TITLE: Stock Reagent Preparation	Revision Date: 07-April-2017	Issue Date: 07-April-2015
Document Number: MIC81800	Status: Approved	
Distribution: Mycobacteria Manual	Page: 1 of 5	
Approved by: Gloria Badari, Director, Corporate Services and Chief Financial Officer	Signed by: (Original Signed Copy in Microbiology)	

PURPOSE:

To standardize the preparation of chemical reagents for AFB processing and smear preparation.

- 3% NaOH, (digestant)
- Phenol-Albumin
- Phenol-Alcohol solution

SPECIAL SAFETY PRECAUTIONS:

- Handle all patient samples and testing reagent using “Routine Practices”
- Please refer to the Northwest Territories Infection Prevention and Control Manual, March 2012
- Prior to testing all patient are to be identified as per I-0500 Use of Two Patient Identifiers.

Note: Perform all solution prep in the fume hood in Core Lab (by the urinalysis bench).

Phenol:

- Stock solution Phenol 90%.
- Extremely caustic and is rapidly absorbed through skin.
- Avoid all contact with skin. Review MSDS before handling.

NaOH:

- Caustic. Review MSDS before handling.

PREPARATION OF 3% NaOH (sodium hydroxide) DIGESTANT

Reagent purpose: Used in the processing step for sample digestion and decontamination.

Supplies and Instructions for preparing the Working 3% NaOH solution:

Step	Action
Materials:	
• 500 mL Graduated cylinder • 1000 mL Graduated cylinder • 4000 mL Erlenmeyer flask • Parafilm	• 5N NaOH stock reagent • WHMIS Label • Sharpie pen, black. • Funnel
• Distilled Water	
Preparation of Working 3% NaOH solution:	
1	Perform all work in the chemical fume hood in Core Lab. Always pour base into water.
2	Measure 1700 mL distilled water using 1000 mL graduated cylinder. Pour into 4 L Erlenmeyer flask.
3	Measure 300 mL of 5N NaOH in a 500mL graduated cylinder.
4	Using a funnel, pour the NaOH into Erlenmeyer flask.
5	Cover with a piece of Parafilm. Gently swirl to mix.
6	Label with WHMIS label. Date and initial label with a Sharpie pen
7	Carry into TB lab. Store on bench top at Room Temp.
8	Perform QC on new batch. Write on label when QC was performed.
9	When QC is satisfactory transfer to amber working solution dispenser jar. Label with WHMIS label, date made and QC'd, and initials.

Quality Control of NaOH working solution:

Refer to **MIC81900 QC of Mycobacteria Reagents & Culture** procedure.

PREPARATION OF PHENOL-ALCOHOL (5% Phenol in 70% Ethanol).

Reagent Purpose: Direct & culture smear fixing, to render AFB non-viable before staining.

Supplies and Instructions for preparing the Fixing Phenol-alcohol solution:

Step	Action
Materials:	
	• Pre-measured beaker - 56mL marked off (premeasure the volume with water) • 1 L graduated cylinder (1000mL) • 1 250 mL graduated cylinder • 99% Isopropyl Alcohol • 90% phenol • Distilled water
Preparation of:	
1	Perform all work in the chemical fume hood in Core Lab. Put on PPE.
2	Place Phenol in a secondary container on a cart, and transport to fume hood.
3	Work over an absorbent pad to catch any drips
4	Prepare 70% alcohol: Pour 737 mL of Isopropyl Alcohol into a graduated cylinder and transfer to a brown glass working solution bottle.
5	Measure 207 mL of distilled water and add to the working solution bottle.
6	Carefully pour 56 mL of phenol into the premeasured beaker.
7	Add phenol to the diluted alcohol in the glass bottle, using a funnel to avoid spills.
8	Wipe bottle rim. Screw the cap on firmly and gently invert to mix. Be sure the bottle is properly labeled and add the date the solution was made.
9	Return the phenol to the storage cupboard as in step 2.

Quality Control of Phenol-Alcohol:

None.

PREPARATION OF ALBUMIN-PHENOL

Reagent purpose: Provides protein to help adhere MGIT or LJ culture growth to slides. Phenol helps to render AFB non-viable.

Supplies and Instructions for preparing the Albumin-Phenol solution:

Step	Action
Materials:	
	• Rabbit or bovine serum albumin • Phenol • Sterile distilled water • Sterile syringes (5 mL, 30 mL) • TB pipetter • Dropper bottle • Sharpie pen, black.
Preparation of:	
1	Perform all work in the chemical fume hood in Core Lab. Wear PPE.
2	Dispense 4.0 mL Bovine Serum Albumin into a clean dropper bottle using a sterile 5mL syringe.
3	Use the TB pipettor, adjust to 100 µL and dispense 0.1 mL Phenol into the dropper bottle.
4	Add 20 mL of sterile distilled water into the dropper bottle using a 30 mL sterile syringe.
5	Mix. Label the dropper bottle with “Albumin-Phenol”, your initials and date.
6	Carry into TB lab. Store in the TB BSc.

Quality Control of Phenol-Albumin:

None.

REFERENCES:

- Ontario Mycobacteriology Bench Manual, Central Public Health Laboratory, 2003.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	6-Feb-15	Initial Release	L. Driedger