THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

REAGENT PREPARATION

Module 6: Preparation of plain Löwenstein-Jensen (LJ) media

Date:

Presenter:

Venue:

Overview of learning content

- Rationale for use of LJ
- Requirements and Equipment
- Steps involved in LJ media preparation
- Quality Control
- Labelling and Storage of media
- Documentation
- Summary points

EXERCISE (5MINS)

1. What is the principle of growing MTB on LJ culture?

Rationale for use of LJ

- LJ Media is used because it:
  - Supports good growth of small numbers of mycobacteria
  - Allow for differentiation based on colony pigment and morphology
  - Inhibits the growth of contaminants
  - Economical and simple to prepare from ingredients that are readily available
  - Does not need CO_2 incubator

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Requirements for LJ preparation

Reagents/Chemicals:

- Magnesium citrate
- Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)
- Malachite green dye
- 70% Ethanol
- Malachite green dye
- Asparagine
- Glycerol
- Reference strains (rapid grower like *M. fortuitum* and slow growers like *M.tb* H37Rv sTRAIN)
- Sterile distilled water

Equipment:

- Analytical balance sensitive to 0.1 g
- Weighing masses of 10g, 20g and 100g
- Inspissator
- Racks
- Dispenser device
- Bunsen burner
- Water bath
- Homogenization equipment
- Autoclave
- Hand brush
- Biosafety hood

Requirements for LJ preparation

Materials:

- 1000 mL sterile graduated cylinder
- 2000 mL sterile graduated cylinder
- 2000 mL sterile wide mouth conical flask
- 500 mL sterile conical flask
- 100 mL sterile graduated cylinderVolumetric Flask
- Sterile Funnel
- Weighing paper
- Clean glass bottles
- Spatula
- Autoclave tap
- Sterile gauze

1. PPE

Personal Protective Equipment: What must be worn when you work in the laboratory.

Eye Protection

Lab Coat

Long Pants

Closed Toed Shoes – no exposed skin around feet

Lab gloves – when required

Steps to preparing LJ medium

1. Prepare mineral salt solution and malachite green solution
2. Autoclave solutions
3. Cleaning of eggs
2. Homogenize whole eggs
4. Combine solutions and homogenized eggs
5. Dispense and cap tightly
6. Slant and inspissate
7. QC

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Preparation of mineral salts

Distilled water	600
L-Asparagine	3.6g
KH_2PO_4	2.4g
Magnesium citrate	0.6g
$MgSO_4.7H_20$	0.24g
Glycerol	12ml

- Weigh and measure reagents as indicated in table
- Dissolve in distilled water
- Label container
- Autoclave at 121°C for 20 minutes
- Incubate overnight before use
- Incase not needed for use store at 2-8°C

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Preparation of Malachite green solution

- Weigh Malachite green dye 2.0 g
- Measure Sterile distilled water 100.0 mL
- Dissolve the dye in sterile distilled water by gently swirling the cornical flask.
- Transfer the dye to the universal bottles and appropratelly label them and autoclave at 121°C for 20 minutes to sterilze.
- Store at room temperature for 6 months

Cleaning of Eggs

- Inspect fresh hen's eggs for cracks (<2 days old)
- Soak eggs in soap solution for 30 minutes
- Scrub thoroughly with brush
- Rinse with clean water
- Soak eggs in 70% ethanol for 15 minutes
- Place eggs on drying pan and pass a flame
- Crack eggs in a sterile graduated cylinder upto a desired volume
- Homogenize the eggs

70% ethanol for 15 minutes

Rinsing with clean water

- Cracked and homogenized eggs filtered through a sterile tissue/gauze

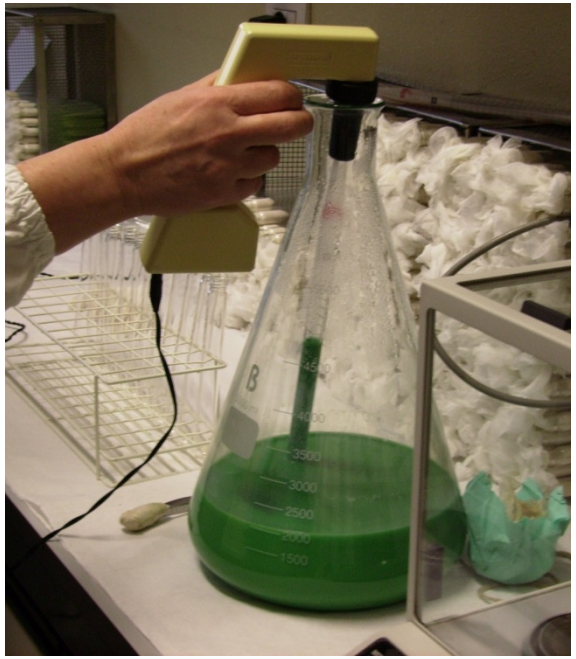
- To 1000ml homogenized eggs add 600ml mineral salts and 25ml malachite green
- Mix thoroughly

Measuring Malachite green

Adding Malachite green to homogenized eggs

Aliquoting of LJ media

- Connect the media dispenser machine in the laminar flow hood into the flask containing media
- Adjust the media dispenser to dispense 5ml for universal containers
- Dispense media into the universal bottles and cap immediately



Inspissation

- The inspissator should be switched on prior such that it attains a temperature of 85 °C
- Place the tubes containing media in the inspissator in a slanted position
- Cover the bottles with an inspissator blacket
- Coagulate the media for 45 minutes at 85 °C
- Check quality by beating one slant against a table. If the media disaggregates, the inspissation should be extended by more 5 minutes
- L own at room temp t

LJ slants in the inspissator

Inspissator

Quality control: Visual inspection

- **Inspect all tubes**
  - Discard tubes that are discoloured, have poor texture or bubbles following inspissation

Color:

- Different shades of green colour: poor homogenization or presence of material residues in the tubes
- Dark green: low pH (acidic)
- Light green/yellow: high pH (alkaline)

Texture:

- If the medium disintegrates easily, the inspissation temperature might have been too low; not suitable for culture inoculation

Homogeneity:

- If bubbles: excessive temperature
- If clumps: poor homogenization

Quality control

- Quality check:
  - *M. fortuitum - growth in 48hrs - fast growers (co*
  - *H37Rv - slow growers e.g. MTB (more than 3 wks)*

MTB growth on LJ MEDIA

- Sterility check:
  - Place a random sample (10%) of the batch at 37 °C for 48hrs
  - Evaluate each slant for growth
  - Calculate contamination percentage
    - % contamination = $\frac{\text{Number of slants contaminated}}{\text{Total number of slants prepared}} \times 100$
- If batch % contamination exceeds 0-5% notify Technical supervisor and prepare anew one

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Labelling and Storage

- Label the media tray with batch number, date of preparation, date of expiry, person who prepared and identity of media
- Store plain LJ media up to 6 months at 2-8 ^{o}C.
- If there is shortage of fridge space, store plain media at 37°C in an incubator for 3 months.

Key factors for successful LJ medium preparation and trouble-shooting

- Eggs used to prepare LJ medium must be fresh (<2 days old) and from hens that are not treated with antibiotics
- Time and temperature of inspissation are crucial and must be controlled:
  - Quality of egg medium deteriorates when coagulation is done at too high a temperature or for too long
  - Discoloration of coagulated medium may be due to excessive temperature
- Little holes or bubbles on the surface of the medium indicate faulty coagulation
  - Cap media tightly before inspissating to help prevent bubbles

Assessment

1. What are the major steps involved in the preparation of media?
2. Why should eggs be from hens that are not under antibiotic treatment?
3. Why shouldn't the eggs cracks be used?
4. What are the quality checks performed on media before use?

Summary points

- Use only clean sterile glassware
- Ensure eggs are fresh and antibiotic-free
- Use good aseptic technique when preparing LJ medium
- Pay close attention to time and temperature during inspissating
- Inspect all prepared media carefully; always perform and document QC

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp
- Laboratory Diagnosis of Tuberculosis by Sputum Microscopy | The Handbook | Global Edition
- TB AFB Smear Microscopy Trainer Notes

https://www.aphl.org/programs/infectious_disease/tuberculosis/TBCore/TB_AFB_Smear_Microscopy_TrainerNotes.pdf

Acknowledgments