infection that occurred between 1985 and 1999. Sixteen infected microbiologists performed the following procedures with *N. meningitidis*: examining Petri solid medium plates (50%), subculturing isolates (50%), and performing

serogroup identification (38%). Fifty-six percent of the 16 laboratory-acquired infections were due to serogroup B and 44% of the infections were due to serogroup C. Fifty percent of the cases were fatal (Sejvar, 2005).

After the analysis was completed, CDC received three additional reports of laboratory-acquired infections in 2002. All three staff members had worked with *N. meningitidis* on the open bench; two of the staff members had worked behind a standard splash shield (Sejvar, 2005). All of the occupational infections were acquired in the microbiology laboratory; the infections occurred after working with *N. meningitidis* cultures on the open bench, not from handling body fluids from meningitis patients.

Route of Transmission

N. meningitidis is transmitted from person to person by droplets of respiratory or throat secretions; close and prolonged contact is required for community-acquired infections (World Health Organization, 2003). In the laboratory setting, *N. meningitidis* is transmitted by aerosol or droplets. In the 31 cases reported, only the individual actually subculturing the isolate, preparing the suspension, or setting up antibiotic susceptibility tests on the open bench were infected. Sejvar points out that some of the routine procedures conducted by the infected workers have not traditionally been considered high-risk for the generation of aerosols, e.g., transferring culture with a loop. Two of the infected workers used a splash shield (CDC, 2005); apparently, a splash shield is not sufficiently protective. CDC points out that the use of face shields or respirators cannot be evaluated from the information provided, and emphasizes use of the biosafety cabinet for manipulation of isolates from sterile sites.

Host Factors

Additional host risk factors are suspected in only two of the 31 LAIs with *N. meningitidis* and both cases were fatal. One infected microbiologist had an upper respiratory infection at the time of exposure; this may have contributed to susceptibility (CDC, 1991) or possibly lead to contaminated hands touching mucous membranes. Another microbiologist was 1.5 meters tall and worked standing up at the bench. It was suggested that short stature increased the risk of work on the bench since the manipulations were closer to the breathing zone of the infected individual (Paradis, 1994). In the 31 case reports, the only documented lapse from established laboratory procedure occurred during the preparation of a laboratory strain of *N. meningitidis* for electron microscopy. A technician did not wear gloves to prepare negative stains, which requires adding 0.1 ml of a suspension of *N. meningitidis* to 0.9 ml of a formalin solution (Bhatti, 1982).

Incident Reporting and Prevention

There is only one anecdotal report of a “mishap.” The husband of one microbiologist told the ambulance crew that his wife had a mishap in the laboratory with a bacterial culture from a meningitis patient. No additional information is available. The microbiologist died and the incident was not reported to the laboratory director or coworkers (CDC, 1991). This reinforces that parenteral or mucosal exposures to *N. meningitidis* require prompt reporting and treatment. Microbiologists who find, after their cultures are identified, that they have inadvertently manipulated invasive *N. meningitidis* isolates in a manner that could induce aerosolization or droplet formation (including plating, subculturing, and serogrouping) on an open bench top should also consider antimicrobial chemoprophylaxis (CDC, 2002). In order to help prevent *N. meningitidis* infections, training for clinical microbiologists should emphasize use of the biosafety cabinet and personal protective equipment, provide information on the benefits and limitations of vaccination, and underscore that incidents must be reported promptly. Current information to guide the occupational health program in dealing with exposure incidents, and counseling on vaccination is available on the web at www.cdc.gov/mmwr/preview/mmwrhtml/rr5407a1.htm

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ABSA thanks the many of you have generously contributed to the Richard C. Knudsen Memorial Fund. The proceeds from this fund will be used to establish an award to honor Rich's memory. Those wishing to make donations to this fund should make their checks payable to the American Biological Safety Association. Please add a notation to the memo line that the check is to be used for the Richard C. Knudsen Memorial Fund. Checks should be mailed to ABSA, 1200 Allanson Road, Mundelein, Illinois 60060-3808.