THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

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

Training on LJ culture method

Module 11a: Principles and Protocols of MTB ISOLATE STORAGE

DATE:

VENUE: SRL, Uganda

FACILITATOR:

Content outline

- Purpose and principle of isolate storage
- Material required and Suitable storage containers
- Preservation methods
- Procedure for Harvesting Pure Isolate
- Isolate retrieval

Purpose of isolate storage

Purpose

- To preserve the viability and ensure intact genetic composition of MTB for future testing such as repeat DST testing and molecular epidemiology testing

Materials required

Storage media

- 7H9 media enriched with OADC
- LJ media
- 25% Glycerol

materials and equipment

- -20 and -80 ° C freezers
- Sterile Cryo-tubes
- Disposable loop
- Pasteur pipettes
- Rack
- Bijou bottles
- sterile Distilled water
- 1.0McFarland
- Sterile bead
- Vortex

OADC supplement role

- Oleic Acid

Important in mycobacterial metabolism

- Albumin (bovine)

Binds free fatty acids which may be toxic to mycobacteria

- Dextrose

Energy source

- Catalase

Destroys toxic peroxides

- Polyoxyethylene stearate (POES)

Enhances growth of M. tuberculosis and assists in providing a uniform inoculum

Harvesting Pure Isolate overview

- Do necessary identification work up & confirm the purity
- Ensure growth is consistent with M.TB prior to storing.
- Store isolates at room temperature in the dark (preferred) or at 2–8 ° C in the refrigerator until you do harvesting.
- Store all isolates grown on the LJ culture in numerical order based on Laboratory study ID or code.
- Use colonies showing good, confluent & pure growth of M.TB on LJ slant within 10-15 days of first appearance.
- Older cultures will not provide reliable long term viability.

Storage on LJ Media in cryovials

1. Work in a certified BSC II.
2. Aseptically, add some sterile glass beads in a labelled bijou bottle properly labelled
3. Using a sterile plastic loop, scoop a portion of the growth from the LJ slant, paying attention not to scrap media.
4. Deposit the colonies into the bijou bottles by passing the loop through the glass beads.
5. Add a few drops (4) of Sterile Distilled Water
6. Tightly screw the cap and vortex for 5-10 seconds.

Storage on LJ Media in cryovial cont'd

7. Aseptically add sterile distilled water drop wise while comparing with McFarland Standard #1.

8. Inoculate approximately 50ul (2 drops using Pasteur pipettes) of the emulsified isolate onto two labeled LJ storage cryo-vials.

9 Incubate at 37°C until growth is visible (≈ 2-3 weeks).

10. Transfer the LJ storage cryovials containing visible growth initially at -20°C and then to -80°C freezer after two days under an identified location on Storage worksheet

11. Update storage database.

Group exercise (5mins)

1. You have been contacted as a researcher in TB lab to provide biorepository expertise, Describe how to go about this

Storing from Solid Cultures into Liquid medium

1. Retrieve 7H9 cryovials from the refrigerator and Label them.

2. Inoculate approximately 50ul (2 drops using pastuer pipettes) of the emulsified isolate into one labeled cryovial.

3. Tightly close and vortex for 5-10 seconds.

Storing from Solid Cultures into Liquid medium

3. Incubate inoculated cryovial in the incubator at -37^{o}c for 2-3 weeks and observe for evidence of growth

4. Store the cryovials initially at -20^{0}C freezer for two days and then finally transfer into the -80^{0}C freezer for long term storage.

5. Register the Mtb isolates stored by their Lab numbers on the storage worksheet and storage database.

Retrieval of Isolates

Retrieval process

1. Permission should be sought from management before isolates can be retrieved
2. Obtain an Isolate retrieval form and fill it out.
3. The retrieved isolates are deleted from the database.
4. Subculture Isolates following retrieval onto LJ slant and incubate at 37^{o}C for 3-4 weeks until growth is visible.
5. Store portion of grown isolates and return back into their ORIGINAL positions

Assessment

1. List materials and the different media used in isolate storage
2. Give reasons why isolate storage is necessary
3. List safety precautions to take during isolate storage
4. What McFarland standard is used when making bacterial suspensions?

Summary

- Storage preserves the viability of MTB for future testing
- Storage media include; LJ media and Middle brook 7H9 broth enriched with OADC supplement, 25% glycerol
- Store the cryovial initially at -20^{0}C freezer for two days and then finally transfer into the -80^{0}C freezer for long term storage.
- Ensure to return all the retrieved isolates after subculture

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