Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

Biosafety in the Mycobacteria Lab: Are you really safe?

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In your daily work, are you aware of the risk category and associated biosafety risks of all microorganisms you knowingly and may unknowingly work with?

A. Yes, I put on my biosafety risk assessment glasses daily when I enter the lab and think about all associated biosafety risks with every culture, test, and task I perform.

B. I try, but I'm not completely sure what the "risk category" and "associated biosafety risks" are for all microorganisms I may work with.

C. There is too much work to be done to think about risk categories and biosafety risk assessments unless it's spelled out in our SOP's.

D. I expect my employer to define all biosafety risks related to my job and to take responsibility for preventing lab acquired infections.

E. Answers C and D

Goal is Zero Risk

"*Persons working in clinical diagnostic laboratories are exposed to many risks ……. whether laboratorians work in microbiology or elsewhere in the laboratory, the …… (clinical) laboratory is a challenging environment. The more that laboratorians become aware of and adhere to recommended, science-based safety precautions, the lower the risk.* ***The goal of a safety program is to lower the risk to as close as possible to zero****, although zero risk is as yet unattainable as long as patient specimens and live organisms are manipulated. Protection of laboratorians, coworkers, patients, families, and the environment is the greatest safety concern*."

https://www.cdc.gov/mmwr/pdf/other/su6101.pdf 2012

WCLN 2017 Biosafety Risk Assessment Survey

- 16 of 84 (19%) labs process specimens for AFB culture
- 14 of 16 labs perform all work-up of AFB isolates in a Class II BSC using BSL-3 practices (e.g. controlled access to the area when working, decontamination of all waste, wear a solid front gown with cuffed sleeves, gloves, and a N-95 respirator/PAPR). The 2 labs that didn't respond to this question are full service hospital labs.
- 8 of 16 labs stated they were BSL-3 facilities, but only 2 labs met all the criteria listed in the BMBL 5th Edition for a BSL-3 laboratory

Why All the Fuss About Biosafety in the Mycobacteria Lab?

WISCONSIN TUBERCULOSIS STATE TOTALS							
	2010	2011	2012	2013	2014	2015	2016
CASES	55	70	71	50	48	69	40
RATE*	1	1.2	1.2	0.87	0.83	1.2	0.69
*CASES/100,000							

Low case rate and therefore likelihood of exposure is low, but pathogenicity is high. There is no safe level of exposure since inhalation exposure to as few as 1–10 organisms can cause disease.

Reasons Why Unexpected Laboratory Associated Infections (LAI's) Occur:

- Failure to perform biosafety risk assessments and use appropriate mitigations
- Failure of equipment, (e.g. disruption of directional air flow in room, inadequate volumes of air exchanges (6-12), BSC not properly maintained, etc.)
- Failure to properly train staff how to use equipment (e.g. BSC)
- Staff fail to follow SOPs, or there are no detailed SOP's (e.g. incorrect doffing of PPE, competencies are not monitored, etc.)

Does your lab provide and document in depth training on proper use of a BSC?

A. Yes and we have a detailed SOP and perform competency testing
B. Staff are trained but we do not have a detailed SOP for BSC use or documentation
C. Staff are expected to learn by observation of senior staff
D. New staff are expected to have this training in their school curriculum or previous job experience
E. We need to address this

CDC TRAIN Free Training

- *Fundamentals of Working Safely in a Biological Safety Cabinet*
- *Fundamentals of Centrifuge Safety*
- *Fundamentals of Chemical Fume Hood Safety*
- *Biosafety: So Much More than PPE*

The above courses can be found at:

https://www.train.org/cdctrain/home

LAI's May Be Complicated by Drug Resistance.

- 69 Verified Cases of Tuberculosis in Wisconsin in 2015.
- 60 patients had culture susceptibility testing performed.

***M. tuberculosis* complex First-Line Drug Susceptibility Testing**[§]	
Susceptible to all first-line drugs	49
Resistant to INH (0.2 ug/ml) only	2
Resistant to both INH concentrations	2
Resistant to rifampin only	0
Resistant to ethambutol only	0
PZA resistant	1 (*M. bovis*)
poly-resistant	1 (EMB &PZA)
Multi-drug resistant (MDR)[#]	4
non-viable, unable to perform	1
TOTAL	60

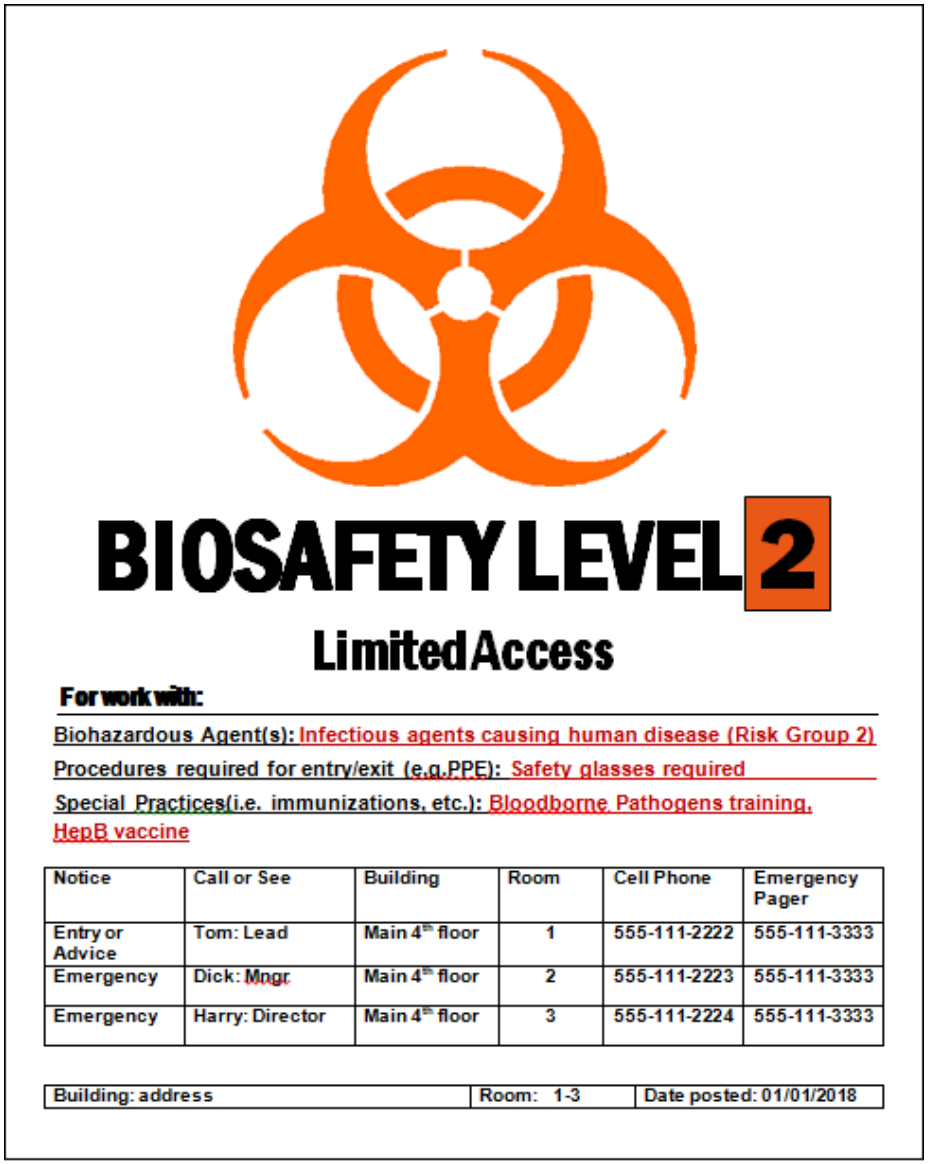
(§)TB First-Line Drugs tested: isoniazid (INH) 0.2 and 1.0 ug/ml, rifampin 1.0 ug/ml, ethambutol 5.0 ug/ml, pyrazinamide (PZA) 100 ug/ml.
(#) MDR = resistant to at least INH and rifampin.

BSL-2 w/BSL-3 Practices, or BSL-3

Since there is no safe level of exposure *M tuberculosis*, a hierarchy of controls must be employed to reduce exposures:

- Safe work practices: BSL-2, BSL-2 plus BSL-3 practices, BSL-3
- Use of containment equipment (e.g. BSC, centrifuge with sealed carriers/cups)
- Specially designed laboratory facilities with directional air flow and ideally a separate room from the general microbiology lab
- Decontamination of all lab waste (e.g. autoclave on-site, or sealed and transported in leak proof containers)
- Biohazard sign posted at entrance listing the BSL level, a responsible person's name, a phone #, and requirements to enter the space

Develop all policies and practices related to safety using a biosafety risk assessment process that is documented in the laboratory's (bio)safety manual.



BIOSAFETY LEVEL 2

Limited Access

For work with:

Biohazardous Agent(s): Infectious agents causing human disease (Risk Group 2)

Procedures required for entry/exit (e.g.PPE): Safety glasses required

Special Practices(i.e. immunizations, etc.): Bloodborne Pathogens training, HepB vaccine

Notice	Call or See	Building	Room	Cell Phone	Emergency Pager
Entry or Advice	Tom: Lead	Main 4th floor	1	555-111-2222	555-111-3333
Emergency	Dick: Mngr	Main 4th floor	2	555-111-2223	555-111-3333
Emergency	Harry: Director	Main 4th floor	3	555-111-2224	555-111-3333

Building: address	Room: 1-3	Date posted: 01/01/2018

Biohazard Sign is Posted at the Lab Entrance

2015: 48 % posted sign, BSL level and contacts included 43%

2017: 80% posted sign, BSL level and contacts included 41%

TB Lab Safety Criteria

- The mycobacteriology laboratory should be enclosed and separated by closable doors from other laboratory sections.
- Directional air flow in the room from clean to less clean is encouraged to provide zones of containment where air flows toward work spaces in which higher-risk laboratory procedures are conducted. Air handling systems within the microbiology laboratory suite should be adjusted to direct airflow from the corridor into the microbiology laboratory and from the general microbiology laboratory into separate and enclosed tuberculosis, mycology, and virology specialty laboratories.

TB Lab Safety Criteria (cont.)

- All clinical samples submitted specifically for TB testing must be handled by persons using PPE consisting of a laboratory coat and gloves
- All work must be performed in a BSC that is certified at least annually. This includes removing primary specimens from outer biohazard bags and any aliquoting for various tests in other lab sections.
- Access into the mycobacteria lab should be restricted to staff only.
- Hands-free sinks are required for biosafety level BSL-3 facilities and are recommended for BSL-2 facilities.
- Tuberculosis laboratories that manipulate cultures (e.g. ID/AST) should ideally meet BSL-3 requirements.

TB Lab Safety Criteria (cont.)

- BSL-2 facilities that elect to process clinical specimens for *MTB:*
  1. Should perform a biosafety risk assessment to determine that work with *M. tuberculosis* can be conducted safely in a separate, closed BSL-2 laboratory using BSL-3 practices and procedures
  2. Exhaust air from the room is vented to the outside of the building
  3. The laboratory director approves the practice

  NOTE: If any of these conditions cannot be met, a BSL-3 facility is recommended for culture manipulation.
- Specimens submitted for routine cultures and Gram stain, especially sputum and other respiratory specimens, may contain tubercle bacilli and must be handled with care regardless of whether or not acid-fast bacillus (AFB) cultures were ordered. A biosafety risk assessment is recommended.

TB Lab Safety Criteria (cont.)

- A solid-front, disposable gown with snug (knit) cuffs is routinely worn
- Gloves are worn whenever there is reasonable risk of contamination of skin from spray, splatter or droplets during aerosol-generating procedures a N95 respirator or PAPR is recommended and staff must comply with OSHA respiratory program requirements
- Use centrifuge with removable sealed carriers or sealed cytocentrifuge rotor/shield assembly, and move to BSC prior to opening
- With BSC running, decontaminate surface of anything in the BSC prior to removal (e.g. containers with supplies)

TB Lab Safety Criteria (cont.)

- Disinfectants – Use intermediate level disinfectant that is EPA approved and labelled tuberculocidal https://www.education.nh.gov/instruction/school_health/documents/disinfectants.pdf
- BSC - Use plastic backed absorbent toweling soaked with disinfectant on the work surface
- Decant liquids into a splash proof container - chemically disinfect or autoclave before discarding
- Doff PPE before leaving the laboratory – document training and monitor competency

  (Beak method for gloves)
  https://www.youtube.com/watch?v=BOAb_cy3HxM&feature=youtu.be
- Develop all policies and practices related to safety using a biosafety risk assessment process that is documented in the laboratory's (bio)safety manual.

- Identify the biologic agent(s)
- Identify personnel affected throughout the workflow
- Review process / SOP for any biologic risks
- Assess waste stream for biologic risks and biosecurity
- Determine the likelihood and consequences of any risk, and necessary controls.
- Develop strategy to mitigate risks
- Evaluate staff competency and utilization of PPE and safety equipment
- Review risk assessment with staff and management, and document
- Evaluate effectiveness of risk mitigation
- Review annually or reassess when appropriate

Assess Plan Mitigate Reassess

Primary Controls:

- Engineering Controls
- Personal Protective Equipment
- SOP's
- Leadership

Laboratory Changes in the Last Year (N=79)

Laboratory Change	Percent of labs that experienced
New staff*	74%
New procedure/method/instrument*	68%
New facility or facility remodel*	11%
Work with new infectious agent*	5%

***All of these impact risk and require the performance of a biosafety risk assessment. 54% performed related assessments.**

Risk Assessment Xpert® MTB/RIF Assay

GeneXpert MTB/RIF assay is a **nucleic acid amplification (NAA) test** which simultaneously **detects DNA of *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampin (RIF) (i.e. mutation of the rpoB gene)** in less than 2 hours.

Procedure	Process Step	Potential Hazards	Initial Risk Level	Control (Mitigation)	Residual Risk Level
	AFB specimens previously tested that are positive for M tb by PCR are eligible for testing by Xpert Mtb/RIF assay. Patient specimen is mixed with specimen processing reagent to deactivate it, then added to the Xpert cartridge, the lid snapped closed, the cartridge placed in the GeneXpert instrument, and all subsequent test reactions occur in the sealed cartridge. https://www.youtube.com/watch?v=Q4IE28Um3XA			**General mitigation: see facilities, training files, competency assessments and SOPs Occurrence management reports and accident reports for any unusual events**	
	Biological Agent: Mycobacteria tuberculosis	**Risk group 3 agent associated with serious human disease.**	**Medium**		
	Personnel affected: staff that work in the BSL-3 mycobacteria lab, and staff that work proximal to the GeneXpert instrument in the BSL-2 bacti lab.				

	<u>Engineering controls</u>: BSL-3 lab and BSC located in BSL-3 lab.			**BSL-3 lab is monitored continuously for proper air flow. BSC is certified annually, interior surfaces are disinfected per SOP, and staff are trained to work in BSC to optimize protection of this control**	
	<u>Process</u>: AFB specimens previously tested that are positive for M tb by PCR are eligible for testing by Xpert Mtb/RIF assay. Initial specimen processing and PCR testing is performed in a BSL-3 lab using established SOPs.	**Concentrated specimens for Xpert test contain viable M tb organisms, risk group 3. RIF resistant M tb strains may also be resistant to other anti-mycobacteria antibiotics.**	**Medium**	**All initial processing of specimens submitted for AFB testing are performed in a BSL-3 lab with appropriate SOP's, and engineering and PPE controls: BSL-3 facility, BSCs, lab coats, gloves, eyewear, respirators, and appropriate disinfection of surfaces and waste.**	**Low**

	Prepare the Xpert cartridge: An aliquot of the primary concentrated specimen, sample processing reagent, Xpert cartridge, and needed supplies for specimen transfer is placed in a BSC in the BSL-3 lab.	**Potential droplets and aerosols at time transfer of the primary concentrated specimen to a 15ml screw cap tube, addition of sample reagent (5-8% sodium hydroxide and 10-15% isopropanol (per SDS)), vortexing of capped tube for 10 seconds, incubate 15 minutes at room temp vortexing again at halfway mark, and opening tube to obtain an aliquot with provided transfer pipette. Note that NaOH and Etoh have cidal properties, but procedure does not guarantee specimen nonviable.**	**Low**	**BSL-3 facility, BSC, lab coat, gloves, eyewear, respirator, dirty to clean workflow in BSC. Disposal of ancillary supplies in discard container in BSC which is autoclaved prior to removal from the BSL-3 lab.**	**Low**
	Inoculate Xpert cartridge	**Open cartridge in BSC. Open specimen tube to obtain an aliquot with provided transfer pipette and inoculate with a disposable transfer pipette into the sample chamber of the cartridge. Close the cartridge lid firmly.**	**Low**	**BSL-3 facility, BSC, lab coat, gloves, eyewear, respirator, dirty to clean workflow in BSC. Disposal of ancillary supplies in discard container in BSC and red MERI infectious waste barrels and contents are autoclaved prior to removal from the BSL-3 lab.**	**Low**

	Disinfect outside of Xpert cartridge	**Before removing from the BSL-3 facility, wipe down the Xpert cartridge with Caviwipes (mycobactericidal with 3 minutes of contact time).**	**Low**	**BSL-3 facility, BSC, lab coat, gloves, eyewear, respirator, dirty to clean workflow in BSC. . Disposal of Caviwipes in red MERI infectious waste barrel which is autoclaved prior to removal from the BSL-3 lab.**	**Very low**
	Place Xpert cartridge in custom transfer tray.	**Before removing from the BSL-3 facility, Wipe all sides of transfer tray with Cavicide wipes, then transport to GeneXpert instrument in adjacent BSL-2 bacti lab.**	**Very low**	**BSL-3 facility, BSC, lab coat, gloves, eyewear, respirator, dirty to clean workflow in BSC. . Disposal of Caviwipes inred MERI infectious waste barrel and contents are autoclaved prior to removal from the BSL-3 lab.**	**Very low**
	Transport Xpert cartridge in custom transfer tray to GeneXpert instrument work area in adjacent BSL-2 lab.	**Exit the inner door of the BSL-3 facility into the anteroom and place the custom transfer tray onto the cart on the clean side of the laboratory. Wash hands and change safety glasses before exiting to the BSL-2 laboratory space (normal exit procedure)**	**Very low**	**BSL-2 facility, lab coat, gloves, eyewear.**	**Very low**

	<u>Likelihood and consequences of any risk in BSL-2 lab</u>: M. tb is a risk group 3 microorganism. Primary risk is exposure to aerosols, and lessor risk due to contact by droplet and splashes, and handling positive culture materials.		**Very low**	**Likelihood for exposure is low when specimens and cultures are handled using specified engineering controls, PPE, and lab practices specified in SOPs.**	**Very low**
	<u>Engineering controls</u>: BSL-2 lab			**BSL-2 facility with negative air pressure to adjacent hallways and clerical work areas.**	
	Removal of Xpert cartridge from custom transfer tray and insertion into GeneXpert instrument.	**Contact hazard: Remove cartridge, from custom transport tray, insert cartridge in instrument, instruct instrument to start test.**	**Very low**	**BSL-2 facility, lab coat, gloves, eyewear. Remove gloves and wash hands post handling cartridge and custom carrier.**	**Very low**
	<u>Waste Stream</u>: Discard Xpert cartridge after test is complete	**Discard Xpert cartridge in red MERI infectious waste stream in BSL-2 lab**	**Very low**	**BSL-2 facility, lab coat, gloves, eyewear. Remove gloves and wash hands post handling cartridge.**	**Very low**

	Training and competencies:			**Initial training, review of related SOPs, documentation, annual review of SOPs, and periodic competencies.**	
	Review risk assessment with staff and management, and document.				
	Evaluate effectiveness of risk mitigation				
	Review annually and/or reassess when appropriate.				

Discussion

- What TB lab waste should be autoclaved?
- Can you bring papers out of the BSL3 or BSL2 TB lab and where do they go? Can I recycle papers from the TB lab?
- Discuss safety concerns with using a cytocentrifuge for TB.
- What are the gloving recommendations?
- Can I use a Bunsen burner for fixing slides or what alternate options are available (e.g. hot plate or phenol)?
- How are specimens with AFB culture and smear ordered handled in other areas of the clinical lab, eg cell counts, manual prep of smears for hematology, cytospin slides, chemistry analytes, aliquots for various other tests and sendouts?
- Has a biosafety risk assessment been performed for all manipulations and testing performed outside the mycobacteria lab?

Biosafety Resources

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th ed. 2009

Revised edition coming by 2019 will have section specific to clinical laboratories

https://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories, MMWR, Supplement/Vol.61, January 6, 2012

https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

Biological Safety: Principles and Practices, Fifth Edition 2017

http://www.asmscience.org/content/book/10.1128/9781555819637

Mycobacterium tuberculosis: Assessing Your Laboratory. APHL 2013

https://www.aphl.org/aboutAPHL/publications/Documents/ID_2013Aug_Mycobacterium-Tuberculosis-Assessing-Your-Laboratory.pdf

Tuberculosis Laboratory Biosafety Manual. WHO 2012

http://apps.who.int/iris/bitstream/10665/77949/1/9789241504638_eng.pdf

Where do we go from here?

Not many regulations, but LAIs are real…