

MGIT CULTURE

Module 5: Preparation of sputum processing reagents for TB culture

Date:

Venue:






Name:

OUTLINE




- Media preparation area
- Equipment/ materials
- Preparing reagents
- Labelling and Storage
- Quality control

Preparing the media preparation are

General

-  Keep the environment as clean as possible; wipe all hard surfaces with disinfectant daily
-  Mop the floor with water containing disinfectant
-  Use good aseptic technique
-  Keep door closed during work
-  The water used to prepare media and reagents must be distilled

General

-  Use sterile glassware and equipment
-  Use reagent analytical grade chemicals and reagents
-  Store all chemicals and powders properly

Choice of glassware and bottles

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



Polypropylene
glass ware



MG/PP/005, Version 1.0,
Effective date: 01-Jun-
2019



Verification of balance

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



Weighing
balance



Weights

Cleaning of glassware

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



Reagents to prepare

- 4% Sodium hydroxide
- 2.9% Sodium citrate
- 0.067M Phosphate buffer (PBS),
pH 6.8

Digestant(NaOH)

- Sodium hydroxide and sodium citrate may be mixed, sterilized and stored in sterile screw-cap bottles for up to 6 months
- Once the NALC is added to the NaOH/Na citrate solution, then working solution is good for 24 hours

Prepare 1 litre of NaOH/Na citrate digestant

- Bottle 1: Add 20 g NaOH to 0.5 litre distilled water (4% NaOH)
- Bottle 2: Add 14.5g Na citrate to 0.5 litre distilled water (2.9% Na citrate)
- Autoclave 15 minutes, 121 °C, 15 psi
- Combine the 2 solutions in a 1 litre bottle

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

0.067M Phosphate buffer, pH 6.8

- To prepare the buffer solution, mix 450 ml of solution A (Di Sodium Hydrogen Orthophosphate) with 450 ml of solution B (Potassium Dihydrogen Orthophosphate)
- Check the pH using the pH meter or PH strips
- Adjust the pH to 6.8 by using:
 - Solution A to raise the pH
 - Solution B to lower the pH

0.067M Phosphate buffer, pH 6.8

- May aliquot for single use
 - 40 ml each tube for neutralization step
 - 2-3 ml each tube for pellet re-suspension
- Sterilize in the autoclave at 121 °C for 15 minutes at 15 psi (115 kPa)
- Label, cool to room temperature and store at 2-8

°C

Expiration period = 1 year

Labelling

- Labels must include the following information:
 - Name of the reagent prepared (PBS)
 - Date prepared (10/DEC/2018)
 - Expiration date (10/DEC/2019)
 - Prepared by (JAMES)
 - Storage conditions (2-8° C)

Documentation

Reagent Preparation

Name of reagent: NaOH-Na Citrate working solution	Lot number: 08001	Date of preparation: 09/15/2008	Total Amount Prepared 2 liters	Expiration Date: 03/15/2009	Prepared by: Kyle
Ingredients	Supplier/catalog number	Lot number	Amount added	Technologist	Date
NaOH	Fisher # S320-500	074014	40 g	KT	9/15/08
Tri-sodium citrate- dihydrate	Fisher # S279-500	080291	29 g	KT	9/15/08
DI Water	CDC	NA	2 liters	KT	9/15/08

Instructions for preparation:

Using a digital balance weigh out the NaOH and Na citrate in the amounts indicated above.

Measure and add the water specified above to the powders in an appropriately sized beaker.

Place beaker on a magnetic stirrer and use a stir bar to mix well.

Aliquot desired amounts into clean glass or polypropylene bottles with caps tight

Label all bottles with name of reagent, lot number, date of preparation, expiration date and technologist.

Autoclave at 121°C for 15 minutes at 15 PSI.

Cool to room temperature, store at 2-8°C

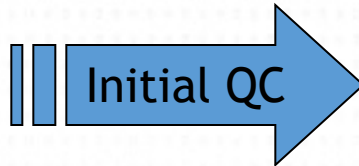
Storage

- Attempt to prepare only a 2 month supply at a time to ensure reagents are always fresh
- If NaOH and PBS are stored at room temperature, store them in a cool, dark area or store these reagents at 2-8 °C
- 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

Quality control of processing reagents

- Ensure sterility of sodium hydroxide/sodium citrate and NALC by sampling on completion of each processing run
- Sub-culture a sample of PBS used on Brain Heart Infusion or blood agar on completion of each processing run
- Aliquot 2ml of the prepared reagents and incubate at 36 to 39 °C for 24hrs to check for turbidity

Quality control for initial sterility and cross-contamination check during processing

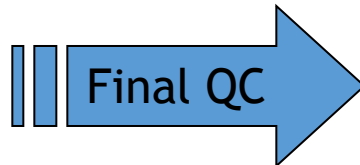


Sub-culture PBS to LJ slant and BHI agar or blood agar



LJ & BA sub-cultures

Add 1-2 ml to each sediment after decanting



Sub-culture PBS to LJ slant and BHI agar or blood agar



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Reference Laboratory

Summary

- Reagents and media used for TB specimen processing and QC include:
 - 4% Sodium hydroxide (NaOH)
 - 2.9% Sodium citrate
 - 0.067M Phosphate buffer, pH 6.8
 - Blood agar /Brain heart infusion agar
- Ensure balance is level and verified
- Measure ingredients accurately
- Label and store appropriately
- Document all preparation and QC!

Assessment

- List all the materials and equipment required for preparation of sputum processing reagents for TB culture.
- List steps involved in preparation of the digesting reagents used to process sputum samples.

References

Acknowledgments

