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MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

LOWENSTEIN JENSEN CULTURE METHOD

(LJ culture and isolate storage)

Module Four: Introduction to LJ Culture

DATE:

VENUE: SRL, Uganda

FACILITATOR:

OUTLINE

- Background
- Principle of LJ method
- Reagents and materials used
- Advantages of the method
- LJ culture sample processing techniques
- Limitations

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BACKGROUND

- Lowenstein Jensen media is **a glycerated egg-based medium** selective in nature used for the cultivation and isolation of *Mycobacterium* species.
- Inoculation of both solid and liquid media are considered the "Gold Standard" for TB culture

- LJ Supports growth of most mycobacteria
  - Growth can be quantified
  - Colony morphology and pigmentation can be seen

Principle of the method

- LJ culture method is based on the principal of **cultivating slow growing mycobacteria organism** on a medium containing a variety of inorganic salts which provide essential substances for the growth of *mycobacteria*. These include:

- Glycerol added that gives an abundant source of carbon and energy to the slow growing *Mycobacteria*
- Asparagine added to promote the initiation of growth and increase the growth rate.
- Egg yolk as a source of lipids for the metabolism of *mycobacteria*.
- malachite green dye acting as a Partial inhibitor of bacteria other than MTB.

Advantages of LJ culture method

- Allows morphology to be visualized, mixed cultures of mycobacteria can be detected
- detection ability of up to 10-100 viable bacilli/ml of specimen
- Colony count can be performed
- Less contamination rate
- less expensive

Limitations of the LJ culture method

- Long TAT (up to eight weeks)
- Requires further tests for confirmation (Microscopy and/or biochemical and/or serological).
- Negative culture results do not rule-out active infection by mycobacteria.

Reagents and materials used

LJ media preparation

- Mineral salts ;

  Magnesium Sulfate, Monopotassium Phosphate, Sodium Citrate,
- Malachite Green
- L-Asparagine
- Whole Egg,
- glycerol

Equipment and materials

- Incubator
- Incubation trays
- Culture bottles
- Insipisator
- Media dispenser
- Blender/plunger rods
- Bunsen burner
- Laminar flow

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LJ culture sample processing techniques

Purpose

- Decontamination of bacteria other than mycobacteria.
- liquefaction of mucous and organic debris in the specimen.

Decontamination methods

- NALC-NaOH method
- Petroff's method

Processing procedure: Quality control

Prepare a worksheet for each batch of specimens processed

List the numbers of all specimens processed in the batch

Record the date and name of the technician performing the work

List lot numbers and expiration dates for reagents used

Record date of preparation of NALC/NaOH to ensure that it has been prepared on day of use

Record identity of equipment used during processing procedure

Before beginning processing, ensure that regular maintenance on the BSC and the safety centrifuge has been performed and documented

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NALC/NaOH method: Drawbacks

- NALC is expensive and loses activity rapidly
  - NALC must be freshly prepared
  - Extreme agitation of NALC in the presence of NaOH will result in lost activity

- Excessive exposure of the sample to NaOH in the NALC/NaOH decontamination mixture can cause over-killing of mycobacteria.

If stronger decontamination is needed, the starting concentration of NaOH may be increased to 6% (rather than increasing the time of exposure)

- To reduce excessive contamination
- To handle heavily contaminated specimens

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Storage overview of Isolates

- storage is performed to retain the grown M.tb isolates for future purposes.
- Many different solid media are available for cultivating and storing mycobacteria. These include :LJ media, 7H9 and Glycerol

- There is no general consensus on which medium is best for routine isolation.

- Attention must be given to;
  - purity of chemical components used for Media preparation
  - Storage equipment used

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ASSESSMENT

1. State the principal of the LJ culture method?
2. List the advantages of LJ culture media?
3. Mention the purpose of Sample processing?

Summary

- TB culture is more sensitive than AFB microscopy
- TB culture using solid medium allows good growth of most mycobacteria, growth can be quantified, and colony morphology and pigmentation can be examined
- Solid-based growth detection is slower hence more sensitive
- Examine cultures (solid) for growth weekly

REFERENCES

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