THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

MGIT CULTURE

Module 13: BACTEC™ MGIT™ 960 culture positive follow up

Date:

Venue:

Presenter:

Outline

- Overview of BD MGIT 960 Instrument
- Removing positive/Negative cultures from the instrument
- Printing MGIT 960 unloaded positives report
- Preparing and reading ZN AFB smear
- Identification tests.

Overview of BD MGIT 960 Instrument

- Drawers designated as A, B & C

- Sample Measurement Module (SMM)
  - **Tube Rack** – "stations"
  - **Detector Assembly** – 16 detectors for each row. Moves Left to Right.
  - **Status Indicators** – three lamps on drawer
  - **Station Status LED** – indicators on each station. Colors: red, green or orange

MGIT 960 barcode scanner

- Located on the front of the instrument to provide ability to scan tube labels for specimen identification

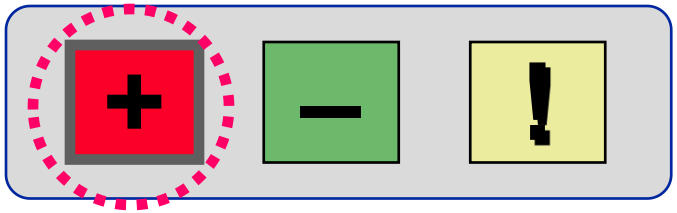
- Turns on automatically whenever the system is ready (and expecting) to scan a barcode

LCD display and keypad

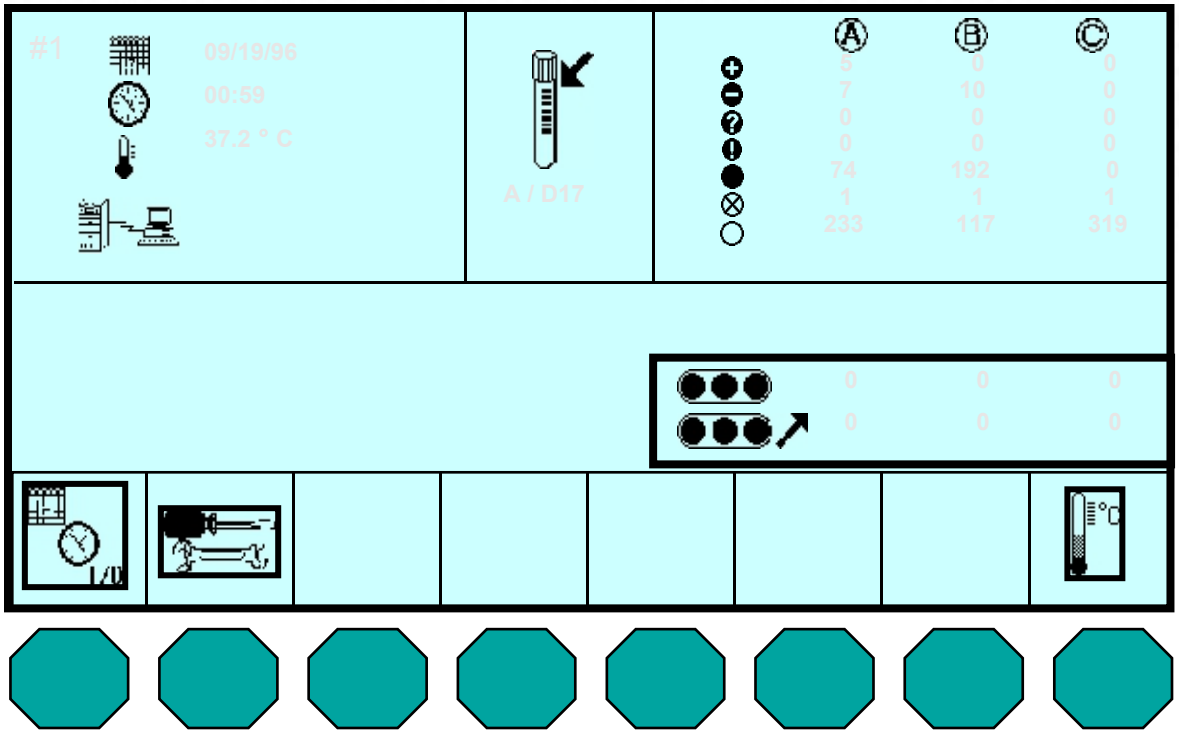
- Presents information about system status and function key definitions
- Each display shows icons representing the current soft key assignments at the bottom of the screen

Notification of positives on drawer

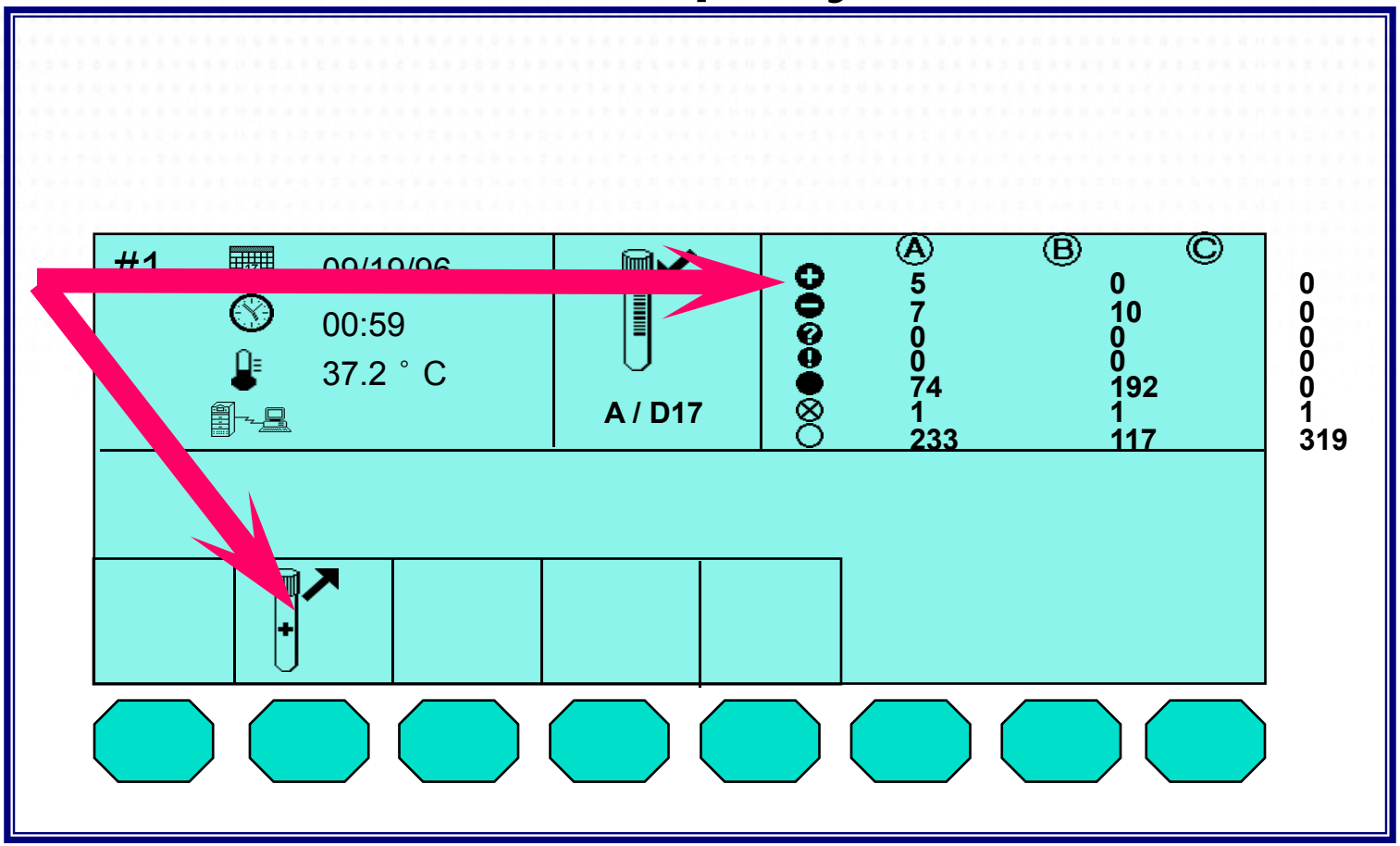
- Indicator lamp on drawer illuminates
- Audible alert sounds



BACTEC™ MGIT™ 960 Instrument Interface

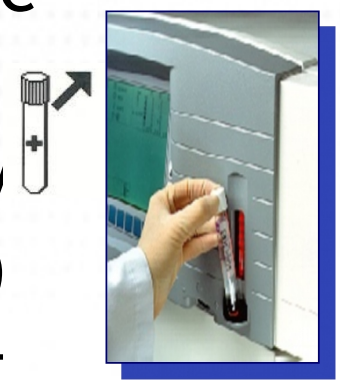


Notification of positives on LCD display



Instrument positive cultures

- Open the drawer on which the positive indicator light is illuminated
- Press the “remove positive tubes” key
- Positive station LEDs will light (green)
- Remove the tube from the instrument
- Scan the barcode
- Visually check tube for flaky, clumped growth
- Repeat until instrument beeps 3 times signaling all positives have been removed



Print unloaded positive report

- From the status screen, press the “print reports” soft key
- Press the soft key that corresponds to the “Unloaded Positive Tubes”
- Ensure that all tubes listed on the report correspond to the tubes removed
- Write date positive on the MGIT tube with permanent marker

Unloaded positives report

BACTEC MGIT 960

Unloaded Positives Report

Instrument Number	Current Date/Time	Temperature A	Temperature B	Temperature C	Software Version	Page Number
2	03-28-2011 15:44	36.6°C	36.9°C	36.7°C	V3.06C	1

Tube Position	Accession Number	Sequence Number	Growth Unit	Tube Status	TTD	Date Positive	Protocol Length	Start of Protocol
A/F02	2011487014	430171889894	4723	+	7;9	03-26-2011	41	03-18-2011 14:45
A/F04	2011487016	430171889878	331	+	9;5	03-27-2011	41	03-18-2011 14:45
A/E20	2011487012	430171889907	5492	+	7;13	03-26-2011	41	03-18-2011 14:45

Unloaded positives report

BACTEC MGIT 960
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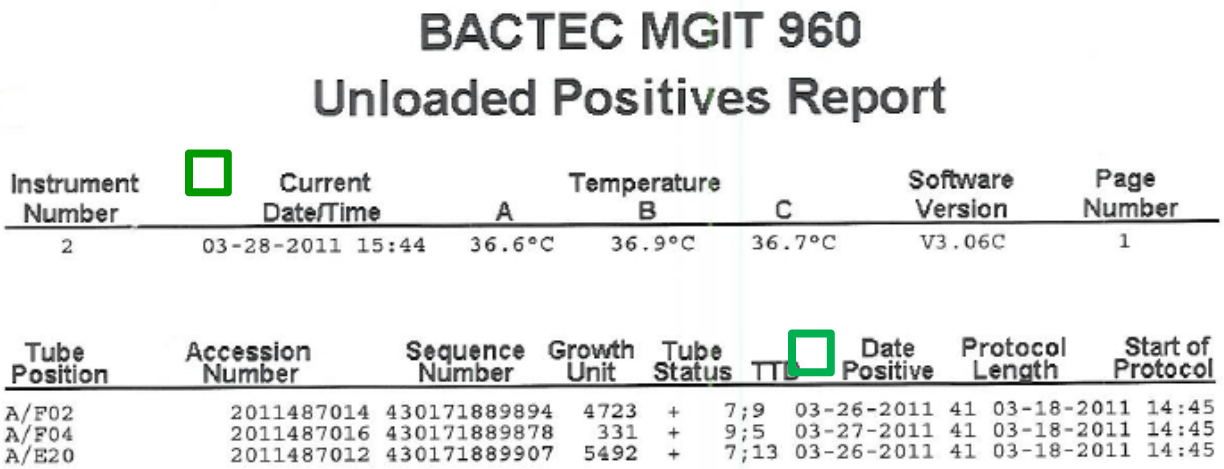
Time to detection (TTD) must be ≥ 2 days

- If TTD is less than 2 days, it is likely to be contaminated or an NTM

Unloaded positive report



BACTEC MGIT 960

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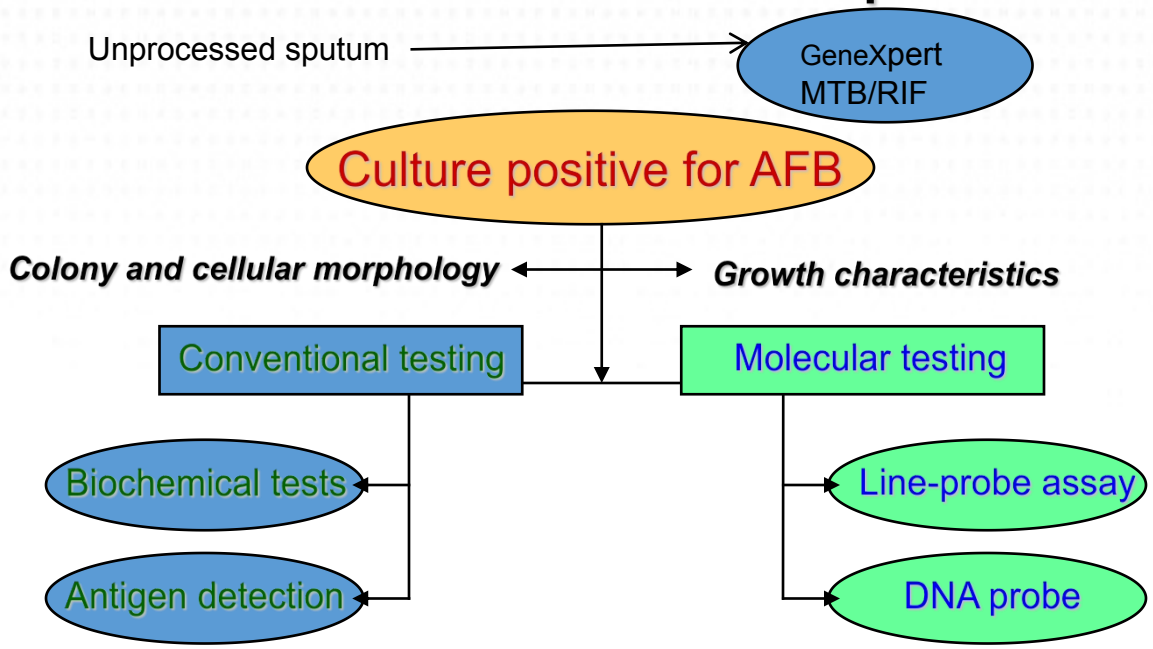
The MGIT culture must be 1 - 5 days old to be used as a seed for DST inoculation. The day it is determined to be positive by the instrument is day 0.

Members of the MTBC

- *M. tuberculosis*
- *M. africanum*
- *M. canetti*
- *M. bovis*
- *M. bovis* BCG vaccine variant
- *M. caprae*
- *M. microti*
- *M. pinnepedii*

Identification methods for the *M. tuberculosis* complex



TB conventional identification

- Morphology (colonies/cellular)
- Biochemical tests
- Antigen detection (new product)

AFB positive?

- Prepare a smear of the positive MGIT 960 tube
  - Label a new clean glass slide with specimen ID #, source (MGIT) and date
  - Work in a BSC, wear appropriate PPE
    - Potential high concentration of *M. tb*

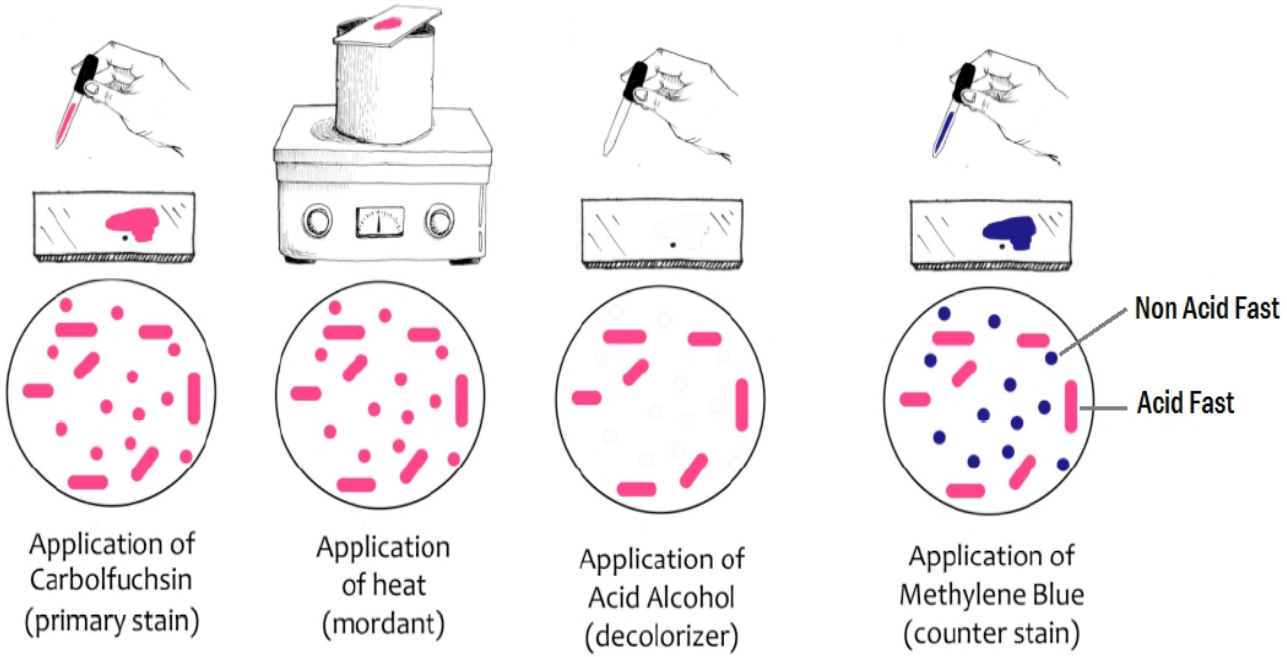
Acid-Fast Staining Principles

- Property of acid fastness is based on the presence of mycolic acid in their cell wall
- Primary stain binds to cell wall mycolic acid.
- Intense decolourisation does not release primary stain from the cell wall of AFB
- Counterstain provides contrasting background

Mycobacterium tuberculosis

Acid-Fast Staining Principles Cont'd

Principle of ZN in stepwise description



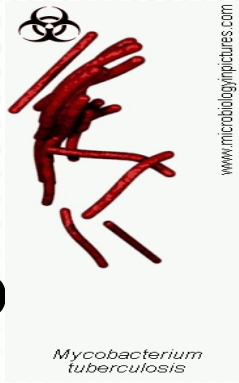
AFB smear preparation

- Do not shake or invert the tube
- Add a drop of fixative(Formal milk) on microscope slide
- Using a sterile transfer pipette add a drop of MGIT broth from the bottom of the tube to slide with fixative
- Spread it into a 1 x 2 cm oval smear
- Allow to dry INSIDE the BSC
- Fixative is usually based on bovine/rabbit serum and Formal milk

Heat fix and stain with Ziehl –Neelsen reagents

- Heat-fix the smear using a hot plate (65-80 ˚C for 2 hours or with a flame (pass through 3-4 times)
- ZN staining
  - Carbol fuchsin - heated to steaming - 5 minutes
  - 3% Acid alcohol - 3 minutes
  - Methylene blue - 1 minute

Reading AFB smear



- Examine the stained smears using the 100x o objective
- Scanning a series of three parallel sweeps the length of the smear, or by scanning a series of nine parallel sweeps the width of the smear
  - Smears scanned in this manner will give about 60 to 100 consecutive fields in one sweep with a 100x objective
- Looking for red bacilli, possible serpentine cords, on a blue background

Definitive identification

- An AFB + smear with serpentine cording is NOT a definitive identification system for reporting *M. tuberculosis* complex
  - There are over 100 species of mycobacteria that are all AFB +
  - *M. kansasii* also exhibits serpentine cords
  - Therefore, it should be used in conjuction with other tests

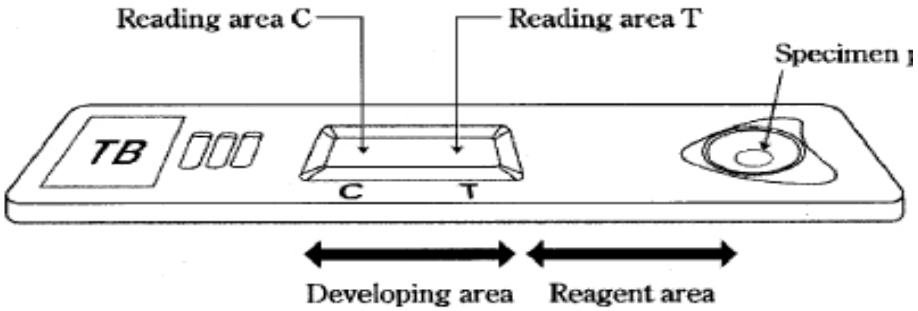
Definitive identification options

- Chromatographic lateral flow immunoassay
  - Capilia,SD Bioline, or MTBc
- Line Probe Assay
  - CM or MTBDR+
- GeneXpert MTB/RIF

Antigen detection using chromatographic immunoassay

Procedure:

- *Note: Unlike HIV rapid tests, this test must be inoculated, read and discarded inside a BSC!*
- A 100 µl suspension of growth from liquid or solid medium is added to the specimen well and, if belonging to the TB complex, will react with the Ab to MPT64
- The test is read after 15 minutes



Test procedure….

- Perform test in a BSC; wear appropriate PPE
- Label test cartridge
- Dispense 100 µl of the well-mixed MGIT culture with a transfer pipette on the specimen placing area of the cartridge
- Examine the reading area of the cartridge after 15 minutes

Note: Test results must be interpreted no later than 60 minutes after inoculation

Quality control (MPT64)

- Done each day test is performed and documented on log sheet
- Positive control
  - Well-characterized MTBC strain (ACCT)
- Negative control
  - Well-characterized strain or reference strain of non-tuberculous mycobacteria (NTM)
    - *M. avium*, *M. fortuitum*, etc. growing in liquid media

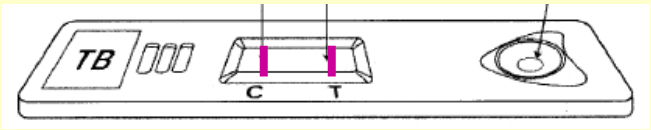
Interpretation of Results

- Positive
  - Formation of a purple to red line on the reading areas labeled [T] and [C] of the cartridge
- Negative
  - Formation of a purple to red line on the reading area labeled [C] of the cartridge but not [T]
- Invalid
  - If no line is observed on the reading area [C], technical errors or product damage has occurred. In this case the test should be considered invalid and repeated using a new cartridge

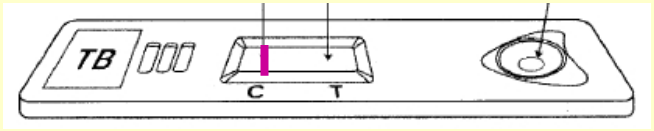
Antigen detection using the chromatographic immunoassay

Interpretation:

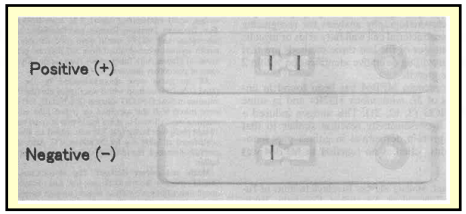
MTB complex – two purple-reddish bands at test and control sites



NTM or other bacterial species – one purple-reddish band only at control site



Note: The test cannot be interpreted if there is no band at the control site



Inoculate a Blood-Agar (BA) plate for contaminants Growth

- Pull out one blood agar plate from the fridge, (this procedure can be done immediately the tube turns MGIT positive)
- Using a marker pen, invert and divide the plate up to 8 sections at the bottom depending on the number of samples to be inoculated.
  - **NOTE:** In case of an excessively turbid tube, inoculate on one plate.
- Label each of the sections with the Laboratory serial numbers of the patient's culture to be inoculated there.

… cont

- Open one positive MGIT Culture at a time
- Inoculate one loop-full of the contents on the correspondingly labeled portion of the BA plate.
- Seal the plate with tape Incubate at 37°C for 24 hrs
- Remove the plate from the incubator, read the BA results
- Record the results in appropriate format
- Dispose of the BA plate(s)

Assessment

- Describe the procedure for unloading Positive cultures from the MGIT machine.
- Describe a process of confirming MTB from positive MGIT cultures.

Summary

- BACTEC MGIT 960 – decreased time to detection for growth
- Prepare a smear to confirm MGIT tube growth is AFB positive and rule our contaminants
- Identify all AFB growth as MTBC before setting up DST
  - Rapid ID system – lateral flow
  - Molecular identification

Acknowledgement