THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

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

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

Module 8: Requirements for submission, acceptance and processing of isolates for DST

Date:

By:

Uganda Supranational Reference Laboratory

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

Learning objectives

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

- Understand the submission process for isolates received for DST
- Understand the criteria for acceptance or rejection of isolates
- Understand how TB isolates are prepared for DST

Module outline

- Source of isolates
  - Referred to DST testing laboratory from another laboratory
  - Grown in DST testing laboratory
- Criteria for acceptance or rejection of isolates
  - Labelling, leakage
  - Contamination
- Preparing the isolate for DST
  - Identification
  - Molecular testing

Source of isolates: External (1)

- Isolates referred to DST testing laboratory from another laboratory
  - Best to process sputum specimens and perform culture in laboratories near to where specimen is collected
    - Contaminants may overgrow TB in sputum specimens during transport time and conditions
    - Isolates more likely to survive transportation conditions
    - However, biosafety requirements must be strictly followed to transport isolates

Source of isolates: External (2)

- Species identification and DST are best performed at National TB Reference Laboratory or other Quality Assured referral laboratories
  - Transport time and need to re-grow isolate delays DST reporting time

- Isolates and relevant and patient information must be accurately transcribed and transported to the NTRL for DST

Source of isolates: Internal

- Isolates grown in DST testing laboratory
  - Information on patient has already been documented when specimen received for culture
  - Internal database or log books provide information on previous tests performed on this same patient
    - Direct smear results on current isolate
    - Results on tests performed on specimens from this patient
    - Specific issues seen with previous specimens or isolates can be addressed
      - Example: growth found only on either liquid or solid media
  - No need for transport
    - Faster turn-around time for reporting DST results
    - Isolates contained within same laboratory; biosafety issues easier to address

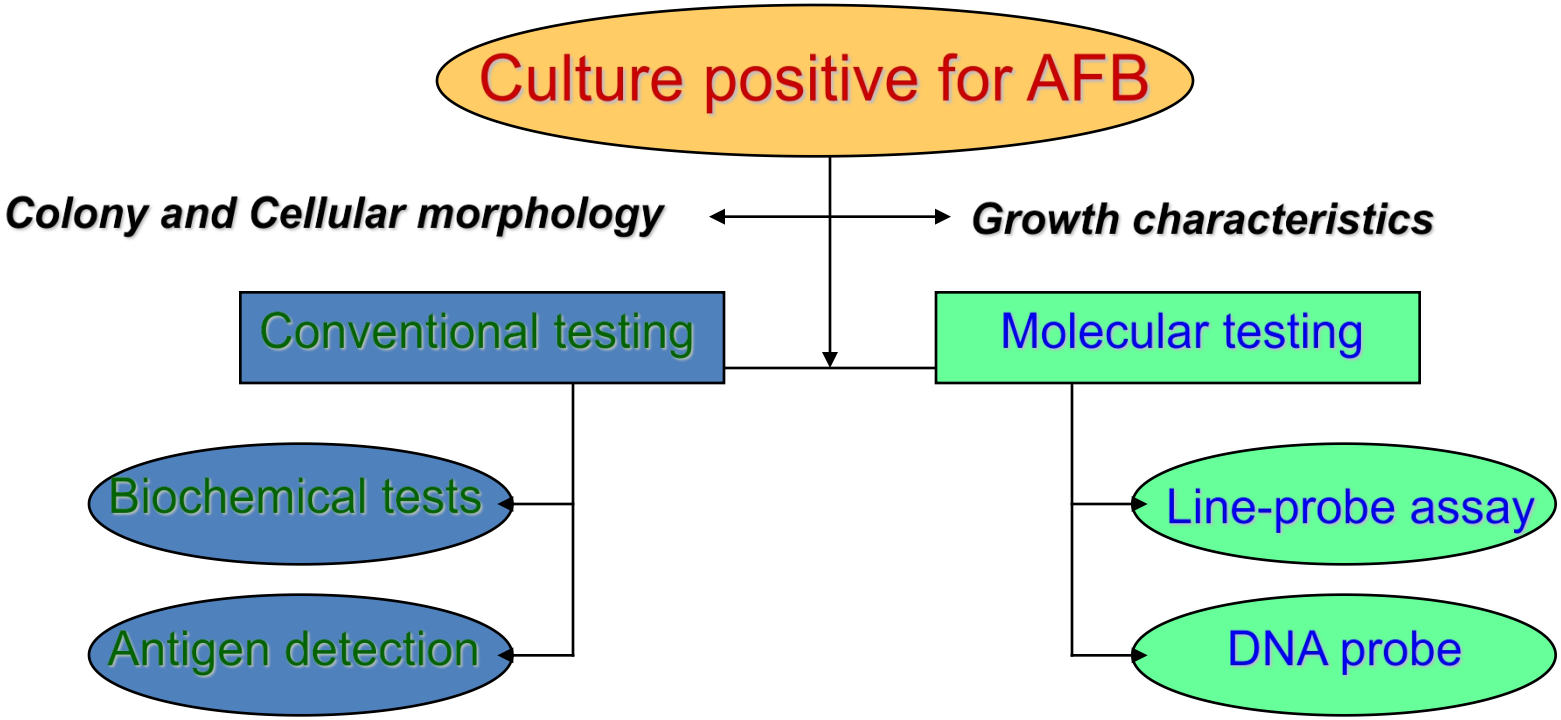
Criteria for rejection of an isolate

- Do not process; contact the submitter to resubmit isolate for the following reasons:
  - No label on tube
  - Improper or leaking container
  - Isolate overgrown with contaminant
    - Caution: Opening tube with mould can contaminate BSC and other specimens
- Isolates may be processed if minor questions can be clarified
  - Misspellings between tube and request slip
  - Contact submitter to clarify questions

Preparation of isolate for DST

- Identification of isolate as belonging to the *M. tuberculosis* complex
- Ensure that the TB isolate is not contaminated
- Advantages of molecular or liquid medium DST
- Preparation of cells for molecular testing
- Preparation of cells for DST in liquid broth in the MGIT system

Isolate must be identified as MTBC before performing DST



Non-tuberculous mycobacteria can be resistant to anti-TB drugs, and testing can result in inappropriate patient therapy

Growth of contaminants on solid media

- LJ medium contains bacteriostatic agent
  - Malachite green inhibits most environmental and normal flora
  - Important to remove liquid in bottom of LJ before inoculation. Loosen cap for 1st week of incubation
    - Limited exposure to malachite green, so contaminants may eventually grow in this liquid
- Contaminated LJ medium
  - If contamination is slight, attempt to isolate TB colonies; transfer to new LJ
  - If totally contaminated, request a new specimen

Growth of contaminants in MGIT tubes

- Contaminated MGIT tubes usually become positive very early:
  - 24 – 48 hours
  - Rapid growers such as *M. avium-intracellulare* complex (MAC) may be positive in 3–4 days
- Visual observation:
  - Homogeneous turbidity
- Non-acid-fast bacteria may be seen in AFB smea of MGIT tube
  - With or without AFB
- Subculture to blood agar plate
  - Growth in 24–48 hours

DST performed using solid media

- Possible problems with egg-based media (LJ)
  - Potency of drugs may be diminished during inspissation
  - Components of the eggs (phospholipids, proteins) affect some drugs
- Middlebrook 7H10 agar with OADC
  - OADC is expensive
  - Lot-to-lot variations in quality have been reported
- DST on solid media requires 3 to 4 weeks of incubation

Gold standard for DST

- Agar proportion method using Middlebrook 7H10 with quality assured OADC
  - Considered the standard method for TB DST in the USA and many European countries
- Most first- and second-line drugs can be accurately tested for susceptibility or resistance using this method
- However, because of the long turn-around time, the method is more appropriate for surveillance than for individual patient treatment

Advantages of molecular or liquid medium DST

- Molecular methods
  - Can be performed within 1–2 days
  - Identification of TB is included in LPA
  - Detection of MDR TB is quite reliable
  - Accurate results may be obtained even with mixed culture
  - However, not all drugs are currently available
- Liquid medium DST using the MGIT system
  - Results ready within 7–10 days
  - Currently validated for all 1st-line drugs including PZA and some 2nd-line drugs

Preparation of isolates for MGIT DST

- Isolates can be grown either on solid or liquid media prior to being tested in the MGIT DST
  - Need healthy cells in logarithmic phase of growth
  - Need fairly light inoculum so that drug concentrations are not overwhelmed

- Growth should be AFB-positive and identified as a pure culture of the MTB complex before further testing

Inoculum from the MGIT tube (1)

- Adhering to the following recommended timeframe is important for accurate results
  - The day a MGIT tube is positive by the instrument is considered **Day 0.**
  - The tube should be kept incubated for at least one more day (**Day 1**) before DST
    - May be incubated in a separate incubator at 37 ºC ± 1 ºC
  - A positive tube may be used for DST up to (**Day 5**)
    - A tube that has been positive for more than 5 days

Inoculum from the MGIT tube (2)

- If growth in a tube is on **Day 1** or **Day 2**, vortex to break clumps, let big clumps settle for 10 minutes and use the supernatant undiluted for inoculation of the drug set.

- If growth is on **Day 3, 4 or 5**, vortex to break clumps, let big clumps settle for 10 minutes and then dilute 1.0 ml of the positive broth with 4.0 ml of sterile saline. This will be a 1:5 dilution. Use

noculum from growth on solid medium (1)

- Use growth on solid medium within 15 days of appearance of positive growth
- Transfer a large amount of growth into a tube with broth or saline and glass beads; vortex the tube for 1–2 minutes to break the clumps – the turbidity should be > McFarland #1.0
- Let the suspension stand undisturbed for 20 minutes, then transfer the supernatant into another sterile tube

noculum from growth on solid medium (2)

- Let this tube stand undisturbed for another 15 minutes then transfer the supernatant into another sterile tube – the turbidity should > McFarland #0.5
- Adjust the turbidity of this suspension down to McFarland #0.5 by adding sterile saline; do not make it below this standard
- Dilute the above suspension 1:5 by adding 1.0 ml of the suspension to 4.0 ml of sterile saline; mix well and use it as the inoculum for drug susceptibility testing

Summary: Isolates for DST

- Isolates can either be grown in the DST testing laboratory or referred from another laboratory
- Isolates should not be tested if they are unlabelled, leaked, or overgrown with mould
- DST using molecular or MGIT system methods have much shorter turn-around times than DST using solid media
- For phenotypic DST, growth should be AFB-positive, and identified as a pure culture of the MTB complex

Assessments

- List the sources of isolates for MGIT DST
- What are the criteria for rejection/acceptance of isolates for MGIT DST

References

- BACTEC® MGIT 960™ System User's Manual. Becton Dickinson Company. 2004/06 Document number MA-0117. Revision E
- Global Laboratory Initiative (GLI) http://www.gliquality.org/
- www.who.int/tb

Acknowledgments

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

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

gli
global laboratory initiative
advancing TB diagnosis

KNCV
TUBERCULOSISFOUNDATION

KOFIH
Korea Foundation for International Healthcare

DEPARTMENT OF HEALTH AND HUMAN SERVICES - USA

CDC

World Health Organization

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