MUCOGEST™

Cat. no. Z130	MucoGest™ 50, 250mg	20 tubes/box
Cat. no. Z131	MucoGest™ 100, 500mg	20 tubes/box

INTENDED USE

Hardy Diagnostics MucoGest™ is composed of dithiothreitol, a mucolytic agent recommended for use in the digestion and decontamination procedures of sputum and other clinical specimens for the recovery of mycobacteria and fungi.

SUMMARY

MucoGest™ is composed of the mucolytic agent, dithiothreitol. Dithiothreitol serves as a mucolytic agent by disrupting disulfide bonds in the mucus of sputum. Its use as a sputum digestant was described by W.W. Cleland in 1963.[5] The reagent was later evaluated by Reep and Kaplan, who found it to be successful as a mucolytic agent for sputum specimens in the recovery of acid-fast bacilli. Reep and Kaplan also determined dithiothreitol to be useful in liquifying sputa for mycological culture.[10]

Recovery of Mycobacteria:

Although MucoGest™ itself has no inhibitory effect on bacteria, it improves the recovery of acid-fast bacilli by breaking down mucous components of sputum. Specimens intended for the recovery of acid-fast bacilli are first treated with a MucoGest™-TB Base mixture. The specimen is then treated with phosphate buffer and bovine serum albumin.

TB Base Digestant (Cat. no. U22) is composed of sodium hydroxide which serves as a decontaminant by inhibiting the growth of non-acid-fast contaminants.

Phosphate Buffer (Cat. no. U10) lowers the specific gravity, as well as gently neutralizes the specimen after decontamination.

Bovine Serum Albumin (Cat. no. Z81) enhances growth of mycobacteria. It also assists in adhering the sediment material to the slide or solid media and increases the volume of material for culture.

Recovery of Fungi:

MucoGest™ can also be used as the liquifying agent for the recovery of fungi from mucoid specimens. When recovering fungi, however, sodium citrate (Cat. no. U23 or X47), rather than sodium hydroxide (contained within TB Base Digestant), is used as the diluent. Reep and Kaplan found that sodium hydroxide produced fungal toxicity effects on specimens, thereby decreasing the recovery of important pulmonary mycotic disease agents.[10] Use of sodium citrate eliminates deleterious effects on the specimen and allows for the successful recovery of mycological cultures.[10]

Once treated with the MucoGest™-sodium citrate component, specimens are treated with phosphate buffer and bovine serum albumin, respectively.

FORMULA

MucoGest™ 50	
Dithiothreitol	250.0mg/tube

MucoGest™ 100	
Dithiothreitol	500.0mg/tube

STORAGE AND SHELF LIFE

Storage: Upon receipt store MucoGest™ at 2-8ºC. Do not use if there are signs of decontamination or if the expiration date has passed. Protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: Specimen should be collected according to protocol established by the user laboratory. Avoid contamination of the specimen with oral or nasal secretions. Transport specimens to the lab without delay. The specimen should be refrigerated if processing will be delayed.

Method of Use: **Work within a biological safety cabinet and wear gloves.**

For Recovery of Mycobacteria:

1. Add 5ml of TB Base Digestant (2.94% sodium citrate/4% sodium hydroxide) to the MucoGest™ tube. Swirl to dissolve.

2. If preparing MucoGest™ 50, add contents of step 1 (above) to 45ml of TB Base Digestant.*
If preparing MucoGest™ 100, add contents of step 1 (above) to 95ml of TB Base Digestant.*

* Proceed to step 3 below.

For Recovery of Fungi:

1a. Add 5ml 2.94% sodium citrate to a MucoGest™ tube. Swirl to dissolve.

2a. If preparing MucoGest™ 50, add contents of step 1a (above) to 45ml of 2.94% sodium citrate.

Note: Cat. no. X47 fill is 50ml.

If preparing MucoGest™ 100, add contents of step 1a (above) to 95ml of 2.94% sodium citrate.

Note: Cat. no. U23 fill is 100ml.

3. Transfer sputum to a 50ml, aerosol-free, screw-capped centrifuge tube. Transfer no more than 10ml of sputum per 50ml tube. This will ensure that the volume of specimen to be processed never exceeds one-fifth the volume of the tube. When processing the entire specimen, divide the specimen into separate centrifuge tubes and combine the sediments after centrifugation (step 8 below).

4. To the specimen, add a volume of MucoGest™ solution prepared in step 2 and/or step 2a, equivalent to but not more than the amount of the specimen. Avoid touching the lip of the specimen container with reagent bottles. Tighten caps firmly.

5. Vortex specimen until liquified (five to twenty seconds).

6. Allow the vortexed specimen to sit at room temperature (15-30ºC.) for 15 minutes. Do not allow specimen to sit longer than 20 minutes before adding diluent.

7. Add at least 30ml (per 50ml centrifuge tube) of pH 6.8 buffer or water. Tighten cap and swirl to mix.

8. Centrifuge at 3000rpm (1800-2400xg) for 15 minutes.

9. Under a biosafety cabinet, carefully decant the supernatant into a splash proof container containing a cold sterilant. Wipe the lip of the container with disinfectant. Do not allow the disinfectant to enter the tube.

10. Aseptically aliquot one or two milliliters of 0.2% bovine albumin or sterile distilled water to each sediment. **Gently** shake by hand to mix. Gentle shaking decreases chance of creating aerosols.

Note: pH adjustment is not necessary.

11. Using a sterile capillary pipette, mix sediment. Additionally, a 1:10 dilution may be cultured when culturing for AFB. Dilute the suspension 1:10 by adding 0.5ml of suspension from step 10 (above) to 4.5ml sterile distilled water.

12. The undiluted and dilute sediment suspensions are used to inoculate media for isolation and for susceptibility testing. Place two drops on the surface of each medium. Make a smear by placing one drop of the undiluted specimen with albumin on a slide and allow it to dry thoroughly before staining.

INTERPRETATION OF RESULTS

See listed references or Hardy Diagnostics Technical Information Sheets for the interpretation of growth on various

media designed to isolate mycobacteria or fungi.

LIMITATIONS

MucoGest™ is a liquifying agent and has no inhibitory effect on bacteria.

Occasional specimens are so contaminated with resistant bacteria, such as *Klebsiella* spp. or *Pseudomonas* spp., that the decontamination process is not effective and the contaminating bacteria will overgrow the slower growing mycobacteria. A selective medium with antibiotics, such as Lowenstein Jensen, Selective or Middlebrook 7H11, Selective can be used to decrease the growth of contaminating organisms.

Timing is important during the digestion process. A digestion time of longer than 15 minutes should not be used. Many *Mycobacterium* spp. are killed by over decontamination.

No more than 10ml of mucopurulent material should be processed in a tube at one time. Sputum specimens should be representative of good sputum samples. Material should not resemble saliva. Never use a preservative or fixative with the specimen.

It is recommended that TB Base and Phosphate Buffer be used in small containers (50ml or less) in order to prevent back-splash contamination. Contamination can occur by touching the rim of the reagent bottle to the rim of the centrifuge tube or when pouring liquid into the centrifuge tubes; as liquid pours out, air and droplets rush back into the container.

This product is only a part of the overall identification scheme. It is recommended that biochemical and serological tests be performed on pure cultures for complete identification. For more information, see appropriate references.[1-3]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as vortex mixer, biological safety cabinet, centrifuge tubes, slides, media, loops, incinerator, incubators, pasteur pipettes, etc., as well as serological and biochemical reagents, are not provided.

Additional supplies that are required but are not provided:

- TB Base Digestant (Cat. no. U22) for acid-fast culture digestion-decontamination;
  TB Base Digestant is composed of 4% sodium hydroxide / 2.94% sodium citrate.
- 2.94% Sodium Citrate (Cat. no. U23 or X47) for fungal culture digestion.
- Phosphate Buffer, pH 6.8 (Cat. no. U10).
- Bovine Serum Albumin (Cat. no. Z81).

QUALITY CONTROL

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

MucoGest™ should appear as a white, crystalline powder.

MucoGest™ (Cat. no. Z130).

REFERENCES

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3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. Kent, P.T., et al. 1985. *Public Health Mycobacteriology: A Guide for the Level III Laboratory*, U. S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA.

5. Cleland, W.W. 1964. Dithiothreitol: *A New Protective Reagent for SH Groups*, Biochemistry; Vol. 3, No. 4.

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7. Hirsch, S. Roger, et al. 1969. *Sputum Liquifying Agents: A Comparative In Vitro Evaluation*, J. Lab and Clin. Micro.

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9. Reep, B.R. and W. Kaplan. 1972. *The Effect of Newer Tubercle Bacillus Digestion and Decontamination Procedures on Fungi Causing Pulmonary Diseases, Mycopathologia and Mycologia Applicata*; Vol. 46:325-334.

10. Reep, B.R. and W. Kaplan. 1972. *The Use of N-acetyl-L-cysteine and Dithiothreitol to Process Sputa for Mycological and Fluorescent Antibody Examinations*, H.L.S.; Vol. 9, No. 2.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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