THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

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

Module 12: Trouble shooting of LJ DST results

Venue:

Presenter:

Date:

Introduction and Objective

Introduction

This module details the steps taken when troubleshooting discordant and invalid/noninterpretable LJ DST results.

Objective

By the end of the module participants should be able to troubleshoot discordant and non interpretable LJ DST results

Module Outline

- Use of correct equipment and reagents for LJ DST
- Troubleshooting of non-interpretable LJ DST results.
- Causes of false sensitive and false resistance results in a DST lab.
- Reasons for repeating DST in the laboratory.

Exercise (5 minutes)

- Joseph a laboratory technologist receives a presumptive MDR sample in the laboratory and tests it on LPA, Gene xpert and 1st LJ DST as per the Laboratory's algorithm. The results for all the tests are described in the table below:

GENE XPERT	LPA	1ST line phenotypic DST
MTB DETECTED HIGH, RIFAMPICIN RESISTANCE DETECTED	Isoniazid: RESISTANT Rifampicin: RESISTANT	Pyrazinamide; SENSITIVE Isoniazid; SENSITIVE Rifampicin; SENSITIVE Ethambutol; SENSITIVE

Exercise (5 minutes)

- Explore all possible causes of such a discordant result and how you would manage such discrepancies in your laboratory?

The right equipment/materials 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

- Drug reconstitution:
  - Use sterile distilled/deionized water and other diluents.
  - 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 un-used portions.

Use the correct inoculum

- Start with the right inoculum
  - Pure cultures only
  - Fresh cultures
    - Slant ≤ 14 days
  - 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

DST troubleshooting: Non-interpretable results

***Contaminated result*;**

- Inoculum contained mixed growth of *MTB* contaminants.
- Materials used were not sterile.
- Cross contamination during manipulation.

***Heavy growth on growth control*;**

- Inoculum was heavy.

LJ DST RESULT; NON INTERPRETABLE RESULTS

- ***No growth on growth control:***
  - Inoculum was too light
  - Inoculated culture isolate was too old containing only dead *Mtb* cells.
- ***Growth on PNB:***
  - Inoculum contained NTM

LJ DST TROUBLESHOOTING

- **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 bacteria suspension added to the drug containing tubes?
  - Drug not reconstituted properly
  - A high drug critical concentration in the drug containing media
  - Organism could be borderline-resistant

LJ DST TROUBLESHOOTING

- **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 LJ media.
  - Organism could be borderline-resistant

LJ DST Troubleshooting: when to retest

- Un-interpretable results
- Mono-resistance (especially RIF, EMB & PZA, CAP, AMK)
- Results disagree with previous testing from the same patient.
  - Borderline resistance/susceptibility?
- QC organisms fail to give expected results
- Unexpected high number of resistant isolates in a batch.

Assesment

1. List 5 precautions one should take to ensure that accurate DST results are produced in the lab.
2. A turbid McFarland suspension beyond 1.0 can cause a false resistant LJ DST result True/False
3. List 4 causes of false resistant LJ DST results.
4. List 4 causes of false sensitive LJ DST results.

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 drugs.

References

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

Acknowledgments

KOFIH
Korea Foundation for International Healthcare
한국국제보건의료재단

KNCV
TUBERCULOSISFOUNDATION

gli
global laboratory initiative
advancing TB diagnosis

DEPARTMENT OF HEALTH AND HUMAN SERVICES - USA

CDC

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

World Health Organization

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