THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

MGIT CULTURE

Module 9: BACTEC™ MGIT™ 960 system growth detection

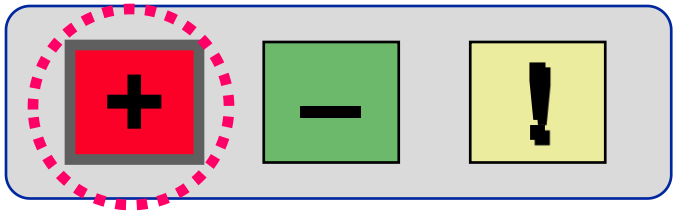
Venue:
Name:
Date:

Outline

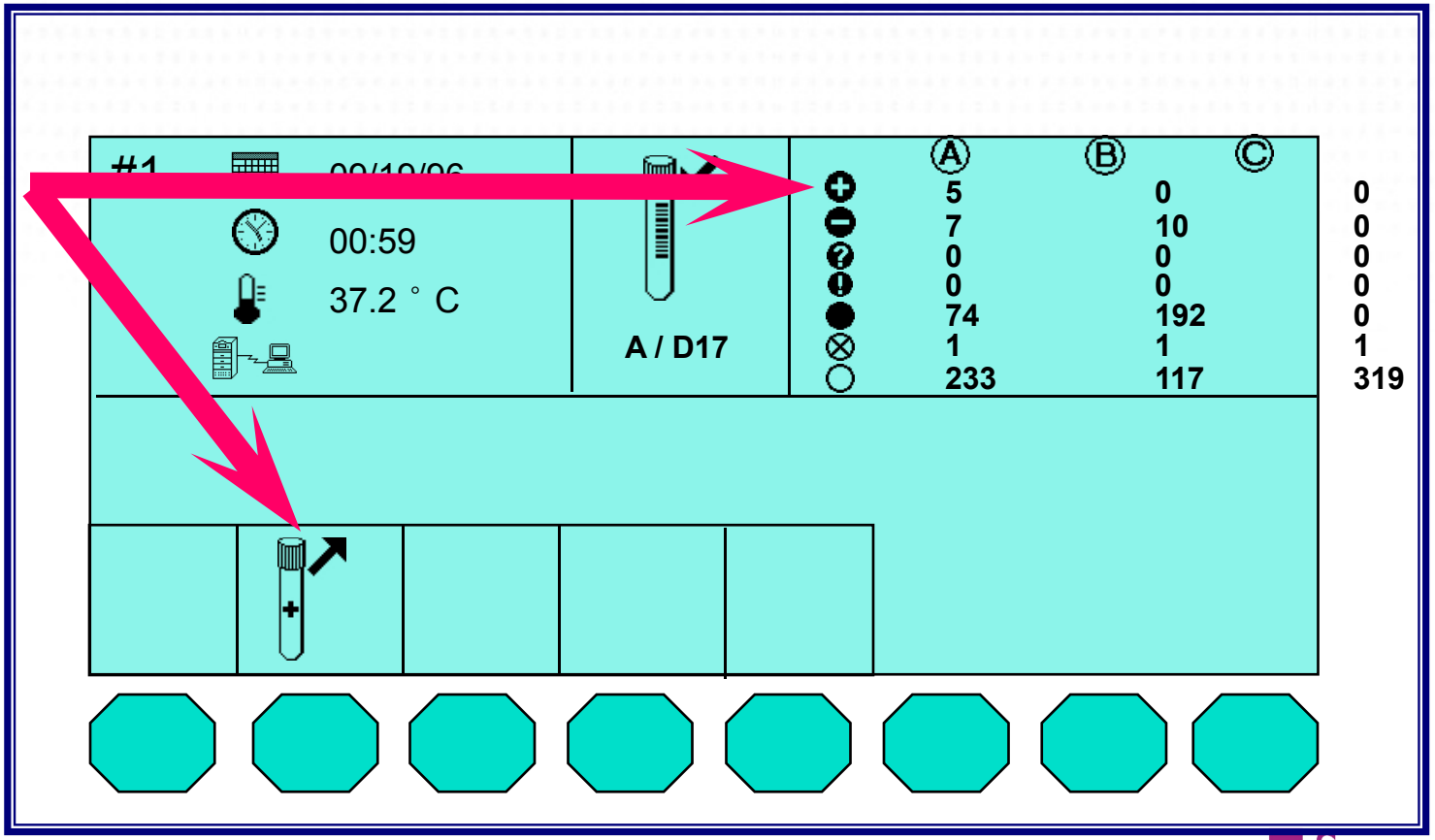
- Removing cultures from the instrument
  - Positives
  - Negatives
- Troubleshooting
- Media quality control
- Frequently asked questions

Notification of positives on drawer

- Indicator lamp on drawer illuminates
- Audible alert sounds



Notification of positives on LCD display

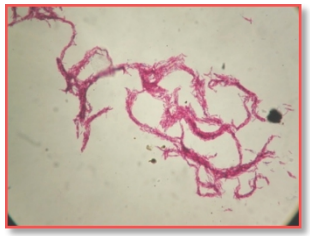


Removing positive tubes

- The drawer with positive tubes always has a red light indicator with a '+' sign
- Select the option '**Remove positive tubes**'. The lights at the positions of all positive tubes will blink
- Remove one of these tubes and scan the barcode
- The lights of this tube position will stop blinking

Instrument-positive cultures

- Remove the tube from the instrument
- Scan the bar-code
- Visually check tube for flaky, clumped growth
- Prepare smear in BSC
- Perform AFB smear using ZN stain
- Confirm presence of corded



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Instrument-positive cultures may be contaminated

- Turbid growth suggests contamination
  - Microorganisms other than AFB can grow and be detected as positive by the instrument
- Contaminated culture may also contain AFB
  - Prepare smear in BSC
  - Perform AFB smear using ZN stain

- If culture contains AFB plus contaminant
  - Re-process the contaminated suspension
  - Inoculate into fresh MGIT tube
  - Refer to MGIT User Manual for details

[Causes and prevention of contamination will be covered in a later module]

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False-positive cultures

- If no organisms are seen on AFB smear
  - Re-scan the tube
  - Replace in drawer to allow tube to complete test protocol
  - Must be re-entered within 5 hours of removal
    - Re-sets positivity algorithms
    - Retains previous test readings and protocol

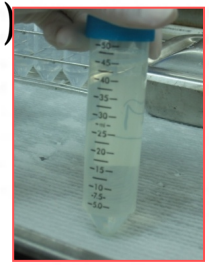
Note: If MGIT tube contains visible signs of growth and nothing appears on the smear, the specimen may be washing off of the slide during the staining process. Add a drop of phenolized rabbit serum or BSA (Bovine Serum Albumin) to the smear when specimen is added, then dry, heat-fix and stain.

False positives

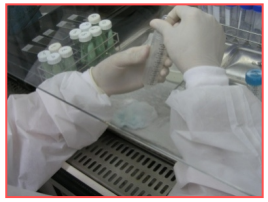
- If MGIT tube is fluorescing and no AFB is seen on the smear, the specimen may be washed off the slide during the staining process
  - Add a drop of phenolized rabbit serum or BSA (Bovine Serum Albumin) to the smear when specimen is added.
  - Remember to rinse smears with a gentle stream of water NOT a rapid flowing stream of water

Potential cause of false positives

- During processing, pH 6.8 phosphate buffer (PB) must be used to neutralize the NaOH
  - Dilute the NALC/NaOH with PB up to 50 ml before centrifugation
  - Resuspend the pellet with PB up to 1–3 ml after centrifugation



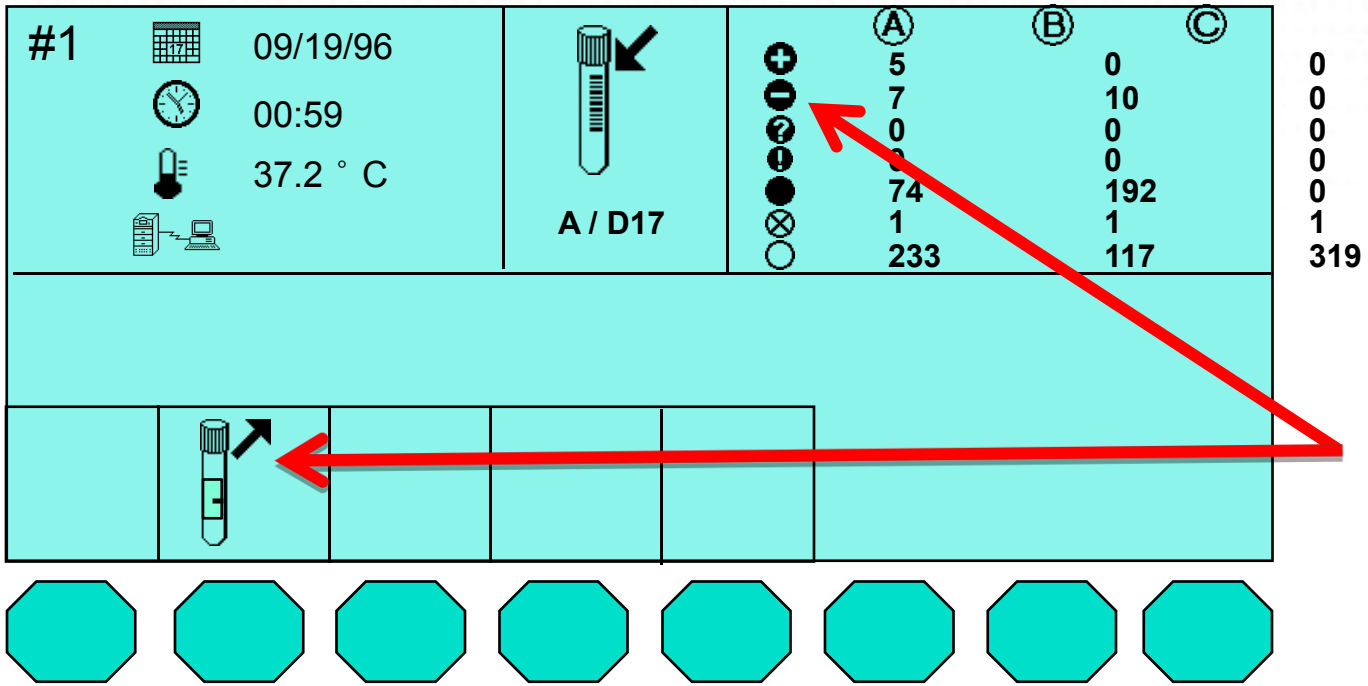
- If there is insufficient neutralization
  - pH remains high
  - May result in false fluorescence
  - Instrument reads tube as positive



(High pH may also delay or inhibit growth of TB resulting in a false negative culture.)

Instrument-negative cultures

If MGIT tube has not been flagged as positive before the end of the 42-day protocol, instrument will signal culture as negative



Removing negative tubes

- The drawer with negative tubes always has a green light indicator with a '-' sign.
- Open the drawer with the tubes to be removed. Select the option **'Remove negative tubes'**. The lights at the positions of all negative tubes will blink.
- Remove negative tubes one by one, each tube should be removed and scanned individually. Continue to do so until all tubes have been removed.
- Close the drawer only when you have removed **all highlighted negatives.**

Instrument-negative cultures

- Remove the tube from the instrument
- Scan the bar-code
- Visually check tube for flaky, clumped growth

Potential causes of false negatives

- TB will be killed by:
  - Extended exposure to NaOH during processing
  - High pH (insufficient neutralization during processing)
- Growth may be inhibited by:
  - Addition of too much PANTA
  - Loss of CO_2 from MGIT vial headspace
    - Keep MGIT tubes upright during inoculation
    - Ensure tube caps are tight during incubation

Troubleshooting (1)

Decreased or no recovery of mycobacteria

- Check decontamination procedure
  - Exposure to NaOH, time, concentration
  - Centrifugation
    - Speed, time, temperature
  - Decanting of supernatant
    - Inadequate decant affects pH of media
    - If not done carefully, pellet may be lost during decantation
  - PANTA concentration
    - Too high ☾ growth inhibition

Troubleshooting (2)

Delay in the time to detection

- Specimen quality?
- Smear positivity?
  - Fewer organisms present in specimen will take longer to grow to detectable levels
- High NaOH concentration?
  - Lethal effect
  - High pH of inoculum
- Longer exposure time to NaOH/NALC?

Troubleshooting (3)

Delay in the detection time

- Centrifugation
  - Speed too low ☾ poor sedimentation
    - Fewer organisms in inoculum
  - Time and temperature ☾ lethal effect

- Purity and concentration of reagents, water
  - Water may contain inhibitory substance

- Glassware and other items
  - Residual cleaning agents can be toxic

Instrument reports: Printing Reports

- After removing tubes, print out the report.
- Match the barcode on the removed tubes with the ones on the report
- Transfer the lab accession number to the corresponding barcode on the printed out report
- When completed, select the option **'OK'** to clear memory of the unloaded tubes as reflected on the screen of the machine.

Instrument reports

- The BACTEC 960 will print the following reports:
  - Unloaded positives
  - Unloaded negatives
  - Unloaded ongoing
  - Instrument inventory
  - Quality control

Identification of specimens

- Printed reports list the *MGIT tube bar-code number*
- Need to link MGIT tube bar-code to the *laboratory specimen number*
  - Tube can also be labelled with a bar-code specifying the laboratory specimen number (if available)
  - Instrument can read a second bar-code
  - Second bar-code would be listed on printed report
- If no specimen bar-code label, specimen number must be linked somehow with MGIT tube bar-code number
  - Handwritten on report next to the tube bar-code number

Reading MGIT 960 tubes manually

- In the event of instrument failure or off-line incubation, manual reading may be done
  - Wood's lamp
  - Other UV light source with bulb of good intensity
- Do not read in darkened rooms or rooms with bright sunlight
- Always wear protective UV goggles

Reading MGIT 960 tubes manually

- Place tubes directly on top of a Wood's lamp with long-wave bulb
- Tilt slightly so that the tubes are read at a slight angle
- Place positive and negative controls at end of row to give visual assistance in reading
- Place "suspect" tube between positive and negative control tubes
  - May want to skip rows in test tube rack to facilitate reading

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BACTEC MGIT 960 tube quality control (QC)

MGIT tube quality control

- Each shipment or new lot of MGIT medium should be QC tested upon receipt
- New reagents should not be used until they have passed QC testing
- All activities should be performed in a BSC
- QC procedures are found in MGIT manual and will be demonstrated in the laboratory session

Summary

- BACTEC MGIT 960 provides rapid time to detection for growth and detection
- With good technique, the result has faster time to detection and greater sensitivity
- QC of media is required with each new lot or new shipment

Assessment

- What could be the possible causes of false:

-Positive results.

-Negative results.

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp

Acknowledgement