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 11: Interpreting and Reporting of LJ DST results

Venue:

Presenter:

Date:

Introduction and Objective

Introduction

The module details the principle and procedure of how to interpret LJ DST results.

Objective

By the end of the module, participants should have acquired the knowledge and skills for proper interpretation and reporting of LJ DST results.

Module Outline

- Introduction to reading and interpretation of LJ DST media.
- WHO/IUATLD grading scale for LJ DST.
- Interpretation of LJ DST results.
- Recording and Reporting of LJ DST

Exercise (5 minutes)

- Brainstorm on some of the potential consequences of reporting false sensistive and false resistant LJ DST results.

Introduction to LJ DST drug interpretation

- Slants should be read after 4 weeks of incubation as provisional results and after 6 weeks of incubation for definitive interpretation.

- Before interpreting LJ DST results, first confirm whether the media used to set the patient isolates passed QC.

Introduction to LJ DST drug interpretation

- In LJ DST proportional method, the number of colonies growing on drug free medium is compared to the number of colonies on drug containing medium and the proportion of resistance bacilli are determined.

Introduction to LJ DST interpretation

A drug containing slant that has no growth or a growth less than the number of colonies on the growth control is reported as a sensitive case.

A drug containing slant that has a growth equal too or higher than the number of colonies on the growth control is reported as a resistant case.

Introduction to LJ DST interpretation

- If on day 28 the ratio of the number of colonies on the medium containing the anti- TB agent to the number of colonies on the control medium is 1% or greater, then the culture is resistant.
- If there are no colonies on the medium containing the anti-TB agent and Control 1 (10–2 dilution) has confluent growth, the strain can be reported as susceptible without further reading.

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Introduction to LJ DST interpretation

- All Ethambutol and PNB results are interpreted at day 28 to avoid reporting false resistance and false positive results respectively.
- Except for the above circumstances, all other results can be reported after 40 days (6 weeks).

Morphology of *M TUBERCULOSIS* ON LJ media

LJDST/PP/011, Version 1.0,
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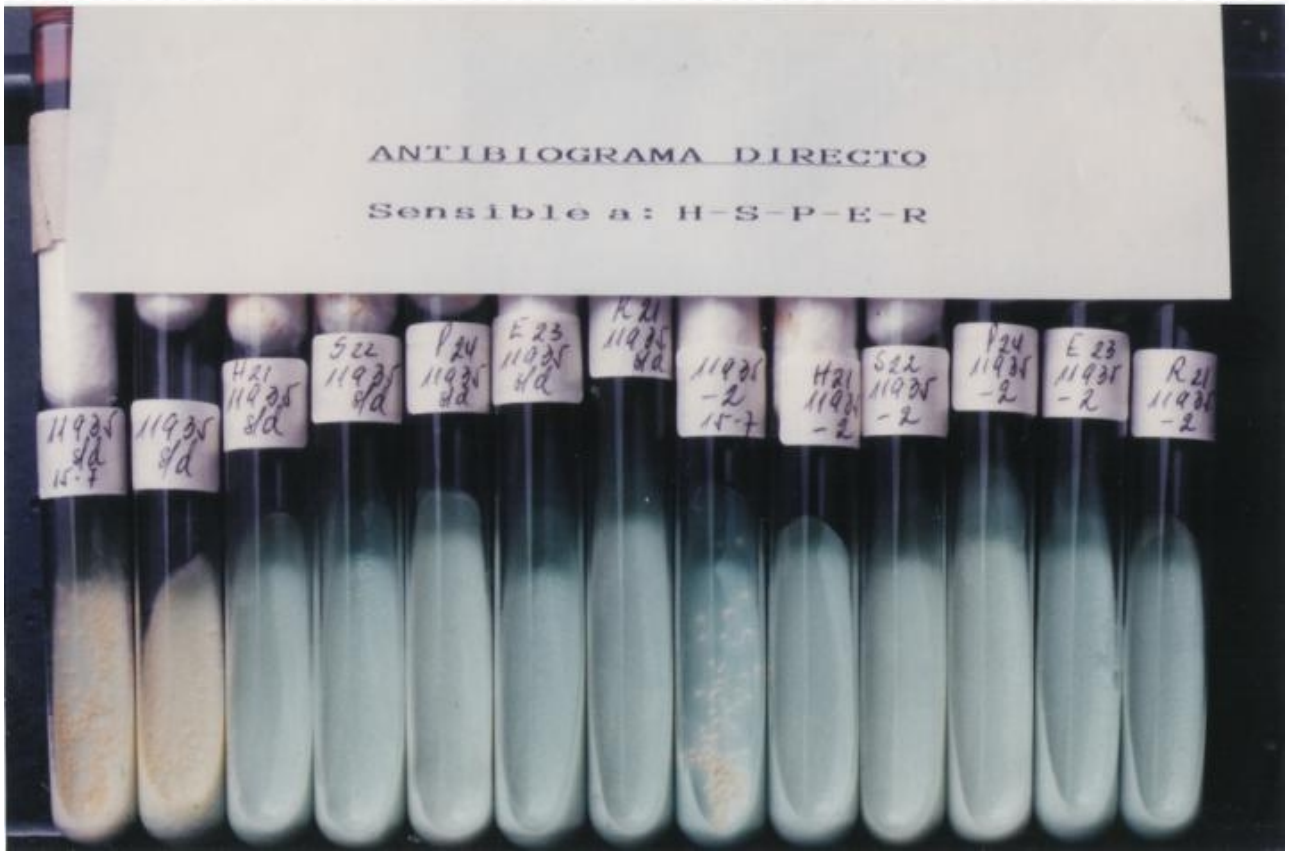
Example of a susceptibility testing by proportion method



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Examining LJ DST Cultures

- Count the total number of colonies growing

on the different tubes.

Control 1 usually has confluent growth; Control 2 should have countable colonies (about 20-100 colonies).

If two tubes have been inoculated as controls, then an average of the colony count for the two is used for calculations.

- A percentage is calculated using the following formula.

$$\frac{\text{Number of colonies on the medium containing the anti-TB agent}}{\text{Number of colonies on Control 1 (10–2 dilution)}} \times 100\%$$

Examining LJ DST cultures

- **Example 1**

Growth control (10–2 dilution) has 80 colonies;

The tube with the anti-TB agent has 6 colonies;

$$6/80 \times 100 = 7.5\%,$$

Therefore, the strain is resistant.

Note:

- The number of colonies grown on Control 1 must be approximately 100 times the number of colonies on Control 2; this indicates that the 1:100 dilution has been prepared properly.

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Examining LJ DST Cultures

- Do not count colonies that are growing only on the upper part of the slant because this indicates that the anti-TB agent has been inactivated in that portion.
- Do not consider slants with mixed growth of MTB population and contaminants.

Examining Cultures

- If Control 2 has confluent growth, this indicates that the medium has been over inoculated, and the test should be repeated.
- In this situation, growth on the medium containing the anti-TB agent could be due to over inoculation, and the culture should not be interpreted as resistant.
- However, if there is no growth on the medium containing the anti-TB agent, the test can be reported as susceptible.

IUATLD/WHO LJ DST grading scale

Reading (colonies)	Report
Contaminated	Contaminated
1 – 50	Actual number
51 -100	1+
More than 1+, but still numerable (101 – 299)	2+
300-500 (innumerable colonies)	3+
>500 (confluent growth)	4+

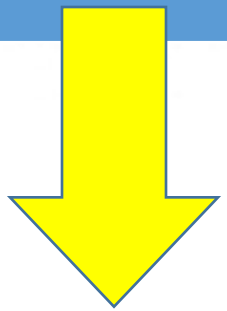
Interpretation

Growth of 20 colonies on GC and no growth on drug containing slant

Sensitive to drug

Interpretation

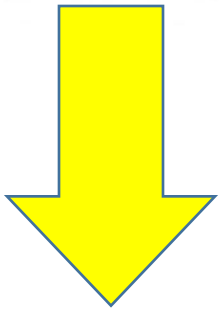
Growth of 1+ on GC and growth of 2+ on Rifampicin slant



Resistant to Rifampicin

Interpretation

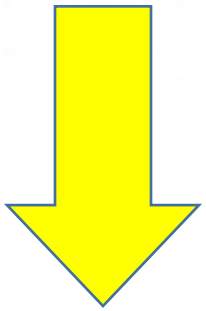
No Growth on GC and growth of 1+ on kanamycin slant



Invalid result- Repeat

Interpretation

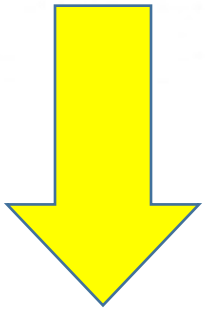
GC slant contaminated and growth of 2+ on Levofloxacin, Moxifloxacin and Amikacin



Invalid result- Repeat

Interpretation

Growth of 2+ on GC, growth on PNB and growth of 1+ on Rifampicin slant



Invalid result- Repeat

Reporting of LJ DST results

- Results of the tests should be reported only as "susceptible" or "resistant" to facilitate use by the clinician.
- Results should be reported as soon as they are available. Results can be reported after 4 weeks if there is growth on the medium containing the anti-TB agent and all other parameters such as quality control are satisfactory.

Assesment

1. Describe the principle behind LJ DST result interpretation.
2. When do you tell that an isolate is susceptible to a specific drug.
3. When do you tell that an isolate is resistant to a specific drug.
4. At what point are Ethambutol and PNB results interpreted.
5. List two possible scenarios of invalid DST results

Summary

- LJ DST interpretation is based on the principle of comparing *Mtb* growth on the growth control with growth on the drug containing slant.
- Any growth on the drug containing media that is equal or more than the growth on the growth control is considered as the 1% resistant population that is clinically significant.

References

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

Acknowledgments

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