
remel

TB DIGESTANT (4% SODIUM HYDROXIDE)

INTENDED USE

Remel TB Digestant (4% Sodium Hydroxide) is recommended for use in qualitative procedures for digestion and decontamination of clinical specimens prior to inoculation of media for isolation of *Mycobacterium* species.

SUMMARY AND EXPLANATION

The majority of clinical specimens submitted to the microbiology laboratory for the isolation of acid-fast bacilli are contaminated with more rapidly growing commensal microbial flora.¹ Also, respiratory specimens, such as sputum, contain mucin which may trap microorganisms. Such specimens require liquefaction to release the mycobacterial cells prior to inoculation of media. The N-acetyl-L-cysteine (NAC) method is the most widely used procedure for digestion and decontamination of specimens for recovery of *Mycobacterium* spp.² TB Digestant has also been used successfully in Petroff's sodium hydroxide digestion method.³

PRINCIPLE

TB Digestant decontaminates the specimen by reducing or eliminating commensal microbial flora and digests respiratory secretions, releasing mycobacterial cells for detection in stains and cultures.

REAGENTS (CLASSICAL FORMULA)*

Sodium Hydroxide (CAS 1310-73-2)..... 40.0 g
Demineralized Water (CAS 7732-18-5) 1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

DANGER! CORROSIVE, may cause burns or irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact.

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. Refer to Material Safety Data Sheet for additional information.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature until used.

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.⁴⁻⁶

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, 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) NAC 50 (REF R21076), NAC 100 (REF R21079), Sputagest 50 (REF R21096), or Sputagest 100 (REF R21099), (11) 2.94% Sodium Citrate (REF R21262), (12) Phenol red solution (0.4% in 4% NaOH), (13) HCl, concentrated, (14) Demineralized water, (15) Vortex mixer, centrifuge.

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.^{5,6}

NAC/Digestant or Sputagest/Digestant Method

Reagent Preparation:

- Prepare a digestant solution by combining equal volumes of 4% Sodium Hydroxide and 2.94% Sodium Citrate.
- To 5 ml of digestant solution, add NAC 50, NAC 100, Sputagest 50, or Sputagest 100. Swirl mixture to dissolve.
- If using NAC 50 or Sputagest 50, transfer the dissolved 5 ml digestant mixture to 45 ml of digestant solution (equal volumes of 4% Sodium Hydroxide and 2.94% Sodium Citrate).
- If using NAC 100 or Sputagest 100, transfer the dissolved 5 ml mixture to 95 ml of digestant solution.

Specimen Processing:

1. Transfer 10 ml or less of specimen to a sterile centrifuge tube and add an equal volume of 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.⁵

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 the tube in an aerosol-free centrifuge cup. Centrifuge the tube at $\geq 3000 \times g$ 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 the lip of tube with disinfectant and recap.
10. Resuspend the sediment (pellet), adding a small amount of Phosphate Buffer, if necessary.
11. Use the sediment to prepare smears and cultures.
12. Refer to Clinical Microbiology Procedures Handbook or the CDC manual for further instructions 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).^{3,4}

Petroff's Method¹

Reagent Preparation:

- Prepare HCl/phenol red indicator by combining 20 ml of phenol red solution (0.4% in 4% NaOH) and 85 ml of concentrated HCl with demineralized water to make 1000 ml.

Specimen Processing:

1. Add an equal volume of 4% Sodium Hydroxide to 10 ml or less of specimen in a sterile centrifuge tube.
2. Mix the contents on a Vortex mixer to digest and let stand for 15 minutes. (Note: Specimens should remain in contact with 4% Sodium Hydroxide for only 15 minutes. Overprocessing of specimen results in reduced recovery of mycobacteria.)⁵
3. Place tube in an aerosol-free centrifuge cup. Centrifuge at $3000 \times g$ for 15 to 20 minutes.
4. Pour off supernatant into a splash-proof discard container filled with a suitable disinfectant. Do not allow the disinfectant to flow into the tube. Swab the lip of tube with disinfectant and recap.
5. Neutralize the sediment (pellet) by adding HCl/phenol red indicator, dropwise, until indicator changes from red to persistent yellow.
6. Mix sediment to resuspend.
7. Use neutralized sediment to prepare smears and cultures.
8. 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).^{1,6}

QUALITY CONTROL

All lot numbers of TB Digestant have been tested and found to be acceptable. Testing of control organisms should be performed in accordance with the quality control procedures established by each laboratory following applicable regulatory agencies. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. Sodium hydroxide 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

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PACKAGING

REF R21266, 4% Sodium Hydroxide..... 250 ml/Btl

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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