THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

REAGENT PREPARATION

Module 11b: Preparation of Middlebrook

DATE:

Presenter:

Venue

Overview of learning content

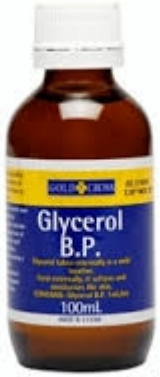
- Materials and Equipment required for preparation
- Procedure
- Quality Control (QC)
- Storage and Labelling

Requirements

Reagents/Chemicals:

- Middlebrook powder
- Glycerol
- OADC enrichment media
- Distilled water

Middlebrook powder



Glycerol

Equipment:

- Weighing Balance
- Autoclave

Weighing balance

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Requirements

Materials:

- Cryovials
- Duran bottles
- Measuring cylinder

Miscellaneous:

- Gloves
- Laboratory coats
- Closed shoes

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Preparation of Middlebrook (7H9) media

- **Preparation of 1 liter**
  - Suspend 4.7g of the Middlebrook powder in 900ml of distilled water.
  - Add 2ml of glycerol.
  - Autoclave the solution at 121^{o}C for 15 minutes
  - Allow to cool to 45^{o}C, then add 100ml of OADC enrichment media.
  - Aliquot into 250 ml sterile duran bottles
  - Incubate for 72 hours at 37 ^{o}C for sterility check
  - Label and store at 2-8^{o}C

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Preparation of Middlebrook (7H9) media

- **Aliquoting media in cryovials**
  - Retrieve one duran 250ml bottle containing sterile middlebrook from the refrigerator.
  - Aliquout 1 ml into cryovials

Quality control

- Incubate prepared media at 37ºC for at 37ºC 72 hours. Discard the whole solution if it becomes cloudy.
- Inoculate on BA to rule out contamination

Labelling and storage

- Label
  - Name of reagent
  - Date of preparation
  - Expiry date
  - Preparer
- Store at 2-8^{o}C for 3 months.

Assessment

1. List the major materials needed for the preparation of middlebrook media?
2. How do you perform QC on Middlebrook media?
3. What are the storage conditions for Middlebrook media?

Module summary

- Materials and Equipment required for preparation
- Procedure
- Quality Control (QC)
- Storage and Labelling

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