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 9: Setting of LJ DST isolates

Venue:

Presenter:

Date:

Introduction and Objective

Introduction

•This module details the steps involved in setting LJ DST from clinical isolates.

Objectives:

At the end participates should be able;

•To prepare the bacterial suspension of MacFarland 1.0 turbidity and make the required serial dilutions.

•To set LJ DST from bacterial suspensions of clinical isolates.

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Module Outline

- Overview of LJ DST
- Materials required for LJ DST.
- Preparation of 1.0 McFarland bacteria suspension
- Preparation of bacteria dilutions
- LJ DST Inoculation

Over view of LJ DST

- This is a proportional DST technique based on determining the 1% MTBc resistant population that is clinically significant.

- It is based on the principle of comparing the growth on drug free media **(growth control)** to the growth on drug containing media.

- The strain is presumed to be resistant when growth of more than a certain proportion of the inoculum **(critical proportion)** occurs on culture media containing a defined drug concentration(**critical concentration**)

Over view of LJ DST

critical concentration

- The lowest concentration of an anti-TB drug in the culture medium at which growth of tubercle bacilli indicates resistance of clinical significance.

critical proportion

- The percentage of tubercle bacilli in the inoculum whose growth on culture media containing the critical concentration of an anti-TB drug signifies the clinical ineffectiveness of that drug.

Over view of LJ DST

Growth control:

- Culture yielded after inoculation of tubercle bacilli on a culture medium *without* any test drug in order to exhibit unrestricted growth.

Materials/Equipment

Reagents/ materials

- Sterile distilled water
- Calibrated pasture pippetes(1ml and 3 ml)
- Khan tubes.
- Culture isolates positive for MTBc.
- Sterile Inoculation loops
- Universal bottles
- Plain LJ media

Materials

PNB growth control (500µg/ml)

MacFarland 1.0

Reference strains(H37Rv)

Equipment

- Biosafety cabinet
- Incubator
- Votexer

Materials required for LJ DST setting

1st and 2nd Line LJ DST media of the following critical concentrations:

DRUG	Critical concentration (µg/ml)
Isoniazid	0.2
Rifampicin	40
Ethambutol	2.0
Moxifloxacin	1.0
Levofloxacin	2.0
Amikacin	30

Work procedure(1)

- ***Prepare 1.0 Macfarland bacteria suspension as below:***
  - With a loop, scrape colonies from all over the culture (try to pick up portions from all colonies and not media because it gives you false turbidity).
  - Use a sterile, small, thick-walled screw-capped glass tube containing 5–7 sterile glass beads (approximately 3 mm in diameter).
  - Gently shake the loop over the beads.

Work procedure(2)

Preparation of 1.0 Macfarland suspension(neat suspension):

- Add some drops of sterile saline or distilled water, shake, add 2 further drops and vortex.

- Let stand for 30 minutes to allow the larger aggregates of bacteria to settle.

- Transfer the homogenous upper part of the supernatant into another sterile tube without disturbing the sediment. ***It should not contain any visible clumps***.

Work procedure(3)

Preparation of 1.0 McFarland suspension (neat suspension):

Adjust the turbidity of the bacterial suspension to match the McFarland standard No.1. it approximates to 1mg of wet bacterial mass/ml

NB;

- If the suspension is too turbid, add some drops of sterile distilled water.
- If the suspension is insufficiently turbid, do not add more cells to the suspension (because mycobacterial cells are difficult to homogenize);
- Let the suspension settle, discard some of the supernatant to concentrate cells, and adjust the turbidity by adding few drops of distilled water.

A Wrong Mcfarland concentration can result into false resistance or false susceptible results?

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Work procedure(4)

Preparation of bacteria dilutions:

- Make serial 10-fold dilutions of the standard suspension by diluting sequentially 1.0 ml of the culture suspension in tubes containing 9 ml of sterile distilled water or normal saline (0.85% sodium chloride).
- Make sure to mix each dilution thoroughly.

Work procedure(4)

Preparation of bacteria dilutions:

- Dilutions of 10–2 (for Control 1) and 10–4 (for Control 2) should be inoculated as the growth controls (that is, LJ medium without any anti-TB agents).
- Some laboratories inoculate duplicate LJ tubes for both Control 1 and Control 2.
- One tube with medium containing each anti-TB agent is inoculated with only the 10–2 dilution.

Work procedure (5)

LJ DST DST Inoculation;

- The volume of the inoculum should be 0.1 ml.
- The inoculum should be placed onto the middle two thirds of the slant avoiding the edges.
- Ensure that the inoculum is spread evenly on the middle part of the slide by slighty tilting the inoculated slant.
- Ensure that all tubes are properly labelled.

Work procedure (6)

Incubation

•After inoculation, the tubes may be placed at an angle and are incubated at 37 OC ± 1 OC.

•Some laboratories incubate the tubes with the screw caps slightly loosened to allow for evaporation of the inoculum.

•Then, after 24-48 hours, the caps are tightened and the tubes are incubated for about 6 weeks.

•Extreme caution should be taken if the caps are left loose since strains of MDR-TB and XDR-TB pose a potentially major biosafety risk.

Work procedure(7)

- Document your work on the LJ DST work sheet/register.
- Read at 4th week, final results for ethambutol and PNB and preliminary results for the rest of the drugs.
- Incubate for more 2 weeks and read final results at 6th week for other drugs.

NB :

1.The inoculated media should always be examined for contamination after 1 week of incubation.

2.Interpretable results for all drugs can be concluded at 4 weeks

Assesment

- Describe the principle behind LJ DST?
- Define Critical concentration and critical proportion as applied in LJ DST?
- What materials are required for LJ DST setting?
- What is the relevance of Macfarland 1.0 in LJ DST?

Summary

- *LJ DST technique is based on the principle of comparing growth on drug containing media to the growth on drug free media to ascertain the 1% clinically significant MTBc population.*

- A Wrong McFarland concentration can result into false resistance or false susceptible results?

References

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

- https://www.who.int/tb/publications/2018/WHO_technical_drug_susceptibility_testing/en/

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