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BIOSAFETY RISK ASSESSMENT: A CASE STUDY IN ASSESSING AN INSTRUMENT WITH BRUCELLA IN MIND

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The American Society for Microbiology (ASM) recently released their Biological Safety guideline as part of their Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases, found at https://www.asm.org/index.php/guidelines/sentinelguidelines. This guideline is a tremendous resource to clinical laboratories, especially Microbiology departments where staff may manipulate and culture specimens that could contain biological threat agents. When using these guidelines, laboratories should perform a biosafety risk assessment of their laboratories, procedures, and instruments to ensure that staff are adequately protected from hazards that may be present. The guideline itself and resources it references provide an excellent start to this process. To aid in performing biosafety risk assessments, the Bureau of Public Health Laboratories (BPHL) offers a free Standard Operating Procedure (SOP) and related worksheets that may be used to efficiently and thoroughly assess one's lab. These can be found at http://www.floridahealth.gov/programs-and-services/public-healthlaboratories/biosafety/risk-

(Continued on page 2)

Inside this issue:

BIOSAFETY RISK ASSESSMENT: A CASE STUDY IN ASSESSING AN INSTRUMENT WITH BRUCELLA IN MIND (CONTINUED)

(Continued from page 1)

assessment.html. The Association of Public Health Laboratories (APHL) offers additional examples of risk assessment templates with its "APHL Risk Assessment Best Practices" document found at https://www.aphl.org/programs/preparedness/Biosafety-andBiosecurity/Pages/BB-Resources.aspx.

To see how one might use one of these tools to assess a laboratory instrument and its associated procedure, BPHL's Instrument/Method Worksheet is used here to assess the hazards of a Bacteriology instrument and the procedure for its use, including offering risk mitigation recommendations taken from laws, rules, guidance, standards, and scholarly research articles. References are given where applicable to better understand what resources gave rise to this hazard identification and recommendations.

Consider the following scenario:

- You open a bacterial culture plate with colonies growing on the agar, pull off a few similar colonies with a sterile swab, and mix the colonies into a plastic tube containing saline.
- You vortex mix this tube and check that the solution has the right density. You place the tube in a holder.
- You use a micropipette to aspirate a small amount of saline/colony solution from the tube, add it to another tube of saline and then place this second tube into the same holder.
- This instrument uses tube-specific reagent devices with transfer tubes attached to them. You orient two reagent devices on the holder so that the transfer tubes are placed into their respective saline/colony solutions.
- The holder is placed into the instrument, and a vacuum is used to mix the saline/colony solutions and draw the solution into the reagent devices via the transfer tubes. The instrument cuts the transfer tubes and seals the reagent device.
- The holder is then moved into an incubator in the instrument and read at a given interval. The instrument uses results for each reagent device's wells in conjunction with a database of potential reagent results and associated organisms to give you a likely organism or susceptibility for the associated culture.

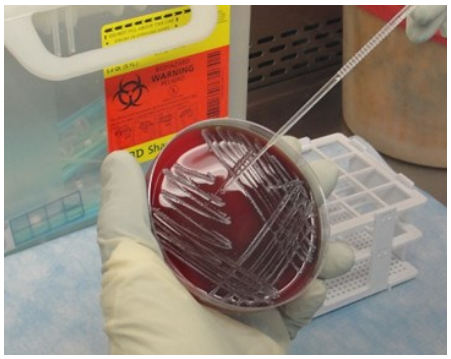
(Continued on page 3)

BIOSAFETY RISK ASSESSMENT: A CASE STUDY IN ASSESSING AN INSTRUMENT WITH BRUCELLA IN MIND (CONTINUED)

(Continued from page 2)

Where are the hazards here? Is there potential for sharps hazards, splashes, aerosolization, leaking, breakage, or contamination of biohazardous material? Working through the Instrument/Method Worksheet with this procedure in mind, we can understand many of the potential hazards as well as recommended mitigation strategies.

What could make a splash or generate aerosols of potentially infectious substances?

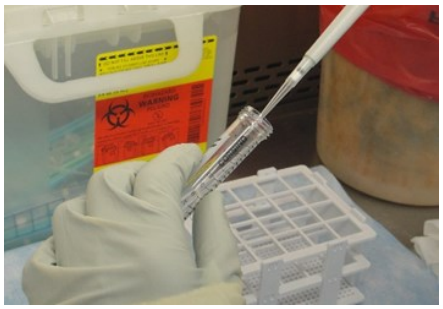


- You open a container[1] (the culture plate).
- You use a sterile swab to remove colonies from the plate, which mimics the action of using an inoculation needle or loop.[2]
- You mix the saline/colony solution using a vortex mixer.[3]
- You pipette saline/colony solution using a micropipette.[4]
- You use a vacuum-assisted device[5] in drawing the saline/colony solution through the transfer tube from its plastic tube into the reagent device in the instrument.

What else could impart energy to the microbial suspension[6] (the saline/colony solution), potentially generating some splashes and aerosols:

- You mix the saline/colony solution with the swab.
- You place the saline/colony solution tubes into the holder.

Knowing this procedure could generate splashes and aerosols of infectious agents, what can we do?



- Perform as much of the procedure as possible in a biological safety cabinet (BSC) to contain aerosols.[7] As part of this, use the vortex mixer in a BSC.[8]
- Use aerosol-resistant pipette tips with barrier filters.[9]
- Touch pipette tips to the inside of the well or tube before pressing the delivery plunger.[10]
- Ensure tubes are tightly sealed prior to mixing with a vortex mixer.[11] (Note: Do not seal a tube with a cap or

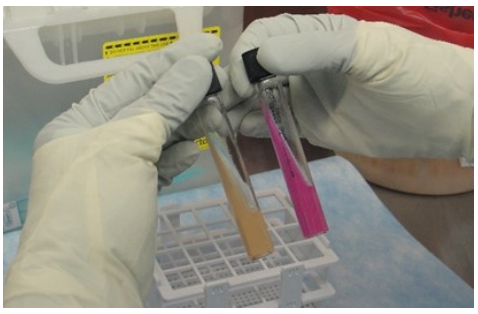
(Continued on page 4)

(Continued from page 3)

other covering that will also be used with other tubes. Using the same cap or covering with multiple tubes can lead to cross-contamination.)
- Wait for a few seconds before opening caps after vortex mixing or shaking.[12]
- Locate instrument and its procedure as far away from other instruments and people as possible, especially areas of high traffic.[13]
- If you suspect that a specimen being tested contains a Select Agent,[14] exercise extreme caution. It is recommended to not use automated instruments with specimens that are suspected to contain select agents. Instead, follow the rule-out and refer protocols from http://www.asm.org/index.php/guidelines/sentinel-guidelines before referring specimens to public health laboratories.
- Physicians and other patient care staff should flag patients or individual specimens if there is a suspicion that the patient may have a pathogen that poses a high risk to laboratory staff, even if the pathogen does not pose a significant risk to patient care staff and other patients. An example would be *Brucella, which is a bloodborne* pathogen in the eyes of patient care staff but a significant laboratory hazard when aerosolized or cultured. When alerted by the flag or notes, the laboratory can use an alternate algorithm for relevant specimens. This algorithm may include using additional controls like following the protocols for suspected biological threat agents and emerging infectious diseases, using a BSC for all specimen and related culture manipulation, working in a BSL-3 laboratory, wearing respirators, and other additional controls.

With those recommendations understood, just how big of a risk could this instrument and its procedure pose to lab staff? Is there a chance that, given current testing algorithms, *Brucella suis* could be on that culture plate? While not frequently encountered, this bacterium is endemic in Florida. It's a risk group 3 organism and has a low inhalation infectious dose.[15] It would certainly be at a high concentration in this procedure since you're manipulating a culture.[16]

If *Brucella and other risk group 3 or low inhalation infectious dose agents* are a risk, then what else can we do?



- Use an alternative instrument or procedure that poses a lower risk. In this case, do not use this instrument and instead use traditional biochemical tests in a BSC if *Brucella or other agents of concern are* suspected. Using additional biosafety controls

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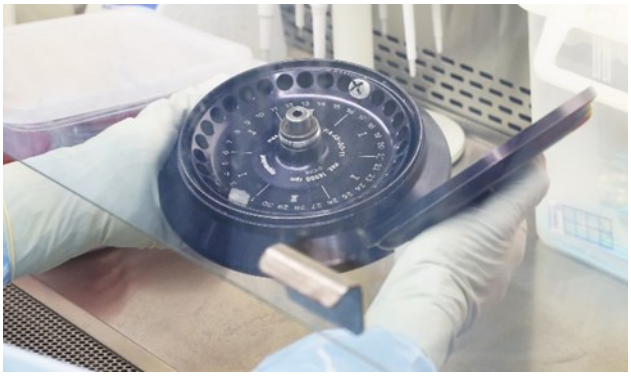
like the BSC might be done at a minimum based on "trigger points." Trigger points are specific circumstances or situations that cause an event (using additional controls) to occur. They may include colony growth or staining characteristics, growth from normally sterile sites (such as blood, CSF, body fluids, lymph node & other aspirates), unusual test results, etc.[17]

Regardless of suspicion of higher risk agents like *Brucella, what is the* minimum that we should consider to be safe given the hazards of this procedure and instrument?

- Wear gloves (nitrile, latex, chloroprene, or other suitable material – not vinyl) and lab coat, gown, smock, or uniform designated for lab use while working with potentially infectious materials.[18]
- Ensure instrument safety shields and containment devices are in place at time of use.[19] This would include any bench-fixed splash shields that may be used.
- If work is not conducted in a BSC or behind a splatter guard (such as an instrument safety shield or bench-fixed splash shield), use eye and face protection to protect against splashes or sprays.[20] Examples include safety glasses, goggles, mask, and face shield.



- Disinfect instrument surfaces per scientific literature and manufacturer documentation using a disinfectant appropriate for the potential agents and the instrument.[21] Perform this disinfection regularly or as recommended.
- Fill using mechanical devices (e.g., transfer pipettes). Never decant (pour).[22] This should be easily met if the procedure is followed as described wherein a pipette is used.
- Handle sample trays and sample plates with caution, and cover them when not being sampled to prevent spillage.[23]
- Secure specimen containers, culture tubes, shell vials, and other cylindrical vessels in racks whenever possible.[24]
- If spills occur, immediately disinfect work surface and instrument surfaces per scientific literature and manufacturer documentation using a disinfectant appropriate for the potential agents and the instrument. For spills that occur outside of a BSC and involve agents that pose a risk via the inhalation route, consider leaving the area immediately and not reentering for 30 minutes (air

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change rate ≥12 air changes per hour) to 60 minutes (air change rate <12 air changes per hour or unknown).[25]
- Use commercial plastic-backed bench paper in BSCs and on laboratory work benches to absorb falling droplets.[26]
- Consider effluents of clinical analyzers to be contaminated. One could include used saline/colony solutions and reagent devices as effluents.[27]
- While handling, storing, and transporting effluents, use containers constructed to contain all contents and prevent leakage.[28]
- Dispose of effluents per federal, state, and local regulations.[29]

It is likely that a bacteriology lab using such an instrument already has many of these biosafety controls in place, but analyzing the procedure and instrument using this method or others ensures that laboratory management have considered important associated hazards and recommended controls to ensure the safety of lab staff. This is important for all testing in a laboratory and especially important if the procedure may involve large volumes of specimen,[30] concentrated/cultured specimen, or suspected biological agents with low inhalation infectious doses. Try assessing your lab's instruments and procedures with BPHL's Instrument/Method Worksheet or other assessment tools and see how your current biosafety controls compare to what is recommended.

References.

1. APHL Template for Public Health Laboratory Risk Assessment for Ebola Virus Disease (EVD) Testing, https://www.aphl.org/programs/preparedness/documents/aphl-template.pdf –page 6; CDC (Centers for Disease Control and Prevention) MMWR (Morbidity and Mortality Report) Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories, http://www.cdc.gov/mmwr/pdf/other/su6101.pdf – page 9; U.S. Department of Health and Human Services (HHS)/CDC/National Institutes of Health (NIH) Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, http://www.cdc.gov/biosafety/publications/bmbl5/ – page 36
2. CDC MMWR Guidelines for Safe Work Practices – page 8
3. APHL Template for Public Health Laboratory Risk Assessment for Ebola Virus Disease (EVD) Testing – pages 5 and 6; CDC MMWR Guidelines for Safe Work Practices – pages 9 and 28; BMBL – page 14; CDC Brucellosis Reference Guide: Exposures, Testing, And Prevention (Updated February 2017), https://www.cdc.gov/brucellosis/pdf/brucellosi-reference-guide.pdf (page 10) states that "widespread aerosol generating procedures include, but are not limited to: centrifuging without sealed carriers, vortexing, sonicating, or accidents resulting in spillage or splashes (i.e. breakage of tube containing specimen)." One could expect that pouring is like accidents resulting in spillage or splashes.
4. APHL Template for Public Health Laboratory Risk Assessment for Ebola Virus Disease (EVD) Testing – page 6; CDC MMWR Guidelines for Safe Work Practices – pages 8 and 28; BMBL – page 14; CDC Brucellosis Reference Guide: Exposures, Testing, And Prevention (page 10) states that "other manipulations such as automated pipetting of a suspension containing the organism, grinding the

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specimen, blending the specimen, shaking the specimen or procedures for suspension in liquid to produce standard concentration for identification may require further investigation (i.e. inclusion of steps that could be considered major aerosol generating activities)."
5. CDC MMWR Guidelines for Safe Work Practices – page 25
6. BMBL – page 14
7. CDC Guidance for U.S. Laboratories for Managing and Testing Routine Clinical Specimens When There is a Concern About Ebola Virus Disease, http://www.cdc.gov/vhf/ebola/healthcare-us/laboratories/safe-specimenmanagement.html
8. CDC MMWR Guidelines for Safe Work Practices – page 65
9. CDC MMWR Guidelines for Safe Work Practices – page 29
10. CDC MMWR Guidelines for Safe Work Practices – page 29
11. CDC MMWR Guidelines for Safe Work Practices – page 29
12. CLSI (Clinical and Laboratory Standards Institute) M29-A4 (Vol. 34 No. 8) Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition, https://clsi.org/standards/products/microbiology/documents/m29/ – page 33
13. CDC Guidance for U.S. Laboratories for Managing and Testing Routine Clinical Specimens When There is a Concern About Ebola Virus Disease
14. A complete list of select agents can be found at http://www.selectagents.gov/SelectAgentsandToxinsList.html.
15. American Biological Safety Association (ABSA) Risk Group Database at https://my.absa.org/tiki-index.php?page=Riskgroups; https://www.canada.ca/en/public-health/services/laboratory-biosafetybiosecurity/pathogen-safety-data-sheets-risk-assessment/brucella-b-abortus-bcanis-b-melitensis-b-suis-material-safety-data-sheets-msds.html; BMBL – page 127
16. CDC Brucellosis Reference Guide: Exposures, Testing, And Prevention (page 10) includes "enriched material (e.g., a *Brucella* isolate, positive blood bottle)" and "reproductive clinical specimen (e.g., amniotic fluid, placental products)" as specimen types that may contain a high concentration of *Brucella*
17. APHL ASM Clinical Laboratory Preparedness and Response Guide (Blue Book), https://www.aphl.org/aboutAPHL/publications/Documents/WORK_BlueBook.pdf – page 140; CDC MMWR Guidelines for Safe Work Practices – page 63
18. CDC MMWR Guidelines for Safe Work Practices – pages 33, 66, 69, and 73; CLSI M29-A4 – page 21; BMBL – pages 36 and 37
19. CDC MMWR Guidelines for Safe Work Practices – pages 33, 66, 69, and 73
20. CDC MMWR Guidelines for Safe Work Practices – page 17
21. CLSI M29-A4 – page 63
22. CDC MMWR Guidelines for Safe Work Practices – pages 33, 66, 69, and 73
23. CDC MMWR Guidelines for Safe Work Practices – pages 33, 66, 69, and 73
24. CDC MMWR Guidelines for Safe Work Practices – page 28
25. CLSI M29-A4 – page 72
26. CDC MMWR Guidelines for Safe Work Practices – page 29
27. CDC MMWR Guidelines for Safe Work Practices – pages 33, 66, 69, and 73
28. CLSI M29-A4 – page 59
29. CDC MMWR Guidelines for Safe Work Practices – pages 33, 66, 69, and 73
30. CLSI M29-A4 – pages 43 and 73

Biosafety risk assessment and lab biosafety training

The Bureau of Public Health Laboratories biosafety outreach officer (BOO) is currently offering a course in biosafety risk assessment and laboratory biosafety to clinical laboratory institutions. The training consists of two sessions that are approximately one hour each and offered on-site at no charge to the facility. The first session discusses biosafety risk assessment and the second session focuses on biosafety in the clinical laboratory.

Biosafety risk assessment is a systematic process of evaluating the potential risks involved in a laboratory procedure and determining the measures needed to manage any gaps or risks identified. The BOO has created standard operating procedures and resource documents to assist clinical hospital laboratories in biosafety risk assessment and laboratory biosafety. This session will train clinical laboratory personnel how to use these documents to perform risk assessments in their laboratory.

The second session is for anyone who works in the laboratory or is responsible for a safe working environment. Topics include general laboratory biosafety, the use of biological safety cabinets (BSCs), choosing correct personal protective equipment, proper use and removal of gloves, and spill cleanup.

For more information or to schedule training, contact Ed Kopp at 813-233-2260 (Edgar.Kopp@flhealth.gov).

Chemical Threat (CT) Preparedness Training

The CT laboratory coordinators continue to reach out to the health and medical community by offering training for CT preparedness at hospitals and county health departments (CHDs). This training covers chemical terrorism awareness and the collection of clinical specimens after a chemical terrorism event. Hospital and CHD staff play an important role in the response to a chemical exposure event when clinical specimens are collected for analysis. For your convenience and to increase participation, this training can be presented at your facility. Each course lasts approximately one hour with one 15-minute break between courses. Training manuals, "hands-on" exercise materials, and CT preparedness kits will be provided. This training is recommended for physicians, nurses, epidemiologists, emergency department personnel, phlebotomists, hospital and health department laboratory personnel and others who may collect clinical specimens. Contact the CT laboratory coordinators in your region for more information (see the Bureau of Public Health Laboratories Directory for contact information).

Laboratory Response Network (LRN) Training—Biological Defense

The Bureau of Public Health Laboratories is currently offering an LRN sentinel laboratory training course at no cost at your facility. This training follows the American Society for Microbiology (ASM) Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases. Scheduling the training at your facility is a relatively easy process. Determine when you would like to have the training and how many people will be attending. A time will be set up that is convenient for all. The training materials are provided as well as the biodefense reference manuals for your laboratory.

The training syllabus includes: an overview of the LRN; biosafety risk assessment and biosafety for the clinical laboratory; the ASM protocols for ruling out potential bioterrorism agents and how to refer a sample to the state LRN Public Health Reference Laboratory when a bioterrorism agent cannot be ruled out; and an introduction to the CDC Select Agent Program.

Please contact Betty Wheeler at 904-791-1568 (Betty.Wheeler@FLhealth.gov) to schedule a class for your facility.

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