Biosafety and Risk Assessment for New Molecular Methods

Michael Pentella, PhD, D(ABMM)
Michael.pentella@state.ma.us
Director, Massachusetts Bureau of Laboratory Sciences

Disclosures

- Dr. Pentella has no financial relationships with any manufacturer mentioned in this presentation

Risk Assessment: Predict, Identify, & Mitigate Risk

- Consider:
  - Agent involved
  - Agent Concentration in sample/suspension
  - Suspension Volume
  - Generation of Aerosols, Droplets or Droplet Nuclei
  - Protocol Complexity
  - Use of Sharps

When to perform the risk assessment?

- Before work begins
- Whenever there is a move or renovation
- New employees
- Change in reagents
- **New equipment**
- Repeat when changes are to made in practices, employees or facilities

Before the instrument arrives

- Determine the biosafety level for placement of the new equipment
  - Think about service of the equipment
- Include safety in the new equipment implementation checklist
- Waste disposal process

Steps to complete Risk Assessment

A. Identify agent hazards and perform an initial risk assessment, place the findings in writing

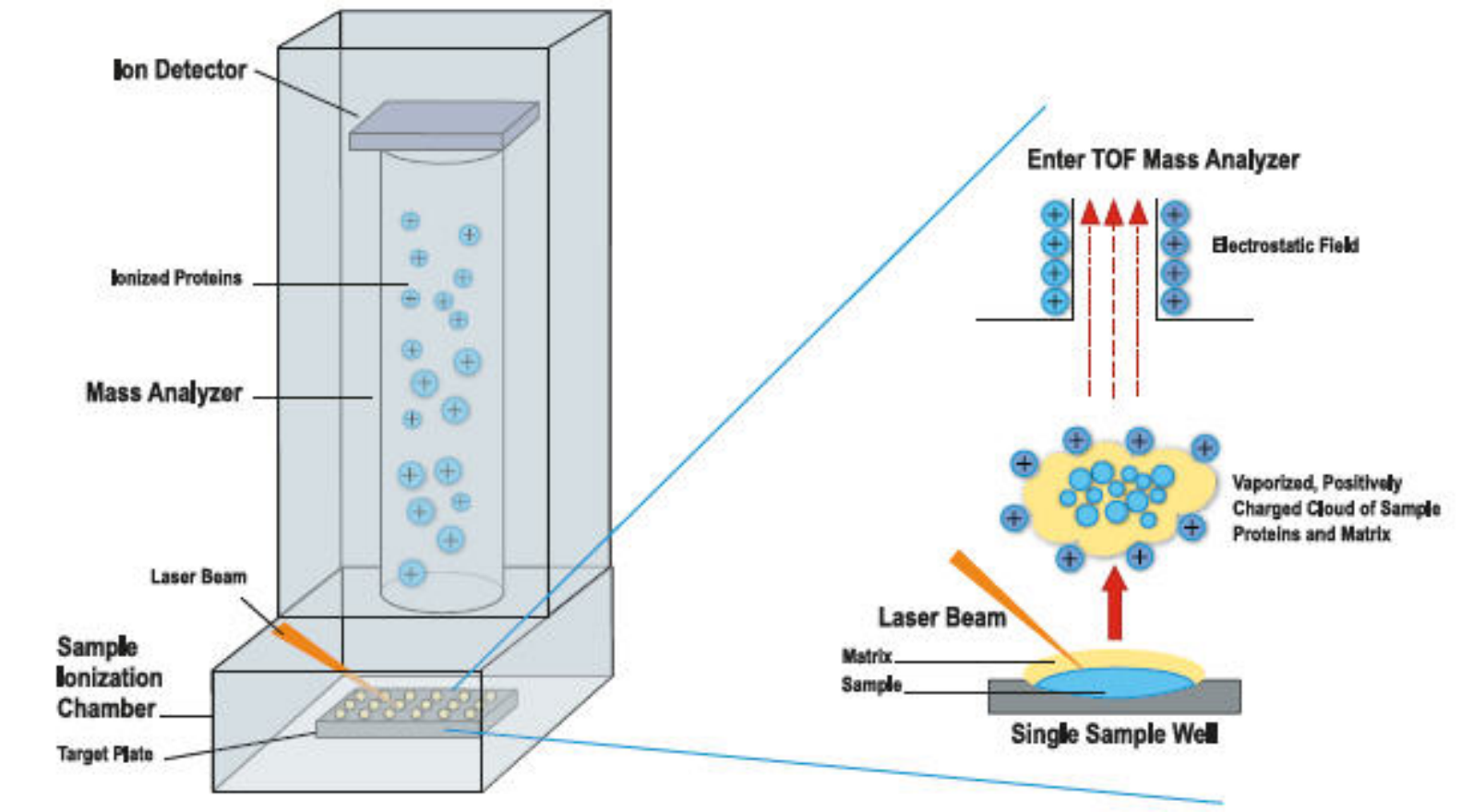
B. Identify lab procedure hazards, place the findings in writing

C. Review assessment with staff and management

Example: Maldi-TOF

- Matrix Assisted Laser Desorption Ionization-Time of Flight) Mass Spectrometry to measure a unique molecular fingerprint of an organism
- Purchased in 2014 and planned to use for both BSL-2 and BSL-3 labs

Closed system but what happens inside the box?



https://www.google.com/search?q=maldi+tof+bacterial+identification&rlz=1C1CHFX_enUS490US491&espv=2&biw=1400&bih=915&tbm=isch&imgil=5eOpCOpFQZnmNM%253A%253BJv0G3IKn2cd6QM%253Bhttp%25253A%25252F%25252Fwww.mayomedicallaboratories.com%25252Farticles%25252Fcommunique%25252F2013%25252F01-maldi-tof-mass-spectrometry%25252F&source=iu&pf=m&fir=5eOpCOpFQZnmNM%253A%252CJv0G3IKn2cd6QM%252C_&usg=__DtpZvXOI2Ndtnuj2tIg4bz7Hlxo%3D&ved=0CEsQyjc&ei=Z4lKVajgJuLdsATt0oDYCw#imgrc=5eOpCOpFQZnmNM%253A%3BJv0G3IKn2cd6QM%3Bhttp%253A%252F%252Fwww.mayomedicallaboratories.com%252Fimages%252Farticles%252Fcommunique%252F2013%252F01-maldi-tof-mass-spectrometry%252Fmaldi-tof.jpg%3Bhttp%253A%252F%252Fwww.mayomedicallaboratories.com%252Farticles%252Fcommunique%252F2013%252F01-maldi-tof-mass-spectrometry%252F%3B611%3B344

Risk Assessment of MALDI workflow

- In considering the use of MALDI-ToF for pathogenic bacteria:
  - Where in the work flow is the MALDI utilized?
  - What are the biosafety measures for the analysis?
  - The MALDI would be either exclusively used in a BSL-3 OR pathogens inactivated in BSL-3 prior to moving to BSL-2

General Steps in the MALDI-ToF Process

Steps

1. Suspect organism
2. Select colony or broth
3. **Preparation of sample for target plate**
4. Place on instrument
5. Generate profile spectrum

Risk Assessment

1. Determine Biosafety Level
2. Risk of splash/splatter
3. **Risk of splash/splatter**
4. Closed system - no risk at time of testing
5. No risk

Preparation of sample for target plate

- 3 Options:
  1. **Direct method**: colonies are applied directly on target as a thin smear then covered with alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix
  2. **Extended method**: colonies are applied directly on target as a thin smear then covered with 70% formic acid and finally HCCA matrix
  3. **Tube extraction method**: organisms are extracted by ethanol, formic acid, and acetonitrile prior to creating a smear and overlaying with HCCA matrix.

Drawbacks of inactivation

- Inactivation may alter the physicochemical properties of the pathogen leading to protocol specific changes of the mass spectral fingerprints.
- Changes vary depending on inactivation method used.
- Inactivation can affect the accuracy of identification.
- Inactivation is relevant in the process of compilation of commercial databases.

Mycobacteria is more complicated…

- Protocol
  - Heat kill Mycobacteria at 100°C for 30 minutes
  - Extraction using 100% ethanol, silica beads, acetonitrile, and 70% formic acid
- Must document that the protocol is successful

Colonies on Solid Media vs. Broth

- Solid culture: Add 300 µL water to a screw cap microtube, then add colonies with a loop.
- Liquid culture: Pipette 1.2mL of the liquid culture into a screw cap microtube. Centrifuge in sealed rotor at >13,000 rpm for 2 minutes. Remove supernatant with a pipette.
  - Add 300 µL water to the tube and vortex 5-10s to mix.
- The next step for both is the 30 minute heat kill and they are treated the same from there.

TB Preparation of Extraction

Procedure	Potential Hazards	Process Step	Control
Preparation of Organism for MALDI-ToF Testing	Aerosolization/ Splash/ Splatter	1. 300 µL sterile water and 2 col of suspect TB 2. Heat at 100 degrees celsius for 30 min 3. Add 900 µL of 100% ethanol and vortex 4. Centrifuge 13,000 RPM for 2 min 5. Remove supernatant with pipette-repeat steps 4&5 6. Move to BSL-2 lab 7. Air Dry pellet at room temp 8. Add Silica beads + 25 µL Acetonitrile 9. Vortex 1 min 10. Add 25 µL of 70% formic acid and vortex 11. Centrifuge as above	1. BSL-3 using BSC, PPE and screwed capped tube with o-ring 2. Viability study confirmed kill 3. Use goggles 4. Use sealed rotor 5. BSL-3 practices 6. BSL-2 practices 7. BSL-2 practices 8. BSL-2 practices 9. BSL-2 practices 10. Use chemical precautions

Heat Inactivation Study A – 8 wks

	N=	No heat Growth Control	No heat Growth Control	# Heated at 95-100°C for 30 min w/growth:		# Heated at 95-100°C for 60 min w/growth:	
		MGIT	7H10	MGIT	7H10	MGIT	7H10
MTC	17	17	17	0	0	0	0
M. bovis	3	3	2*	0	0	0	0
MAC	1	1	1	0	0	0	0
M. kansasii	1	1	1	0	0	0	0
M. fortuitum	1	1	1	0	0	0	0
M. abscessus	1	1	1	0	0	0	0
M. gordonae	1	1	1	0	0	0	0
Sterile water	1	0	0	0	0	0	0

*1 M. bovis isolate unheated grew only in MGIT at 7 weeks

Heat Inactivation Study B–11 wks

using100 degrees Celsius for 30 min

Isolate	No. Strains	Viability Media	Final Result 11 week incub
MTBC	11	MGIT	No Growth
MAC	1	MGIT	No Growth
M. abscessus	1	MGIT	No Growth
M. fortuitum	1	MGIT	No Growth
Untreated MTBC Growth Control	2	MGIT	Growth week 1
Sterile water	1	MGIT	No Growth

Verification of Results on MALDI

Organism	Number Tested	Number Verified
M. tuberculosis complex	29	29
M. avium complex	64	64
M. abscessus	39	38 (1 *M. cheloni*?)
M. gordonae	20	20
M. fortuitum	13	12 (1 *M. mageritense*?)
M. kansasii	16	16
M. xenopi	6	6
M. marinum	5	5
M. chelonae	6	6

Both colonies and broth cultures used for validation – only total number provided

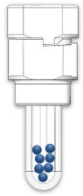
*? Isolates submitted for Sequencing

Covaris M220 Focused-ultrasonicator using Adaptive Focused Acoustics (AFA)

- Treatment system delivers acoustic energy to the sample
- Safety System protects users from contact with acoustic energy
- Computer Software provides control
- Covaris AFA Mycobacteria Preparation
  - Reduces prep time to 2 min per sample
  - Requires fewer manipulations
  - Uses formic acid:acetonitrile:water (35:50:15)

Covaris Protocol 4 minute / 4 step / 0 open-close

STEP 1:
Centrifuge microTUBE prefilled with beads

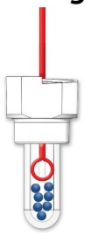


Centrifuge at 300 RCF For 10 seconds to pellet beads

STEP 2:
Add reagent

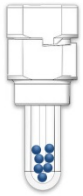
Add 100 uL of Extraction Solvent

STEP 3:
Add biomass
Add reagent



Add 1 µL inoculation loop with biomass

STEP 4:
AFA



AFA at 40W PIP, 50% DC, 200 CPB for 120 sec

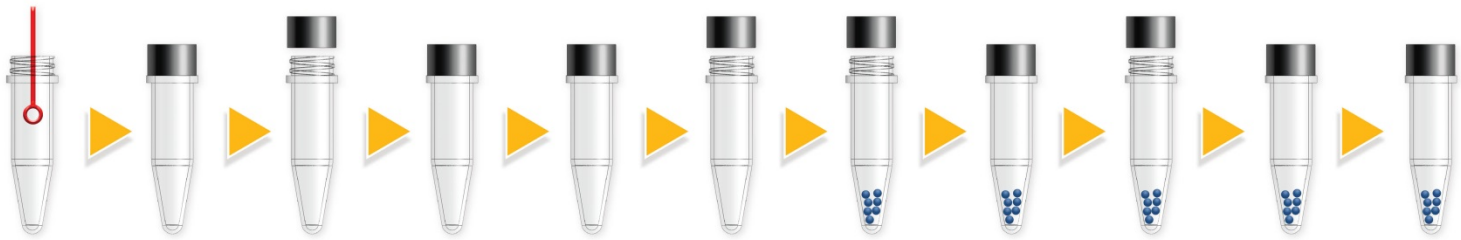
Centrifuge 13,000 RCF for 2 min

MALDI-TOF

Spot 1 µL of supernatant on MALDI target.

Conventional Protocol 45 minute / 10 step / 3 open-close

MALDI-TOF



Add colony + H_2O

Heat 100°C 30 min

Add EtOH

Vortex

Spin 2X

Decant, dry pellet

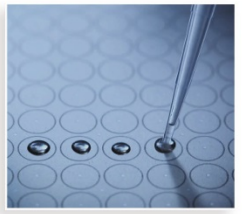
Add beads

Vortex

Add formic acid

Vortex

Spin



Spot 1 µL of supernatant on MALDI target.

Covaris M220 Viability study Phase I

Organism	No. Isolates	Growth – **Heated for 30 min at 100 C in Covaris with water	Growth – **Not Heated Covaris AFA prep
MTB Complex	11	0	0
M. avium complex	1	0	0
M. fortuitum complex	1	0	0
M. abscessus	1	0	0
Sterile Water	1	0	0
Untreated MTBC* (no treatment, positive control)	2	2 Growth in MGIT at one week	0

*Two MTB complex strains were run as a growth control
**All cultures held in MGIT for 11 weeks

Covaris M220 Phase II

- Evaluating sample prep method on 75 clinical isolates prospectively to determine if comparable results for identification
- To date, have completed over 30 isolates (5 MTB complex)

Biosafety Program Overview

1. Perform risk assessments
2. Select safety practices based on risk assessment
3. Write biosafety competencies
4. Provide safety orientation and ongoing training
5. Perform regular audits and monitor compliance (safety committee)
6. Discuss with Occupational Health Program
7. Create a culture of safety

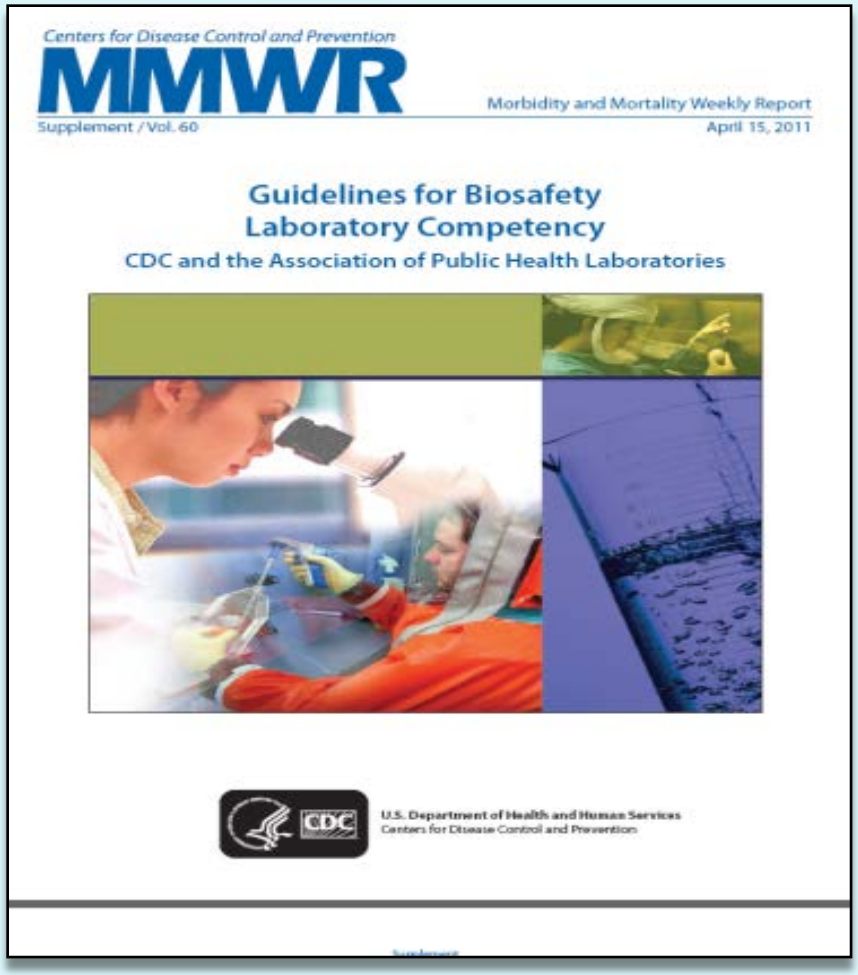
Lab Safety Practices

- Personal Protective Equipment
- Disinfectant
  - Daily Disinfectant BSC, Counters and Centrifuge
- Capped Centrifuge tubes
- Splash Proof Containers
- Use Of UV lights
- Use disposable loops
- Allow slides to dry in BSC
- Spill Clean-up procedure

http://www.cdc.gov/mmwr/preview/mmwrhtml/su6101a1.htm?s_cid=su6101a1_w

Biosafety Competencies

- Connect competencies to required skills
  - Skill Domain I: Potential hazards
  - Skill Domain II: Hazard controls
  - Skill Domain III: Administrative controls
  - Skill Domain IV: Emergency preparedness and response



Intent of Competencies

- Define essential competencies needed by laboratory personnel to work safely with biologic materials and other hazards commonly found in biologic laboratory
- Reduce the risk of exposures at all levels
- Provide essential base-line information for a format to develop facility specific competencies
- Target audience is the laboratorian

Tasks to Link to Biosafety Competencies

- Review the competencies
- Select the competencies from each domain that are applicable to the lab based on the risk assessment

Laboratory Biosafety Competency Assessment Form – Entry Level

Name:_____Date:_____

Skill Domain	Biosafety Competency – abbreviated from the Guidelines for Biosafety Laboratory Competency	Competency Level Ranking	Importance	Frequency	Comment
I Bio 3a	Describe PPE used when handling biologic materials				
II PPE 1	List PPE required for general laboratory entry				
II PPE 2	Describe specific PPE to be used for each procedure				
II PPE 4a	Demonstrate proper donning and doffing of gloves and gown				
II PPE 4b	Describe the limitations of PPE				
II Decon 3e	Describe routine surface decontamination procedures				
II Decon 1	Describe waste segregation procedures				
II Decon 2a	Describe proper disposal of different types of biological waste				
III Occ Health 4	Describe signs and symptoms following exposure				
III Risk Mgmt 3	Describe the risk assessment process				
IV Emer Resp 2	Describe reporting requirements for emergencies				
IV Drills	Participate in drills and exercises				

Reviewed by:_____Date:_____

Legend:

Competency Level: Entry Level: Laboratory Scientist or Medical Technologist; **Midlevel**: Chief/Lead Scientist or Medical Technologist, Laboratory Specialist or Laboratory Manager; **Senior Level**: Laboratory Manager, Chief Technologist, or Hospital or Clinical Director.

Competency Level Ranking:

1 = Awareness: You have no training or experience.

2 = Basic: You have received basic training.

3 = Intermediate: You have repeated successful experiences.

4 = Advanced: You can perform the actions associated with this skill without assistance.

5 = Expert: You can train others in this competency

Importance to the Position:

1 = An important competency for position

2 = Neutral

Frequency Competency Performed:

D = Daily W = Weekly M = Monthly R = Rarely A = As Needed

Reference: Delany J., J. Rodriguez, D. Holmes, M. Pentella, K. Baxley, and K. Shah. CDC/APHL Laboratory Biosafety Competencies for the BSL-2, BSL-3, and BSL-4 Laboratories. MMWR, Supplement, April 15, 2011. http://www.cdc.gov/mmwr/pdf/other/su6002.pdf

Laboratory Biosafety Competency Assessment Form – Midlevel Name:_____Date:_____

Skill Domain	Biosafety Competency – abbreviated from the Guidelines for Biosafety Laboratory Competency	Competency Level Ranking	Importance	Frequency	Comment
I Bio 3a	Demonstrate correct use of PPE for handling bio materials				
II PPE 1	Monitor availability of PPE				
II PPE 2	Demonstrate use of specific PPE required for each procedure				
II PPE 4a	Demonstrate proper donning and doffing of gloves and gown				
II PPE 4b	Describe the limitations of PPE				
II Decon 3e	Implement routine surface decontamination procedures				
II Decon 1	Implement waste segregation procedures				
II Decon 2a	Demonstrate proper disposal of different types of bio waste				
III Occ Health 4	Describe signs and symptoms following exposure				
III Risk Mgmt 3	Conduct a risk assessment				
IV Emer Resp 2	Implement plans and policies for reporting emergencies				
II.D-3c	Describe proper use of autoclave				
IV Drills	Implement drills and exercises				

Legend:

Competency Level: Entry Level: Laboratory Scientist or Medical Technologist; **Midlevel**: Chief/Lead Scientist or Medical Technologist, Laboratory Specialist or Laboratory Manager; **Senior Level**: Laboratory Manager, Chief Technologist, or Hospital or Clinical Director.

Competency Level Ranking:

1 = Awareness: You have no training or experience.

2 = Basic: You have received basic training.

3 = Intermediate: You have repeated successful experiences.

4 = Advanced: You can perform the actions associated with this skill without assistance.

5 = Expert: You can train others in this competency

Importance to the Position:

1 = An important competency for position

2 = Neutral

Frequency Competency Performed:

D = Daily W = Weekly M = Monthly R = Rarely A = As Needed

Reference: Delany J., J. Rodriguez, D. Holmes, M. Pentella, K. Baxley, and K. Shah. CDC/APHL Laboratory Biosafety Competencies for the BSL-2, BSL-3, and BSL-4 Laboratories. MMWR, Supplement, April 15, 2011. http://www.cdc.gov/mmwr/pdf/other/su6002.pdf

Laboratory Biosafety Competency Assessment Form – Senior Level

Name: _____ Date: _____

Skill Domain	Biosafety Competency – abbreviated from the Guidelines for Biosafety Laboratory Competency	Competency Level Ranking	Importance	Frequency	Comment
I Bio 3a	Evaluate PPE for handling bio materials				
II PPE 1	Determine PPE required for general lab entry				
II PPE 2	Determine procedures for use of specific PPE				
II PPE 4a	Develop procedures for personnel to comply with sequence				
II PPE 4b	Ensure personnel's knowledge of limitations of PPE				
II Decon 3e	Develop routine surface decontamination procedures				
II Decon 1	Establish waste segregation procedures				
II Decon 2a	Develop protocols for biological waste disposal				
III Occ Health 4	Ensure personnel's knowledge of signs and symptoms				
III Risk Mgmt 3	Ensure risk assessment is performed				
IV Emer Resp 2	Develop plans and policies for reporting emergencies				
II.D-3c	Describe proper use of autoclave				
IV Drills	Develop drills and exercises				

Reviewed by: _____ Date: _____

Legend:

Competency Level: Entry Level: Laboratory Scientist or Medical Technologist; **Midlevel**: Chief/Lead Scientist or Medical Technologist, Laboratory Specialist or Laboratory Manager; **Senior Level**: Laboratory Manager, Chief Technologist, or Hospital or Clinical Director.

Competency Level Ranking:

1 = Awareness: You have no training or experience.

2 = Basic: You have received basic training.

3 = Intermediate: You have repeated successful experiences.

4 = Advanced: You can perform the actions associated with this skill without assistance.

5 = Expert: You can train others in this competency

Importance to the Position:

1 = An important competency for position

2 = Neutral

Frequency Competency Performed:

D = Daily W = Weekly M = Monthly R = Rarely A = As Needed

Reference: Delany J., J. Rodriguez, D. Holmes, M. Pentella, K. Baxley, and K. Shah. CDC/APHL Laboratory Biosafety Competencies for the BSL-2, BSL-3, and BSL-4 Laboratories. MMWR, Supplement, April 15, 2011. http://www.cdc.gov/mmwr/pdf/other/su6002.pdf

Link RA to Competencies

Process Step	Control	Competency
1. 300 µL sterile water and 2 col of suspect TB	1. BSL-3 using BSC and PPE	1. Work in BSL-3, use of BSC, PPE
2. Heat at 100 degrees celsius for 30 min	2. Viability study confirmed kill	
3. Add 900 µL of 100% ethanol and vortex	3. Use goggles	3. PPE required
4. Centrifuge 13,000 RPM for 2 min	4. Use sealed rotor	4. Use of centrifuge
5. Remove supernatant with pipette-repeat steps 4&5	5. BSL-3 practices	5. Work in BSL-3
6. Move to BSL-2 lab		
7. Air Dry pellet at room temp	6. BSL-2 practices	6. PPE for BSL-2
8. Add Silica beads + 25 µL Acetonitrile	7. BSL-2 practices	
9. Vortex 1 min	8. BSL-2 practices	8. Use of vortex
10. Add 25 µL of 70% formic acid and vortex	9. BSL-2 practices	
11. Centrifuge as above	10. Use chemical precautions	

Centers for Disease Control and Prevention
MMWR
Morbidity and Mortality Weekly Report
Supplement / Vol. 64 / No. 1
May 15, 2015

Competency Guidelines for Public Health Laboratory Professionals

CDC and the Association of Public Health Laboratories

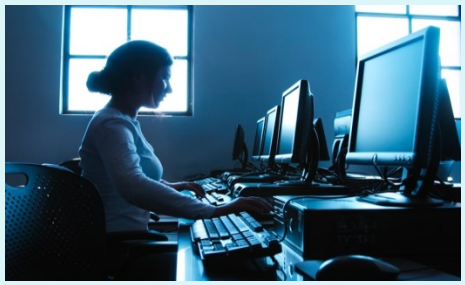
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Slide Courtesy of John Ridderhof, CDC/OPHSS/CSELS

http://www.cdc.gov/mmwr/preview/mmwrhtml/su6401a1.htm?s_cid=su6401a1_e

Perform Safety Education & Training

- Based on RA and competencies design the training that is needed.
- Determine what outside training is available and what site specific training is needed.
- Consider the best format for the training
- Write materials and exams for in house training

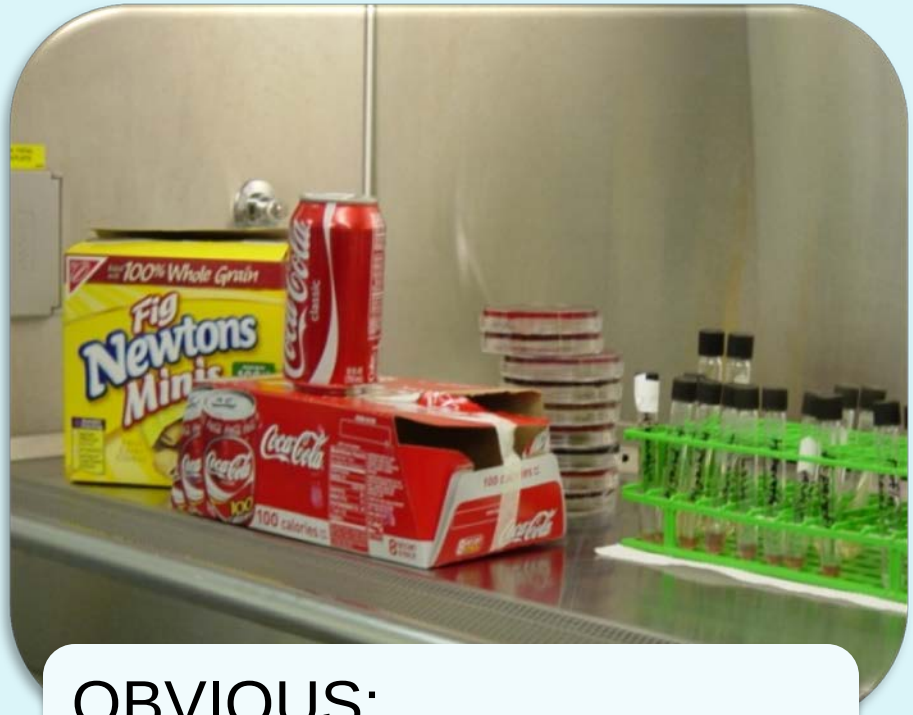


Following up on the biosafety plans

- **Exercise** the procedures
- **Audit** the program by self audits, internal audits, external audits
- **Monitor** staff and equipment performance
- **Mandate Reporting and Follow up** on accidents, incidents, and near misses
- **Revise** the plans accordingly
- **Discuss** biosafety at regular meetings

Safety Audits

UNSAFE PRACTICES



<u>OBVIOUS</u>:
Food in work area

<u>LESS OBVIOUS</u>: Boxes blocking air flow in BSC

Use a biosafety checklist

YES	NO	Standard	Resources	Comments
☐	☐	**Is basic PPE provided for all personnel working in the laboratory? (basic PPE includes gloves, laboratory coats or gowns, protective eyewear or face protection, etc.)**	http://www.cdc.gov/HAI/prevent/ppe_train.html	Any observation made during audit

APHL Biosafety Checklist

- Checklist consists of 6 sections:
  1. Risk Assessment
  2. Selection of Safety Practices
     a. Biosafety Level
     b. Engineering Controls
     c. Personal Protective Equipment (PPE)
     d. Laboratory Practices
  3. Biosafety Competencies
  4. Safety Orientation and Training
  5. Audits, Monitoring and Safety Committee
  6. Administrative Controls

http://www.aphl.org/AboutAPHL/publications/Documents/ID_BiosafetyChecklist_42015.pdf#search=tb%20biosafety

Examples of Checklist Questions

1. Has the person performing the risk assessment received training and are they experienced in risk assessments?
2. Is there a written procedure for appropriate donning and doffing PPE including laboratory coats, gloves, protective eyewear, face shields, N95 and/or PAPRs?
3. Are the Biosafety Laboratory Competencies used for annual staff reviews?

Examples of Checklist Questions

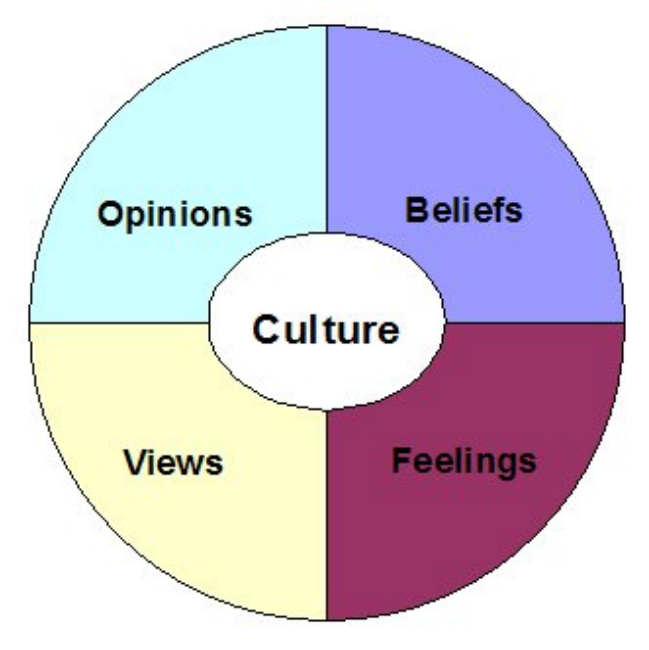
4. Do all new personnel receive safety training before they begin working in their assigned laboratory?
5. Are internal safety audits performed at least annually and after significant safety breaches?
6. Are biohazard signs posted by the entrance of laboratories where infectious agents are processed and tested and in other areas where indicated?

Address concerns from labs not impacted

- Make Biosafety a topic for lab-wide meetings
- Take every safety question/concern seriously
- Communicate about the testing so that everything is transparent.

Building a culture of safety

- Need a commitment from administration and lab leadership
- Have regular communication about safety issues



Thank you

- Massachusetts TB Lab Section: Jasmine Guillet, Paul Elvin, and Tracy Stiles