THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

MGIT CULTURE

Module 8: 0verview of liquid culture using the BACTEC™ MGIT™ 960 system

Date:
Venue:
Presenter:

Outline

- Principle of MGIT Culture
- Indicators for MGIT Culture
- Equipment required
- Reagents in the MGIT tube
- Instrument overview
- Loading the inoculated MGIT tube into the instrument

Principle of the analysis

- The BACTEC™ MGIT™960 liquid culture system is based on fluorescence detection of mycobacterial growth in clinical samples other than blood.
- The MGIT 7ml tubes contain modified middle brook 7H9 broth and a fluorescent sensor at the bottom which responds to the concentration of oxygen.
- (An enrichment, MGIT OADC (Oleic acid, Albumin, Dextrose and Catalase) or
- MGIT 960 Growth Supplement, is added to make the medium complete. This Growth

….CONT (Principle of the analysis)

- Supplement is essential for growth of many mycobacteria, especially those belonging to *M*

- *Tuberculosis* complex. Addition of the MGIT PANTA is necessary to suppress contamination)

- Initial concentration of dissolved oxygen quenches the emissions from the compound and little fluorescence can be detected. **Later,** (On inoculation with a specimen), actively respiring microorganisms consume the oxygen which allows the compound to fluorese.

….CONT (Principle of the analysis)

- MGIT tubes are incubated into the BACTEC™ MGIT™960 system which monitors the tubes for increasing fluorescence for positive samples **or 6 weeks for negative ones (and *flags a tube as negative if no flourescence is detected within 6 weeks (42 days*)**

- Positive samples are usually detected from 4 days onwards.

Indications for MGIT Culture

- Diagnosis of TB
- MDR follow-up

EQUIPMENT REQUIRED

- 1000ul adjustable pipette
- Sterile barrier filter pipette
- Mycobacteria Growth Indicator Tubes.
- BACTEC™ MGIT™960 System
- Biological safety Cabinet
- Light Microscope
- Disposable 50ml falcon tubes
- Centrifuge with a minimum of 3000x g force with safety shield

EQUIPMENT REQUIRED (…cont)

- Vortex mixer, Fridge, water bath
- Timer
- Petri dishes
- Markers
- Incubator (35 – 38^{0}C)
- Microscope slides
- Universal bottles
- Sterile plastic loops
- Slide warmer
- Pencil

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Reagents

- BACTEC MGIT960 growth Supplement
- BBL MGIT Tube (7 ml)
- MGIT PANTA Antibiotic Mixture
- ZN reagents
- BA reagents
- Formal Milk

BACTEC MGIT 960 reagents

1. MGIT 960 Growth supplement
2. BBL™ MGIT PANTA
3. BBL MGIT Tube (7 ml)

BD Diagnostic Systems

MGIT 960 Growth supplement

- **Oleic Acid**
  - Important in mycobacterial metabolism
- **Albumin** (bovine)
  - Binds free fatty acids which may be toxic to TB
- **Dextrose**
  - Energy source
- **Catalase**
  - Destroys toxic peroxides
- **Polyoxyethylene stearate** (**POES**)
  - Enhances growth of *M. tuberculosis* and assists in providing a uniform inoculum

BBL MGIT PANTA

- Antimicrobial mixture:
  - **P**olymyxin B – Gram negatives
  - **A**mphotericin B – Yeast, fungi
  - **N**aladixic Acid – Gram negatives
  - **T**rimethoprim – Gram negatives and positives
  - **A**zlocillin – Gram negatives and positives

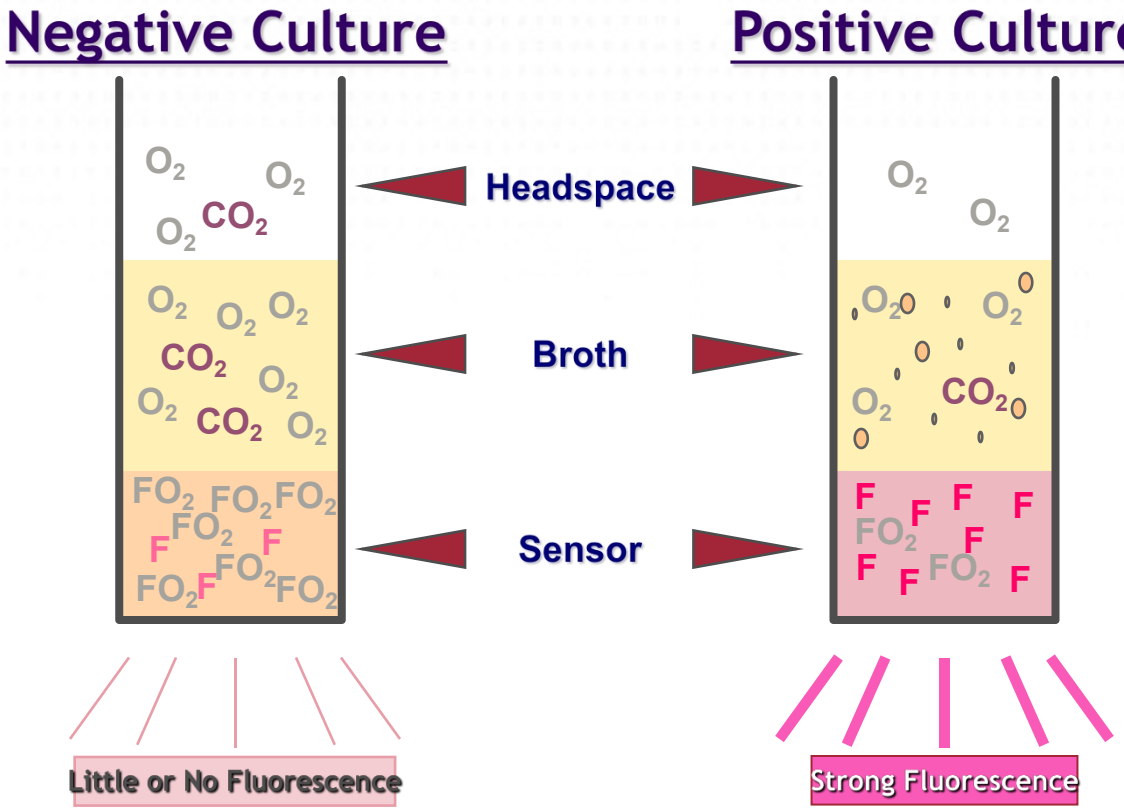
- Suppresses non-mycobacterial growth

BBL MGIT tube (7ml)

- Modified Middlebrook 7H9 broth base
- Fluorescent indicator detects O_2 level
  - Embedded in silicon in bottom of tube
- Bacteria use O_2 during growth
  - O_2 released from indicator in tube bottom
  - Fluorescence indicates growth of bacteria

O_2 bound to indicator = no fluorescence

O_2 consumed during bacterial growth = fluorescence



Inoculated tubes ready to incubate

- Place tubes in rack and carry to instrument for loading
  - To prevent breakage due to dropping tubes, always transport tubes in rack

- Load tubes into instrument on same day as processing/inoculation

BACTEC MGIT 960 system

- Incubates cultures at 37 ^{o}C
- Continuous monitoring of fluorescence
- Includes bar-code scanning
- 3 drawers with total of 960 tube capacity
- Simple 4-step operating procedure

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BACTEC MGIT 960 system

- Simple workflow
  - Instrument automatically directs placement of each tube into instrument
  - Instrument indicates positives/Neg with both a visual and an audible signal as they occur

- Automated quality control of instrument
  - Performed continuously to ensure precise and reliable operation.

BACTEC MGIT 960 system

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Step 1: **Open the desired drawer and select workflow on LCD display**

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BACTEC MGIT 960 system

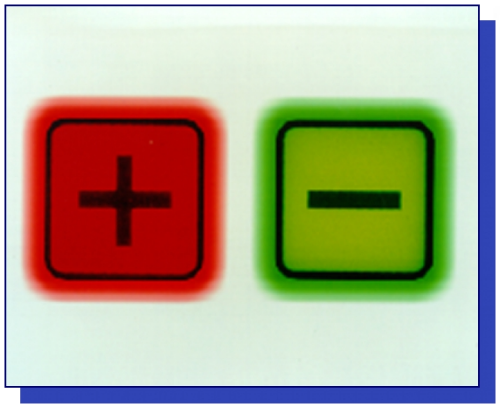
Step 2: **Scan pre-affixed bar-code on tube**

BACTEC MGIT 960 system

Step 3: **Load where indicated by solid green LED light in drawer and close the drawer**

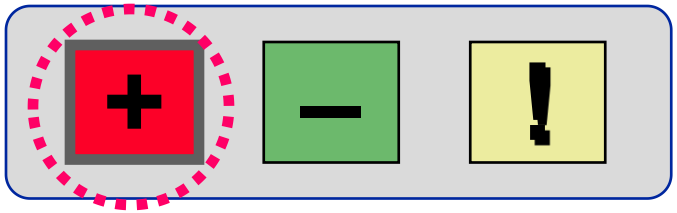
BACTEC MGIT 960 system

Step 4: **Remove positives and completed negatives as they occur (icons appear on each drawer)**

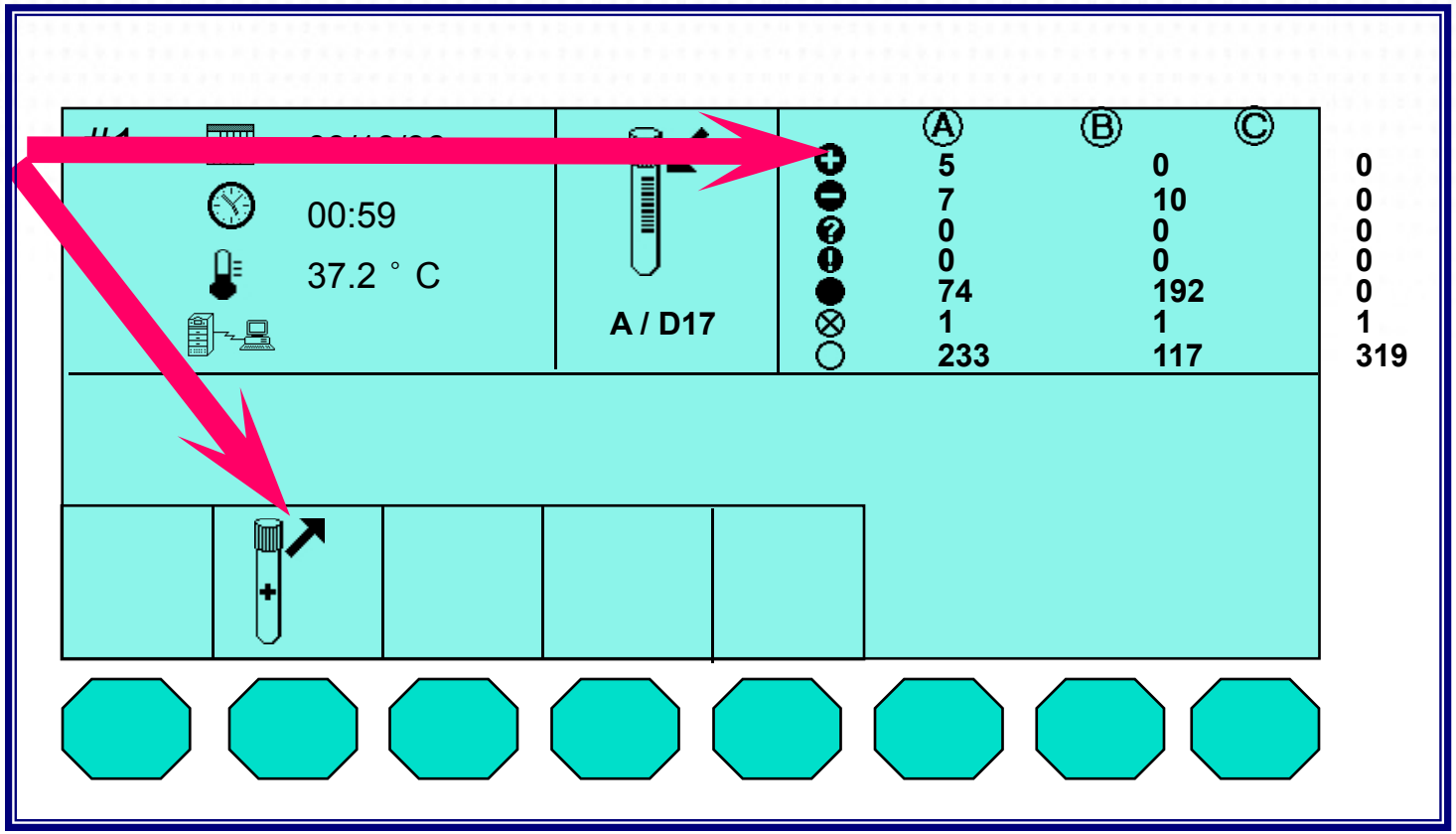


Notification of positives on drawer

- Indicator lamp on drawer illuminates
- Audible alert sounds



Notification of positives on LCD display



Detection system

- Detector board moves under tubes once an hour to scan for fluorescence
  - Data evaluated and tube positivity established based on software algorithms

Length of incubation

- Protocol length for growth detection can be from 1 to 56 days
  - Default protocol length is 42 days for detection of TB from processed sputum specimens

- Specimens with no growth after 42 days can be reported as negative
  - Subcultures or AFB stains are not required unless flakes of growth are seen in tube

BACTEC MGIT 960 Maintenance

Daily

Check temperature readout on LCD display

Check temperature of internal thermometer

Test all three external indicator lamps on all three drawers

For each drawer, test red and green light-emitting diode (LED) lights

Check printer paper supply

Print out "Quality Control Report.“

BACTEC MGIT 960 maintenance

Monthly; more often if dusty environment

Clean and replace filters

As needed

Replace fluorescence calibrators prior to expiration

Quality Assurance Activities Overview

- Do not use expired product
- Reconstituted PANTA must be used within 5 days
- Do not store MGIT tube after addition of PANTA mixture but should immediately be inoculated with the specimen
- Make sure all additions are made inside the biosafety cabinet.
- Do not open several tubes at a time
- Open MGIT tube for as short a period of time as possible
- Recap the tube tightly

… CONT

- Do not add more than 500ul of specimen as this may disturb the pH of the medium and cause false fluorescence
- lot tested reagents before use
- Include routine IQC in processes

Summary

- BACTEC MGIT 960 system provides rapid time to detection for TB growth detection
- 960 is easy to use with little hands on time; frees staff to do other tasks
- 960 has built-in quality control for instrument detectors, resulting in minimal maintenance for staff

Assessment review

- Describe the principle of MGIT Culture?
- When is MGIT Culture requested for?
- List 8 equipment required for MGIT culture?

Acknowledgement

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