UGANDA Supranational® Reference Laboratory

Timely Accurate Diagnostics for TB Free Africa

Training on Proficiency Testing Scheme (Microscopy PT)

Module 3: Reagents, materials, and equipment

Date

Uganda Supranational Reference Laboratory

Contents outline

- Reagents
- Positive specimen
- Negative specimen
- Materials and equipment
- Preparation of artificial sputum
- Preparation of 0.4% Sodium hydroxide

Reagents, materials and equipment

- **Reagents**

- 40% Formaldehyde
- 4% NaOH
- Distilled water
- Artificial sputum

Reagents, materials and equipment

- **Positive specimen**
  - (Fresh specimens, no more than 2 days old, are preferred)
  - Amount: 3 ml or more; AFB load: >2+ AFB by Ziehl-Neelsen direct smear;
  - .Thickness: Watery (less mucous) specimens are preferred to increase consistency.
  - Color: White to light green; blood stained specimens should be avoided

- **Negative specimen**
  - artificial sputum (in house prepared)
  - fresh specimens, no more than 2 days old, are preferred)
    - Amount: 5 ml or more; Color: white to green;
    - Thickness: Watery (less mucous) specimens are preferred to increase consistency
    - **Note**: An AFB negative specimen with 20 or more white blood cells per field is preferred.

Reagents, materials and equipment

•Materials and equipment

- Microscope Slides
- Slide mailers
- Slide boxes
- Slide transport box
- Biosafety cabinet
- Microscopes (Fluorescent and Bright field)
- Vortex
- Water bath at 55-60°C
- Distilled water
- Centrifuge
- Sticker labels

Reagents, materials and equipment

- **Preparation of 0.5L of Artificial sputum**
- Weigh 5.0g of methylcellulose and 500ml sterile distilled water
- Mix to dissolve by using a Bunsen flame to facilitate the dissolving process.
- Emulsify the 50mls of egg albumen **(do not include the egg yolk).**
- Add emulsified egg to the mixture when methylcellulose is dissolved
- Autoclave for 20 minutes at 121 C
- Leave to cool and inoculate on Blood Agar for 48 Hours.

Reagents, materials and equipment

- **Preparation of 1L of 4% NaOH**
- Weigh 40 g NaOH pellets
  - NB. NaOH is caustic: avoid skin contact.
- add 1000 mL of Distilled Water.
  - NB. mixture will become warmer.
- Mix until complete dissolution of the pellets.
- Autoclave at 121ºC for 15 minutes

Exercise

- List at least two QC checks done during (**two minutes).**
  - preparation of reagents and
  - materials used in Microscopy PT preparation

Assessment

- List three safety equipment and materials required for Microscopy PT preparation.
- What are qualities of a good source of a positive specimen used in Microscopy PT preparation?
- What is the use of artificial sputum in Microscopy PT preparation?

References

- External Quality for AFB Smear microscopy by IUATLD
- ISO 13528:2005, *Statistical methods for use in proficiency testing by interlaboratory comparisons*
- ISO 15189, *Medical laboratories — Particular requirements for quality and competence*
- ISO Guide 34, *General requirements for the competence of reference material producers*
- ISO Guide 35, *Reference materials — General and statistical principles for certification*
- *ISO/IEC 17043 First edition 2010-02-01*
- Guide 34, ISO Guide 35 and ISO 13528 (homogeneity and stability)
- ISO/IEC Guide 98-3, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement* (GUM:1995)
- ISO/IEC 17011:2004, *Conformity assessment — General requirements for accreditation bodies accrediting conformity assessment bodies*
- ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

Acknowledgments

KOFIH
Korea Foundation for International Healthcare
한국국제보건의료재단