

REAGENT PREPARATION

Module 9: Preparation of sputum processing Reagents for TB culture

Date:

Presenter:

Venue:

Overview of learning content

- General precautions before preparation
- Materials and Equipment required for preparation
- Selecting of right glassware
- Procedures for preparation
- Quality Control (QC) of freshly prepared reagents
- Labelling and Storage of reagents

EXERCISE (5MINS)

1. Discuss the reagents used in sputum processing and the principle behind.

General precautions

- Keep the environment as clean as possible; wipe all hard surfaces with disinfectant daily
- Mop with water containing bleach or other disinfectant
- Use good aseptic technique
- Keep door closed during work
- The water used to prepare media and reagents must be deionor
- Use sterile glassware and equipment
- Use reagent grade chemicals and reagent
- Store all chemicals and powders proper



Requirements for reagent preparation

- Reagents/Chemicals:
 - 6% NaOH Pellets
 - Potassium phosphate monobasic anhydrous KH_2PO_4
 - Sodium phosphate dibasic heptahydrate $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$
 - Sodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$)
- Equipment:
 - Weighing balance sensitive to 0.1 g
 - Weighing masses of 10g, 20g and 100g



Weighing balance **Supranational[®]**
Reference Laboratory



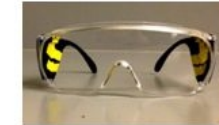
Requirements

- **Materials:**
 - Volumetric Flask
 - Funnel
 - Weigh paper
 - Clean glass bottles
 - Labels
 - Spatula

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



• PPE

Choice of glassware and bottles

- Re-usable glass tubes, flasks or bottles, or autoclavable polypropylene with screw-caps
- Resistant boro-silicate laboratory glass



Verification of balance

- Ensure the balance is level and use a soft brush to remove any debris
- Ensure balance has been verified with analytical weights and documented
- Use a weigh boat or weigh paper



Cleaning of glassware

- Clean glassware in hot soapy water with bottle brushes
- Remove all debris and residue
- Rinse repeatedly in hot water (4 times)
 - to remove all soap residue, which can affect pH and alter prepared reagent
- Rinse in deionized water (2 times)

Can be dried in a hot air oven

Reagents used in sample processing

- 4% or 6% Sodium hydroxide (NaOH)
- 2.9% Sodium citrate
- 0.067M Phosphate buffer, pH 6.8
- N-Acetyl-L-cystine (NALC)

Preparation of 6% NaOH

- Perform a balance check using masses of 10g, 20g, and 100g respectively and record
- Add 2000ml of distilled water to 120g NaOH pellets
- Mix until dissolution of pellets (mixture can get warmer)
- Transfer solution into a labelled 2000ml bottle
- Autoclave at 121°C for 20 minutes, with screw cap loose by ¼ turn
- Leave solution to cool before you transfer to the refrigerator

for storage

Preparation of 2.9% ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$)

- Perform a balance functional check using standard weights
- Add 2000ml of distilled water into 58g of sodium citrate and mix
- Transfer the mixture into a labelled 2000ml bottle
- Autoclave at 121°C for 20 minutes, with screw cap loose by $\frac{1}{4}$ turn

Leave solution to cool before you transfer to the refrigerator

for storage

Preparation of Phosphate Buffered Saline

- Perform a balance functional check using 10g, 20g, and 100g respectively
- Add 4500ml of distilled water into 39.8 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, and 20.6 g of KH_2PO_4
- Mix to complete dissolution and Check the pH using a pH meter or Litmus paper (Range 6.5-7.2)
- Transfer the mixture into a labelled 2000ml bottle
- Autoclave at 121°C for 20 minutes, with screw cap loose by $\frac{1}{4}$ turn

Leave solution to cool before you transfer to the refrigerator for

N-Acetyl-L-Cystine (NALC)

- NALC is purchased as a powder
- Weigh NALC
 - Amount determined by volume of NaOH/Na citrate solution
- Place in sterile screw-cap tube
- Label and store at room temperature

Total volume of NALC-NaOH/Na citrate (ml)	4% NaOH (ml)	2.9% Na citrate (ml)	NALC (g)
50	25	25	0.25
c100	50	50	0.50
200	100	100	1.00
500	250	250	2.50
1000	500	500	5.00

Labelling of Reagents

- Labels must include the following information:
 - Name of the medium
 - Date prepared
 - Expiration date
 - Prepared by
 - Storage conditions

Quality control of processing reagents

- Incubate a small aliquot overnight at 37°C. Discard the whole solution if it becomes cloudy
- Inoculate also on blood Agar overnight to rule out any contamination
- pH should be in between an acceptable range of 6.5 to 7.2

Storage

- **Storage for reagents**
 - PBS and 2.9% Sodium Citrate at 2-8°C up to 3 months,
 - 6% NaOH at 4°C up to 6 months
 - NALC can be stored at room temperature in a cool, dry, dark area. Ensure each individual tube is tightly capped; moisture will cause clumping and cause the NALC to stick to the sides of the tube making it difficult to add to the

NaOH

Assessment

- What PPE is required in preparation of sample processing reagents?
- What reagents are needed for sample preparation?
- Why is it important to select the right glassware?
- Why do we use distilled water?

Module Summary

- Reagents and media used for TB specimen processing and QC include:
- Ensure balance is level and verified
- Measure ingredients accurately
- Label and store appropriately
- **Document all preparation and 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

