



# MGIT CUTURE

## Module 6: Processing and inoculation of sputum specimens

**Date:**

**Venue:**

**Name:**

# Outline

- Principle of processing sputum specimens
- Digestion and decontamination procedure
  - N-Acetyl-L-cysteine (NALC) - sodium hydroxide
- Inoculation of processed specimens onto liquid media
- Quality control of processing



# Principle of processing(1)

- Sputum specimens are viscous materials contaminated with normal flora
- Processing involves pre-treatment of the sputum specimens



# Principle of processing(2)

- Digestion: to free the TB bacilli from the mucus, cells or tissue in which they may be embedded
- Decontamination: to eradicate normal flora that grow more rapidly than TB and would interfere with the ability to recover TB
- Homogenization of the digested materials
- Concentration of the TB bacilli by centrifugation before smear preparation and medium inoculation



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# Most common processing methods

## 1. N-acetyl-L-cysteine/sodium hydroxide method (NALC-NaOH) method

- Mildest decontamination method; however, still kills about 33% of mycobacteria present
- Can be used with both liquid and solid media

## 2. Petroff's sodium hydroxide (NaOH) method

- Harsher method; can kill up to 70% of mycobacteria in specimen
- Useful with highly contaminated specimens
- Not recommended for use with liquid media

# N-Acetyl-L-cysteine-Sodium hydroxide method

- Sodium hydroxide (NaOH)
  - Decontaminating agent
- Sodium citrate
  - Binds the heavy metal ions that might be present in the specimen that could inactivate the NALC
- N-Acetyl-L-cysteine (NALC)
  - Mucolytic agent allows NaOH to be used at a lower concentration (1%), aids in liquifying sputum to release the AFB and better decontaminate the specimen from normal flora



# NALC/NaOH method: Principles and advantages

- In this method, the final concentration of NaOH in the specimen is less than with the Petroff method
  - Starting NaOH concentration is 4%, and the final NaOH concentration is 1% (vs. a final concentration of 2% NaOH with the Petroff method)
  - Inclusion of the mucolytic agent NALC makes possible more rapid digestion at the lower concentration of NaOH
  - Less tubercle bacilli are killed with this method due to lower NaOH concentration, resulting in higher rates of culture positive specimens
- Specimens processed with this method are suitable for inoculation onto both solid and liquid media (since pH is adjusted with phosphate buffer)



# NALC/NaOH method: Disadvantages

- NALC is expensive and loses activity rapidly
  - NALC must be prepared each day of use
  - Extreme agitation of NALC in the presence of NaOH will result in lost activity
- As with the Petroff method, NaOH exposure time must be strictly controlled to prevent over-kill of TB
- If stronger decontamination is needed, the starting concentration of NaOH may be increased to 5% or 6% (rather than increasing the time of exposure)
  - To reduce excessive contamination
  - To handle heavily contaminated specimens



# Processing procedure: Quality control

Prepare a worksheet for each batch of specimens processed

- List the numbers of all specimens processed in the batch

- Record the date and name of the technician performing the work

- List lot numbers and expiration dates for reagents used



# Quality control

- Record date of preparation of NALC/NaOH to ensure that it has been prepared on day of use
- Equipment used
- Before beginning processing, ensure that regular maintenance on the BSC and the safety centrifuge has been performed and documented



# NALC/NaOH procedure preparation

- Process clinical specimens as soon as possible or refrigerate them at 2-4 °C
- Prepare a written check list of materials required and arrange supplies, reagents and specimens in a BSC
- Work in sets equivalent to one centrifuge load (8-16 specimens at a time)
- Cover the working area with absorbent material and spray it with suitable disinfectant



# Preparation of NALC/NaOH

Prepare digestant

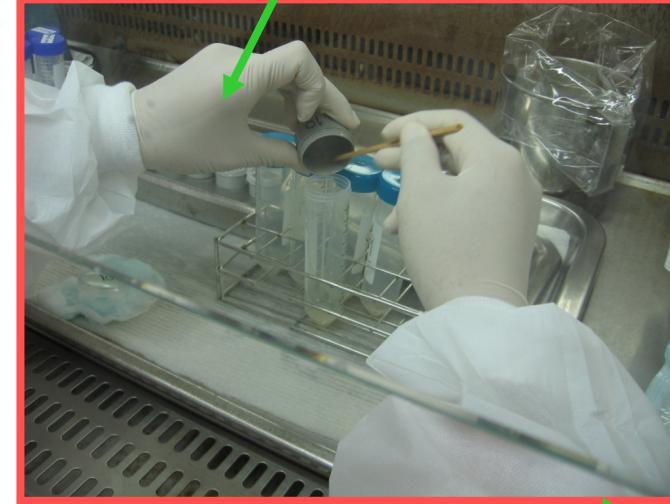
Add 0.5 grams of N-acetyl-L-cysteine (NALC) to each 100 ml of NaOH (50 ml) /Na citrate (50 ml) needed

Only prepare enough for one day



# Beginning the processing procedure

- Collect sputum in 50 ml sterile, plastic, screw-capped centrifuge tubes
  - Use a stick or loop to transfer viscous sputa
- Transfer 10 ml of sputum into 50 ml sterile, plastic, screw-capped centrifuge tubes (if less than 10 ml, use the whole specimen!)
- If desired, the volume of each specimen can be brought up to 10 ml with sterile phosphate buffer (pH 6.8)



# Beginning the processing procedure

- It is easier to add the appropriate volume of NALC-NaOH in the next step if the volume of specimen is standardized



# NALC/NaOH procedure

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# NALC/NaOH procedure 2

- Note volume of specimen (A), add an equal volume of NALC-NaOH solution (B) and tighten cap
- Gently vortex each tube at a moderate speed for not more than 20 seconds (C)
- Invert each tube 5 times to ensure that the NALC-NaOH solution contacts the entire inner surface of the tube
  - Avoid extreme vortexing or shaking since this action can inactivate the NALC
- Let the tubes stand at room temperature (20-25 °C) for 15 min for decontamination

OR

- Put the tube onto an orbital shaker for 15 min at a slow speed of 400 rev/min.



# DO NOT Prolong Exposure To NaOH!

- NaOH exposure time must be strictly limited to 15 minutes to prevent over-kill of TB
- If stronger decontamination is needed, the starting concentration of NaOH may be increased to 5% or 6% but the time of exposure should not be extended



# NALC/NaOH procedure 3

Dilute the specimen with pH 6.8 phosphate buffer (A) to the 50 ml mark (B)

Recap the tubes tightly and invert several times (C)

Either individual aliquots or a dispenser bottle could be used to add the phosphate buffer

The phosphate buffer reduces the action of NaOH and lowers the viscosity and pH of the mixture

Sterile distilled water cannot be used instead of the phosphate buffer because the final pH would be too high for use in the MGIT system



# NALC/NaOH procedure 4

In the BSC, load the diluted specimens into aerosol-free safety centrifuge cups

Centrifuge at 3000 x g for 20 min at 4 °C

- 3000 x g = critical for efficient concentration of the tubercle bacilli that are unevenly dispersed in the specimen
- 20 min at 4 °C = critical to ensure that heat generated during centrifugation does not kill the tubercle bacilli



# Reconstitution of working MGIT PANTA

- In a biosafety cabinet reconstitute one 15ml vial of BACTEC MGIT 960 growth Supplement into one vial of lyophilized MGIT PANTA
- Mix until all the MGIT PANTA powder has completely dissolved



# Reconstitution of working MGIT PANTA

- Label the prepared MGIT PANTA mixture with the initials of the technologist, date of preparation and expiration date
- Reconstituted PANTA must be stored at 2-8°C and used within 5 days.



# Inoculation of MGIT vials

In an appropriate BSC add 0.8 ml of the reconstituted PANTA into labelled MGIT tube

Ascertically transfer 0.5 ml of the processed sediment to a 7ml MGIT tube with PANTA

- ❑ Do not add more than 0.5 ml of specimen
- ❑ Work on one sample at a time to reduce the risk of cross contamination

Tightly re-cap the tube and invert 3-5 times to mix well



# Inoculation of MGIT vials

- The inoculated MGIT-tubes are now taken to the Bactec MGIT 960 instrument for incubation.
- Document all the specimens processed on the given worksheets/register



# How to minimize cross-contamination and maintain the oxygen concentration in the media

- ➊ Open tubes one at a time and
- ➋ Open tubes for short a time as possible.
- ➌ Do not leave more than one MGIT tubes uncapped at the same time.
- ➍ Wipe tubes' exterior and caps with mycobacteriocidal disinfectant such as lysol
- ➎ Let the suspension stand for 20 minutes undisturbed.
- ➏ Using a sterile pipette.



# Procedure for preparation and inoculation of MGIT tubes with inoculum from solid media.

- Prepare 0.5 McFarland from colonies solid media may of NOT >15 days from the first appearance of positive growth
- Let this preparation stand for another 15 minutes undisturbed
- Avoid taking any growth that has settled on the bottom



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The turbidity of this suspension should be greater than McFarland 0.5 standard.

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# ....Cont

- Dilute 1.0 ml of this suspension in 4.0 ml sterile distilled water and mix well
- Follow the Steps of Innoculation of the mixture as those of Processed Sputum.



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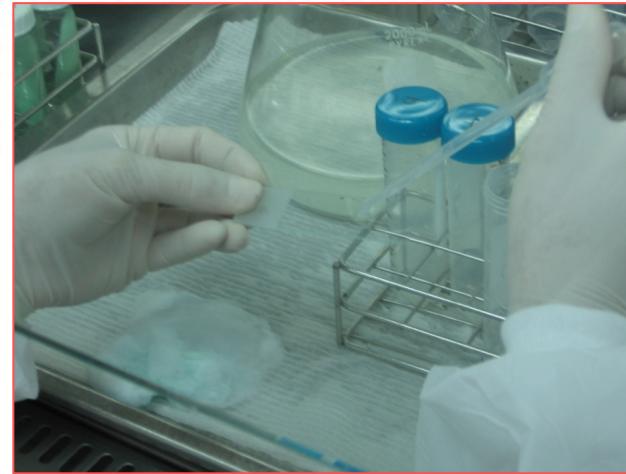
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# Final steps in specimen processing

- Smear preparation
- Storage of sediment
- 
- Clean-up of BSC after processing



# Summary points

- Sputum specimens must be processed;
  - Digestion
  - Homogenization,
  - Decontamination
  - Concentration
- Most common processing method presented:
  - N-acetyl-L-cysteine/sodium hydroxide (NALC-NaOH) method (liquid and solid medium)
  - Inoculation onto both solid and liquid media is the “Gold Standard” for sensitive growth detection



# ASSESSMENT REVIEW

- List all the materials and equipment required for MGIT culture
- List steps involved in Processing and inoculation of sputum specimens
- Describe the procedure for preparation and inoculation of MGIT tubes with:
  - Processed sputum.
  - inoculum from solid media.



# Acknowledgments



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To eliminate TB

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