remel

PHOSPHATE BUFFER M/15 (pH 6.8)

INTENDED USE
Remel Phosphate Buffer M/15 (pH 6.8) reagent is recommended for use in qualitative procedures for neutralization of clinical specimens in the digestion/ decontamination procedures for mycobacteriological and sputum mycological cultures.

SUMMARY AND EXPLANATION
In 1965, Krasnow and Kidd used Phosphate Buffer M/15 (pH 6.8) in a benzalkonium chloride procedure for decontamination of specimens prior to culture for *Mycobacterium*.[1] Kubica et al. also used Phosphate Buffer M/15 in their procedure for digestion and decontamination of specimens for mycobacterial cultures.[2]

PRINCIPLE
Phosphate Buffer M/15 (pH 6.8) neutralizes the continued action of the 4% Sodium Hydroxide, or other decontaminating agent, and lowers the viscosity of the mixture.[5]

REAGENTS (CLASSICAL FORMULA)*
Disodium Phosphate (CAS 7558-79-4) ..... 4.7 g
Monopotassium Phosphate (CAS 7778-77-0) .. 4.5 g
Demineralized Water (CAS 7732-18-5) ..... 1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE
This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use.

PRODUCT DETERIORATION
This product should not be used if (1) there is evidence of contamination, (2) the color has changed, (3) the expiration date has passed, or (4) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT
Specimens should be collected and handled following recommended guidelines.[3,5]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Mycobacteriological safety equipment, (7) Disinfectant, (8) 50 ml sterile, graduated, plastic, centrifuge tubes, (9) Pipettes, (10) TB Base Digestant (R21268) or 2.9% Sodium Citrate (R21262), (11) NAC 50 (R21076), NAC 100 (R21079), Sputagest 50 (R21096), or Sputagest 100 (R21099), (12) 10% KOH (R21230), (13) Sterile demineralized water.

PROCEDURE
Follow established laboratory safety procedures when working with acid-fast cultures and specimens. Consult appropriate references when necessary for detailed procedural information on specimen processing and media inoculation.[3,5]

Mycobacteriological Culture

Reagent Preparation:
- Add NAC 50, NAC 100, Sputagest 50, or Sputagest 100 to 5 ml of TB Base Digestant (REF 21268). Swirl to dissolve. Add the dissolved 5 ml mixture to 45 ml of TB Base Digestant for use with NAC 50 and Sputagest 50 or to 95 ml of TB Base Digestant when using NAC 100 or Sputagest 100.

Specimen Processing:
1. Transfer 10 ml or less of sputum to a sterile, 50 ml centrifuge tube and add an equal volume of the NAC or Sputagest/digestant solution.
2. Tighten the cap and invert the tube ensuring that the solution contacts all inside surfaces of the tube and cap.
3. Mix the contents for approximately 20 seconds on a Vortex mixer.
4. Allow the mixture to stand at room temperature for 15 minutes. Specimens should remain in contact with the decontaminating agent for only 15 minutes. Overprocessing may result in reduced recovery of mycobacteria.[3]
5. Swirl the tube periodically to assist in mucolytic action.
6. Add Phosphate Buffer M/15 (pH 6.8) to the 50 ml mark.
7. Recap the tube tightly and invert several times to mix the contents.
8. Place tube in an aerosol-free, sealed centrifuge cup. Centrifuge at ≥3000 x g for 15 to 20 minutes.
9. Pour off the supernatant into a splash-proof discard container filled with a suitable disinfectant. Do not allow the disinfectant to flow into the tube. Swab lip of tube with disinfectant and recap.
10. Resuspend the sediment (pellet), adding a small amount of Phosphate Buffer if necessary.
11. Use sediment to prepare stains and/or cultures.

12. Refer to Clinical Microbiology Procedures Handbook or the CDC manual for further procedures in the digestion/decontamination process and recommended guidelines for processing other specimen types (e.g., gastric lavage, laryngeal swabs, tissue, blood, and other body fluids).[4,5]

Mycological Culture for Sputum[6]

Reagent Preparation:

- Add NAC 50, NAC 100, Sputagest 50, or Sputagest 100 to 5 ml of 2.94% Sodium Citrate (REF 21262). Swirl to dissolve. Add the dissolved 5 ml mixture to 45 ml of 2.94% Sodium Citrate for use with NAC 50 and Sputagest 50 or to 95 ml of 2.94% Sodium Citrate when using NAC 100 or Sputagest 100.

Specimen Processing:

1. Transfer 10 ml or less of sputum to a sterile, 50 ml centrifuge tube and add an equal volume of the NAC or Sputagest/digestant solution.
2. Tighten cap and invert the tube ensuring that the solution contacts all inside surfaces of the tube and cap.
3. Mix tube on Vortex until each specimen is liquefied, or almost liquefied, if culturing for *Pneumocystis carinii*. Consult appropriate references for procedures for specific fungi or yeast.[3,5]
4. Dilute mixture to 50 ml mark with sterile demineralized water or Phosphate Buffer M/15 (pH 6.8). Tighten cap and mix by swirling or inversion.
5. Place tube in an aerosol-free centrifuge cup. Centrifuge at 2100 x g for 15 minutes.
6. Pour off the supernatant into a splash-proof discard container filled with a suitable disinfectant. Do not allow the disinfectant to flow into the tube. Swab lip of tube with disinfectant and recap.
7. Mix to resuspend the sediment.
8. Use 0.1 ml of the sediment to inoculate culture media. A portion of the sediment is examined microscopically in 10% KOH and other smears may be maintained for future staining.
9. Incubate cultures at 30°C for 4 weeks or longer.

QUALITY CONTROL

All lot numbers of Phosphate Buffer M/15 (pH 6.8) have been tested and have been found to be acceptable. Testing of control organisms should be performed in accordance with the quality control procedures established by each laboratory following their state and local regulatory agencies. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. Sodium hydroxide (TB Base Digestant) must be used cautiously because it is only somewhat less harmful to tubercle bacilli than to the contaminating organisms. Allowing specimens to remain in contact with decontaminating solution for longer than 15 minutes may result in reduced recovery rates of *Mycobacterium* spp.[3]

BIBLIOGRAPHY

1. Krasnow, I. and G.C. Kidd. 1965. Am. J. Clin. Pathol. 44:238-240.
2. Kubica, G.P., A.J. Kaufmann, and W.E. Dye. 1963. Am. Rev. Respir. Dis. 87:775-779.
3. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken. 2003. Manual of Clinical Microbiology. 8th ed. ASM, Washington, D.C.
4. Kent, P.T. and G.P. Kubica. 1985. Public Health Mycobacteriology, A Guide for the Level III Laboratory. U.S. Dept. of H.H.S. and CDC, Atlanta, GA.
5. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed. ASM Press, Washington D.C.
6. McGinnis, M.R. 1980. Laboratory Handbook of Medical Mycology. Academic Press, New York, NY.

PACKAGING

Phosphate Buffer M/15 (pH 6.8):

REF R21256, 50 ml/Btl ..... 10 Btl/Pk
REF R21249, 250 ml/Btl ..... EA
REF R21248, 500 ml/Btl ..... EA

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

CAS (Chemical Abstracts Service Registry No.)

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