

Training on *Mycobacterium tuberculosis* drug susceptibility testing (first and second line LJ DST)

Module 6: Isolate requirements for LJ DST

Venue:

Presenter:

Date:

Introduction and Objective

Introduction

- This module details the characteristics of isolates to be used in LJ DST and their different sources.

Objectives

By the end of the module ,Participates should;

- Know the qualities of a good isolate for LJ DST
- Understand different sources of isolates to be used

Module Outline

- Source of isolates for LJ DST
- Criteria for acceptance or rejection of isolates
- Preparing the isolate for DST

Exercise (5 minutes)

- List the qualities of a good isolate for LJ DST and Why?

Sources of isolates for LJ DST

- There are two sources of isolates for LJ DST:

- 🌐 External sources

- 🌐 Internal source

- ***External sources:*** Samples/Isolates referred to DST testing laboratory from another laboratory.

- ***Internal sources:*** Culture isolated that are recovered within the DST testing laboratory

Sources of LJ DST culture isolates

External sources:

- 🌐 Positive culture Isolates are more likely to survive transportation conditions.
- 🌐 Biosafety requirements must be strictly followed to transport isolates for DST.
- 🌐 Isolates and relevant patient information must be accurately transcribed and transported to the DST testing laboratory.
- 🌐 Species identification and DST are best performed at National TB Reference Laboratory or other Quality Assured referral laboratories.

Criteria for rejection of isolates from external sources

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



Preparation of isolates for LJ DST

- Use growth on solid medium within 14 days of appearance of positive growth
 - Species Identification test should be performed before use of isolates.
- Transfer a large amount of growth into a tube with distilled water/saline and glass beads; vortex the tube for 1-2 minutes to break the clumps and leave the suspension to settle for 10 minutes.

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
 - Lot-to-lot variations in quality have been reported.
- DST on solid media requires 3 to 4 weeks of incubation

Assesment

- List some of the sources of isolates for LJ DST.
- What circumstances can result into referred isolates being rejected by the lab they are referred to?
- Positive LJ culture isolates that qualify for DST should be set on LJ DST before antigen test is done: True/False.
- Positive LJ culture isolates should always be more than a month from the day of culture positivity before setting on LJ DST: True/False.

Summary

- Isolates can either be grown in the DST testing laboratory or referred from another laboratory.
- Isolates should not be tested if they are un-labelled, leaked, contaminated/overgrown with moulds.
- Growth of MTBc should first be confirmed before LJ DST is set.

Summary

- Positive LJ culture isolates that are to be set on LJ DST should be less than 14 days.

References

- GLI TB training package
<http://www.stoptb.org/wg/gli/trainingpackages.asp>

Acknowledgments

