

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

- (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 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 121°C 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

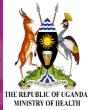
- List three safety equipment and materials required for Microscopy PT preparation.
- What are qualities of a good source of a positive specimen used in Microscopy PT preparation?
- What is the use of artificial sputum in Microscopy PT preparation?





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



















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