THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on Tuberculosis Drug and Susceptibility Testing (MGIT DST – Liquid Method)

Module 11: BACTEC MGIT 960 DST troubleshooting

Date:

By:

Venue:

Module outline

- Equipment and reagent use
- Error reports for DST set
- DST troubleshooting
- Summary

The right equipment used correctly

- Start with the right equipment/supplies
  - Use only sterile tubes and pipettes
  - Sterile glass beads to break up clumps in inoculum from solid media
  - Calibrated pipettes
    - Drug addition to tubes
  - 0.5 McFarland standard with same tube diameter as inoculum tube and same media
    - Organism in 7H9, use 7H9 blank in densitometer
  - Vortex well and allow clumps to settle
    - Do not select inoculum from bottom of tube or over inoculate tubes with clumps of organisms

Use the correct reagents

- PZA test medium and MGIT 7 ml tubes are not interchangeable when testing SIRE and PZA DST
- PZA supplement and SIRE supplement are not interchangeable
- Do not use supplement other than that supplied with the drugs – no PANTA or other growth supplement
- Drug reconstitution:
  - Use sterile distilled/deionized water
  - Ensure drug dissolves completely
  - Store reconstituted drug at -20 ^{o}C up to 6 months, but do not exceed original expiration date
  - Use thawed, reconstituted drug the same day; discard any unused portions

Use the correct inoculum

- Start with the right inoculum
  - Pure cultures only
  - Fresh cultures
    - Slant ≤ 14 days
    - Positive MGIT tube
      - Day MGIT 960 called ‘positive’ plus 1 day to 5 days from date of positivity
  - Homogeneous inoculum
    - Use sterile glass beads and vortex to break up organism clumps
    - Use only supernatant for inoculation
  - Too high or too low an inoculum may give erroneous results or un-interpretable results
    - Prepare dilutions according to procedure
    - Use accurate pipette to add inoculum to tubes

Error report for DST set

- Some conditions occur that may affect the test results; the instrument will report this as an error (X)
  - GC tubes become positive in less than 4 days
    - Inoculum too heavy
  - GC tubes remain negative up to 21 days
    - Inoculum too light

Invalid DST sets 'X'

- Growth control is not positive within 13 days (SIRE) or 21 days (PZA):
  - Assumption is made that set is under-inoculated
- Growth control is positive too soon (< 4 days):
  - Assumption is made that set is over-inoculated or contaminated
- ANY tube is missing in carrier

DST interpretation of DST results

- Invalid results ‘X’
  - Initial inoculum was too heavy
    - If working from MGIT tube, ‘Day’ 3 to 5 must be diluted
    - If working from solid media
      - Tube must be allowed to settle – over-inoculation and minimize aerosols
      - Adjust suspension from solid to 0.5 McFarland
    - Initial specimen not vortex-mixed completely; clumps still present in suspension
  - Growth Control suspensions for SIRE and PZA are different (1:100 vs 1:10)

DST troubleshooting: Non-interpreted results

- Instrument took too long to interpret results (SIRE >14 days; PZA >21 days)
  - Initial inoculum too light
    - Precise pipettes must be used for dilutions and addition to tubes
      - Not transfer pipettes
    - If working with solid media
      - Media may have been picked up, which interferes with reading 0.5 McFarland
  - Initial culture too old
    - Not in proper growth phase

DST troubleshooting (1)

- **False susceptible**
  - Organism inoculum is too light
    - Starting suspension must be > 1.0 McFarland
  - Suspension not homogeneous
  - Culture too old
  - Growth control dilution was too heavy
    - Growth control grew too fast
  - Was proper amount of supplement added to tubes?
    - Was supplement properly stored?
  - Drug not reconstituted properly
  - Too much drug added to tubes
  - Are tube caps on tight? If not, oxygen can leak into tube
  - Only PZA medium and PZA supplement can be used for PZA test
  - Organism could be borderline-resistant

DST troubleshooting (2)

- **False resistant**
  - Culture is contaminated or mixed culture
  - Organism inoculum was too heavy
  - Organism was not properly diluted
  - Suspension was not homogeneous or had clumps
  - Growth control dilution was too light
    - Growth control grew too slowly
  - Drug was not reconstituted properly
  - Drug was not stored properly
  - Drug was not added to tube
  - MGIT tube was not mixed properly after the addition of the organism suspension
  - Organism could be borderline-resistant

DST troubleshooting (3)

- Perform definitive identification
  - Perform ZN smear on positive MGIT tubes
  - Identify MTBC
    - Some NTMs show resistance to anti-mycobacterial drugs

DST troubleshooting (4)

Verify purity of inoculum

- Confirm inoculum purity
  - ZN smear
    - Record cellular morphology on worksheet
      - MTBC mixed with NTM
    - Contamination (fungus and bacteria)
  - Blood plate for contamination check
  - Middlebrook 7H10
    - Rule out mixed mycobacterial cultures

Status X: error/Indeterminate results

- Verify resistance profile
  - Any resistance to a drug
  - Confirm resistance
    - Drug not added to tube
    - Less than 0.1 ml of drug added to tube
    - Inoculum selected from the bottom of seed tube or clumps were not allowed to settle
  - Inoculate a MGIT 960 seed tube
  - Reset DST panel with resistant drug

ZN on growing drug-containing tubes

- If Middlebrook 7H10 is not available to confirm mycobacteria purity
  - ZN on resistant drug-containing tubes
    - Verify that only one type of cellular morphology is present
    - Still need to reset DST to verify drug was added to tube

DST troubleshooting: When to re-test

- Un-interpreted results
- Mono-resistance (especially RIF, EMB & PZA)
- Results disagree with previous testing from the same patient
  - Borderline resistance/susceptibility?
- Patient is a new untreated case and shows resistance
- QC organisms fail to give expected results
- Unexpected high number of resistant isolates in a batch

Troubleshooting summary

- Organism suspension must be homogeneous, without clumps
- Care must be taken when preparing dilutions
- Drugs must be reconstituted and stored properly
- Sterile tubes and pipettes must be used
- Accurate pipettes must be used, not disposable transfer pipettes
- QC of DST is critical to ensure test is functioning properly
  - Should be performed on all new lots of DST reagents and MGIT tubes and weekly with each batch of testing

Assessment

- List the possible causes of;
  - False resistance DST results and ways to troubleshoot them
  - False susceptibility DST results and ways troubleshoot them

References

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