remel

# SPUTAGEST (50 and 100)

**INTENDED USE**
Remel Sputagest (50 and 100) are mucolytic agents 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 the recovery of acid-fast bacilli are contaminated with more rapidly growing commensal microbial flora. Also, respiratory specimens such as sputum and bronchial lavage often contain mucin which may trap acid-fast bacilli. Such specimens require liquefaction to release the mycobacterial cells prior to inoculation of media.[1] The dithiothreitol technique for digestion of sputum specimens was introduced by Cleland in 1964.[2] Reep and Kaplan evaluated dithiothreitol and N-acetyl-L-cysteine and reported both agents successfully liquefy and decontaminate clinical specimens for acid-fast cultures.[3] In further testing, they demonstrated dithiothreitol could be used with sodium citrate as a diluent to liquefy specimens for the recovery of fungi.[4]

**PRINCIPLE**
Dithiothreitol serves as a mucolytic agent by disrupting disulfide bonds in the mucous of sputum, gastric lavage, and other body fluids.

**REAGENTS (CLASSICAL FORMULA)***
**Sputagest 50:**
Dithiothreitol (CAS 3483-12-3) ..... 50.0 g
Demineralized Water (CAS 7732-18-5) ..... 1000.0 ml

**Sputagest 100:**
Dithiothreitol (CAS 3483-12-3) ..... 100.0 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**
Store product in its original container at 2-8°C until used. After reconstitution, Sputagest (50 or 100) may be stored at 2-8°C for a maximum of 30 days. 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.[5,6]

**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) TB Base Digestant (REF R21268) or Sodium Citrate 2.94% (REF R21262), (11) Phosphate Buffer M/15 (pH 6.8) (REF R21256), (12) 10% KOH (REF R21230), (13) Sterile demineralized water, (14) 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]

**Mycobacteriological Culture**
**Reagent Preparation:**
Add Sputagest (50 or 100) to 5 ml of TB Base Digestant. Swirl to dissolve. Add the dissolved 5 ml mixture to 45 ml of TB Base Digestant for use with Sputagest 50 or to 95 ml of TB Base Digestant when using Sputagest 100.

**Specimen Processing:**
1. Transfer 10 ml or less of specimen to a sterile, 50 ml centrifuge tube and add an equal volume of 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.[1]
5. Swirl the tube periodically to assist in mucolytic action.
6. Add Phosphate Buffer (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, sealed centrifuge cup. Centrifuge the tube at ≥ 3000 x g for 15 to 20 minutes.

9. 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 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 Clinical Microbiology Procedures Handbook or the CDC manual for further instructions in the digestion/decontamination process and guidelines for processing other types of specimens (i.e., gastric lavage, laryngeal swabs, tissue, blood, other body fluids).[1,6]

**Mycological Culture for Sputum[6,7]**
**Reagent Preparation:**
Add Sputagest (50 or 100) to 5 ml of 2.94% Sodium Citrate and swirl to dissolve. Add the dissolved 5 ml mixture to 45 ml of 2.94% Sodium Citrate for use with Sputagest 50 or to 95 ml of 2.94% Sodium Citrate when using 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 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 each tube on a Vortex mixer 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 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. Examine the sediment microscopically 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 Sputagest (50 and 100) 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.[5]

**BIBLIOGRAPHY**

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**PACKAGING**
REF R21096, Sputagest 50..... 50 ml/Btl, 6/Pk
REF R21099, Sputagest 100..... 100 ml/Btl, 6/Pk

**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) |

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**12076 Santa Fe Drive, Lenexa, KS 66215, USA**
**General Information: (800) 255-6730 Technical Services: (800) 447-3641 Order Entry: (800) 447-3635**
**Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128**
**Website: www.remel.com Email: remel@remel.com**