Laboratory Safety: Work Practices for *Mycobacterium tuberculosis*
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

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 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

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

Minimum requirements to conduct work in the laboratory:

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

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

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

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.
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

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

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

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

**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,

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

- 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 *Mycobacterium 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?

- 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.

Notes:

So, what is 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 *Mycobacterial 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

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?
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)
- [x] 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

**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

**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.

<table>
<thead>
<tr>
<th>Risky activities that can generate droplets and droplet nuclei:</th>
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<tbody>
<tr>
<td>• Vortexing</td>
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<tr>
<td>• Pouring liquid cultures and supernatant fluids</td>
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<tr>
<td>• Using fixed-volume automatic pipettors</td>
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<tr>
<td>• Mixing liquid cultures with a pipette</td>
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<tr>
<td>• Preparing specimen and culture smears</td>
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<tr>
<td>• Dropping tubes or flasks containing cultures</td>
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<tr>
<td>• Spilling suspensions of bacilli</td>
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<tr>
<td>• Breaking tubes during centrifugation</td>
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<tr>
<td>• Heat fixing smears</td>
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</table>
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)

- 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.

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

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.

Download a poster from CDC: [http://www.cdc.gov/healthcare/infectioncontrol/prevent/ppe.html](http://www.cdc.gov/healthcare/infectioncontrol/prevent/ppe.html)
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

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

- 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 a 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

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 forming 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

- 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

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 (ACH) 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

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 (ACH). 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 protection and PPE, and make sure that you are not picking up any broken glass with your hands.
5.4 Post-Exposure Management

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?

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
- [x] In the BSC
- [ ] On the bench top after waiting 5 minutes.
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:


Notes:

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