Laboratory Practices

September 24, 2020
Laboratory Exposures in Minnesota, 2002–2018

- 31 Laboratory Exposures
  - 17 *Brucella* exposures, ≥ 138 people exposed, 2 lab acquired infections
  - 5 *Burkholderia*, 1 *Coxiella*, 5 *Francisella*, 2 *Coccidioides*, 1 *Histoplasma*
MLS Labs Need Biosafety Training

- Training approaches
  - Web training on Risk Assessments
  - In-person regional conferences
    - 1st year just laboratorians
    - 2nd and 3rd year laboratorians and infection control practitioners
  - Site visit assessments
  - Yearly Challenge Set
Biosafety Risk Assessment

- Examines *likelihood* and *consequence* of exposure
- Specimen collection to disposition
- Patient admission to discharge
- Mitigate risks
  - Risk is never zero
Challenges in the Clinical Setting

- Risk of Samples is unknown
- Unfamiliar with agent (rare agents)
  - Work conducted on open bench before risk is known
- Lack of time or money for training (limited staff)
  - Unsafe practices
  - Assumption that BSC and PPE are effective
- Lack of management support
  - PPE usage not always enforced
- High stress
  - Critical nature of work
  - High workload and fast pace
- Limited staff and resources leads to more stress
  - High workload, insufficient BSCs, facility/infrastructure issues
Hierarchy of Controls

- **Elimination**: Physically remove the hazard
- **Substitution**: Replace the hazard
- **Engineering Controls**: Isolate people from the hazard
- **Administrative Controls**: Change the way people work
- **PPE**: Protect the worker with Personal Protective Equipment

*Lab practices are included here.*
Engineering Controls
What is Wrong?
How Does a Class II A2 BSC Work?

**Class II, Type A2**
Air In-flow 70% Recirculated vs. 30% Exhausted

- **HEPA Filtered Air**
- **Contaminated Worksurface Air**
- **Contaminated Room Air**
BSC Best Practices

- Turn on BSC and run for at least 5 minutes
- Decontaminate work surface before and after
- Slow arm movements, perpendicular to the sash
- Minimize moving in and out of the BSC
  - Work clean to contaminated
  - Don’t over load the BSC
  - Work at least 4 inches inside the grill

Preferred BSC operating location:
- Isolated from other work areas
- Removed from high traffic areas
- Away from laboratory entry doors
- Away from lab HVAC exhaust and supply vents
- 12-14” away from ceiling and walls
Administrative Controls
- Standard Microbiology Practices
- Standard Operating Procedures
- Leadership
- Biosafety Manual
- Creating a “Culture” of biosafety
High Risk Activities Identified

- Sniffing plates
- Generating aerosols
- Centrifuging /vortexing
- Making slides
- Inoculating biochemicals
- Not using or improper use of BSC
Aerosols
Procedures That May Generate Aerosols

- Performing catalase test
- Inoculating biochemicals
- Aspirating blood from blood culture bottles
- Pipetting
- Mixing
- Centrifugation
- Grinding
- Vortexing
- Pouring

- Opening lyophilized cultures
- Flaming loops
- Sonicating
- Loading syringes
- Tracheal intubation
- Non-invasive ventilation
- Wound manipulation
- Nebulizer treatment
Biothreat Agent Identification

**Gram Negative Bacilli/Coccobacilli Rule-Out Algorithm**

1. Slow growing Gram negative bacilli/coccobacilli
2. Growth on MAC?
   - **YES**
     - Catalase positive?
       - **YES**
         - Indole negative?
           - **YES**
             - Oxidase positive?
               - **YES**
                 - No hemolysis on BAP?
                   - **YES**
                     - Follow ASM *B. pseudomallei* guidelines
                   - **NO**
                     - Urea negative?
                       - **YES**
                         - Follow ASM *Y. pestis* guidelines
                       - **NO**
                         - Satellite negative?
                           - **YES**
                             - Follow ASM *Brucella* guidelines
                           - **NO**
                             - Gray, translucent, non-hemolytic colonies on BAP?
                               - **YES**
                                 - Follow ASM *B. mallei* guidelines
                               - **NO**
                                 - Satellite negative?
                                   - **YES**
                                     - Follow ASM *F. tularensis* guidelines
3. **NO OR POOR GROWTH**
   - Catalase positive?
     - **NEGATIVE OR WEAK POSITIVE**
     - Oxidase negative?
       - **YES**
         - Grows better on CHOC than BAP?
           - **YES**
             - Satellite negative?
               - **YES**
                 - Follow ASM *F. tularensis* guidelines
A trigger point is a recognized combination of diagnostic findings that can be used to determine when to heighten the precautions or conditions that a sample or culture is handled under.

For example a trigger point would be used to determine when to begin working with an organism in a biological safety cabinet.
Some Trigger Points

- Slowly growing, tiny colonies at 24–48 hours with Gram stain showing Gram-negative rods or Gram-negative coccobacilli
- Slow growth in blood culture bottles (i.e., positive at ≥48 hours), with Gram stain showing small Gram-negative rods or Gram-negative coccobacilli

Some Trigger Points

- Growth only on chocolate agar
- Rapid growth of flat, nonpigmented, irregular colonies with comma projections and ground-glass appearance
- Gram stain showing boxcar-shaped, Gram-positive rods with or without spores

- PPE must be used properly to provide protection
- PPE can be used to build redundancy in protection
- PPE only protects the person wearing it, the hazard is still present
MALDI-TOF Risk Factors

- Many labs greatly reduce or stop doing Gram stains when relying on the MALDI-TOF
  a) Gram stain morphology is perhaps the most important trigger point
- It is difficult to use different reference software on the MALDI-TOF
  a) Most labs have CA and RUO software but they cannot be used simultaneously, and the lengthy steps are impractical under current workloads*
  b) At MDH we tested *B. thuringiensis* on the security database and it identified as *B. anthracis*, the RUO called it *B. cereus*, all with scores above 2.0
  c) MDH has also had the security database identify *B. anthracis* but the RUO identified *B. cereus* (although with a lower score)
- Open bench sample preparation
  a) Samples are prepped on an open bench before they are potentially identified as something more dangerous