THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagnostics for a TB Free Africa

Training on Proficiency Testing Scheme GeneXpert DTS

Module 7: DTS Inactivation and stock preparation

Venue: Srl Uganda

Presenter:Namutebi Joanita

Date:13/4/2022

Introduction

Genexpert PT panels are deigned to be used when they are in a non viable form to eliminate the risk of exposing testing personnel to Laboratory acquired infections

Learning Objectives

By the end of this module participants should be able:

- To understand the procedure of DTS inactivation
- To know the equipment used in DTS inactivation
- To understand the process of DTS inactivation verification

Module content

- Unloading Positive cultures
- Purity check
- Procedure for DTS inactivation

Prepation for the Inactivation Process

- Determine procedure workflow and schedule to complete all procedure steps.
- Prepare and label materials (i.e., media, reagents, supplies)- may be done in a BSL2 space.
- Move labeled materials to the TB containment room (using a laboratory cart) where manipulation of cultures and isolates are done.
- Record media and reagents lot number and expiration dates on the Reagents and Media Log

Grow Reference Strains in MGIT Culture

Select at least eight strains of mycobacterial species from the permanent stocks in freezer vials.

- **2 strains of pan susceptible MTB**
- **4 strains of RIF resistant MTB**
- **2 species of NTM**

Label 4 MGIT tubes for each MTB isolate.

Label 2 MGIT tubes for each NTM isolate

- Total of 28 MGIT tubes
- ****Duplicate or quadruplicate inoculation will depend on how many panels you need to make**

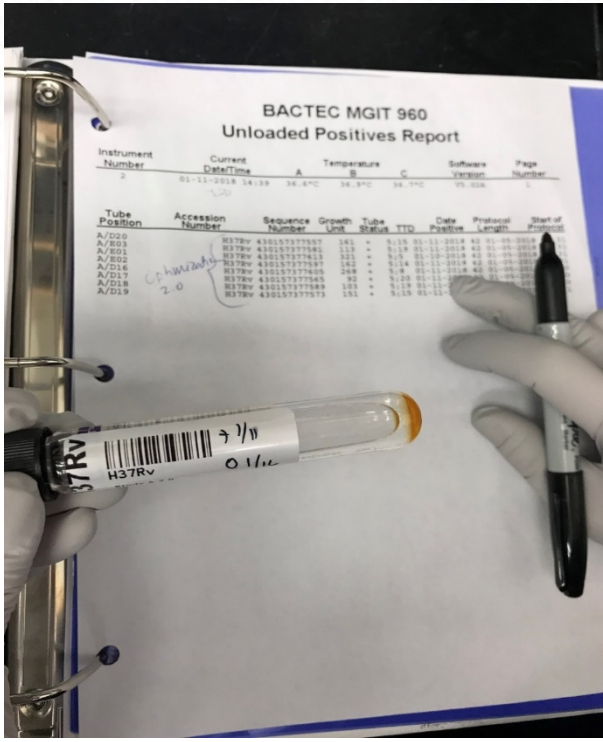
Inoculating MGIT Culture

- Using a repeat pipette, add 0.8 ml of MGIT supplement to each MGIT tube.
- transfer one drop from the 0.25 ml of the thawed freezer isolate to its labeled MGIT tube
- Tighten cap and gently invert tube 2-3 times to mix.
- Decontaminate exterior of tubes and load into MGIT 960 Instrument

**Note: Work with one tube at a time to avoid cross-contamination.

Unloading MGIT Positives

- Unload tubes that were flagged positive
  - Place tubes in a rack.
  - Write date of positivity on each MGIT tube.
  - Unloaded Positive Report,
    - Initial and file in the laboratory file.



Incubation of the Unloaded Positive tubes

- Incubate unloaded positive MGIT tubes in a 35-37C incubator for 4 to 6 days.

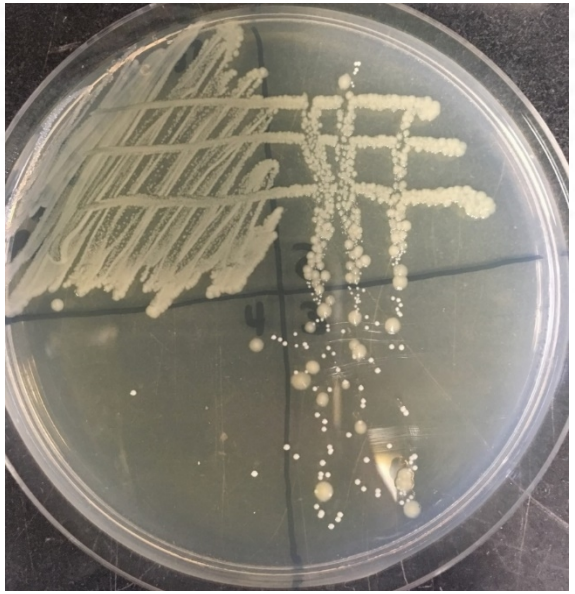
- Store MGIT cultures at 2-8C while waiting for the other cultures to flag positive.
  - The wait should not be longer than 30 days.

Check Purity of MGIT Culture

- Set up BSC for work
- Place rack of positive MGIT cultures inside the BSC
- Vortex each tube for one minute
- Allow tubes to stand for 10 minutes, Set timer
- Label one 7H11 plate or LJ Culture for each MGIT culture

Streak for Isolation and Purity

- Using a sterile 10 µl disposable loop, transfer one loopful of the MGIT culture onto one 7H11 plate
- Streak plate for isolation
- Discard loop into discard container with disinfectant
- Incubate at 35-37C for 2-3 weeks
- Check plates for growth at least once per week.



Interpretation of Purity Check Plates

- Examine growth to confirm that the morphology of the colonies is consistent with the selected species of mycobacteria.
- Discard DTS stock that grow mixed cultures on the 7H11/LJ and/or colonial morphology is not consistent with the expected morphology for the selected species of mycobacteria.
- Record results on the Purity Check Log

Heat Inactivation

- Heat-inactivate all positive MGIT cultures at the same time
- Check oven temperature reading is at 80-85C
- Place rack of MGIT cultures inside the oven
- Close oven door tightly and wait for temperature to stabilize at 80-85C
  - **Note: Oven temperature typically drops when door is opened; wait for temperature to reach 80C before starting the timer

Timing and Documentation

- Start timer for 30 minutes
- After 30 minutes have elapsed, verify oven temperature is 80-85C.
  - Record temperature and time on logs
  - Do not open the oven
- Set timer for additional 30 minutes
- Once a total of one hour has elapsed, verify temperature is 80-85C

Preparing Stock –Adding Beads

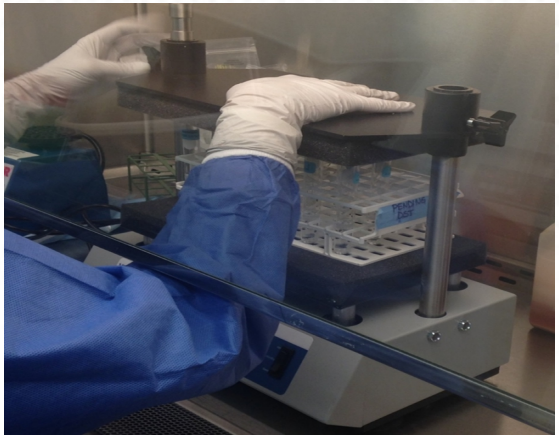
- Place rack of inactivated MGIT culture tubes inside the BSC.
- Make sure the cultures have been allowed to cool to room temperature
  - For each MGIT culture, prepare one tube containing 5-10 sterile 3 mm glass beads.

Preparing Stock –Adding Beads

- Add the glass beads to the MGIT culture tube.
  - Work with one culture tube at a time to avoid cross-contamination.
  - Gently remove the cap MGIT tube at 45-degree angle and slowly pour in 5-10 pieces of sterile 3 mm glass beads while holding the mouths of the two tubes together.
  - Discard the bead tube in designated waste container (do not re-use).

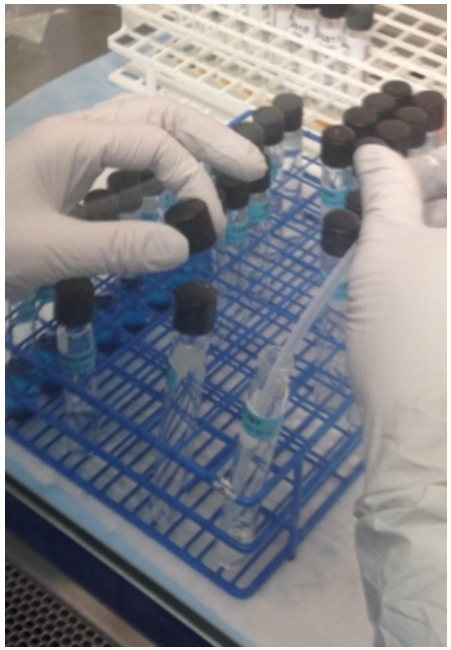
Preparing Stock – Vortexing

- Tighten cap of the MGIT tube
- Vortex tube for 5 minutes. (Ensure full vortex is obtained.)
- Allow tubes to sit for 10 minutes undisturbed, for clumps to settle.



Preparing Stock – Combining

- Label one 50-ml sterile conical tube for each isolate
- Carefully pipet off all the liquid above the beads and transfer fluid to the labeled 50-ml conical tube.
  - Using sterile transfer pipette
  - Work with one culture tube at a time to avoid cross-contamination.
- Combine the liquids from the same isolate in the same conical tube.



Combined stock

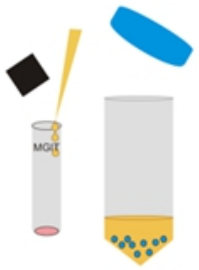
- The combined suspensions of each isolate will be the stock solution to use for performing inactivation verification and for preparing dilutions.

Inactivation Verification -Inoculate MGITs

- Prepare one MGIT tube for each heat-inactivated stock solution
  - Label MGIT tubes with: Stock number, name of the isolate, date of inoculation
- Add 0.8 ml of MGIT supplement to each MGIT tube.

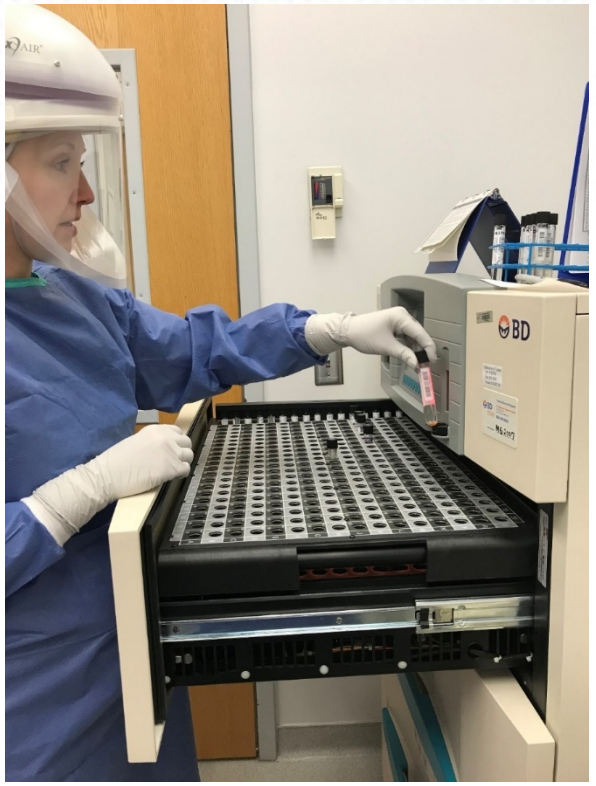
Inactivation Verification -Inoculate MGITs

- Invert heat-inactivated stock solution 2-3x to mix.
- Transfer 0.5 ml of the stock from each isolate to its labeled MGIT tube.
- Cap tightly and invert MGI times to mix



Inactivation Verification –Load and Incubate MGITs

- Load inoculated MGIT tubes in the MGIT instrument
- Leave tubes in the instrument for two 42-day cycles
  - Total of 84 days or until flagged positive by the MGIT instrument



Inactivation Verification –MGIT Negatives

- Remove negative tubes when the first 42-day cycle is completed.
  - Print Unloaded Negative Report from the MGIT instrument.
  - File report in the DTS Preparation binder.

Inactivation Verification –MGIT Negatives

- Scan tubes back into MGIT 960 instrument to start the second 42-day cycle.
- Remove negative tubes when the second 42-day cycle is completed.
  - Print Unloaded Negative Report from the MGIT instrument.

Inactivation Verification -MGIT Negatives (Pass)

Negative culture after the 84days of incubation are an indicator of successful inactivation therefore:

- The prepared stocks do not have viable organisms
- Can proceed with panel preparation

Inactivation Verification –MGIT Positives

Scan out positive MGIT tubes, if any.

- Print Unloaded Positive report.
- File report in the DTS Preparation binder.

Inactivation Verification -MGIT Positives(Failure)

Discard the stock solution for any viability test that is flagged positive by the MGIT instrument.

- Growth in MGIT tube indicates that the heat-inactivation procedure failed and the organism is still viable and must not be used to prepare DTS panels.

Group exercise

- How would you go about failed verification of inactivation?

(Group activity)

Prepare Dilution of the Stock for DTS Pre-test

Prepare materials and place inside the BSC.

- Five cryovials (4-ml capacity) for each stock solution.
- Eight 50-ml sterile conical tubes,
- Sterile saline solution
- Food coloring

Preparation of stock dilution: Labelling

- Label each vial with name of isolate, stock ,and aliquot number or letter (A-E).
- label with name of isolate, stock solution number, dilution (1:1000), and date.

Dilution Preparation-- Continued

- Prepare a 1:10 dilution of each heat-inactivated stock solution
  - Pipet 4.5 ml of sterile saline to each labeled 50-ml conical tube
  - Add 5 µl of food coloring to each 50-ml conical tube
  - Transfer 0.5 ml of stock solution to the labeled 50-ml conical tube
  - Vortex tube for 30 seconds

Dilution Preparation-- Continued

Work with one stock solution at a time to avoid cross-contamination

Allow tubes to stand for 10 minutes after vertexing to allow for settling of large clumps to achieve a more homogenous aliquots

- Set timer

DTS Pre-test Aliquoting

- Uncap labeled 4-ml cryovials inside the BSC
  - Place caps in a clean plastic zipper bag.
- Pipet 100 µl of the diluted stock into the 5 labeled cryovials
- Use a repeat pipette

DTS Pre-test Aliquoting

- Keep pipette tip in upper 1/3 of the suspension when aspirating the diluted stock
  - Avoids disrupting larger clumps that settled to the bottom of the tube
  - **Work with one diluted stock at a time to avoid cross-contamination.
- Discard the remaining 1:10 dilution

DTS Pre-test Drying

- Allow aliquot tubes to sit open inside the BSC in the TB containment laboratory for 7-10 days

  - After 7-10 days, check if specimen at the bottom of the tubes is dry, then tightly cap all tubes

  - **Ensure that tubes are visually dry before capping

Storage of pretest dried tubes

- Store DTS in the dark at a consistent 2-25°C while waiting for pre-testing

Stock Storage

- Store remaining stock solutions in the refrigerator set at 2-8°C.

Assessment

1. What is the procedure for inactivation verification?
2. What are the storage conditions for the prepared DTS panels?
3. What is the essence of adding food color to the prepared DTS aliquots?

Summary

- Verification of inactivation is very essential in DTS preparation, for safety reasons
- The DTS panels are stored in specific storage conditions to ensure sustenance of the integrity proper drying and thus longevity of the prepared panels upon shipment
- Addition of food color to the prepared DTS aliquots allows for visualization of the tube contents for the prepared items

References

- ISO 13528:2005, *Statistical methods for use in proficiency testing by interlaboratory comparisons*
- ISO Guide 34, *General requirements for the competence of reference material producers*
- ISO Guide 35, *Reference materials — General and statistical principles for certification*
- Guide 34, ISO Guide 35 and ISO 13528 (homogeneity and stability)
- ISO/IEC Guide 98-3, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement* (GUM:1995)
- ISO/IEC 17011:2004, *Conformity assessment — General requirements for accreditation bodies accrediting conformity assessment bodies*
- ISO/IEC 17025, *General requirements for the competence of*

Acknowledgments