SPUTUM DIGESTANT REAGENTS

PRODUCT:

Tube and Bottle Reagents:

Sodium Citrate	R6685
Sodium Hydroxide	R6690
N-Acetyl-L-Cysteine Powder	R6590
Phosphate Buffer, 0.067 M, pH 6.8	R6660
Bovine Serum Albumin, 0.2%	T6215

PURPOSE:

Sputum digestant reagents are used to break down the mucous components of sputum and other clinical specimens and to decontaminate the specimen that may contain normal flora in order to allow the slower-growing mycobacteria to grow.

PRINCIPLE:

The recovery of mycobacteria from sputum or other mucous-containing specimens that are contaminated with other organisms is difficult, since mycobacteria species generally grow much slower than other bacterial species. A gentle decontamination and digestant process was developed by Kubica[3,4] in 1963 to enhance the recovery of mycobacteria. Decontamination and digestion must break down the mucous components, kill normal contaminating flora, and allow the mycobacteria to grow. Sodium Hydroxide (NaOH) acts as an emulsifier and a decontaminant, breaking down mucous and inhibiting the growth of contaminants when used with N-Acetyl-L-Cysteine. NaOH also shortens the decontamination time, preserving more viable mycobacteria. The N-Acetyl-L-Cysteine (NALC) is the digestant that breaks up mucopurulent specimens, allowing the sodium hydroxide to come in contact with the contaminating bacteria and releasing the mycobacteria so they may be recovered for culture. Sodium Citrate aids in liquefaction by stabilizing NALC; Sodium Citrate binds heavy metals, allowing NALC to work properly. The 0.067 M Phosphate Buffer is used to gently neutralize and lower the specific gravity of the specimen after the decontamination step. The 0.2% Bovine Serum Albumin is added to the sediment after centrifugation, and is intended to enhance the growth of the mycobacteria species present. It also increases the volume of material for culture and assists in adhering the material to the slide.

FORMULAS:

(1) Sodium Citrate*:
Sodium Citrate Dihydrate ..... 2.94 g
Deionized Water ..... 100.0 ml

(2) Sodium Hydroxide*:
Sodium Hydroxide ..... 4.0 g
Deionized Water ..... 100.0 ml

(3) N-Acetyl-L-Cysteine*:
N-Acetyl-L-Cysteine ..... 250.0 mg
*Digestion formula constituents, see formula for appropriate mixture.

(4) Phosphate Buffer 0.067 M (m/15) pH 6.8:
Disodium Phosphate ..... 0.47 g
Monopotassium Phosphate 0.45
Deionized Water ..... 100.00 ml
Final pH 6.8 ± 0.2 at 25°C

(5) Bovine Serum Albumin Fraction V, 0.2%
Bovine Serum Albumin Fraction V ..... 0.2 g
Sodium Chloride 8.5
Deionized Water ..... 100.0 ml
Final pH 6.8 ± 0.2 at 25°C

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage:

0.2% Bovine Serum Albumin - 2-8°C.

Phosphate Buffer, N-Acetyl-L-Cysteine, Sodium Hydroxide, and Sodium Citrate reagents - room temp (10-25°C).

These reagents should not be used if they are discolored, have developed a heavy precipitate, or if the expiration date has passed. Do not use Bovine Serum Albumin if any signs of contamination are evident.

Limitations: Occasional specimens are so contaminated with resistant bacteria, such as *Klebsiella* or *Pseudomonas*, that the decontamination process is not effective and the contaminating bacteria will overgrow the culture. Sediment material may be redigested using a more alkaline digestion process, or the specimen may be resubmitted and processed using an alternate digestion process such as oxalic acid or a stronger alkaline solution.

Timing is important for digestion to occur. A digestion time longer than 15 minutes should not be used. Many *Mycobacteria* species are killed by over decontamination.

No more than 10 ml of mucopurulent material should be processed in a tube at one time. Similarly, specimens should be representative of good sputum samples. Material should not resemble saliva and should be submitted in a volume > 5ml.

A pH balance is critical and is achieved in the centrifugation step. The timing and speed are important in this step.

If the specimen is excessively mucoid, a few crystals of NALC may be added.

Do not reuse NALC. The reconstituted reagent should not be more than 24 hours old.

If the specimen contains excess blood, the iron in the hemoglobin binds NALC making it impossible to digest. An alternate digestion method must be considered.

PROCEDURE:*

Specimen Collection:

Sputum: Sputum should be collected in sterile 50-milliliter screw-capped centrifuge tubes or sterile sputum cups, preferably disposable ones, that are graduated, showing the 10-milliliter volume (no wax containers). Ten milliliters of sputum should be placed in the container. If a larger volume of sputum is collected, the specimen should be separated into 10-ml volumes. Avoid contamination of the specimen with oral or nasal secretions. The specimen should be refrigerated if processing will be delayed.

Mucoid Gastric Lavage: A neutralized gastric lavage specimen should be collected early morning, fasting. The specimen should be processed as soon as possible. If the specimen cannot be processed immediately, it should be refrigerated with 100 mg of sodium carbonate. Do not use sodium carbonate if the specimen will be processed immediately, as sodium carbonate interferes with the decontamination treatment.

Other Specimens With or Without Mucous-Purulent Material: Refer to standard references.[2,5,6]

Method of Use:

Sputum

1. Just before use, prepare the digestant by dissolving 250 mg NALC in a mixture of 25 ml of Sodium Hydroxide and 25 ml of Sodium Citrate. This reagent should be prepared fresh daily.
2. Transfer 5-10 ml of the sputum specimen into a 50-ml, aerosol-free, screw-capped graduated plastic centrifuge tube. Add to the centrifuge tube a volume of the above digestant solution equal to the volume of the sputum. The final concentration of the NaOH in the tube is 1%.
3. Tighten the cap completely. Invert the tube to coat all of the the inside surfaces with the NALC-NaOH solution. Vortex the centrifuge tube until the specimen is liquefied, approximately 20 seconds. If liquefaction is not complete after this step, agitate the solution, at intervals, during the following decontamination period.
4. Allow the mixture to stand 15 minutes at room temperature, occasionally shaking gently by hand. Do not shake hard enough to aerate the specimen. For viscous specimens, a small pinch of crystalline NALC may be added for better liquefaction. Do not allow the specimen to sit more than 15 minutes, as overprocessing results in reduced recovery of mycobacteria. A small amount of NaOH may be added to increase decontamination, if needed.
5. Fill the centrifuge tube to the 50 ml mark with 0.067 M Phosphate Buffer. Swirl the tube to mix.
6. Centrifuge at least 15 minutes at >3000 X g.
7. Decant the supernatant into a splash-proof discard container containing a suitable disinfectant. Do not allow the top of the tube to make contact with the discard container. Wipe the lip of the tube with disinfectant-soaked gauze (new for each tube), recap.
8. Using a separate sterile pipet for each specimen, add 1 to 2 ml sterile 0.2% BSA fraction V, or 1-2 ml phosphate buffer, and resuspend with gentle shaking or using the pipet. BSA may buffer and detoxify the sediment and increases the adhesion of the specimen to solid media; however, detection times in automated systems may be lengthened.
9. Inoculate appropriate media using a separate dosposable capillary pipette for each specimen to deliver 3 drops to solid