THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on LJ CULTURE METHOD (LJ CULTURE READING AND INTERPRETATION)

Module 7: LJ Culture Reading And Interpretation

DATE:

VENUE: SRL, Uganda

FACILITATOR:

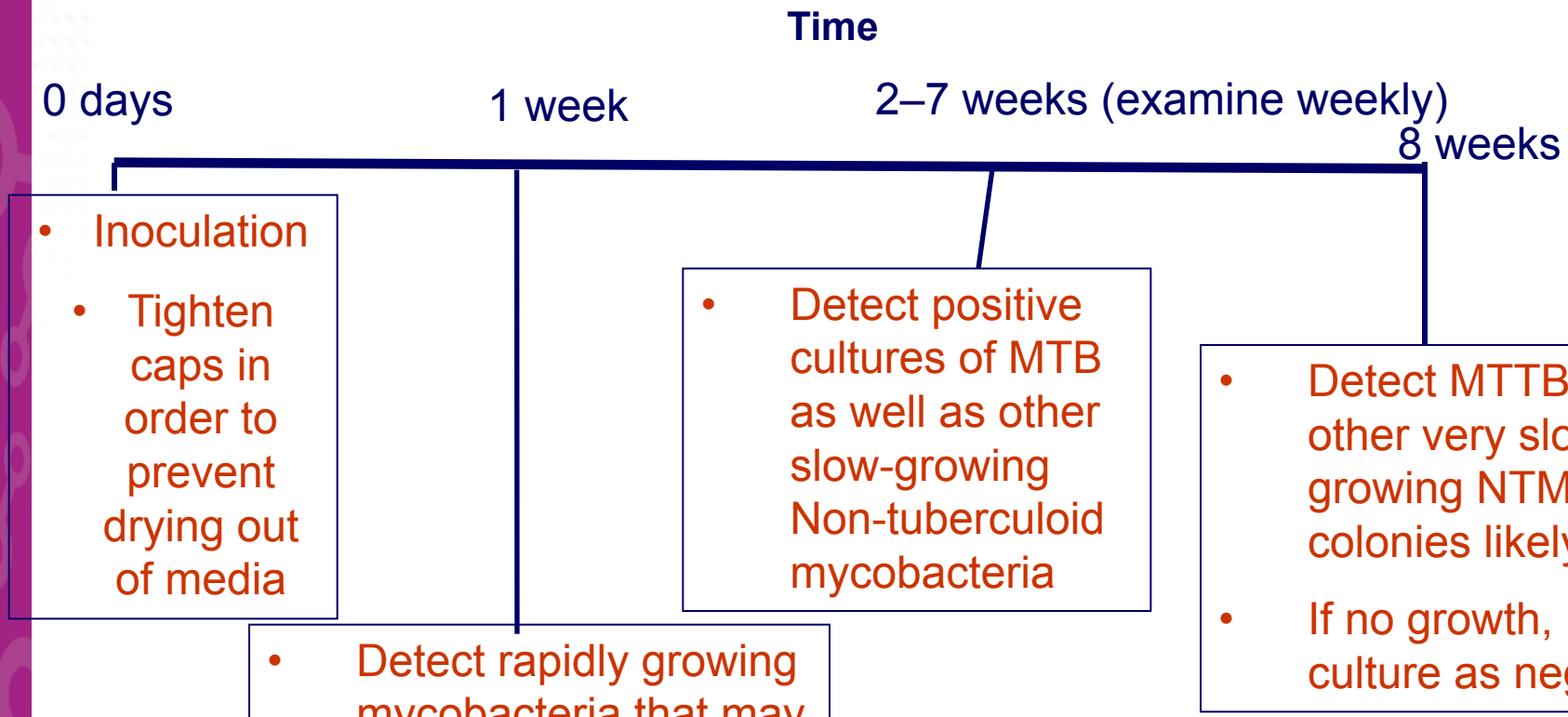
Outline

- Reasons for growth detection
- LJ examination schedule
- Colony morphology; TB or NOT TB
- Culture reading work procedure
- TB identification and confirmation
- Recording and reporting
- LJ slant contamination

Rationale for LJ Culture

- LJ culture is performed to recover mycobacterium as much as possible and its recovery is greater than 95%
- TB culture is more sensitive than acid-fast smear microscopy
- Certain Identification methods for M.tb require Viable mycobacterial cells
- Drug susceptibility tests require viable organisms
- Genotyping of mycobacteria requires large quantities of cells

LJ examination schedule



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Colony morphology; MTB

Visible growth of *M. tuberculosis* on LJ slants usually takes not less than two weeks

Typical colonies on LJ slant are similar in size and shape

- Rough
- Crumbly
- Waxy
- Non-pigmented (cream or ivory colored)

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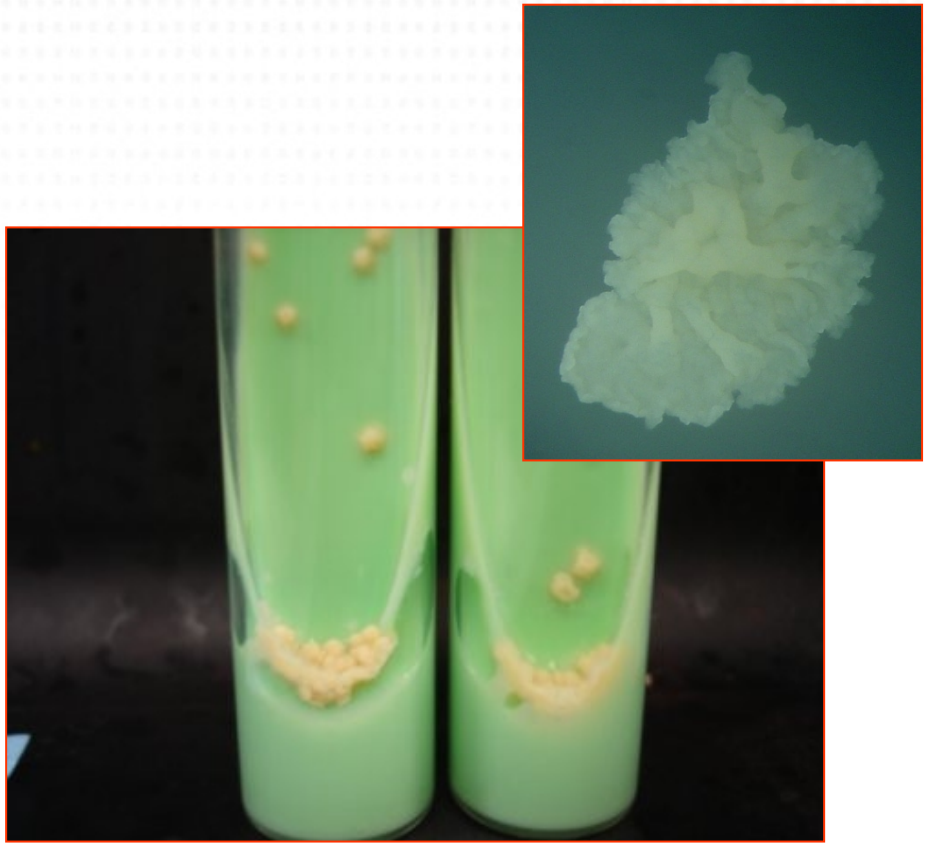
Colony morphology; Other MTBC Species other than MTB

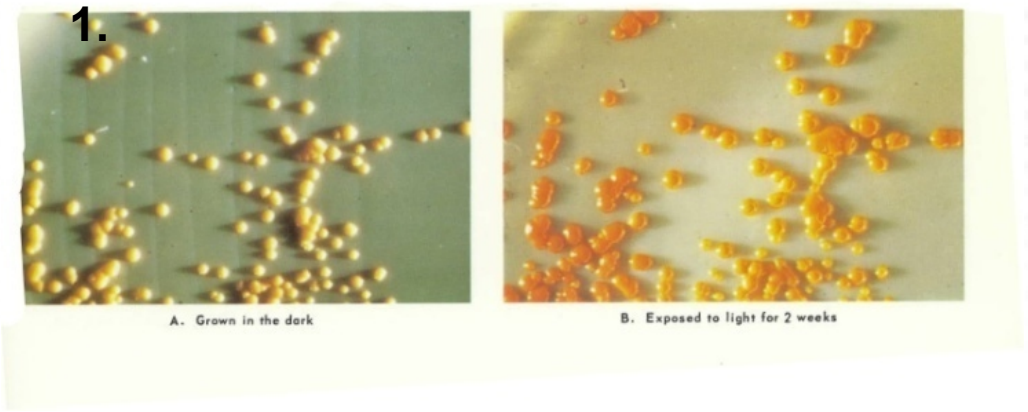
- Typical colonies of *M. bovis* on LJ slant have slightly different growth compared to M.tb
  - Small, round
  - Transparent
  - Wrinkled surface
  - Irregular thin margins
  - Smooth with a slightly granular surface
- Addition of 0.5% pyruvate (instead of glycerol) to the LJ medium facilitates the growth of *M. bovis*



Colony morphology; NON MTB SPECIES

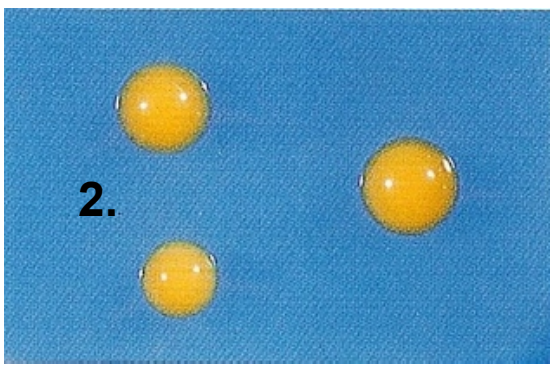
1 **Photochromogens**

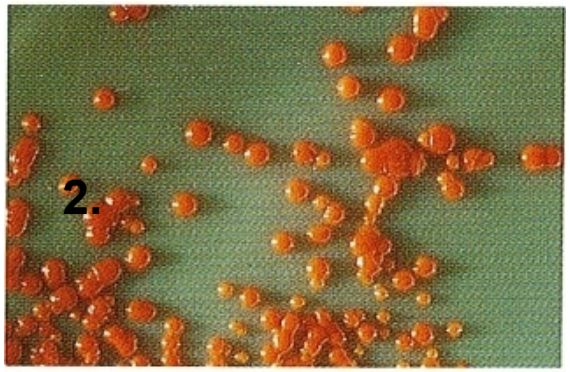
- Colonies are non-pigmented in the dark but turning lemon yellow after exposure to light



2. Scotochromogens

- Colonies are yellow to orange when grown in the light or the dark

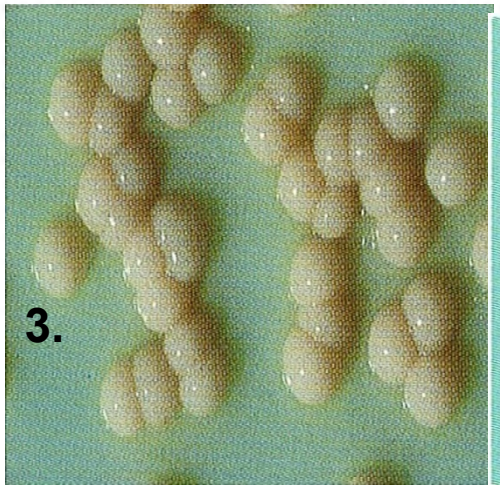


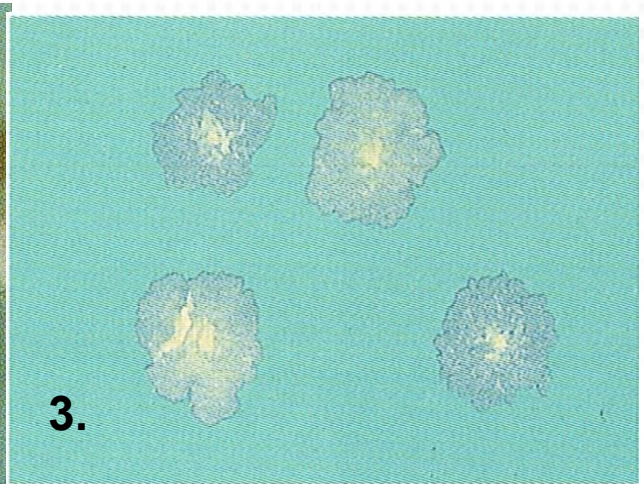


Colony morphology; NON MTB SPECIES

3.Non-photochromogens

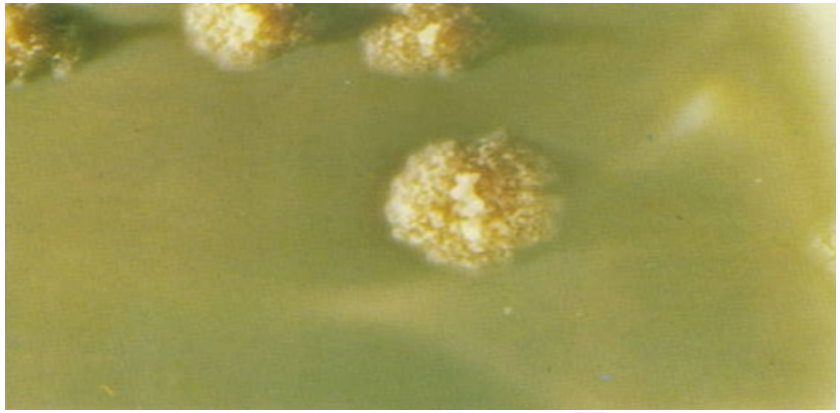
Usually non-pigmented and are unaffected by light





4. Rapid Growers

Organisms are fully mature in 2 -5 days



4.

Colony morphology; MTB or NOT MTB cont'd

Colony morphology is not sufficient to identify mycobacterial complex species

Mixed mycobacterial cultures may be isolated from patient specimens

Mixed mycobacterial cultures can also be the result of environmental contamination of specimens

Work procedure

1. On weekly basis, remove the incubating culture slants on trays to be read starting from the ones making eight weeks to those one week old.

2. Place the trays on the culture reading bench with a reading lamp.

3. Pick a pair of slopes sharing the same accession number at a time (in ascending order)

4. With the aid of an illuminated bulb/lamp, examine for evidence of MTB Complex like colonies.

5. Using the WHO LJ culture grading scale, record growth accordingly.

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Work procedure cont,d

6. Place all positive cultures on a tray labeled with the terms “POSITIVE” and “the week of culture reading” awaiting storage and DST

7. Order for re-decontamination of all samples with both slants contaminated and LJ slants with AFB plus contaminants

NOTE:

All LJ slants that are contaminated at any time of reading (without AFB) or with no growth at 8 weeks are discarded

Recording and reporting(according to WHO/IUATLD and GLI

Reading (colonies)	*Report*
Contaminated	*Contaminated*
No growth	*Negative*
1 – 9 colonies	***Actual number***
10 -100 colonies	***1+***
>100 – 200 colonies	***2+***
>200 colonies (innumerable colonies)	***3+***

LJ SLANT CONTAMINATION

- LJ medium contains bacteriostatic agent
  - Malachite green inhibits most environmental and normal flora
  - Important to remove liquid in bottom of LJ before inoculation
    - Any bacterial growth in the liquid has limited exposure to malachite green and may grow on slant if tube is tipped

- Contaminated LJ medium
  - If contamination is slight, attempt to isolate TB colonies, transfer to new LJ
  - If totally contaminated, request re-decontamination

TB identification and confirmation

Tests used for the identification of MTB include;

- Immunochromatographic assay test
- Molecular assays
- Blood agar
- ZN microscopy
- Biochemical tests

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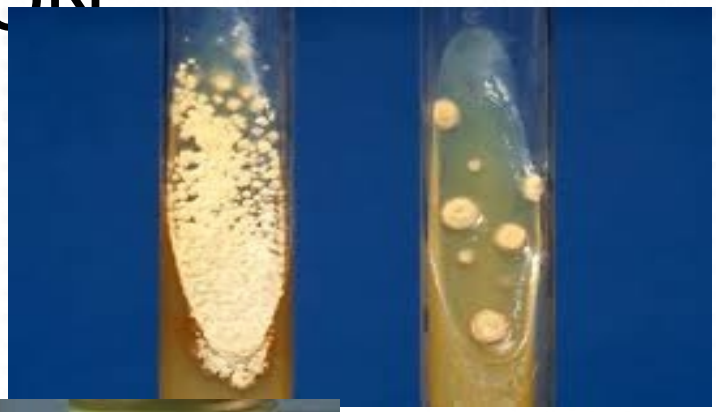
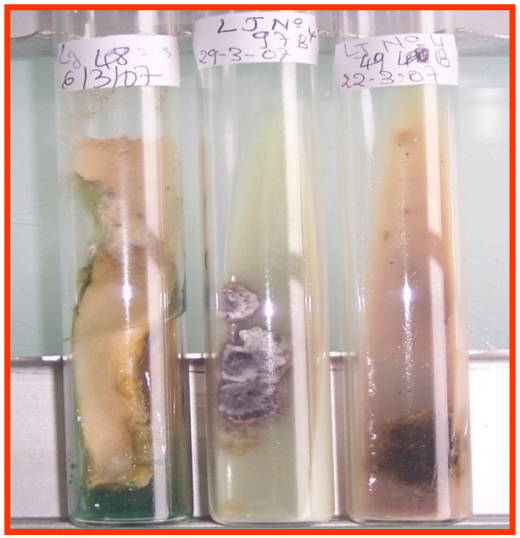
LJ SLANT CONTAMINATION

- Some contaminating organisms cause a significant change in the color of the medium
  - pH of the medium may be altered
  - Contaminant may produce pigment
- some organisms liquefy the medium hence slant collapsing

M.tb cannot be detected under these conditions





Summary

- Typical characteristics of colonies of *M. tuberculosis* on LJ slant are: Rough, Crumbly, Waxy and Non-pigmented
- solid medium allows good growth of most mycobacteria, growth can be quantified, and colony morphology and pigmentation can be examined
- Examine cultures for growth weekly
- Confirm MTB complex by performing Antigen test and NTM by ZN staining (cellular morphology) or molecular method
- Record and send a preliminary report on culture results as soon as growth of typical colonies of AFB is detected

ASSESSMENT

1. What are the key equipment and materials needed during culture reading?
2. What is the characteristic appearance of MTB?
3. what are some of the causes of LJ slant contamination?

REFERENCES

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