Specimen Collection, Handling, Transport and Processing

Specimen Collection, Handling, Transport and Processing

Course Duration: 38:00

Begin Course
1.2 Specimen Collection, Handling, and Transport

Notes:

This training module will discuss the aspects of Specimen Collection, Handling and Transport as they relate to TB testing.
1.3 Specimen Quality Is Important

As with all laboratory testing, it is critical that the specimen submitted be of high quality in order to obtain the highest quality result. APHL has developed a tool to help you assess various aspects of TB testing in your lab. Ensuring quality specimens is included in this tool and the website is included on this slide.

Notes:

http://www.aphl.org/aphlprograms/infectious/tuberculosis/Pages/tbsatool.aspx
1.4 Working with Healthcare Providers

Notes:

Specimen quality begins of course with collection. Health care providers, who are either collecting specimens or instructing patients on how to collect specimens, must clearly understand proper collection methods and how suboptimal collection procedure will affect the result they ultimately use to treat their patient.

It is important to build good working relationships with health care providers. Laboratories also should have a reference manual for providers that includes: specimen type and volume requirements; specimen collection, labeling, storage and transport instructions; and specimen rejection criteria. When the lab does identify persistent issues with specimens submitted, they should provide feedback to the provider.
### 1.5 Specimen Types

<table>
<thead>
<tr>
<th>Respiratory</th>
<th>Non-respiratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Sputum (expectorated, induced)</td>
<td>• Tissue</td>
</tr>
<tr>
<td>• Bronchoalveolar lavage (BAL)</td>
<td>• Body fluids</td>
</tr>
<tr>
<td>• Bronchial wash/brush</td>
<td>• Blood</td>
</tr>
<tr>
<td>• Transtracheal aspirate</td>
<td>• Stool</td>
</tr>
<tr>
<td></td>
<td>• Gastric lavage</td>
</tr>
<tr>
<td></td>
<td>• Urine</td>
</tr>
</tbody>
</table>

Refer to the CLSI M48-A document, *Laboratory Detection and Identification of Mycobacteria*

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**Notes:**

This slide shows specimen types considered acceptable for the isolation of *Mycobacterium tuberculosis*. The CLSI M48-A document, *Laboratory Detection and Identification of Mycobacteria* should be available in your laboratory and it provides a great resource as a comprehensive list of specimen types and recommendations for collection and transportation.
2. Respiratory (Pulmonary) Specimens

Notes:

Now, let's take a look at respiratory specimens.
2.2 Sputum

Sputum is the most common specimen submitted for the detection and isolation of *Mycobacterium tuberculosis*. Sputum comes from a deep productive cough and should contain minimal amounts of oral and nasal material. Sputum can be submitted for several purposes: to establish an initial diagnosis of tuberculosis, to monitor the infectiousness of the patient, and to determine the effectiveness of treatment.

**Notes:**

Sputum is the most common specimen submitted for the detection and isolation of *Mycobacterium tuberculosis*. Sputum comes from a deep productive cough and should contain minimal amounts of oral and nasal material. Sputum can be submitted for several purposes: to establish an initial diagnosis of tuberculosis, to monitor the infectiousness of a patient, and to determine the effectiveness of treatment.
2.3 Sputum Quality

A good sputum specimen will be thick and contain mucoid and mucopurulent material as depicted by the specimen shown on the right. Suboptimal specimens should either be rejected or the results should have a qualifying statement attached. Even good quality sputum specimens of volume less than 3-5 MLs will not give the best results, so negative results obtained from these specimens should have a qualifying statement attached. Sputum specimens that are induced may actually appear thinner and less purulent than a good expectorated sputum. Therefore the lab requisition should indicate when a specimen is induced, so it is not rejected as saliva.
2.4 Sputum Quality

Notes:

Here we see different sputum specimens ranging from a good sputum, seen on the upper left, all the way down to one containing saliva. Saliva, of course, is not an acceptable specimen for tuberculosis testing.
2.5 Indications for Sputum Collection

Indications for Sputum Collection

- Initial diagnosis of TB:
  - Collect a series of three sputum specimens, 8-24 hours apart, at least one of which is an early morning specimen
  - Optimally, sputum should be collected before the initiation of drug therapy
- For release from home isolation:
  - If patient is smear positive and on treatment: Collect sputum until 3 specimens are negative.
- Monitoring of therapy:
  - Obtain, at a minimum, two sputum specimens for culture at least monthly until two consecutive cultures convert to negative

Notes:

The guidelines for frequency of collection for sputum specimens that will be used for initial diagnosis have changed slightly. The most recent MMWR recommendations now say that three sputum specimens should be collected at least 8 hours apart with one of them being a first morning specimen.

First morning specimens are usually considered best for AFB smears since mucus has had the opportunity to collect and concentrate in the lungs overnight.

Laboratorians need to explain to physicians that may routinely send less than three specimens per patient why it is important for treatment.

As with other specimens submitted for culture the specimens should be collected prior to the initiation of drug therapy.

If a patient is on treatment, sputum should be tested until there are three negative smears.

When monitoring patients for the effectiveness of therapy, sputum collection should start at least monthly until the cultures convert to negative.
2.6 Specimen Collection: All Aerosol-Producing Procedures Pose a Risk of Exposure

Notes:

Infectious aerosols are three to five microns in size, and can stay in the air for hours. The greatest risk of TB is from these infectious aerosols. The aerosol is produced as a patient talks, coughs, sings, or sneezes are well recognized as requiring containment.

So, this applies to specimen collection as well, whether it be sputum collection or bronchoscopy. When a patient is suspected or confirmed to have tuberculosis, airborne precautions must be used.
2.7 Specimen Collection

Notes:

Patients suspected of or having TB must be housed in a negative pressure room and everyone who enters the room must use an N-95 respirator. This is especially important during specimen collection. Since we are using respirators, it's important to know that OSHA requires an employer to have a written respirator protection plan and each worker who is assigned to wear a respirator must receive a fit test before the worker is required to wear the respirator in the workplace. And, they must perform a seal check with each use. Fit testing is actually repeated annually. All specimens collected for mycobacteria testing must be collected in a sealed leak proof container.
2.8 Storage and Transport of Sputum Specimens

Notes:

Standard recommendations for most microbiology specimens are that if the specimen cannot be processed within one to two hours of collection, they must be refrigerated. Additionally the specimen should ideally reach the laboratory within 24 hours of collection and be kept cold during transport. It is important that the submitter know to avoid batch shipment. Don’t let them collect all three specimens on a patient and ship them at once. They should ship them as they are collected.
2.9 Pulmonary Specimens Other than Sputum:

There are respiratory and pulmonary specimens other than sputum that are acceptable for the diagnosis of TB. The most common ones submitted are bronch lavages and bronch brushes. As you can see, the minimum volume requirement for these specimens is 3mL, and refrigeration is recommended when there will be a delay from collection to processing. The reason for the refrigeration, or keeping the specimen cold, is to prevent overgrowth of other bacteria and normal flora that may be present. These organisms can quickly outgrow *Mycobacterium tuberculosis* and make it more difficult to isolate, because the doubling time for other common respiratory flora is 15 to 20 minutes, whereas the doubling time for *Mycobacterium Tuberculosis* is 12 to 24 hours.

- The doubling time for other common respiratory flora is 15 to 20 minutes.
- The doubling time for *Mycobacterium tuberculosis* is 12 to 24 hours.
3. Non-Respiratory (Extrapulmonary) Specimens

Notes:

Now, let’s take a look at extrapulmonary specimens that can be submitted for the isolation of *M. Tuberculosis*.
3.2 Extrapulmonary Specimens

The laboratory should expect to receive a variety of extrapulmonary specimens which may be divided into two groups:
- Specimens from non-sterile body sites
- Specimens from normally sterile body sites

- Should be collected in a sterile leakproof container
- Should be transported as soon as possible
- Swabs are generally not acceptable

Notes:
The laboratory should expect to receive a variety of extrapulmonary specimens which may be divided into two groups. Those that are collected from non-sterile body sites, and those that are collected from normally sterile body sites. Once again, they all should be collected in a sterile leakproof container, and they should be transported as soon as possible.

Now, a swab is always an inferior specimen type. The highest quality results will be obtained by using an aspirate or a piece of tissue. Education of physicians on the need to submit fluid or tissue as opposed to a swab should be standard practice for all specimens submitted for culture. Additionally, it might be helpful to get in touch with nurses in surgical suites, and let them know they should not to hand the physician a swab for specimen collection. If there are no policies in place for correcting collection issues, the focus should be on targeted, consistent education for the physicians or other health care providers.
### 3.3 Extrapulmonary Specimen Collection Guidance

<table>
<thead>
<tr>
<th>Specimen from Non-Sterile Body Sites</th>
<th>Recommended Collection Time</th>
<th>Volume Requirements</th>
<th>Collection Frequency</th>
<th>Transport</th>
<th>Recommended for Isolation of MTBC?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric Aspirate</td>
<td>Early morning before patient eats and while still in bed</td>
<td>5-10 ml is optimal; maximum volume is 15 ml</td>
<td>One specimen per day on three consecutive days</td>
<td>Room temperature; if delayed &gt;1 hour neutralize with 100 mg sodium bicarbonate</td>
<td>Yes</td>
</tr>
<tr>
<td>Urine</td>
<td>First morning specimen (void midstream)</td>
<td>10-15 ml minimum; prefer up to 40 ml</td>
<td>One specimen per day on three consecutive days</td>
<td>If delayed &gt;1 hour refrigerate</td>
<td>Yes</td>
</tr>
<tr>
<td>Stool</td>
<td>No recommendation</td>
<td>Minimum volume is 1 gram</td>
<td>No recommendation</td>
<td>Refrigerate if delayed &gt;1 hour; do not freeze</td>
<td>Mainly for diagnosis of disseminated MAC* disease in patients with AIDS</td>
</tr>
</tbody>
</table>

* *Mycobacterium avium* complex

**Notes:**

So, let's look at some specimen collection guidance for these extrapulmonary specimens. For gastric aspirates, it's critical that the specimen be neutralized with 100 mg of sodium carbonate within 1 hour of collection. It can be useful to provide collection kits that include specimen containers that contain the sodium bicarbonate already in them.

It is recommended that 24 hour urine collections not be accepted. It is preferable that 3 consecutive first morning specimens of urine be submitted. Stool is normally submitted for acid fast bacillus culture from AIDS patients suspected of having disseminated MAC. It might be a good policy to include the instruction that submitters call the laboratory first prior to submitting this specimen.
### 3.4 Extrapulmonary Specimen Collection Guidance

<table>
<thead>
<tr>
<th>Specimen from Normally Sterile Body Sites</th>
<th>Volume Requirements</th>
<th>Transport</th>
<th>Recommended for Isolation of MTBC?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral Spinal Fluid</td>
<td>10 ml is optimal; minimum volume is 2-3 ml</td>
<td>As soon as possible at room temperature; do not refrigerate</td>
<td>Usually paucibacillary; culture may have limited sensitivity</td>
</tr>
<tr>
<td>Other Body Fluids (pleural, peritoneal, pericardial, synovial)</td>
<td>10-15 ml is optimal; minimum volume is 10 ml</td>
<td>If delayed, refrigerate</td>
<td>Yes</td>
</tr>
<tr>
<td>Tissues or Lymph Nodes</td>
<td>As much as possible; add 2-3 ml sterile saline</td>
<td>As soon as possible at room temperature (no formalin, preservatives, or fixatives)</td>
<td>Yes</td>
</tr>
<tr>
<td>Blood</td>
<td>10ml preferred, minimum 5 ml. Collect in SPS or heparin tube, no EDTA or ACD</td>
<td>At room temperature, do not refrigerate or freeze</td>
<td>Mainly for diagnosis of disseminated MAC disease in patients with AIDS</td>
</tr>
</tbody>
</table>

**Notes:**

Cerebrospinal fluid is a sterile specimen and requires no refrigeration. So, this is an exception to those criteria we gave before where specimens not received in the laboratory within one to two hours of collection be refrigerated. Spinal fluid typically has low numbers of organisms, and so culture of this specimen may have limited utility. As in the case of stool, blood is also used mainly to diagnose disseminated MAC disease in AIDS patients. It is important that your collection instructions for this specimen type indicate that blood not be collected in EDTA. SPS or heparin tubes are, however, acceptable. CLIA requires each laboratory to develop specimen rejection criteria.
4. Suboptimal and Unacceptable Specimens

Notes:

Suboptimal specimens should either be rejected outright or should be highly discouraged.
Notes:

As we all know, processing specimens that are of poor or suboptimal quality is a drain on the financial and personnel resources of the laboratory, but also results in additional cost to the patient for results that are not of high quality. Laboratories must develop their own set of rejection criteria, and should also develop a list of sub-optimum, highly discouraged specimens.

It is important to note that even when there are problems with specimens obtained by an invasive procedure such as bronchoscopy, lumbar puncture, or surgery, these specimens should not be rejected but the results should be qualified. Also, you don't want to just report a specimen as rejected, it's important to also report why it is being rejected: quantity not sufficient for valid testing, transit time outside of the recommended limit from collection to receipt, etc. The bottom line is that rejection criteria should be established and readily available for reference.
4.3 Possible Rejection Criteria (1)

**Possible Rejection Criteria (1)**

- Labeling of specimen does not match identifiers on requisition form
- Insufficient volume
- Dried swabs in general are not optimal
  - Provide limited material
  - Hydrophobicity of mycobacterial cell envelope inhibits transfer to media
- Pooled sputum or urine
- Sputums left at room temperature for 24 hours

**Notes:**

There are some instances where outright rejection is the proper practice. And these include: any unlabeled or mislabeled specimen, or a specimen that is submitted such as saliva. Dried swabs. Well, while we all know that specimens on swabs are not optimal, we also know that we do get these specimens. Explain to the health care provider that the hydrophobicity of the mycobacterial cell envelope inhibits proper transfer of the organism to the media, and it has the potential to result in a negative culture even if mycobacteria are present in the specimen. Also, pooled sputum or urine that has been collected over the course of the day should be rejected. Also, sputums that have been at room temperature for greater than or equal to 24 hours should be rejected.
4.4 Possible Rejection Criteria (2)

Possible Rejection Criteria (2)

- Broken specimen containers or leaking specimens
- Excessive delay between specimen collection and receipt in the laboratory
- Blood specimens collected in EDTA might be rejected for culture as these inhibit growth of MTB
- Tissue or abscess material in formalin
- Gastric lavage fluid if pH not adjusted within one hour of collection

Notes:

Specimen containers that are leaking or broken present the opportunity for contamination of the specimen, but also they represent a safety hazard to staff. Staff, including staff that maybe opening the deliveries, should be trained in proper safety protocols for these specimens. Laboratories should develop their own delivery interval criteria and may or may not reject specimens based on the length of time from collection to receipt in the lab.

For laboratories receiving specimens from patients who are not at their facility, a time limit longer than 24 hours may be in order due to the logistics of transport. Some labs may set this limit at 48 or 72 hours as long as the specimen is transported under the appropriate conditions. Clinicians and providers should be educated on the reasons behind the requirements for rapid delivery. Also, it's important that tissues submitted in Formalin will inhibit growth. So, the testing of tissues submitted this way should be rejected.
Now, let’s discuss a little bit about specimen transport.
5.2 Transport of Biological Substances (Category B)

**Basic triple packaging system**

- (i) leakproof primary receptacle(s);

- (ii) leakproof secondary packaging containing sufficient additional absorbent material shall be used to absorb all fluid in case of breakage.

  - For cold transportation conditions, ice or dry ice shall be placed outside the secondary receptacle. Wet ice shall be placed in a leakproof container.

- (iii) an outer packaging of adequate strength for its capacity, mass, and intended use.

**Notes:**

Shipping clinical specimens for TB testing qualifies as a Category B biological substance. This means that you have to have basic triple packaging system that includes a leakproof primary container, leakproof secondary packaging that contains sufficient additional absorbent material that will be used to absorb all the fluid in case of breakage. And also, an outer packaging of adequate strength for its capacity, mass, and intended use.
5.3 Transport of Biological Substances (Category B)

Notes:

Remember that those doing the packaging and shipping must be trained and certified in packaging and shipping protocols and be recertified every two years. Labeling is absolutely critical. Certification labels should be visible and properly placed on the package. Unannounced visits by the Department of Transportation do happen. Also, remember that your 24 hour contact person must be available if they are called, or it could actually lead to fines for your institution.
5.4 Sputum Collection Kit

Notes:
Sometimes, it's very helpful to actually provide a specimen collection kit. This slide shows an example of a collection and shipping kit supplied by the Wisconsin State Laboratory of Hygiene. This is a public health laboratory and you can see that they have included a cool pack so that specimens can be kept at an appropriate temperature until it arrives in the laboratory.
Transport of Biological Substances

- Transport of patient specimens is regulated by both the Department of Transportation (DOT) and by International Air Transport Association (IATA) rules.
- Laboratories must have personnel trained in and familiar with these regulations.
- For details regarding these regulations, please see the information provided in the next slide.

Notes:
Transport of patient specimens is regulated by both the Department of Transportation and by International Air Transport Association or IATA rules. Once again, make sure that you have personnel trained and familiar with these regulations, and that they are recertified every two years. For details regarding these regulations, please see the information provided on the next slide.
5.6 Packing and Shipping Guidance

Notes:
This slide gives several resources for information on proper packaging and shipping protocols. All of these resources should be available at your facility.
6. Additional Information

Notes:

Let's cover some additional information as it relates to specimen collection.
6.2 Instructions for Sputum Collection

- Healthcare providers should educate and coach patients on proper specimen production and collection
- Patients should also be informed of the possible infectious nature of his or her secretions
- Specimens should be collected in appropriate tubes that are sterile, clear, plastic, and leakproof (50 ml screw capped centrifuge tubes that can withstand 3000 x g are preferred)
- Proper labeling protocols should be put in place by the laboratories
- Work with TB program to provide instructions for submitters

Notes:

As emphasis to our previous information, proper collection of the specimen is critical. Sputum specimens are frequently collected by the patient at home, therefore it is imperative that health care providers understand the proper way to collect a good sputum specimen and ensure that they are giving clear instruction to the patients. We don't want them to give specimen cups to patients and instruct them to “spit into the cup.” We'll end up with saliva arriving at the laboratory instead of a really good sputum specimen.

Health care providers providing collection supplies to patients so that they can collect specimens at home also need to emphasize that the laboratory will require 2 patient identifiers on the specimen container: name, medical record, birthdate; as long as there are 2. If possible, the provider should label the specimen container ahead of time as well as fill out the test request form in advance, and then instruct the patient to fill in the date of collection.
6.3 Examples for Instructions for Sputum Collection

Notes:

The Pennsylvania Department of Health has a very nice chart with instructions and graphics on the collection of sputum for TB testing. That chart is displayed here on this slide.
6.4 According to current U.S. recommendations, how many sputum specimens should initially be collected and cultured from patients suspected of having TB infection?

Knowledge Check

According to current U.S. recommendations, how many sputum specimens should initially be collected and cultured from patients suspected of having TB infection?

- One
- Five
- Three
- Thirty
According to CDC/ATS/IDSA recommendations, how often should sputum specimens be collected for culture when monitoring TB patients on therapy?

- At least daily
- At least monthly
- At least every 6 months
- Collection of sputum specimens is unnecessary for TB patients on therapy
6.6 All of the following specimen types should be considered as unacceptable EXCEPT:

- Formalin preserved tissue specimens
- Pooled sputum
- Dried swab
- Bronchial wash
6.7 Which of the following is not part of the DOT/IATA packaging requirement for the transport of Category B Biological Substances?

Which of the following is not part of the DOT/IATA packaging requirement for the transport of Category B Biological Substances?

- A triple packaging system that is leak proof and absorbent
- Dry ice to keep specimens frozen
- Visible certification label UN3373
- Specimen record
So, once we have our specimens and they are properly accessioned, we move on to processing.
7.2 Principles of Specimen Processing

Principles of Specimen Processing

- Respiratory specimens (and other specimens from non-sterile sites) require digestion, decontamination, and concentration:

  **Digestion:** Mucolytic agent used to liquefy sputum specimens to release AFB and expose normal flora for decontamination

  **Decontamination:** Toxic agent used to kill rapidly growing normal flora that would otherwise overgrow slow-growing mycobacteria

  **Centrifugation:** Used to sediment bacteria following digestion/decontamination

**Notes:**

Specimen processing of respiratory specimens or other non-sterile specimens includes digestion, decontamination and a concentration procedure. The digestion portion of this process uses a mucolytic agent that liquefies the sputum allowing the AFB that may be present to be released, and also to enhance contact time between the normal flora and the decontamination agent. There are several decontamination agents you can use depending on your population, conditions surrounding transport, etc. The decontaminating agent kills the more rapidly growing rapidly growing normal flora. Then, concentration of the organisms takes place during the centrifugation step.
7.3 Effect of Processing Procedure

**Notes:**

Mycobacteria can also be killed or lost during processing and this can happen at various points in the procedure. The decontaminating agents can kill Mycobacteria if contact time extends beyond the recommended time period. Loss of viability of Mycobacteria can also occur from heat build-up during centrifugation. So, it's very important to use a refrigerated centrifuge and ensure that your relative G-forces are within acceptable parameters. Vigorous decanting or decanting of specimens that do not produce a tight pellet after centrifugation can also result in loss of organisms.
For more information on biosafety practices in the Mycobacteriology laboratory, please review the module on biosafety within this series.

Notes:

Safety is a critical issue during specimen processing. There are many aerosol generating procedures that occur throughout the process including those listed on this slide. This work needs to be performed inside of a biosafety cabinet, but it's important not only to just work inside the biosafety cabinet, but to make sure that the work inside the biosafety cabinet is done properly. Some basic guidelines are that the actions that occur must be performed in a manner that does not disrupt the airflow of the cabinet and you must avoid excess clutter inside the biosafety cabinet which can block the air vents, and therefore, disrupt the airflow.
7.5 Aseptic Techniques: Getting Started

Aseptic Techniques: Getting Started

- Disinfect BSC, centrifuge, and tabletop with tuberculocidal disinfectant (repeat after specimen and culture workup complete)
- Perform all work on absorbent pad soaked with disinfectant to absorb any droplets that may inadvertently occur
- When possible, leave an empty space in the rack between each specimen tube
- Work in sets equivalent to one centrifuge load (e.g., eight specimens at a time)

Notes:

Some good practices to follow when processing TB specimens include: Disinfection of all surfaces both before and after the work. While processing, ensure that there is an absorbent pad soaked with a tuberculocidal disinfectant on the surface of the BSC. This will help to prevent splash and splatter if droplets occur. Splash and splatter from droplets can lead to cross contamination. If you can try to leave an empty space in the rack between specimen tubes, this also will help prevent cross contamination. You should process only the number of specimens that will fit into your centrifuge. If necessary, you may need to process multiple specimens in different batches.
7.6 Aseptic Techniques: Processing

Notes:

Preventing cross contamination while processing is critical. Make sure that you always use a new disposable, sterile pipette at every step. Also, you should never remove the cap from more than one specimen at a time, or there is the likelihood of cross contamination from splashes and aerosols. It's always important to ensure that when you're pouring liquids, the lips of the two tubes or containers never touch. You must use aerosol-safe centrifuge cups, and when decanting the supernatant after centrifuging, make sure it is into a splash proof container that contains an appropriate disinfectant. You can even use a funnel and line it with absorbent material that has been soaked with disinfectant. This will help prevent splash and splatter and aerosol production that occurs when the liquid hits the surface of the funnel.
7.7 Digestion & Decontamination Methods

Several methods are available for digestion and decontamination of clinical specimens:

- **N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH)**
  - Commercially available: Alpha-Tec NAC-PACTM, BD MycoPrep™
- Oxalic acid
- Cetylpyridinium chloride (CPC)-sodium chloride
- NaOH method (Petroff's method)
- Zephiran-trisodium phosphate (Z-TSP)

Notes:

Now, let's take a look of Digestion & Decontamination Methods. As mentioned earlier, there are several approved methods for digestion and decontamination. The N-acetyl-L-cysteine-sodium hydroxide method is probably the most common, and there are commercial kits available for this method. Oxalic Acid is used for decontamination of pseudomonas for specimens from Cystic Fibrosis patients. (CPC)-sodium chloride is most commonly used for decontamination on an international basis. Make sure that the decontamination method your lab is using is compatible with other tests that may be performed. For instance, some nucleic acid amplification tests cannot be done specimens that have been processed with methods other than the NALC- Sodium Hydroxide method.
7.8 NALC-NaOH Method: Principle

- Most common and preferred method
- Rapid and relatively effective in reducing the number of contaminants
- Addition of the mucolytic agent 2% NALC allows effective decontamination with 1% NaOH (less harsh on mycobacteria) (final concentrations)
- Sodium citrate also included in digestion mixture to bind heavy metal ions in specimen that could inactivate N-acetyl-L-cysteine

Notes:

Now, let's take a look at the NALC- Sodium Hydroxide method. As mentioned, the NALC- Sodium Hydroxide method is the most common method. It's effective and it's fairly rapid. In this method the NALC serves as the digestion, and the Sodium Hydroxide serves as the decontaminating agent. The final concentration of the NALC in the processing liquid should be two percent. And, the final concentration of the Sodium Hydroxide should be at one percent. This has been determined to be a concentration that will effectively kill the normal flora, and still allow the mycobacteria to survive, as long as the contact time does not exceed fifteen minutes. Some specimens may contain heavy metals, and these can inactivate the NALC. Therefore, Sodium Citrate is also added to the mixture.
Notes:

Here you see a chart for the easy determination of the volumes of each reagent needed to achieve the necessary final volume of the processing fluid based on the number of specimens you will be processing. These reagent volumes will allow for the final two percent and one percent concentration of NALC and Sodium Hydroxide, respectively. You can prepare the Sodium Hydroxide and Sodium Citrate in advance, but be sure that you do not add the NALC until you are ready to process. Once the NALC is added, the solution must be used within 24 hours or it should be discarded.
**7.10 NALC-NaOH Method: Procedure for Sputum**

**NALC-NaOH Method: Procedure for Sputum**

1. Add equal volume of NALC-NaOH solution to 5-10 ml of sputum in 50 ml plastic screw cap centrifuge tube.

2. Cap tube tightly. Invert tube so that NALC-NaOH solution contacts all inside surfaces of tube and cap; then mix contents for approximately 5-20 seconds with a Vortex mixer.

3. Allow mixture to stand for 15 minutes at room temperature with occasional gentle shaking by hand.

4. Add sterile distilled water or sterile pH 6.8 phosphate buffer to the 50 ml mark on tube. Securely cap tube and mix by inversion.

5. Centrifuge the tubes for 15 min at 3000 x g using aerosol-proof sealed centrifuge cups.

**Notes:**

The first step in processing is to add an equal volume of NALC-NaOH solution to the specimen in a 50cc plastic screw cap centrifuge tube. You don't want to have more than 10 mL of specimen in the tube, because you need to allow for sufficient volume of the buffer to stop the decontamination reaction. If you do have more than 10mL of specimen, please split it into two tubes. Remember, that the specimen may adhere to the cap or the upper portion of the tube, so all of this specimen needs to get included in the decontamination process. Then, cap the tube tightly and invert it to ensure that the digestion/decontamination solution contacts all of the inside surfaces. Vortex for 5 to 20 seconds depending on the viscosity of the specimen. Restricting the amount of time for vortexing is important. You don't want to oxidize your deconning agent and, therefore, decrease its activity. You can design your process to vortex or hand mix the specimens every 5 minutes during the room temperature contact time. This keeps the decontaminating agent more distributed throughout the specimen, and in some cases, it can actually decrease your contamination rates. You want to allow the specimens to stand at room temperature for 15 minutes. So, start your time as soon as you finish your first patient, and proceed by repeating the process for the remaining specimens. Paying attention to the time interval so that
your stop step takes about the same time to complete as did your initial processing step. You do not want your specimens to remain in contact with the de-con solution for much more than 15 minutes, because after this amount time, mycobacteria will start to be killed. At the end of the 15 minutes, add either sterile distilled water or sterile buffer to the 50 mL mark, secure the cap and mix by inversion. This will stop the decontamination process. Remember to do each tube individually and do not remove all the caps at once and add your stop solution. Load your tubes into aerosol-proof centrifuge cups and centrifuge the tubes for 15 minutes at 3,000 X g. You want to make sure that you’re achieving relative centrifugal force and not just using the RPM setting of 3000 X on your centrifuge. It’s vital that centrifugation speed be at 3000 x g. If the tubes do not spin fast enough, it will prevent the organisms from going into the pellet.
7.11 NALC-NaOH Method: Procedure for Sputum

Notes:

Your centrifuge cups or carriers must be opened inside the BSC to ensure that there is no risk of exposure to potential aerosols that may be present after centrifugation. Ensure that during the decanting process that you do not disturb or lose the pellet, because this is where your mycobacteria will be. Also ensure that you do not pour in such a way as to create splash back that can contaminate the lip of the patient tube. After wiping the lip of the tube as directed here, re-suspend your pellet in 1-2 mL of your chosen re-suspension fluid and mix gently to re-suspend the pellet. Then, you can proceed to inoculation of the media and smear preparation.
7.12 NaOH Concentration is Key

Notes:

This slide shows a chart of the necessary volume of each reagent in order to achieve your desired final concentration of Sodium Hydroxide. As mentioned before, the recommended final concentration of Sodium Hydroxide is one percent. However, based on your patient population or your contamination rate, you may need to adjust to a higher concentration. Just keep in mind that once you are around two percent concentration of Sodium Hydroxide, you may be killing mycobacteria and decreasing the sensitivity of culture for those patients with negative smears.
7.13 Timing is CRITICAL

Notes:

The contact time for Sodium Hydroxide and the specimens is a critical factor in isolation of mycobacteria. Make sure that you consider this timing when determining how many specimens you will process in a single batch.
A positive *M. tuberculosis* control is not advised due to the issues with cross-contamination. However, a negative processing control, which can be 10 ml of sterile water or buffer, should be included with each batch of specimens processed. The negative control will put through the entire specimen processing procedure and actually inoculated on to media as well. Double check your specimen numbers on your patient tubes against each piece of media, as well as the slide during the inoculation step.
7.15 Specimen Processing Proficiency

Specimen Processing Proficiency

- Digestion, decontamination, and concentration procedures should only be performed by trained laboratory staff.
- Mycobacteriology laboratories should participate in an approved proficiency testing program.
- Proficiency in culture and identification of MTB may be maintained by digestion and culture of 15-20 specimens per week.

Notes:

So, as with any clinically diagnostic test performed by your laboratory, CLIA requires that you enroll in proficiency testing program by an approved provider. The PT should cover all of the procedures you do for the testing for Tuberculosis. Also, most resources recommend that you perform testing on a minimum of 15-20 specimens per week in order to maintain proficiency. Labs should take this into consideration when deciding to implement TB processing, culture and smear, or whether to actually maintain this testing. The CLSI document states that an individual microscopist should examine 15 smears per week in order to maintain proficiency.
7.16 Definition of Cross Contamination

Definition of Cross Contamination

- **Cross contamination**: The transfer of *M. tuberculosis* complex bacilli (or other mycobacteria) from one specimen to another specimen that does not contain viable bacilli, causing a false positive result.

  - The phenomenon of misdiagnosis of tuberculosis due to cross contamination has been widely reported and has significant clinical and therapeutic impact on the patient.

Notes:

Cross contamination is a serious issue. It can lead to treatment isolation of a patient that does not have tuberculosis. It also unnecessarily expends epidemiologic and lab resources during follow up.
7.17 Reducing the Possibility of Cross Contamination

Notes:

Here, we see some tips for reducing the chance of cross contamination.

Contamination can occur if the same biosafety cabinet is used for multiple procedures and not properly cleaned and maintained. Also important is staff competency. CLIA requires that all staff perform an annual demonstration of competency for all tests they conduct. This annual DOC includes direct observation as one of the methods you use to assess their competency. This is a good time to observe staff as they perform the digestion-concentration process, to make sure they are not demonstrating “procedural drift.”
7.18 Reducing the Possibility of Cross Contamination

Notes:

Here, we see some additional tips to reduce the possibility of cross contamination. Open the tubes very gently so that you avoid aerosol generation. And when you’re adding reagents to the tube, once again, make sure that you are opening only one tube at a time. Change your gloves often. And, try not to place specimens too close to each other in the rack.
8. Specimens from Cystic Fibrosis Patients and Extrapulmonary Sites

Specimen Collection, Handling, Transport and Processing

SPECIMENS FROM CYSTIC FIBROSIS PATIENTS AND EXTRAPULMONARY SITES
8.2 Cystic Fibrosis (CF) Patients

**Cystic Fibrosis (CF) Patients**

- Specimens from CF patients are often heavily contaminated with *Pseudomonas aeruginosa*.
- If it is known or discovered specimen is from a patient with CF or notable media contamination you can process concentrated sediment using only the 5% oxalic acid method.

**Notes:**

Let's take a brief look at specimens from Cystic Fibrosis patients.

Specimens from cystic fibrosis patients are often heavily contaminated with *Pseudomonas aeruginosa*. If you know a specimen was obtained from a cystic fibrosis patient, you can use the 5% oxalic method of decontamination. Or, if you discover during the reading of cultures that the media from a patient is heavily contaminated with *Pseudomonas* you can actually reprocess the sediment with this method.
8.3 Oxalic Acid Processing Method for Specimens from CF Patients

- Add an equal amount of 5% oxalic acid to:
  - 5-10 ml of primary respiratory specimen or
  - NALC-NaOH-processed concentrated sediment
- Vortex specimen and allow to incubate at room temperature for 30 minutes, mixing every 10 minutes.
- Neutralize with buffer solution.
- Concentrate specimen by centrifugation for 15 minutes at ≥ 3000 x g.
- Decant supernatant into splash-proof container and resuspend pellet with buffer solution.
- Inoculate media.

Notes:

As you can see, the process itself is very similar to the NALC-sodium hydroxide method. The steps include the addition of a decontaminating agent, vortexing, incubation at room temp, neutralization, and centrifugation. The critical difference in the process, besides the decontamination solution, is that the incubation is at room temperature for 30 minutes instead of 15. As we mentioned, this method can be used for decontamination of primary respiratory specimens from patients known to have cystic fibrosis. Or if you noticed that your media is contaminated with Pseudomonas, you can de-con the residual sediment once again using the oxalic method.
8.4 Processing Gastric Lavage and Urine

Notes:

Gastric lavages and urine specimens need to be concentrated and then the pellet can go through the decontamination process just like your sputum specimens. So, we want to centrifuge these for 30 minutes at greater than or equal to 3000 x g, discard your supernatant, re-suspend your pellet in sterile distilled water, and then, process as you do for sputum.
8.5 Processing Aseptically Collected Fluids

- Cerebral spinal fluid (CSF), synovial, pleural, peritoneal, pericardial
- No decontamination required.
- Concentrate specimen to maximize the yield of mycobacteria.
- Inoculate directly to culture media.

Notes:
As we heard earlier in this module, specimens from sterile sites do not need to undergo decontamination. You just need to centrifuge the specimen to concentrate any organisms that may be present prior to inoculating it to media.
8.6 Processing Tissue Specimens

Notes:

Some tissue specimens that you get will be Lymph nodes, or lung tissue, or even biopsies. If it's submitted in formalin, then it's unsatisfactory for culture. But as with other sterile sites specimens, no decontamination is required. You want to process the tissue specimens using a sterile tissue grinder, or mortar and pestle. Then, you inoculate it directly to your culture media. Now, cavitary lesions contain high concentrations of TB, so it's critical that all cutting of tissue occur inside the biosafety cabinet.
8.7 Processing Blood or Bone Marrow Aspirates

Notes:

For blood or bone marrow aspirates, the specimens should be inoculated directly into commercial blood medium. They don't normally recommend that you inoculate blood directly onto a solid media. If you have to transport the blood to the lab, prior to being inoculated into your system, make sure that sodium polyanethol sulfate, heparin, or citrate are used as anticoagulants. Blood collected in EDTA or coagulated blood are not acceptable. Also, it's important to know that some broth systems used for TB culture are not approved for blood specimens. Make sure you read your manufacturers package inserts prior to using these media.
9. Principles of Centrifugation

Notes:

Proper centrifugation is important to ensuring good culture results. So, let's take a look at these specifics.
### 9.2 Concentration of Specimens

<table>
<thead>
<tr>
<th>Concentration of Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Since mycobacteria do not readily sediment, centrifugation force and time are important for maximal concentration.</td>
</tr>
<tr>
<td>• Specimens should be centrifuged for at least 15 minutes at 3000 x g.</td>
</tr>
<tr>
<td>• Revolutions per minute (rpm) is a measure of speed for a particular centrifuge head and not a measure of concentration efficiency or relative centrifugal force (RCF).</td>
</tr>
<tr>
<td>• The relative centrifugal force is measured in units and is expressed in multiples of the earth’s gravitational field abbreviated as g.</td>
</tr>
<tr>
<td>• Use of a refrigerated centrifuge at 8 to 10°C may help to eliminate heat build-up which could be lethal to mycobacteria.</td>
</tr>
</tbody>
</table>

**Notes:**

The makeup of mycobacterial cell walls does not lend these organisms to readily settle or sediment. Centrifugation must be done at proper speed and temperature in order to ensure a successful yield of the organism in the sediment that you are going to use for culture and smear. You need to ensure that staff understand the difference between RPMs and g's. RPMs is a measure of the speed of the centrifuge head, but is not a measure of relative centrifugal force which is then expressed as g. You need to use a calculation of RPMs and the radius of the centrifuge head or rotor to determine the g force. Also, centrifugation results in heat buildup, which can be lethal to mycobacteria. Using a refrigerated centrifuge can avoid this. If a refrigerated centrifuge is not available, pre-cooling the metal cups is an alternative.
9.3 Calculation of Centrifugal Force

Formula for calculating a particular centrifugal force is:

$$ RCF = 1.12r \left( \frac{RPM}{1000} \right)^2 $$

...where \( r \) is the radius (distance in mm from the center of rotation to a point within the rotor), and RPM (revolutions per minute) is the speed of rotation.

- This information should be published in the centrifuge manufacturer's instruction manual.

Note: Nomogram attached in Resources section

Notes:

This is the formula used to calculate your relative centrifugal force. This generally only needs to be calculated when you get a new rotor or a new centrifuge. And also, the information should be available in the operator's manual.
10. Quality Indicators

Notes:

Let's take a look at some indicators that can be used to assess the quality of your specimens and specimen processing. The quality indicators in this module will relate directly to the results you see in your cultures.
Notes:
In order to use the quality indicator of contamination rate, we must understand what the term contamination means in this context. So, contamination occurs when inoculated media is completely compromised due to the overgrowth of non-acid fast organisms. Since we now use broth media, in addition to solid media, a single contamination rate is difficult to apply. So we need to calculate the contamination rate for each type of media. Calculate the ratio of pieces of each media discarded due to contamination to the total number of that media inoculated in one month.
10.3 Contamination Rates

Notes:
Since broth media will grow organisms much easier, the acceptable contamination rate for broth is higher than it is for solid media. 7 to 8 percent for broth compared to 2 to 5 percent for solid media. You don't want to have a contamination rate that is too low or at zero either, because this would indicate that the decontamination process is too harsh. It could be a result of the sodium hydroxide concentration being too high, or the contact time for the sodium hydroxide with the specimen is too long. Conversely, a contamination rate that is higher than the recommended rate could indicate a problem with proper specimen collection, transport, or inadequate decontamination process.
10.4 Potential Causes of High Contamination Rates

Potential Causes of High Contamination Rates

- Lack of appropriate sputum collection guidance for patients
  - Such as improper oral hygiene, which can be improved by rinsing the mouth first with sterile water
- Delays in transport
- Lack of refrigeration prior to transport
- Inappropriate storage conditions or processing delays upon receipt in the laboratory
- Decontamination process is ineffective
  - NaOH concentration too low
  - Specimen exposure time to NALC-NaOH too short

Notes:

If you have inadequate training of the health care provider or patient on proper specimen collection and transport, you may end up seeing a high rate of contamination. When monitoring your contamination rate, it is useful to determine if you are seeing high contamination rates on specimens received from specific clients. This can help you target education activities to improve the quality of the specimens and the results obtained from the specimens.

You may also want to check the decontaminating solution to ensure that staff are using the right volumes of each component to make sure the final concentration of the sodium hydroxide is at the appropriate level. Observing staff performing the digestion/decontamination is also important to ensure that the exposure time of the specimens to the sodium hydroxide is not being shortened. This can happen if staff feel rushed, or they have an overwhelming number of specimens, and they start shaving time off of this part in order to save time in the overall process. It might also be a result of staff not fully understanding the process and the reason for each step to be done exactly according to protocol. So, re-education may be in order.
10.5 Quality Indicators: Positivity (Recovery) Rate

Notes:

Calculating your positivity rate is another good quality indicator. It can be used for several reasons. It establishes a baseline for a given population or geographic area. It assists in identifying potential false positive or false negative cultures (MTB). And, it assists in identifying environmental contamination. To calculate your positivity rate, use the number of cultures that grow MTB versus the total number of cultures done and reported in one month.
10.6 Quality Indicators: Positivity (Recovery) Rate

**Notes:**

Each laboratory’s positivity rate is dependent on the population they serve, or even the geographic region in which they are located. Shifts in patient population can affect this rate in both directions. Cross contamination or contaminated reagents can falsely increase your contamination rate.
10.7 What Should I Do if Quality Indicators or Controls Are Out of Range?

What Should I Do if Quality Indicators or Controls Are Out of Range?

- Ensure quality specimens are being received in the laboratory
- Ensure quality reagents and media are being used
- Ensure water used for specimen processing is sterile and filtered
- Ensure laboratory equipment (e.g., incubators, BSCs) is functioning properly
- Ensure protocols are followed
- Ensure staff are trained and proficient

Notes:
If you are monitoring quality indicators, you will have to take action if they are out of range. Once you discover that your quality indicators are out of range, you need to perform an investigation or root cause analysis, so that you can focus your corrective actions. The investigation should look into specimen collection and transport, reagent quality, proper equipment use and maybe most importantly ensuring that staff are properly trained and perform the process enough to remain proficient.
10.8 Why do respiratory specimens require digestion and decontamination?

Knowledge Check

Why do respiratory specimens require digestion and decontamination?

- To kill off AFB for the protection of laboratorians
- To liquefy specimens to release AFB and kill normal flora
- To reduce the number of AFB for counting by smear microscopy
- To prevent cross contamination of specimens during processing
10.9 During specimen processing, which of the following is recommended as best practice?

Knowledge Check

During specimen processing, which of the following is recommended as best practice?

- Work in multiple sets of specimens to maximize throughput
- Always include a positive MTBC control
- Prepare enough NALC for weekly use
- Open and remove caps from tubes one at a time
10.10 You are performing the NALC/NaOH digestion/decontamination step while processing your respiratory specimens. Your timer should be set to:

Knowledge Check

You are performing the NALC/NaOH digestion/decontamination step while processing your respiratory specimens. Your timer should be set to:

- [ ] 15 minutes
- [ ] 25 minutes
- [ ] 5 minutes
- [ ] Timing is not critical at this step
10.11 The oxalic acid processing method might be used for decontamination of specimens under what circumstances?

**Knowledge Check**

The oxalic acid processing method might be used for decontamination of specimens under what circumstances?

- All specimens received >24 hours after collection
- Decontamination of extrapulmonary specimens
- Decontamination of respiratory specimens from cystic fibrosis or other patient infected with *Pseudomonas aeruginosa*
- This is not an acceptable method of specimen decontamination
10.12 What is the optimal centrifugation measurement for processing specimens?

Knowledge Check

What is the optimal centrifugation measurement for processing specimens?

- 3000 RPM (revolutions per minute) for 15 minutes
- 3000 x G (relative centrifugal force) for 15 minutes
- 2000 RPM for 25 minutes
- 2000 x G for 25 minutes
Notes:

Thank you for participating in this training. We’ve covered a lot of information on specimen collection, transport, and processing. The information covered should help you provide the highest quality results from your TB tests.
10.15 References

References (continued)


- A Centrifuge Primer. Published by Spinco Division of Beckman Instruments, Inc., Palo Alto, California. Copyright 1980, Beckman Instruments, Inc.