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Title: DIGESTION AND DECONTAMINATION OF SPUTUM AND GASTRIC ASPIRATE SPECIMENS FOR AFB

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Annual Review	
By	**Date**

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Document History:

Version Number	Reason for Changes	Date
MIC.007.02	Addition of batch size and use of only freshly prepared 10% bleach for surface cleaning/disinfection.	15 May 2008

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PURPOSE

Procedures for preparation of mucoid and bacterially contaminated sputum and gastric aspirate specimens for mycobacterial culture and smear.

PRINCIPLE

Equal volume of a mixture of the mucolytic agent N-acetyl-l-cysteine (NALC) that breaks down mucous to homogenize specimens and 2% sodium hydroxide (NaOH) to decontaminate is added to the specimen. A phosphate buffer is added after 15 minutes to neutralize the NaOH, to prevent the loss of mycobacteria viability that can occur after too long an exposure to NaOH. The mixture is centrifuged, the supernate discarded and the sediment used to plant cultures and prepare smears for AFB stain.

SAFETY

- Observe standard precautions when handling patient specimens to avoid exposure to bloodborne pathogens.
- All specimen processing must be done in the biohazard safety hood and appropriate personal protection worn (gown, gloves, particulate respirator mask).
- Open centrifuge carriers inside biohazard safety cabinet.
- Use care when handling unstained slides to avoid touching infectious material.
- Use only freshly prepared 10% bleach for cleaning/disinfecting surfaces.

MATERIALS

BD Mycoprep Kit (4340863) (Store at 15-25°C)
- NaOH/NALC Digestion Reagent (BD) (2% NaOH)
- Phosphate Buffer (pH 6.8) packets

500 ml distilled water
Blue-capped media bottle
50 ml blue cap plastic centrifuge tubes
Sterile disposable pipettes
Centrifuge with sealed centrifuge carriers
Splash-proof bottle with >1L capacity, containing 100mL of undiluted chlorine bleach
Timer
Vortex

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Preparation of Phosphate Buffer:

1. Add phosphate buffer packet to 500 ml sterile water in a blue-capped media bottle and mix well.
2. Autoclave at 121°C for 15 minutes with a loose cap. Cool to room temperature and tighten cap.
3. Label bottle with expiration date 6 months from date of preparation.
4. Return to refrigerator after use.

Preparation of NaOH-NALC Reagent:

1. Loosen cap of MYCOPREP reagent bottle and squeeze to remove excess air. Retighten cap.
2. Squeeze the NALC ampule in the reagent bottle to break it.
3. Shake gently to dissolve NALC
4. Label bottle with date and time the reagent was mixed. Discard any unused mixture after 24 hours.

DIGESTION PROCEDURE

NOTE: Most instances of cross-contamination occur during specimen processing especially when heavily positive specimens are included. Use careful technique when adding reagents; do not touch the tubes containing specimens with the lip of the reagent bottles.

1. No more than 16 samples should be processed at one time. If >16 samples, process the first 16 and begin processing the remainder once the first 16 have been centrifuged.
2. Place up to 10 ml of specimen to be digested in a 50 ml blue cap plastic centrifuge tube.
3. Add a volume of the NaOH/Nalc digestion mixture equal to the specimen volume. Total volume (specimen plus digestant) cannot exceed 20 ml. **DO NOT TOUCH THE CENTRIFUGE TUBE WITH THE LIP OF NaOH/NALC CONTAINER WHEN DISPENSING.**
4. Mix suspension on the vortex.
5. Start timer for 15 minutes when digestion mixture is added to the first specimen.
6. Mix by occasional gentle shaking (do not vortex).

7. When time has elapsed, add phosphate buffer to the 50 ml mark of the centrifuge tube. **DO NOT TOUCH THE CENTRIFUGE TUBE WITH THE LIP OF BOTTLE.**
8. Mix solution by inverting each tube several times, or vortexing.
9. Check tubes for cracks before loading centrifuge carriers.
10. Centrifuge at full speed (3750 rpm/3273 x g) for 30 minutes in sealed centrifuge carriers. These ensure against aerosol production in case a tube breaks during centrifugation.
11. **Open centrifuge carriers in the biological safety cabinet**. Check carefully for leakage and broken or cracked tubes. Notify supervisor immediately of any breakage or leakage. Record any loss of specimen due to breakage or leakage on Incident Form.
12. Pour supernate into a splash-proof bottle which contains 80-100 ml undiluted disinfectant or bleach. **DO NOT ALLOW THE LIP OF THE TUBE TO TOUCH THE BOTTLE.** Leave approximately 1.5 ml sediment.
13. Inoculate media and prepare slides from sediment. (Refer to MIC.015 PROCESSING OF SPECIMENS FOR MYCOBACTERIA.)
14. Refrigerate remaining specimen for one week.
15. Refrigerate remaining phosphate buffer.

REFERENCES:

Pfyffer, GE, etal. Mycobacterium: General characteristics, Isolation and Staining Procedures. *In*: Manual of Clinical Microbiology, 8th Ed, 2003, ASM Press, Washington, DC.