Mycobacteriology Service Implementation

Module 3
Version 2019

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SPECIMEN PROCESSING

Processing of CSF and Sterile Body Fluid

- During specimen quality assessment check for companion routine bacterial cultures
- Note the volume and appearance of the fluid
- Check the gram stain/culture results
- If the fluid is of sufficient volume, is cloudy or organisms are seen in the gram stain, **process the specimen as non-sterile**
- If the fluid is clear and no organisms are seen in the gram stain, centrifuge the specimen and use the sediment to directly inoculate media and microscope slides

Processing of Nonsterile Specimens

This is a 3 part process

1. Digestion – uses mucolytic agents to liquefy the specimen and release the AFB
2. Decontamination – uses agents toxic to normal flora contaminants to prevent their overgrowth and allow mycobacteria to flourish
3. Centrifugation – used to concentrate AFB in a specimen sediment for inoculation to solid agar and broth culture media

Processing of Nonsterile Specimens

- Digestion/Decontamination reagents are toxic to mycobacteria
- Processing must be precisely timed to limit decreasing the yield of mycobacteria
- Centrifugation creates high heat that also can decrease the yield of mycobacteria
- Must monitor culture positivity to detect if process is too harsh or centrifugation is poor
- Must monitor contamination rates to detect if process reagents are not strong enough

Processing of Nonsterile Specimens

- Basic Processing Steps include:
  - Disinfecting the workspace
  - Lining the workspace with a disinfectant soaked absorbent pad
  - Placing specimen tubes in a rack with a space between each tube
  - Process no more than a centrifuge load of specimens in each processing batch
  - Open and handle only one specimen container at a time

Digestion/Decontamination Methods

- N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) – Principle method
- NaOH
- Oxalic Acid – *Pseudomonas species;* Cystic Fibrosis patients
- Sulfuric Acid – Urines, Gastric Lavage, Watery fluids
- Bleach – May be used for direct smears

General Specimen Processing Preparation

- Perform specimen quality assessment, noting quantity and quality of the specimen
- Contact the primary caregiver prior to processing suboptimal specimens (i.e. QNS may require recollection)
- Let all refrigerated items warm to room temperature
- Make sure that you have sufficient digestion, decontamination and buffer reagents
- Make sure you have sufficient PPE supplies (i.e. gloves for changing if needed)
- Make sure you have adequate labels and usable pen/markers for labeling

General Specimen Processing Preparation

- Prepare the BSC workspace
- Label microscope slides, media, individual reagent vials for each specimen
- Label centrifuge tubes as needed for the specimen (submitted in non-50mL conical centrifuge container), digestion aliquot

General Digestion/ Decontamination Procedure

- Add digestion/decontamination reagent equal to the volume of the specimen to the tube
- Vortex to mix and liquefy the sample (10 – 30 seconds)
- Let stand for 15 – 20 minutes, vortexing intermittently to mix
- Q.S. to 50 mL volume with buffer reagent.
- Centrifuge for 15 minutes at 3000 x g
- Decant the supernatant in to a second 50 mL conical tube; discard
- Re-suspend the sediment with buffer
- Prepare a smear with 1 – 2 drops (~100 uL) and heat fix.
- Inoculate the broth culture media with 0.5 mL of the sediment
- Inoculate solid agar media with 1 – 3 drops of sediment
- Incubate media appropriately for the specimen source

General Processing

- https://www.youtube.com/watch?v=YlFFSbAoCdM
- https://www.youtube.com/watch?v=SG9WbK9rZVI

SPECIMEN PROCESSING METHODS

- Goal: Increase test sensitivity and decrease probability of false positives

N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) Decontamination Method

- NALC = Mucolytic Agent – dissolved in diluent
- Very susceptible to air; must be used within 24 hours
- Additional NALC can be added to very mucoid specimens
- NaOH acts as decontamination reagent; mixed with Na Citrate that binds heavy metal ions that can inactivate NALC

NaOH Decontamination Method

- Normal decontamination method uses a 2% – 3% NaOH method
- NaOH at a FINAL concentration of ≥2.0% can be lethal to mycobacteria (may see decrease culture sensitivity in smear negative specimens)
- 4% NaOH method is used for specimens with contaminating flora resistant to the routine NALC-NaOH method
- Na Citrate buffer may be included as part of commercial solutions
- Sterile physiological saline used in lieu of chemical buffers (M15 Phosphate, N67, etc.)

Oxalic Acid

- 5% Oxalic Acid is used in lieu of the normal reagents
- This method is used with specimens that are contaminated with Pseudomonas species
- Sterile physiological saline is used in lieu of chemical buffers
- Phenol red used to check pH as NaOH is used to neutralize and re-suspend the pellet

Sulfuric Acid

- 4% Sulfuric Acid is used in lieu of the normal reagents
- This method is used with urines and watery body fluid specimens that remain contaminated using alkaline methods
- Sterile water is used in lieu of chemical buffers
- Phenol red used to check pH as NaOH is used to neutralize and re-suspend the pellet

Bleach Decontamination

- Used only for the preparation of direct specimen smears in an emergency situation to ensure non-viability of AFB
- Aliquot of sample should be retained for further studies prior to decontamination procedure
- Strict timing required to avoid destruction of all AFB
- Sterile water is used in lieu of chemical buffers to wash the sediment

SPECIMEN PROCESSING QUALITY CONTROL

- Goal: Improve the ability to detect false test results and increase the accuracy of the test result

Specimen Processing Quality Control

- Leave 1 – 2 spaces between each tube in the batch (this includes split sample tubes for the same sample)
- Never batch process more than a full centrifuge load (8 – 12 specimens)
- Include a Negative Processing Control (10 ml sterile water or buffer) with each batch of specimens processed (***This sample goes through the entire process and media inoculation as the patient samples***)

Avoiding Cross Contamination

- Open the specimen tubes very gently to avoid aerosol generation.
- When adding reagents to the tube, open one tube at a time. Do not keep all the tubes open at the same time.
- Place distance between tubes in the rack
- Change gloves often as needed

- Never set up proficiency test samples in the same batch or just before setting up patient samples
- Allow at least 15 – 20 minutes of airflow exchanges between batches


to be continued