THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

MGIT CULTURE

Module 10: Preparation blood agar

Outline

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

EXERCISE (5MINS)

1. Discuss the importance and use of Blood agar in a mycobacterium laboratory.

Requirements

Reagents/Chemicals:

- Tryptic soy blood agar base
- Distilled water
- 25ml of sterile defibrinated blood

Equipment:

- Weigh Balance
- Autoclave
- Safety cabinet
- Bunsen burner

Blood agar base

Defibrinated blood

Requirements

Materials:

- Petri dishes
- Reagent bottle
- Measuring cylinder

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

2

Preparation of Blood agar media

- To 500ml of distilled water add 20g of Tryptic Soy Blood Agar Base (TSBA)
- Mix thoroughly. (Dissolving occurs during autoclaving).
- Autoclave at 121°C for 15 minutes
- Allow to cool after autoclaving to 45-50°C
- Aseptically add 25ml of sterile whole blood. Mix thoroughly.
- Gently pour the warm blood agar onto the dishes
- Gently pass the flame over the poured agar in the dishes to remove the air bubbles
- Cover the dishes and allow Blood agar to coagulate

Quality control

- **Perform Sterility Check**
  - Randomly select 2 blood agar plates and incubate them at 37°C for 24 hours.
  - If there is no visible growth or haemolysis of the media then the blood agar is sterile and ready for use.

Contaminated BA

Quality control

- **Test to support growth of bacterial contaminants**
  - After sterility check, inoculate two BA plates with a strain of *M. fortiutum*
  - Incubate the plates at 37°C for 24 hours.
  - Observe for a luxurious growth of *M. fortiutum* on both plates.
  - If only one plate shows growth, repeat QC with two other plates.
  - If there is no growth or only one plate shows growth, then QC fails.

  Documentation is key

Labelling and storage

- Label on the bottom top of the dishes (the batch number, date prepared and expiration date, techinitials)
- Store in a refrigerator up to one month

Assessment

1. What are the major materials needed for the preparation of Blood agar?
2. Why is quality control of blood agar important?
3. Why should we pass a flame over the poured blood agar?
4. Where should the labelling of the media be palced? And why?

Summary

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

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