



**Laboratory Safety: Work Practices for *Mycobacterium tuberculosis***

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Work Practices for  
*Mycobacterium tuberculosis***

Course duration: 24:00

[Begin Course](#)

The banner features a background image of a laboratory worker in full PPE (goggles, mask, gloves, and gown) working at a biosafety cabinet. The text is overlaid on the right side of the image. A green button with the text 'Begin Course' is positioned below the course title. A small white box with a black border containing the text 'Course duration: 24:00' is located above the button.

## 1. Introduction

### 1.1 Laboratory Safety



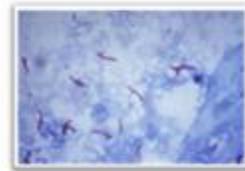
#### Notes:

This training module will discuss safe work practices for performing laboratory tests for the isolation and identification of *Mycobacterium tuberculosis*. In US microbiology labs today, the practice of treating TB specimens with special precautions is well established. And, these protocols have evolved over time.

## 1.2 Agent: *Mycobacterium tuberculosis*

### Agent: *Mycobacterium tuberculosis*

- Infectious dose 1-10 organisms:  
**No safe level of exposure**
- Airborne droplet nuclei can be spread through normal air currents for a long time and throughout a room or building
- There is a risk to laboratorians who process specimens in labs



#### Notes:

Let's cover some general information about the agent *M. tuberculosis* and how it is spread. It is a very infectious agent. Therefore, special work practices are required to handle the specimens and the cultures safely. Aerosol droplet nuclei, which are one to five microns in size, can remain in the air for hours, and reach the alveoli in the lungs and cause infection. Actually, as few as one to ten organisms can cause infection, so there is a recognized risk to laboratorians.

### 1.3 Overview

## Overview

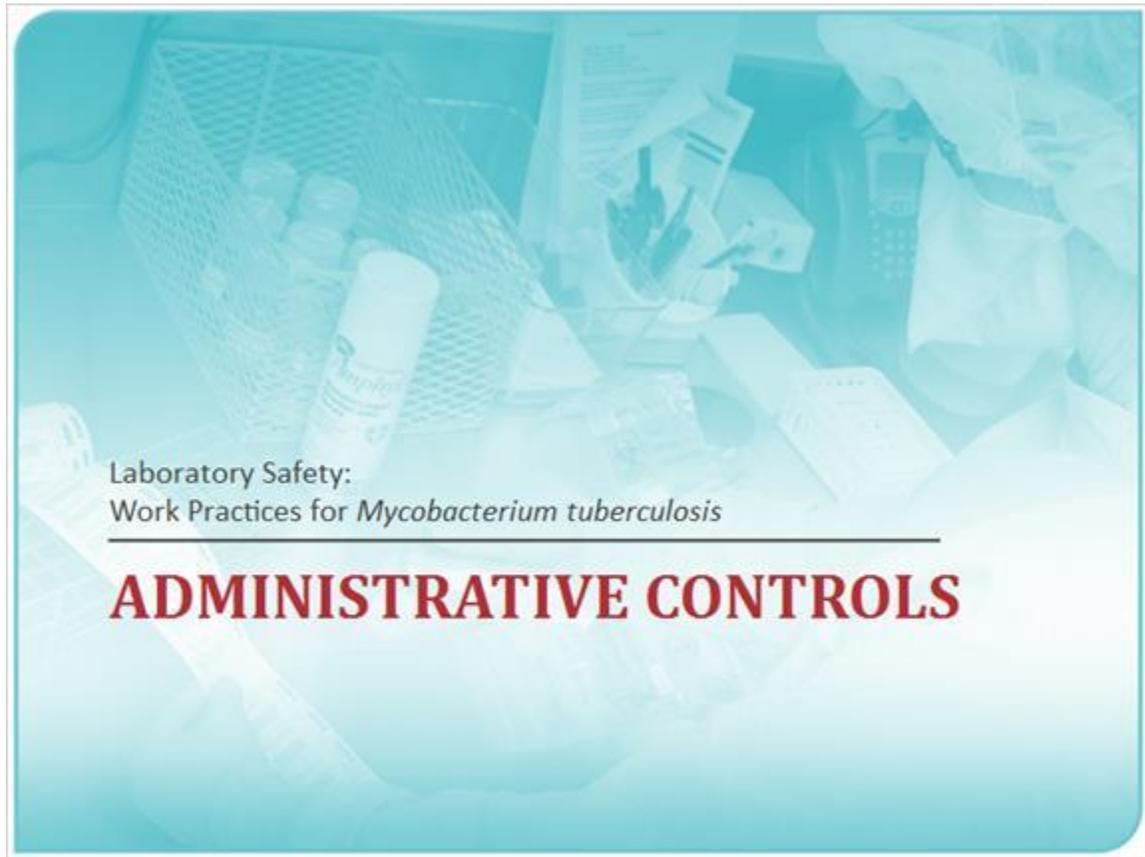
- Administrative Controls
- Engineering Controls
- Personal Protective Equipment (PPE)
- Standard Operating Procedure (SOP)



**Notes:**

In this module, we will cover administrative controls, engineering controls, personal protective equipment, and standard operating procedures. Much of lab safety involves educating staff on how and why it is important to prevent procedural drift and why they need to adhere to safety protocols.

## 2. Administrative Controls



**Notes:**

Let's take a look at some administrative controls associated with biosafety.

## 2.2 Training in Safe Work Practices

### Training in Safe Work Practices

- How well are workers trained for the tasks?
- Do workers meet a level of competency before being allowed to work?
- Training should include:
  - Use of safety equipment
  - Decontamination procedures
  - Spill cleanup
  - Use of autoclave
  - Waste disposal

A photograph showing a person in a white lab coat and green gloves working in a laboratory. The person is holding a glass flask containing a yellow liquid. In the background, there is a white funnel, a glass beaker, and a white dial meter. The person is also wearing a white face mask and safety glasses.

### Notes:

Training is critical for laboratory safety. Staff should be taught how to use the correct safe work practices. They should be observed performing the correct safe work practices. And while working independently, staff should be monitored for following the correct safe work practices. Make sure that your training covers use of safety equipment, decontamination procedures, spill cleanup, use of an autoclave, as well as waste disposal.

## 2.3 Safety Topics for Orientation

**Safety Topics for Orientation  
and Annual Competency**

- Proper and safe handling practices
- Use of biological safety cabinet (BSC)
- Biohazardous waste handling
- Use of autoclave
- Disease symptoms
- Post exposure management
- Reporting exposures and illnesses



### Notes:

The time to start training on safe work practices is during orientation. This slide lists some of the topics that should be covered. We found that formal training on proper use of a biosafety cabinet does not usually occur. So, development of a standard training module is a good way to make sure that all staff are equally trained and prepared to properly use a biosafety cabinet, because if you are not using a biosafety cabinet correctly, you are not protected.

## 2.4 Link to OSH Program

### Link to Occupation Health and Safety Program

Minimum requirements to conduct work in the laboratory:

- General laboratory safety training
- Familiarity with safety guidelines
- Standard precautions
- Training and experience



The image shows a rectangular biosafety sign with a white background and a grey border. At the top, it reads "BIOSAFETY LEVEL 3" in bold black letters. Below that, it says "INFECTIOUS AGENT:" followed by "Mycobacterium tuberculosis" in a smaller font. At the bottom of the sign, there are three distinct icons: on the left, a "No food or drink" sign with a red circle and slash over a plate and glass; in the center, a standard orange biohazard symbol; and on the right, a "NOTICE" sign with a red circle and slash over a hand being washed.

### Notes:

Facilities that offer testing for tuberculosis need to have the support of the Occupational Health and Safety Program. The program can offer training or guidance regarding general lab safety, familiarity with safety guidelines, universal precautions, and also, information regarding the required safe work practices that are needed to conduct work in the laboratory.

## 2.5 Link to OSH Program

### Link to Occupation Health and Safety Program

At a minimum, OSH program includes:

- Offering of Interferon Gamma Release Assay (IGRA) or Tuberculin Skin Test (TST) to those who have risk of exposure to TB (two-step TST on initial hire)
- Ongoing evaluation of the respirator program, based on expected work area

#### Notes:

All staff working up specimens or cultures associated with TB testing, should have a TB test performed routinely. This could be a skin test on hire, and then either a skin test or an Interferon Gamma Release Assay, an IGRA, at regular intervals, typically annually or even every six months based on your institutions policy. Your Occupational Health Program should also conduct ongoing evaluations for the respirator program.

## 2.6 Risk Based on TB Incidence

### Risk Based on TB Incidence

- Frequency of *M. tuberculosis* positive specimens encountered
- Concentration of organisms in specimens
- Number of specimens handled by an individual worker
- Safety practices in the laboratory
- Safety equipment available in the laboratory



### Notes:

It's important to know the incidence of TB in the population you serve. This will give you an indication of how frequently specimens positive for *M. tuberculosis* are processed in the laboratory, the concentration of organisms in the specimens, and the number of multi-drug resistant tuberculosis that is seen. This also gives you an idea for the number of specimens handled by any individual worker. This will help you determine the risk associated with your work and then guide the safety practices that you need.

## 2.7 Chain of Infection



### Notes:

This is a graphic of the chain of infection. If any link in the chain is broken, either by using proper practices, protective equipment, you can prevent an infection from occurring. One of the most important parts in this chain is the susceptible host. It's very important that you create an atmosphere where staff will feel comfortable telling you if they have a condition that makes them more susceptible to infectious diseases, such as being pregnant, or maybe being on medications that would reduce their immune system.

Also, we want to make sure we are doing a proper risk assessment so we can figure out where we can intercept the microorganism from getting into your workers and causing them to become sick. If we can determine how to block the organism at any one time, at any of these steps, we can prevent infection. Or at least, we can effectively manage the infection if it does occur.

## 2.8 Lab Biosafety Plan Components

A presentation slide with a teal header and a light blue background. The header contains the title "Laboratory Biosafety Plan Components". Below the header is a bulleted list of ten components. To the right of the list is a photograph of a silver and black pen resting on a calendar page with dates 15, 22, and 29 visible.

### Laboratory Biosafety Plan Components

- How work is safely performed
- Risk assessment
- Post-exposure management
- Protocol-driven personal protective equipment (PPE) requirements
- Traffic control/access restriction
- Use of safety equipment
- Sanitation (cleaning and disinfection)
- Waste management
- Training
- Medical emergencies

### Notes:

Every laboratory should have a written biosafety program that serves to train staff and is used for reference whenever there are questions. The TB lab biosafety plan should contain all of the components seen here on this slide. Also, you want to make sure that you review it annually, and that you update it as necessary. And remember, anytime revisions or changes are made be sure the staff are trained on any new aspects of the plan.

## 2.9 Exposure Prevention

### Exposure Prevention

Employ a combination of methods:

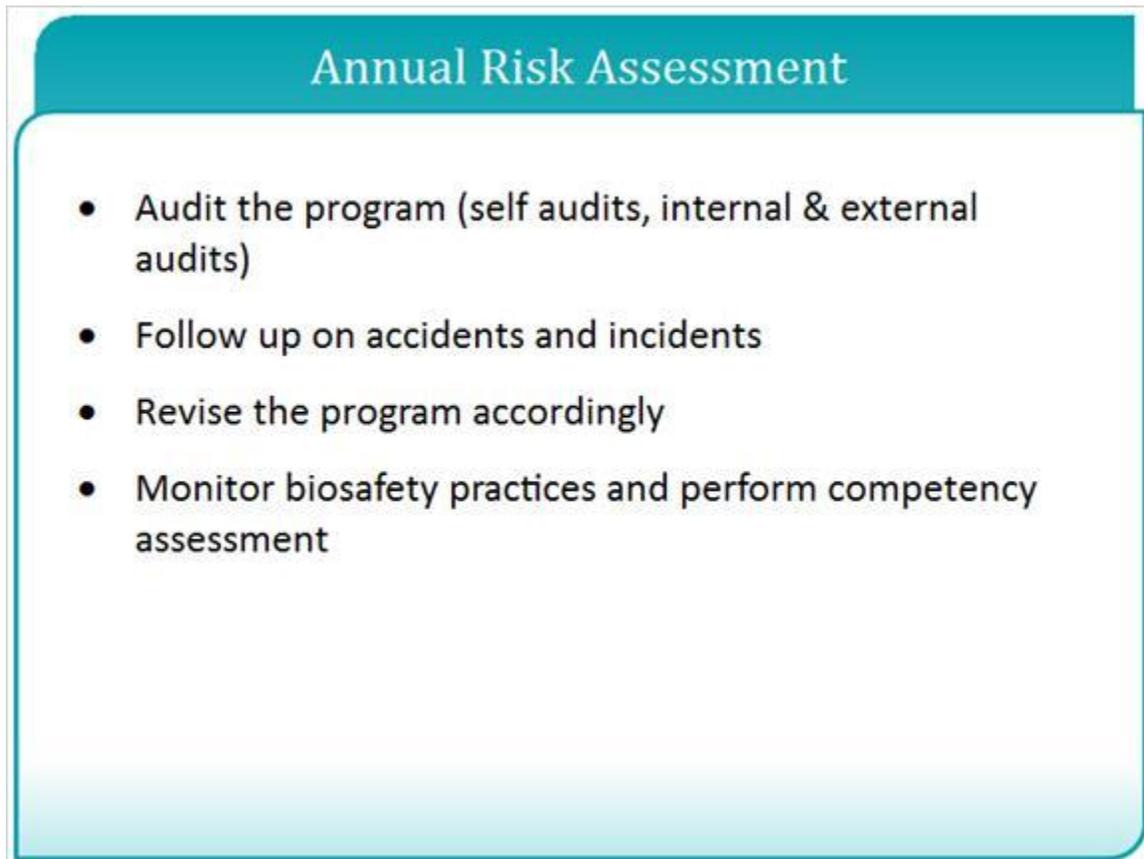
- Safe work practices
- Use of containment equipment
- Specially-designed laboratory facilities



#### Notes:

Every lab should do a risk assessment. What is the incidence of TB in your community? What has been the annual incidence of TB in the past five years? Which task represent a risk to your laboratorians? A knowledgeable assessor is vital to the successful risk assessment. Once you have a risk assessment, you can determine what equipment, PPE, and work practices will best mitigate your risks.

## **2.10 Annual Risk Assessment**

A presentation slide with a teal header and a light blue gradient background. The title 'Annual Risk Assessment' is centered in the header. Below the title is a bulleted list of four items.

**Annual Risk Assessment**

- Audit the program (self audits, internal & external audits)
- Follow up on accidents and incidents
- Revise the program accordingly
- Monitor biosafety practices and perform competency assessment

### **Notes:**

Make sure that you audit your program. You may need to reconduct the risk assessment after accidents or exposures.

## 2.11 Specimen Collection



### Notes:

As we mentioned, the greatest risk of tuberculosis is from the infectious aerosols. So, this even to specimen collection as well, whether it be a sputum or bronchoscopy. Also, when a patient is suspected or confirmed as having tuberculosis, airborne precautions must be used. This means that the patient has to be housed in a negative pressure room, and that everyone who enters the room should use an N-95 respirator, for which they have been fit-tested. This is especially important during specimen collection.

## 2.12 Handling Clinical Specimens

### Handling Clinical Specimens

- Aerosol-producing procedures pose a risk of exposure
- Where can the work be performed, biosafety level 2 (BSL-2) or biosafety level 3 (BSL-3)?
- What PPE are necessary?



#### Notes:

Attention must also be given to the laboratory handling of infectious specimens that can generate similar infectious aerosols. So what is an aerosol? Well, an aerosol is created any time you add energy to a liquid. Now, this energy can take the form of mechanical energy, heat energy, or chemical energy. Also, remember that aerosols are created during tasks we don't typically think of as being risky, such as the creation of a smear. During this process, you have organism, infectious organism, being mixed into a liquid. There has been some controversy on whether or not you can conduct this work in a BSL-2 or have to have a BSL-3. And, we will discuss this in more detail in a few slides.

## 2.13 Handling Clinical Specimens

### Handling Clinical Specimens

- There are differences in literature regarding recommended safety levels for certain mycobacteriology procedures.
- Specimen receipt and log-in can occur on the open bench.
- All aerosol-generating activities (any actions imparting energy into a fluid specimen) must be conducted in a BSC, such as:
  - Surface disinfection of contaminated specimen container
  - Preparation of direct smear
  - Digestion, decontamination, concentration of primary specimen
  - Preparation of concentrated smear
  - Inoculation of culture media

### Notes:

As I mentioned, there are some differences in the literature regarding the recommended safety levels for certain mycobacteriology procedures. So, let's take a look at some of these procedures as points of concern. First, the specimen transport bag should be opened inside a biosafety cabinet, in order to guard against the creation of aerosols, spray and splatter. Specimens that leak during transport must be rejected and a new specimen requested.

Before opening a transport bag, try to observe the specimen for leakage. If the outside of the container is grossly contaminated with the contents of the container, reject the specimen, document the rejection, and request another specimen. If examination shows that the exterior of the specimen container demonstrates minor or just superficial contamination, you can clean the exterior with an appropriate disinfectant before further handling. Opening a specimen container must be done carefully, because splashing, splattering, or even aerosol generation can contaminate the outside of the container.

Wipe the exterior of the container with gauze soaked in a tuberculocidal disinfectant after removing and replacing caps. All aerosols generating activities,

once again any action that imparts energy to a fluid, must be conducted in a biological safety cabinet. For example, any surface disinfection of contaminated specimen containers, preparation of a direct smear, primary specimen digestion, decontamination and concentration, concentrated smear preparation and inoculation of the culture media.

## 2.14 Risky Activities in Other Lab Sections

### Risky Activities in Other Laboratory Sections

- Preparing frozen sections of biopsy specimens
  - Wear an N95 respirator to mitigate the risk
- Cutting or sawing through tissue specimens that have not been fixed
  - Wear an N95 respirator or powered air-purifying respirator (PAPR) during the procedure
- Homogenizing tissues for primary culture
  - Use a BSC

### Notes:

We have to remember that in other parts of the lab such as surgical pathology and histology, there are risks from handling specimens contaminated with *Micobacterium tuberculosis* as well. So preparing frozen sections, cutting or sawing through tissue specimens that have not been fixed, and homogenizing tissues for primary culture all can cause or pose a risk of exposure. These procedures should be done inside a bio safety cabinet.

### 3. Engineering Controls



**Notes:**

Now, let's take a look at engineering controls.

### 3.2 What If a BSL-3 Is NOT Available?



What If a BSL-3 Is NOT Available?

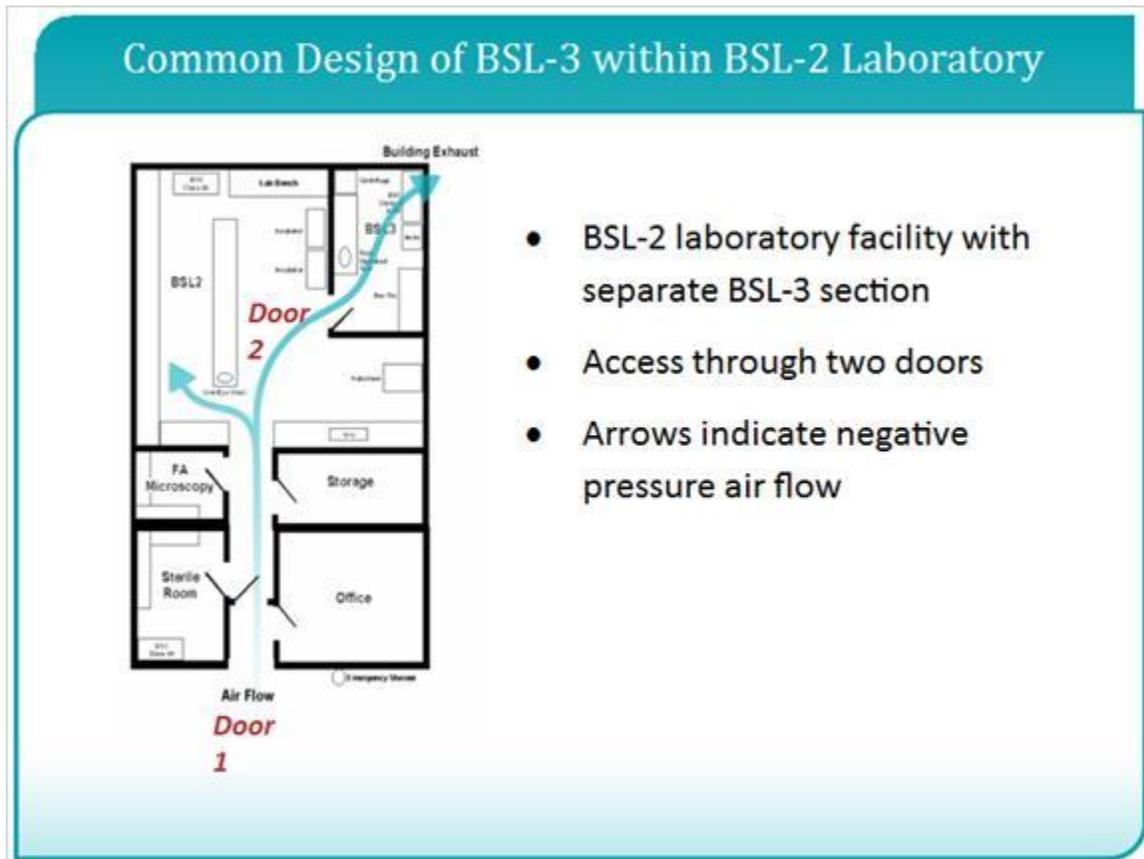
- BSL-2 with BSL-3 practices?
- A risk assessment is essential for determining if work with *M. tuberculosis* can be conducted safely in a separate, closed BSL-2 laboratory using BSL-3 practices and procedures

Performing a risk assessment is absolutely essential.

#### Notes:

So, what if a BSL-3 is not available to you? Can you use a BSL-2 Lab with BSL-3 practices? Retrofitting a BSL-2 facility to accommodate a BSL-3 laboratory is not an option for some facilities that must test for *M. tuberculosis*. The BMBL has removed the language that suggested BSL-3 practices could be done in a BSL-2 lab when working with *Mycobacterium tuberculosis*, as long as biosafety cabinet was used and the air exhausted to the outside of the building. Once again, performing that risk assessment is absolutely essential to determining where you can work with these specimens and cultures, and how to do so safely. Make sure that if you do not have a BSL-3 lab that you do conduct this risk assessment to see if work with *Mycobacterium tuberculosis* can be done in a safe manner by utilizing a closed BSL-2 lab and implementing BSL-3 practices and procedures.

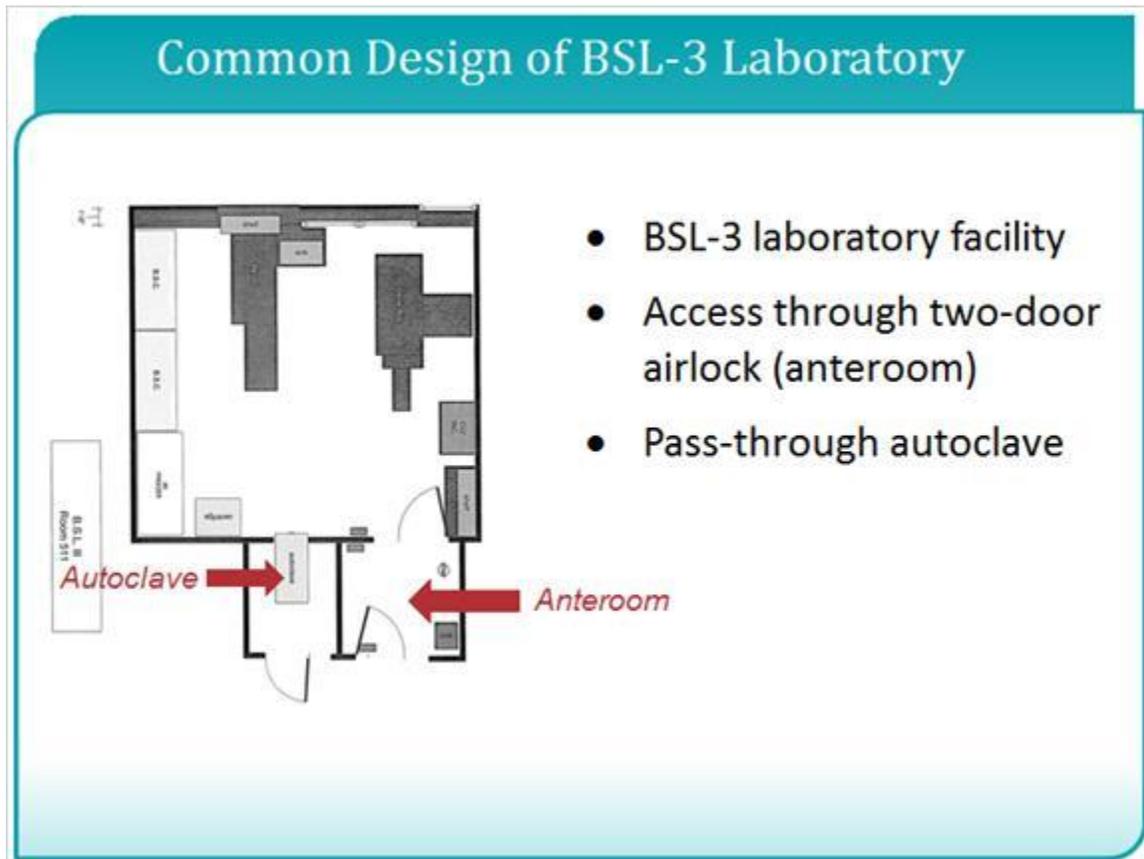
### 3.3 Common Design of BSL-3 within BSL-2 Laboratory



#### Notes:

Here we see a common design of a BSL -3 within a BSL-2 lab. You will notice that you have to access through 2 doors. There needs to be a negative pressure air space between them. And, single pass ventilation system exhausting all the room air to the outside. While a full BSL-3 suite with a dedicated anteroom is available in some clinical labs, the set up you see here is fairly common. There is a large BSL-2 lab that serves as the anteroom to the BSL-3 room. Access to the BSL-3 room must occur by passing through two doors which you see labeled on the diagram. And, there also needs to be a negative airflow condition as you pass through each door into the BSL-3.

### 3.4 Common Design of BSL-3 Laboratory



#### Notes:

Here we see the common design of a BSL-3 suite. Once again, you have access through 2 doors with an air space in between. There are single pass ventilation systems, exhausting all room air to the outside. And typically, you will find a pass-through autoclave associated with this suite.

### 3.5 BSL-3 Laboratory Design

## BSL-3 Laboratory Design

Dedicated, single-pass ventilation system exhausts all room air to outside

- 6-12 air changes per hour (ACH) removes 99% of airborne particulate matter in 23-46 minutes. Time depends on ACH.
- Create negative pressure, airflow should be from “clean” to “less clean” areas
- Laboratory should be kept under negative pressure at all times (alarm for failure of air handling system)
- BSL-3 facility operation should be re-verified at least annually

#### Notes:

A typical BSL-3 lab design would have a dedicated single-pass ventilation system that exhausts all air to the outside. Six to twelve air changes per hour removes about 99% of the airborne particulates in about 23 to 46 minutes. Now the time it actually takes will depend upon your air changes per hour. You want to make sure you are creating a negative pressure airflow, so that it goes from clean to a less clean area. The laboratory should be kept at a negative pressure at all times, even if there is a bio safety cabinet failure. And, the BMBL says "The BSL-3 facility design, operational parameters, and procedures have to be verified and documented prior to operation. And then, you must re-verify and document this re-verification at least annually."

### 3.6 BSL-3 Laboratory Design

## BSL-3 Laboratory Design

- Interior surface of walls, floors, ceiling, and utility penetrations sealed
- Bench tops resistant to acids, alkalis, organic solvents, and moderate heat
- Foot-operated hand washing
- Automatic door closures
- Autoclave



#### Notes:

So, BSL-3 labs have some design features. For decontamination procedures, the interior surface of walls, floors and ceiling are sealed. And, there are sealed utility penetrations. The bench tops are resistant to acids, alkalis, organic solvents, and moderate heat. Typically, there are foot-operated hand washing stations, and automatic door closures. There is an autoclave available in the lab so that infectious waste is contained in the BSL-3 lab. The autoclave must be properly maintained and quality control performed for each run to make certain that sterilization has been achieved.

**3.7 What procedure would be acceptable for performing in an area outside of a BSC?**

## Knowledge Check

What procedure would be acceptable for performing in an area outside of a BSC?

- Smear preparation
- Accessioning specimens for AFB smear and culture
- Processing specimens by NALC/ NaOH
- Inoculating media

**3.8 The AFB section of your laboratory has a certified Class II biological safety cabinet (BSC), and access to the area is restricted via badge entry. Negative airflow is maintained in relation to the hallway. It does not have double-door access but your bio-hazard waste is double bagged and removed from your facility for autoclave disposal. At what biosafety level is your facility operating?**

### Knowledge Check

The AFB section of your laboratory has a certified Class II biological safety cabinet (BSC), and access to the area is restricted via badge entry. Negative airflow is maintained in relation to the hallway. It does not have double-door access but your bio-hazard waste is double bagged and removed from your facility for autoclave disposal. At what biosafety level is your facility operating?

- Biosafety Level 3 (BSL-3)
- BSL-2
- BSL-1

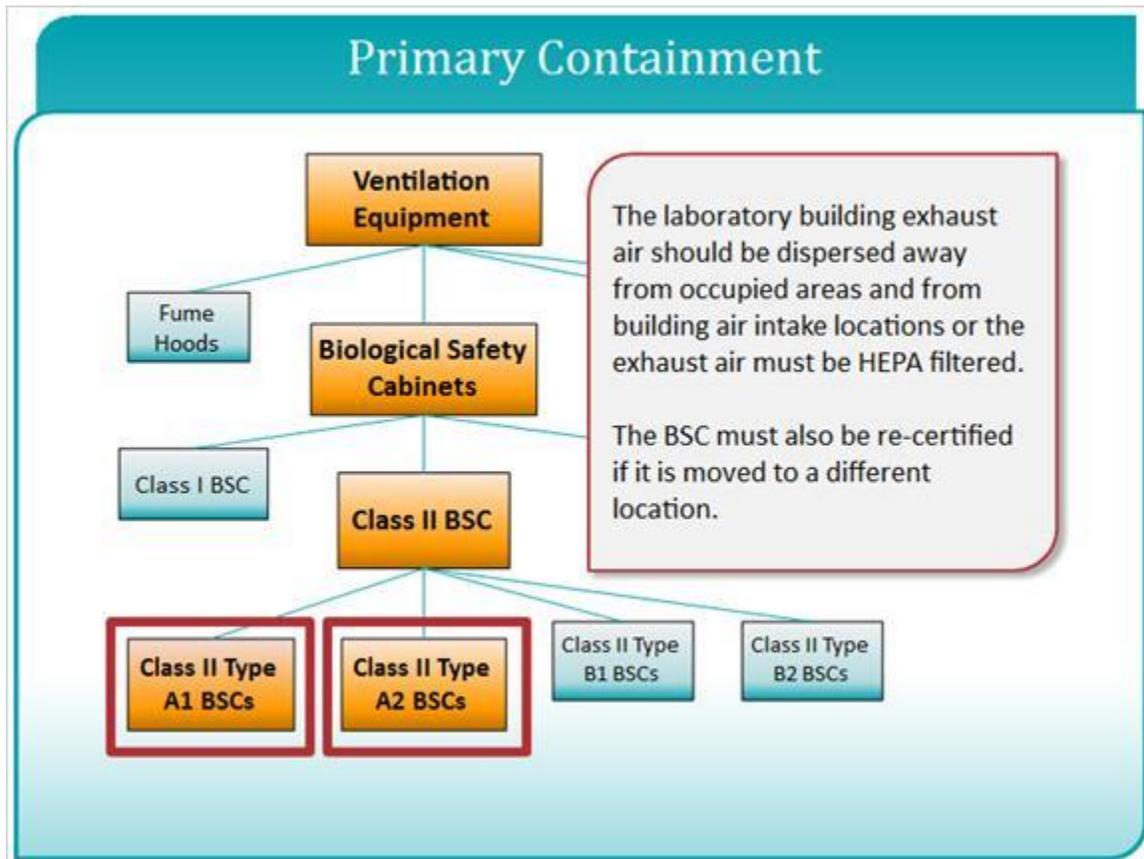
### 3.9 Safe Work Practices: Design



#### Notes:

The door to the TB lab must be kept closed at all times during specimen processing. You should have a biosafety sign posted on the door that states the agents posing a risk, what PPE is necessary, and it should also have emergency contact phone numbers. All work involving the processing of specimens or the manipulations of propagated organism must be performed inside a BSC.

### 3.10 Primary Containment



#### Notes:

So, biosafety cabinets are a primary form of containment. There are many types of biosafety cabinets in either a Class II-A1 or II-A2 BSC should be used in the TB lab. It is not necessary to hard duct the cabinet to the outside. But, you do have HEPA filtered exhaust air from a Class II BSC, and it can safely be re-circulated into the lab environment if the cabinet is tested and certified at least annually and is operated according to manufacturer's recommendations. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered. Also, you want to remember that your biosafety cabinets have to be re-certified if they are moved to a different location.

### 3.11 Containment Equipment: BSC



#### Notes:

Problems with the biosafety cabinets have been a source of converting from a negative TB test to positive for laboratorians. Of particular importance is the proper use of the biosafety cabinets and the training needed to properly work within a biosafety cabinets.

Some basic rules to remember regarding biosafety cabinets: You want to make sure you install the biosafety cabinets away from walking traffic and doors. There should be no storage of items in the biosafety cabinets, and you are working four to six inches back from the front grill. You don't want to block the front grill or the rear grill so that air movement is impaired. Before and after use, you need to clean the surfaces with 10% bleach and follow with 70% alcohol, so that you remove the residual bleach and prevent pitting on the surface. Make sure you move arms in and out slowly, perpendicular to the opening, and don't make sweeping arm movements either while you're working inside the cabinet, and do not sweep your arms outside the cabinet to get supplies and then bring them back in. You want to make sure that you organize the workspace inside the biosafety cabinet such that it does not impede air flow. And, you want to work from clean to a dirty flow.

### 3.12 BSC Maintenance

## BSC Maintenance

- Daily cleaning is just the start
- Read the manual and be familiar with your model's performance characteristics
- Read your annual recertification report and understand what is measured

*Who does your annual preventive maintenance?  
Are they certified?*



#### Notes:

All biosafety cabinets must be certified annually by a trained professional. It's important to remember that staff needs to monitor and understand the Magna-Helix gauge. What you're looking for are large changes in the pressure that could indicate either a hole or a blockage in the filter.

### 3.13 What You Don't Want to See in Your BSC



#### Notes:

OK. This obviously an extreme photo taken for effect, but it does serve as a reminder that managers need to monitor biosafety cabinet use and practices.

### **3.14 TB Specimen Processing: Decontamination and Concentration**

#### TB Specimen Processing: Decontamination and Concentration

Risky activities that can generate droplets and droplet nuclei:

- Vortexing
- Pouring liquid cultures and supernatant fluids
- Using fixed-volume automatic pipettors
- Mixing liquid cultures with a pipette
- Preparing specimen and culture smears
- Dropping tubes or flasks containing cultures
- Spilling suspensions of bacilli
- Breaking tubes during centrifugation
- Heat fixing smears

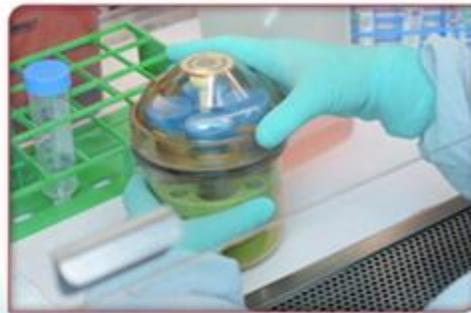
#### **Notes:**

In the TB BSL-3 lab, all work is performed in the biosafety cabinet as previously mentioned. The list you see on this slide serves as a reminder of the type of work activities that can generate droplets and droplet nuclei. Since these activities are being performed inside a BSC, this decreases the risk to the laboratorian.

### 3.15 Containment Equipment: Centrifugation

#### Containment Equipment: Centrifugation

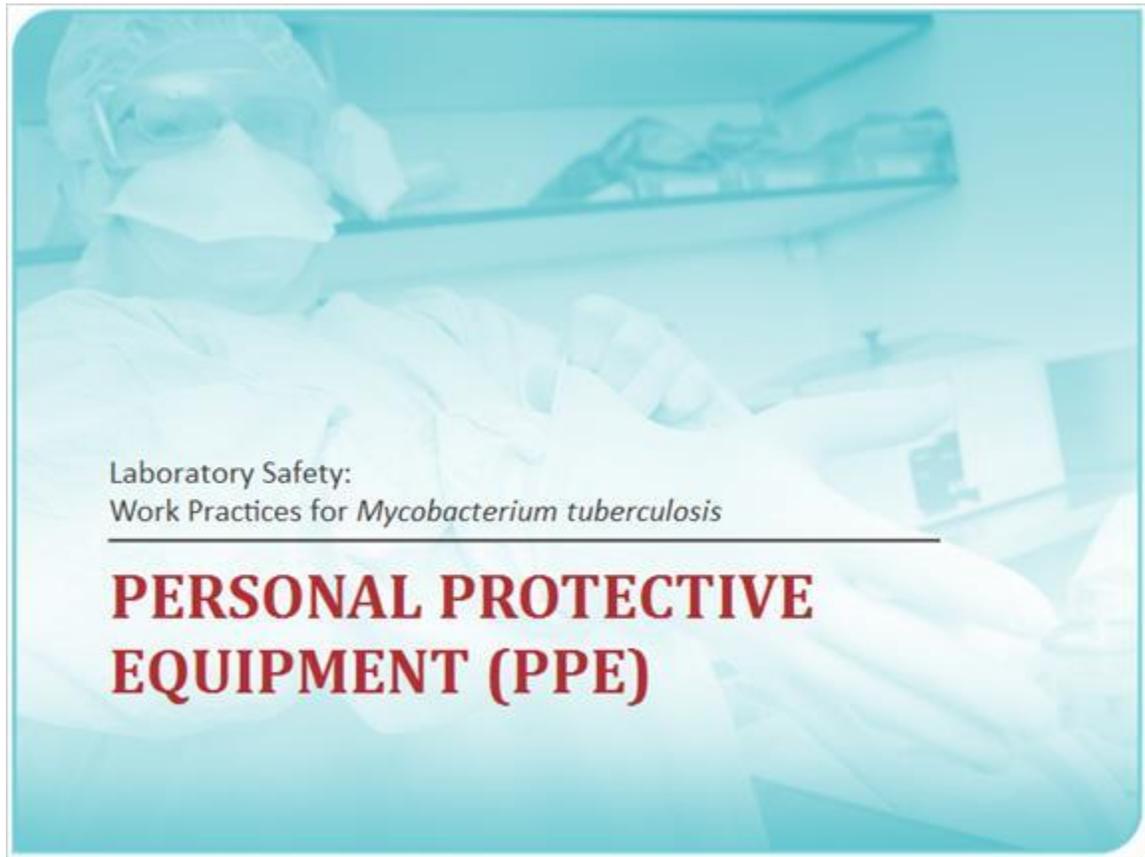
- All culture tubes sealed tightly and placed in centrifuge safety cups inside the BSC
- After centrifugation, open safety cups in BSC



#### Notes:

All culture tubes should be sealed tightly and placed in the centrifuge safety cups inside the biosafety cabinet. And then following centrifugation, those same safety cups should be transported to the biosafety cabinet before you open them.

## 4. Personal Protective Equipment (PPE)



### Notes:

Even though we work inside a biosafety cabinet, personal protective equipment is still necessary.

## 4.2 Personal Protective Equipment (PPE)

### Personal Protective Equipment (PPE)

- Solid front disposable gown with snug (knit) cuffs
- Gloves long enough to overlap gown's sleeves
- Fit-tested respirator (N-95 or equivalent; Powered Air-Purifying Respirator [PAPR])
- Remove all outer protective clothing when leaving the BSL-3 laboratory and place into bags for autoclaving.

A photograph of a person standing in a laboratory, fully equipped with personal protective equipment (PPE). The person is wearing a white hairnet, safety goggles, a white respirator mask covering the nose and mouth, a white long-sleeved gown with a front zipper, and blue nitrile gloves. The background shows laboratory shelves with various items, including bottles and containers.

### Notes:

Solid front disposable gown with snug cuffs must be worn. Also, you have to wear gloves and they must be long enough to overlap the sleeves of the gown. You need to be wearing a respirator, and that respirator must be fit tested. And, you want to remember to remove all outer protective clothing when leaving the BL-3 lab and place that clothing into a bag for autoclaving.

### 4.3 Sequence for Donning PPE

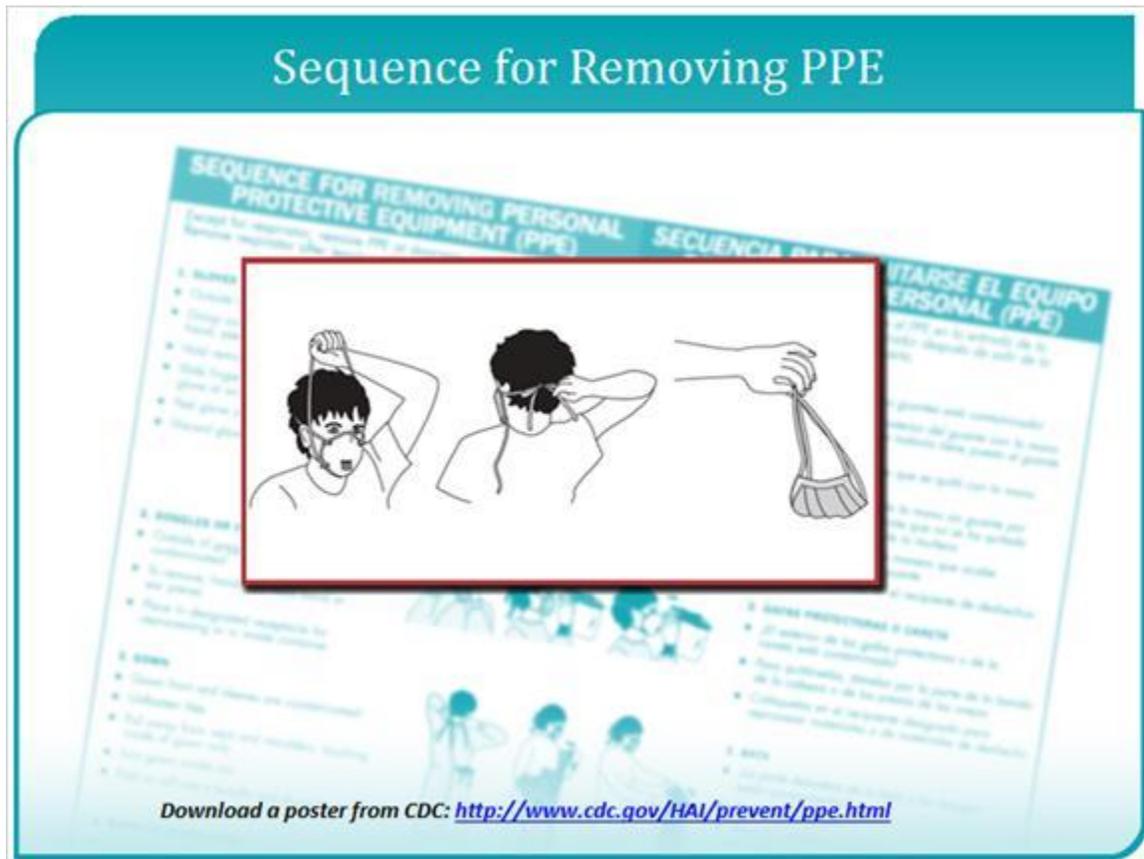
**Sequence for Donning PPE**

*Download a poster from CDC: <http://www.cdc.gov/HAI/prevent/ppe.html>*

#### Notes:

Here we see the sequence for donning your PPE. First you want to make sure you wash your hands. Then, don the PPE in the following order: First, put on your gown. And then, your mask or respirator. Followed by your eye protection. And finally, the gloves. And remember, the gloves must cover those cuffs of the gown. You don't want to see or have any skin exposed.

#### 4.4 Sequence for Removing PPE



#### Notes:

Removing your PPE should occur in the order seen here. Remove your gloves, ensuring that they carefully are turned inside out on themselves. Be sure to resist snapping of the gloves, which can create aerosols of any organisms that might be present. Then, remove your eye protection, followed by the gown. Once again, carefully remove the gown while folding it in on itself, very similar to how you took your gloves off. And then finally, remove your respirator.

## 4.5 Respiratory Protection

### Respiratory Protection

- No BSC is 100% secure
- The National Institute for Occupational Safety and Health (NIOSH) states that respirators provide a greater level of protection
  - Particulate filters are more efficient
  - Can be fit tested
  - Can be fit checked by user to ensure tight face seal
- Respiratory protection program requires SOP, training, storage, inspection, medical review, program evaluation

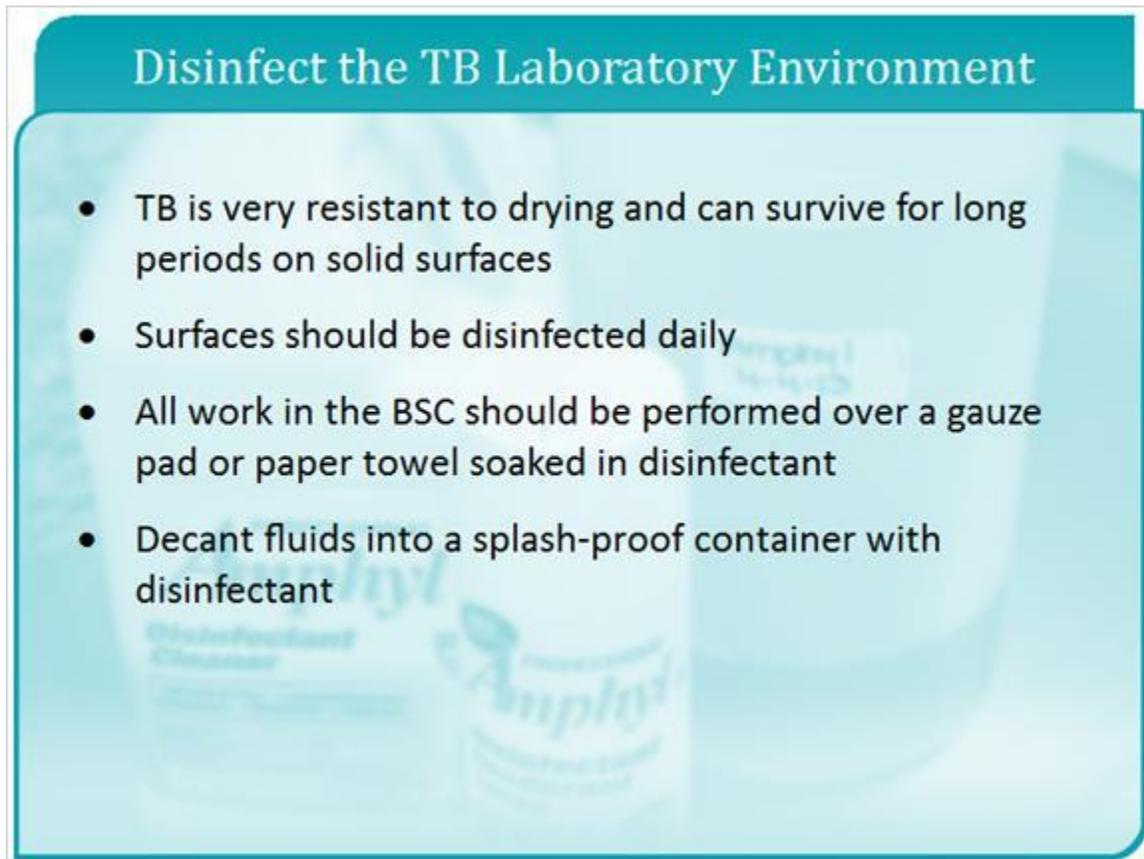


### Notes:

So, you may be wondering, Why do I need to wear a respirator when we are doing all of our work inside a biosafety cabinet? And it is a controversial topic. However, NIOSH is stating that a respirator adds that greater level of protection. Remember, no biosafety cabinet is one hundred percent and an infectious dose of TB is only one to ten organisms. So, wearing a respirator provides greater protection. The particulate filters are more efficient. They can be fit tested. And, they can be fit checked by the user each time to make sure that its making a tight seal on the face.

It's important that when staff are wearing a respirator they are enrolled, or a part of, a respiratory protection program. Now this program requires that you have an SOP, that you conduct training, that there is proper storage and inspection of your respirators, and there needs to be a medical review and a constant program evaluation.

#### 4.6 Disinfect the TB Lab Environment



### Disinfect the TB Laboratory Environment

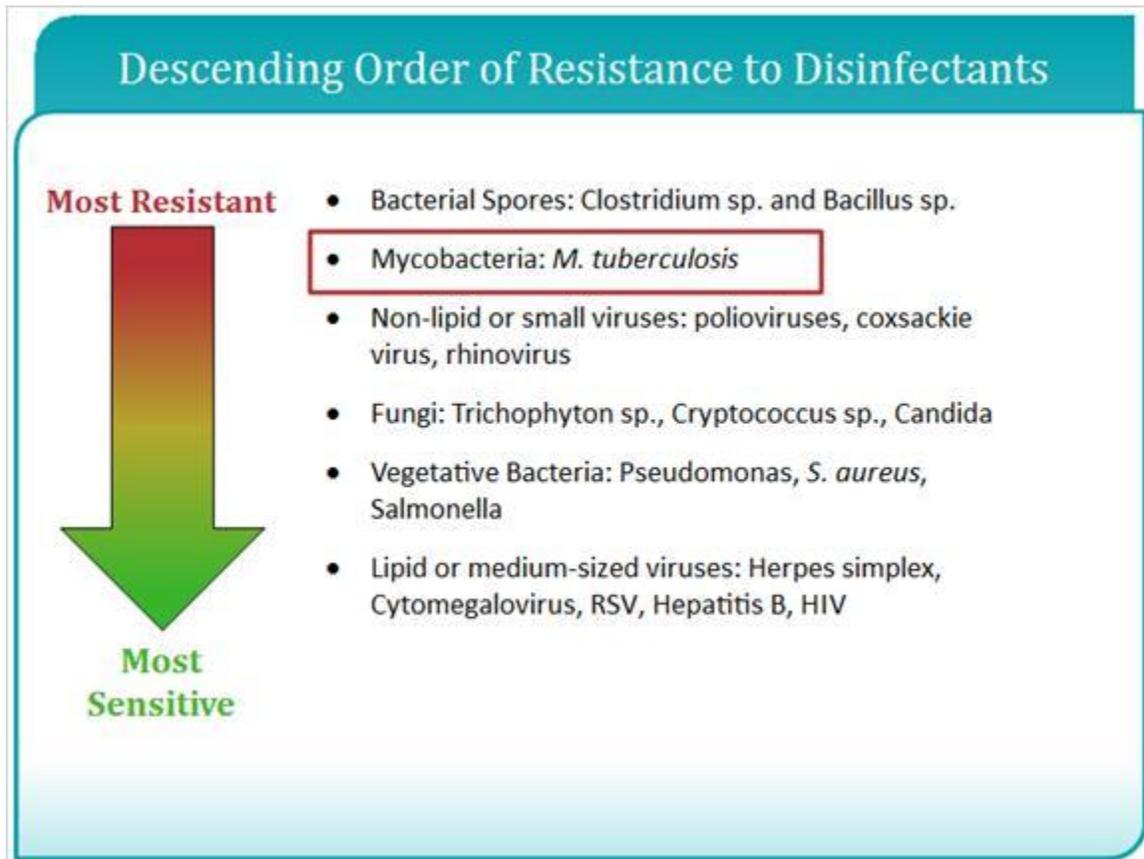
- TB is very resistant to drying and can survive for long periods on solid surfaces
- Surfaces should be disinfected daily
- All work in the BSC should be performed over a gauze pad or paper towel soaked in disinfectant
- Decant fluids into a splash-proof container with disinfectant

#### Notes:

TB is very resistant to drying, and can actually survive on solid surfaces for long periods of time. So you need to make sure that your surfaces are disinfected daily. There is no one universal disinfectant that is effective against all organisms because you have to consider all of the following when you are choosing your disinfectant:

What concentration of the disinfectant? What is the concentration of the pathogen you are trying to kill? Exactly what pathogen is it that you're trying to eliminate? What will be the time of contact between the disinfectant and the material being decontaminated? Also, is there organic material being associated with what you're disinfecting? And you need to think about the environmental conditions, such as PH, temperature, and humidity. But you must have a disinfectant that has tuberculocidal activity.

#### 4.7 Descending Order of Resistance to Disinfectants



#### Notes:

Microorganisms can be ranked according to how easy it is to kill them. As you can see from this ranking, *M. tuberculosis*, is one of the most resistant organisms, very similar to the spore forming *Clostridium* species. Most disinfectants require a period of exposure time in order to work properly. This is especially important for TB, as it is relatively more resistant to disinfectants.

#### 4.8 Select the Right Level of Disinfectant

### Select the Right Level of Disinfectant

- Sterilization: Complete elimination of all forms of microbial life
- High-level disinfection: Destroys all microorganisms except bacterial spores
- Intermediate-level disinfection: Inactivates *M. tuberculosis*, non-spore forming bacteria, most viruses and most fungi
- Low-level disinfection: Kills most bacteria (not TB), some viruses, and some fungi
  - Hospital-type germicides used primarily for housekeeping such as quaternary ammonium compounds ("quats")

#### Notes:

You need to select a disinfectant based on its tuberculocidal activity. So, look for a product that is EPA registered as an "Intermediate-level disinfectant." This means that inactivates *M. Tuberculosis* and non-spore bacteria, most viruses and most fungi. The time to kill *Mycobacterial tuberculosis* is not instantaneous, but it is dependent upon the product. The directions for using the product should be strictly followed as to concentration and exposure time. Some chemicals that are known to have tuberculocidal activity are phenolics, iodophors, chlorine compounds, and alcohols.

#### 4.9 Intermediate-Level Disinfectants

### Intermediate-Level Disinfectants

- Kills *M. tuberculosis* and all other vegetative bacteria, all fungi, and most viruses when directions followed for concentration and exposure time
- Tuberculocidal chemicals include phenolics, iodophors, chlorine compounds, alcohols

**Notes:**

The time to kill *Mycobacterium tuberculosis* is not instantaneous, but it is dependent upon the product. The directions for using the product should be strictly followed as to concentration and exposure time. Some chemicals that are known to have Tuberculocidal activity are phenolics, iodophors, chlorine compounds and alcohols.

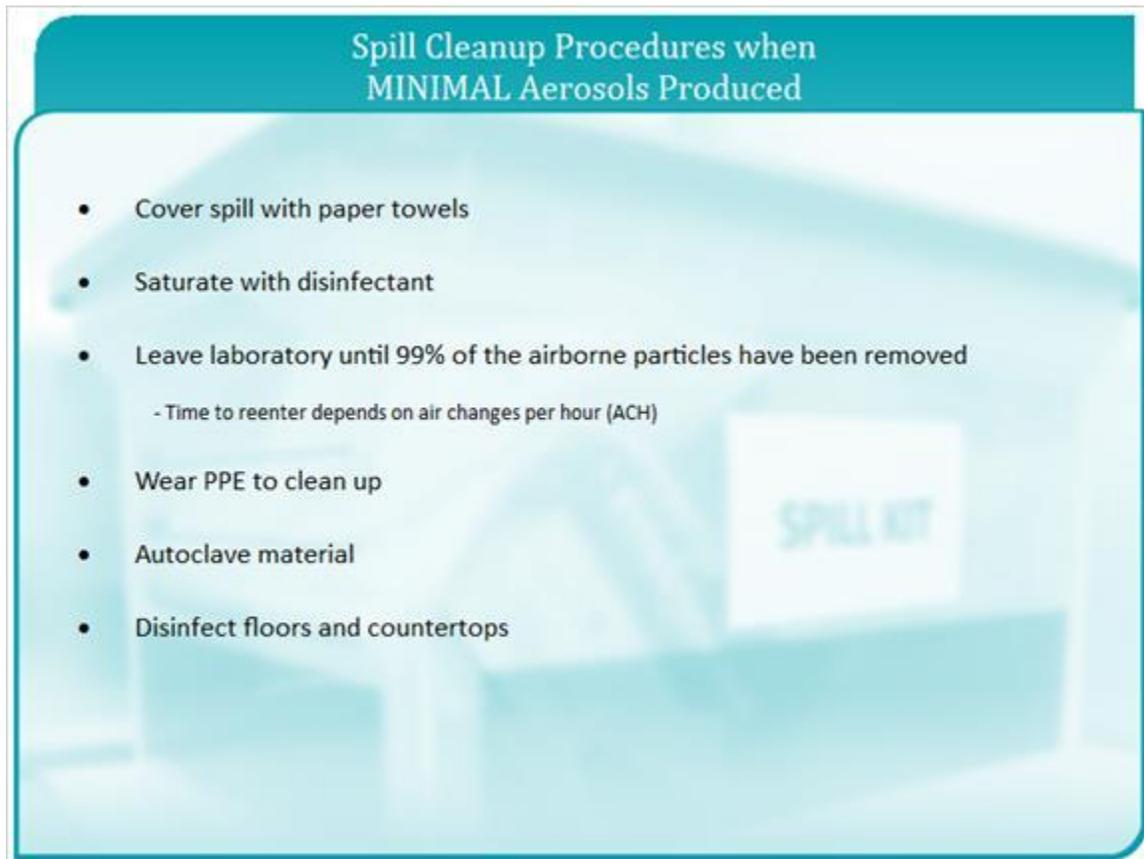
## 5. Standard Operating Procedure (SOP)



### Notes:

Having a good SOP that can be easily referenced by staff is very important.

## 5.2 Spill Cleanup Procedures when MINIMAL Aerosols Produced



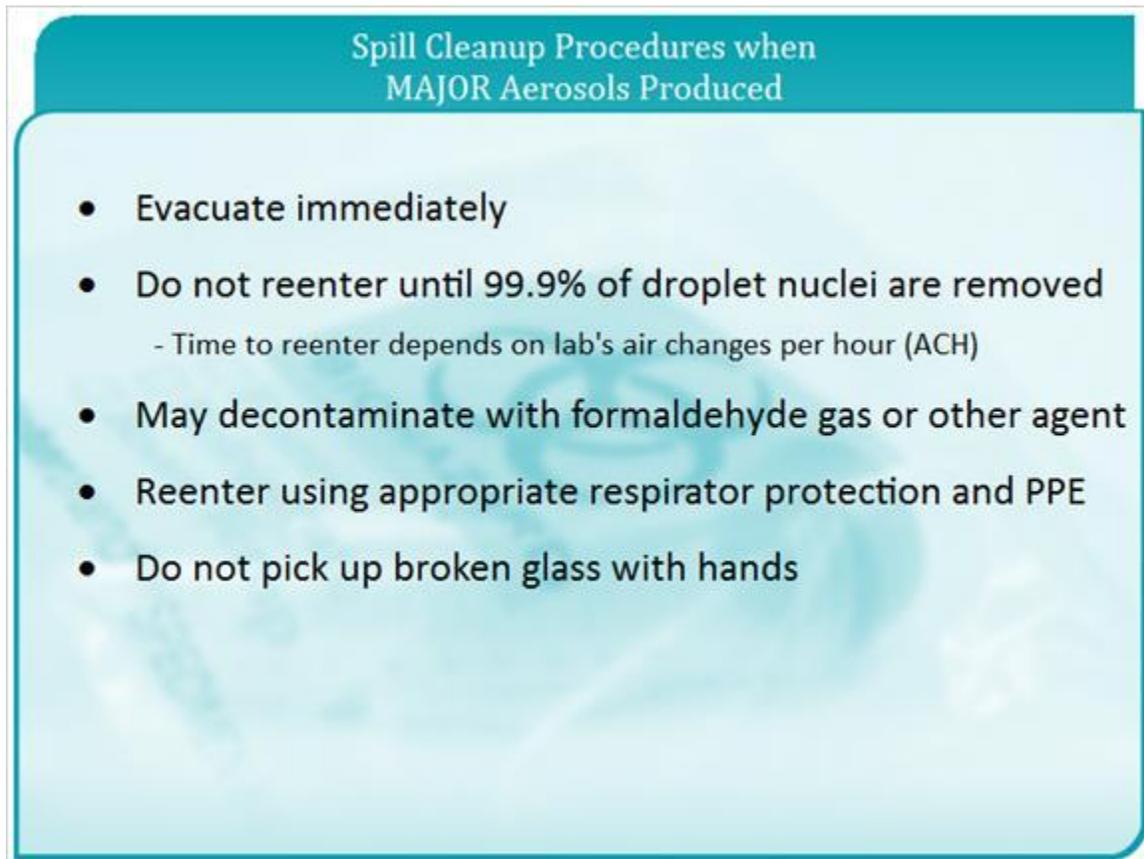
The infographic features a teal header with the title "Spill Cleanup Procedures when MINIMAL Aerosols Produced". Below the header is a list of seven bullet points. The background of the infographic shows a laboratory setting with a spill kit on a counter and a person in a lab coat.

- Cover spill with paper towels
- Saturate with disinfectant
- Leave laboratory until 99% of the airborne particles have been removed
  - Time to reenter depends on air changes per hour (ACH)
- Wear PPE to clean up
- Autoclave material
- Disinfect floors and countertops

### Notes:

All SOPs should include spill response. This is usually designated into two categories. Here we steps for cleaning up a spill with minimal aerosols. So, you want to , first of all, make sure you cover the spill with paper towels, and then saturate those towels with disinfectant. Then leave the laboratory until about 99% of the airborne particles have been removed. So remember, this is going to be dependent upon how many air changes per hour that you have. So, you need to make that determination first, and put that in your SOP. How long will they wait until they reenter the room? You want to make sure you are wearing all of the appropriate PPE to clean up the spill and the material has to be autoclaved. And then, disinfect your floors and countertops.

### 5.3 Spill Cleanup Procedures when MAJOR Aerosols Produced



**Spill Cleanup Procedures when  
MAJOR Aerosols Produced**

- Evacuate immediately
- Do not reenter until 99.9% of droplet nuclei are removed
  - Time to reenter depends on lab's air changes per hour (ACH)
- May decontaminate with formaldehyde gas or other agent
- Reenter using appropriate respirator protection and PPE
- Do not pick up broken glass with hands

**Notes:**

The other category should cover spills where major aerosols are produced. An example would be, you drop a liquid culture of *M. tuberculosis* and it breaks. Here, your first step is to evacuate immediately. Do not reenter until 99.9% of droplet nuclei are removed. Once again, this is dependent upon your air changes per hour. If necessary and it is a large spill, you may have to decontaminate with formaldehyde gas or another agent. Once again, reenter using appropriate respirator and PPE, and make sure that you are not picking up any broken glass with your hands.

## 5.4 Post-Exposure Management



**Post-Exposure Management**

Laboratory Occupational Health Protocol for Infectious Agent Exposure:

- Staff should be trained to recognize symptoms of TB disease
- Report exposure events and illnesses
- Respond to potential exposure events
- Respond to respiratory illness in laboratory workers post exposure
- Initiate diagnostic testing for exposed worker

### Notes:

Does your laboratory have an Infectious Agent Exposure or Laboratory Occupational Health Protocol? It should, and it should include how to report exposure events and illnesses, how the laboratory is going to respond to potential exposure events, how to respond to respiratory illnesses in laboratory workers after they have been exposed, and when do you initiate the laboratory testing of an exposed worker. As we mentioned before, your occupational health and safety should be conducting testing on your laboratorians. So, it can either be a tuberculin skin test or an IGRA.

For laboratories where there is careful documentation of a tuberculin skin tests for a 3-5 year period with no conversions, annual skin testing for employees is appropriate. However, in a laboratory where transmission of tuberculosis has recently occurred, tuberculin skin testing should be repeated every three months until no additional conversions have been detected for two consecutive three-month intervals.

### **5.5 What if There Is an IGRA or TST Conversion?**

#### **What if There is an IGRA or TST Conversion?**

If a staff member converts from IGRA or TST negative to positive:

- Evaluate the BSC, repair and recertify
- Evaluate procedures and techniques
- Retrain and re-educate as indicated

#### **Notes:**

So, what happens if you actually have a conversion? Rarely, a staff member who works with TB may experience a tuberculin skin test conversion or positive results for an IGRA. When this happens, a formal assessment of the equipment, the work practices, the training and education should be reformed. If necessary, you need to repeat your risk assessment. You may need to rewrite some protocols. And after this is done, make sure you reeducate or retrain all staff depending on those changes made.

**5.6 Based on the picture, what is the primary issue with the setup in the BSC?**

### Knowledge Check

Based on the picture, what is the primary issue with the setup in the BSC?



- The BSC is too large, resulting in difficulty decontaminating the working space.
- The BSC is too small, it is required to be large enough to store all supplies and reagents.
- The BSC is too crowded, impeding laminar air flow and efficiency of movement.
- A BSC should not be used for processing sputum specimens.

**5.7 Which statement is true?**

Knowledge Check

Which statement is true?

- Heat fixing does not have the potential to produce droplet nuclei, and can be done outside of the BSC.
- Vortexing does not have the potential to produce droplet nuclei, and can be done outside the BSC.
- Mixing with a pipette does not have the potential to produce droplet nuclei, and can be done outside the BSC.
- All of the above can produce droplet nuclei, and should be performed inside the BSC.

**5.8 After centrifugation, where should conical tubes be removed from the sealed carrier bucket?**

### Knowledge Check

After centrifugation, where should conical tubes be removed from the sealed carrier bucket?

- Directly from within the centrifuge immediately after it stops to prevent re-suspension of the sediment
- In the BSC
- On the bench top after waiting 5 minutes.

**5.9 Which of the following is not a recommended component of personal protective equipment (PPE) for working with *M. tuberculosis*?**

### Knowledge Check

Which of the following is not a recommended component of personal protective equipment (PPE) for working with *M. tuberculosis*?

- N95 particulate respirator
- Surgical mask
- Closed-front gown
- Gloves

## 5.11 Additional References

### Wrap-up

Thank you for your participation!

For additional information on laboratory biosafety, please see the following references:

- Miller, M.J., R. Astles, T. Baszler, K. Chapin, R. Carey, L. Garcia, L. Gray, D. Larone, M. Pentella, A. Pollock, D.S. Shapiro, E. Weirich, and D. Wiedbrauk. Guidelines for safe work practices in Human and Animal medical diagnostic laboratories. MMWR, Supplement, January 6, 2012. <http://www.cdc.gov/mmwr/pdf/other/su6101.pdf>
- 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>

#### Notes:

Thank you for participating in this training. Protection of staff should be of primary concern for your laboratory.