remel

SODIUM CITRATE 2.94%

INTENDED USE
Remel Sodium Citrate 2.94% is a reagent recommended for use in qualitative procedures for digestion and decontamination of clinical specimens prior to inoculation of media for isolation of *Mycobacterium* spp. and fungi.

SUMMARY AND EXPLANATION
The majority of clinical specimens submitted to the microbiology laboratory for isolation of acid-fast bacilli are contaminated with more rapidly growing commensal microbial flora. Also, respiratory specimens often contain mucin which may trap the acid-fast bacilli. Such specimens require liquefaction to release the mycobacterial cells prior to inoculation of media.[1] The N-acetyl-L-cysteine (NAC) method described by Kubica et al. is the most widely used procedure for digestion and decontamination of clinical specimens.[2] Reep and Kaplan further investigated the NAC method for use with specimens submitted for the recovery of fungi.[3] They demonstrated NAC could be used with sodium citrate as a diluent to liquefy specimens prior to the inoculation of mycological media.

PRINCIPLE
Sodium Citrate 2.94% serves to decontaminate the specimen by eliminating or reducing commensal microbial flora.

REAGENTS (CLASSICAL FORMULA)*
Sodium Citrate (CAS 68-04-2) ..... 29.4 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 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.[4,5]

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) TB Digestant (REF R21266), (11) NAC 50 (REF R21076), NAC 100 (REF R21079), Sputagest 50 (REF R21096), or Sputagest 100 (REF R21099), (12) Phosphate Buffer M/15 (pH 6.8) (REF R21256), (13) 10% KOH (REF R21230), (14) Sterile 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.[4,5]

Mycobacteriological Culture[4]
Reagent Preparation:
- Prepare a digestant solution by combining equal amounts of TB Digestant and Sodium Citrate2.94%.
- 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 TB Digestant and Sodium Citrate 2.94%).
- 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 50 ml, sterile centrifuge tube and add an equal volume of NAC/digestant 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.[4]
5. Swirl tube periodically to assist in mucolytic action.
6. Add Phosphate Buffer M/15 (pH 6.8) to 50 ml mark.
7. Recap the tube tightly and invert several times to mix contents.
8. Place the 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 the lip of the 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 stains and/or cultures.
12. Refer to the 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).[1,5]

Mycological Cultures[6]
Reagent Preparation:

- Add NAC 50, NAC 100, Sputagest 50 or Sputagest 100 to 5 ml of Sodium Citrate 2.94% and swirl to dissolve.
- To the NAC 50 or Sputagest 50 mixture, add an additional 45 ml of Sodium Citrate 2.94%. To the NAC 100 or Sputagest 100 mixture, add an additional 95 ml of Sodium Citrate 2.94%.

Specimen Processing:

1. Transfer 10 ml or less of specimen to a 50 ml, sterile centrifuge tube and add an equal volume of the NAC or Sputagest/ Sodium Citrate 2.94% mixture.
2. Tighten the cap and invert the tube ensuring that the solution contacts all inside surfaces of the tube and cap.
3. Mix each tube on a Vortex until the specimen is liquefied, or almost liquefied, if the organism of interest is *Pneumocystis carinii*. Consult appropriate references for procedures for specific fungi.[5,6]
4. Dilute the solution to the 50 ml mark with sterile demineralized water or Phosphate Buffer M/15 (pH 6.8). Tighten the cap and mix by swirling or inversion.
5. Place the tube in an aerosol-free, sealed 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 the lip of the tube with disinfectant and recap.
7. Mix to resuspend the sediment.
8. Use 0.1 ml of sediment to inoculate culture media. Microscopically examine a portion of the sediment in 10% KOH. Other smears may be prepared and maintained for future staining.
9. Incubate cultures at 30°C for 4 weeks or longer.

QUALITY CONTROL
All lot numbers of Sodium Citrate 2.94% 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 their state and local 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 mycobacteria 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.[4]

BIBLIOGRAPHY

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PACKAGING
REF R21262, Sodium Citrate 2.94% ..... 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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