Laboratory Procedures

BBL® MycoPrep™

Specimen Digestion/Decontamination Kit

For Processing of Mycobacterial Specimens

I. INTENDED USE

The **BBL® MycoPrep™** Specimen Digestion/Decontamination Kits are used for the digestion and decontamination of clinical specimens suspected to contain mycobacteria, especially *Mycobacterium tuberculosis*.

II. SUMMARY AND EXPLANATION

The majority of clinical specimens sent to the mycobacteriology laboratory for cultural confirmation of suspected mycobacterial infection (e.g., sputum, bronchial or trachea lavage) are contaminated by rapidly growing normal flora. To maximize the mycobacterial yield, contaminated specimens require treatment with a digestion and decontamination procedure. N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent.[1]

III. PRINCIPLES OF THE PROCEDURE

Sodium hydroxide (NaOH) can be used as both a digestant and decontaminant. As a mucolytic agent, it is most effective at a final specimen concentration of 2%. However, as a decontaminating agent, this concentration is toxic to both contaminants and to some mycobacteria.[1]

N-acetyl-L-cysteine (NALC) is also a mucolytic agent. In the **BBL® MycoPrep™** reagent bottle, the NALC is combined with 2% NaOH. When the reagent is diluted with an equal volume of specimen, it provides effective digestion and decontamination with a final concentration of 1% NaOH, which is less toxic to mycobacteria.[1]

Sodium citrate is included in the reagent to bind heavy metal ions that may be present in the specimen and which can inactivate NALC.[1]

Because NALC loses mucolytic activity in solution, the **BBL® MycoPrep™** reagent contains the NALC component in a sealed glass ampule within the NaOH-citrate solution. The ampule is broken and the reagents gently mixed before use.

Packets of pre-weighed, powdered phosphate salts are included in the **BBL® MycoPrep™** kit for preparation of phosphate buffer solution pH 6.8 and for use in washing the digested-decontaminated specimen. The phosphate buffer decreases the activity of the NALC-NaOH solution and lowers the specific gravity of the specimen before the mycobacteria are recovered by centrifugation.

IV. REAGENTS

BBL® MycoPrep™ Reagent

Approximate Formula* Per L Purified Water

NaOH ..... 20.0 g
Trisodium citrate
($Na_3C_6H_5O_7 \bullet 2H_2O$) 14.5
Each sealed glass ampule within the bottle contains 0.375 g NALC ($C_5H_9NO_3S$).

BBL® MycoPrep™ Phosphate Buffer

Approximate Formula* Per 500 ml Purified Water
Disodium Phosphate
(Na_2HPO_4) ..... 2.37 g
Monopotassium Phosphate
(KH_2PO_4) 2.27
Final pH 6.8

**Adjusted and/or supplemented as required to meet performance criteria*

Precautions: For In Vitro Diagnostic Use

The NALC-NaOH reagent contains strong alkali and causes severe burns. Take off all contaminated clothing immediately. Gloves and eye/face protection must be worn. NaOH is irritating to the eyes and skin. In the event of eye or skin contact, rinse immediately with an eye wash system or tap water for at least 15 min and seek medical advice. If ingested, give milk, egg white or l large amounts of water and seek medical advice. Keep out of reach of children.

Caution: Break ampule close to its center *one time only*. Do not manipulate ampule further as the plastic bottle may puncture and injury may occur.

Storage Instructions: On receipt, store at 15 to 25°C. Do not freeze. Do not open until ready to use.

Product Deterioration: Do not use reagents if ampules are broken or there is visible evidence of deterioration. Do not use phosphate salts if packages are torn or unsealed.

V. SPECIMEN COLLECTION AND HANDLING

Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. All specimens should be handled according to Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) guidelines or local institution guidelines for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

Refer to appropriate texts for details of specimen collection and handling procedures.[1-4]

VI. PROCEDURE

Materials Provided: See "Availability" (Package Insert).

Materials Not Provided: Ancillary laboratory equipment required for this procedure.

Instructions:

1. Prepare the **BBL® MycoPrep™** Phosphate Buffer as needed, by pouring contents of one packet into a 500 ml volumetric flask and fill to line with distilled or deionized water. Check pH with a meter. The solution should be pH 6.8. Transfer the buffer solution to a screw-capped container and, with cap loosened, autoclave at 121°C for 15 min. Cool to room temperature and tighten cap.

2. Using caution not to spill, loosen screw-cap on the **MycoPrep™** Reagent bottle. Locate ampule in bottle, squeeze excess air from the bottle and tighten cap. with bottle in the upright position, squeeze the bottle until the ampule breaks. *(Note: the 150 ml bottle contains two ampules that must be broken).* Shake gently to dissolve the NALC. Avoid excessive agitation. ONCE AMPULE IS BROKEN, USE REAGENT WITHIN 24 H.[1]

3. In a biological safety cabinet, using a sterile, aerosol-free 50 ml centrifuge tube with screw cap, add equal amounts of specimen and activated NALC-NaOH solution (approximately 10 ml of each).

4. Cap the centrifuge tube and mix on a Vortex-type mixer until specimen is liquefied. If specimen is especially viscous, add more NALC-NaOH solution and repeat mixing.

5. Allow mixture to stand at room temperature for 15 min with occasional gentle shaking. Avoid over-treating the specimen.

6. Add the prepared phosphate buffer to the 50 ml mark on the centrifuge tube and mix. Centrifuge for 15 - 20 min at 3000 x *g*.

7. Carefully decant all of the supernatant fluid.

8. Add a small quantity of phosphate buffer of pH 6.8 (e.g., 0.5 to 2.0 ml) and resuspend the sediment. Use the suspension for the preparation of smears and the performance of mycobacteriological procedures.[1-4]

User Quality Control: For each lot or shipment, examine kit components as described under "Product Deterioration". Process a culture containing acid-fast organisms as per established laboratory quality control procedures for mycobacterial specimens.

VII. LIMITATIONS OF THE PROCEDURE

No one method of digestion-decontamination is suitable for all clinical specimens in all situations. When selecting a procedure, choose the mildest procedure that will reduce contamination.

VIII. AVAILABILITY

Cat. No.	Description
4340862	**BBL® MycoPrep™** Specimen Digestion/Decontamination Kit, consisting of ten 75 ml bottles of Reagent (NALC-NaOH Solution) and 5 packages of Phosphate Buffer.
4340863	**BBL® MycoPrep™** Specimen Digestion/Decontamination Kit, consisting of ten 150 ml bottles of Reagent (NALC-NaOH) and 10 packages of Phosphate Buffer.

IX. REFERENCES

1. Kent, P.T., and G.P. Kubica. 1985. public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta.

2. Cernoch, P.L., R.K. Enns, M.A. Saubolle, and R.J. Wallace, Jr. 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

3. Nolte, F.S., and B. Metchock. 1995. *Mycobacterium*, p. 400-437. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken. (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

4. Isenberg, H.D. (ed). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

TECHNICAL INFORMATION: In the United States, telephone Technical Services, toll free, (800) 638-8663.

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