IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

TB PREP KIT™

Cat. no. Z160	TB Prep Kit™	5 tests/kit
	Each kit contains:	
	X45 - TB Base Digestant, 2oz. Polyethylene Bottle, 50ml	5 bottles
	Z160A - N-Acetyl-L-Cysteine (NALC), 2ml Cryogenic Vial, 250mg	5 vials

INTENDED USE

Hardy Diagnostics TB Prep Kit™ is used for the digestion and decontamination of clinical specimens suspected to contain *Mycobacterium*, especially *Mycobacterium tuberculosis*. Sputum decontamination reagents are used to break down mucous components of sputum and to decontaminate the specimen of normal flora in order to allow slower growing mycobacteria to grow.

SUMMARY

The recovery of mycobacteria from sputum or other mucous containing specimens contaminated with other organisms is difficult, since mycobacteria generally grow much slower than other bacterial species. Decontamination and digestion of the mucous components kills contaminating normal flora and allows slower growing mycobacteria to grow.

Sodium hydroxide (NaOH), in the TB Base Digestant, acts as an emulsifier and a decontaminant, breaking down mucoid material and inhibiting the growth of contaminants. N-Acetyl-L-Cysteine (NALC), when combined with NaOH, facilitates decontamination by further digesting mucopurulent specimens which allows the NaOH to penetrate. Sodium citrate, in the TB Base Digestant, aids in the liquification by binding heavy metals, thus stabilizing NALC and allowing it to work properly. Phosphate Buffer lowers the specific gravity of the specimen and gently neutralizes the specimen after decontamination. Bovine Serum Albumin is added to the sediment after centrifugation to enhance the growth of mycobacteria. Bovine Serum Albumin also assists in adhering the sediment material to the slide or solid media and increases the volume of material for culture.

REAGENT FORMULA

TB Base Digestant (X45):	
NaOH (4% Solution)	50%
Sodium Citrate (2.94% Solution)	50%

Final pH 13.5 +/- 0.5 at 25ºC.

NALC (Z160A):	

N-Acetyl-L-Cysteine	250.0mg

STORAGE AND SHELF LIFE

Storage: TB Prep Kit™ is stored at 15-30ºC. Allow product to warm to room temperature before use. Do not use these reagents if they are discolored, have developed a heavy precipitate, or if the expiration date has passed. Do not freeze or overheat.

The expiration date 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 incubation times as stated below.

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 Precautions for blood. 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." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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 M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

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

PROCEDURE

Specimen Collection: Collect 10ml of specimen in a sterile 50ml screw-cap centrifuge tube (Cat. no. 227261) or graduated disposable sputum cup. If a larger volume of sputum is collected, the specimen should be separated into 10ml volumes. 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.

1. Prepare digestant solution daily by dissolving 250mg of NALC Reagent per 50ml of TB Base Digestant. Prepare only what can be used in 24 hours.

2. Transfer 5-10ml of sputum specimen into a 50ml, aerosol-free, screw-capped polypropylene centrifuge tube (aerosol free and graduated, Cat. no. 227261). If more than 10ml of specimen is submitted select 10ml of the most purulent, bloody or mucoid portion.

3. Add an equal volume of digestant solution to the sputum. If the specimen is bloody, do not add more than 8ml of digestant solution. Avoid touching the lip of the specimen container with reagent bottles. Tighten caps firmly.

4. Vortex the centrifuge tube for no more than 30 seconds.

5. Allow the centrifuge tube to sit at room temperature (15-30ºC.) for 15 minutes, to decontaminate the specimen. Do

not allow specimen to sit longer than 15 minutes.

6. Fill centrifuge tube within 2cm of the top of the tube with Phosphate Buffer (Cat. no. U10, X31 or X43). Recap the tube tightly, and invert several times until solution is mixed. Avoid touching the lip of the specimen container with the reagent bottle.

7. Centrifuge at least 15 to 20 minutes at 3000Xg.

8. Wipe the top of the tube with disinfectant.

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

10. Resuspend the sediment in 1-2ml of 0.2% Bovine Serum Albumin (Cat. no. Z81), to increase the adhesion of the sample to the slide. Swirl to mix. Use a loop or applicator stick to prepare a slide over a 1 x 2cm area.

11. Dilute the suspension 1:10 by adding 0.5ml of suspension from step 10 above, to 4.5ml sterile distilled water (Cat. no. K187).

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.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

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. Sediment material may be redigested using a more alkaline digestion process, or the specimen may be resubmitted and processed using an alternative digestion method. A selective medium, with antibiotics such as Lowenstein-Jensen Selective (Cat. no. C25) or Middlebrook 7H11 Selective (Cat. no. C38), 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.

A proper pH must be maintained throughout the reaction. An alkaline pH is critical for decontamination and must be maintained before and during the centrifugation step. Timing and speed are important during centrifugation. A neutral pH after centrifugation is critical to the viability of the mycobacteria, and must be achieved by the addition of the proper amount of Phosphate Buffer.

If the specimen is excessively mucoid, a few additional crystals of NALC may be added.

Do not reuse NALC. The reconstituted reagent should not be more than 24 hours old, since oxygen exposure will render it ineffective.

If the specimen contains excess blood, the iron in the hemoglobin binds the NALC making it impossible to digest. An alternate digestion method must be considered.

It is recommended that TB Base Digestant 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.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, pasteur pipets, slides, other culture media, Phosphate Buffer (Cat. no. X31 or X43), Bovine Serum Albumin, 0.2% (Cat. no. Z81), sterile distilled water (Cat. no. K187), sterile saline (Cat. no. K248), centrifuge tubes (Cat. no. 227261), graduated disposable sputum cups, vortex mixers, biological safety cabinets, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

- Check for signs of contamination or deterioration.
- TB Base Digestant Red is not a growth medium.
- The product is tested for its ability to decontaminate.

The following procedure is routinely used for testing at Hardy Diagnostics:

QC Procedure:

1. Prepare a 1ml suspension, equivalent to a McFarland 0.5 opacity standard (Cat. no. ML05), of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in sterile saline (Cat. no. K248).

2. Mix 1ml of TB Base Digestant to the suspension and incubate aerobically at 35ºC. for ten minutes.

3. Neutralize the suspension by adding 2ml of Phosphate Buffer; vortex and streak 0.01ml onto a Blood Agar plate (Cat. no. A10).

4. Incubate aerobically at 35ºC. Both *P. aeruginosa* and *S. aureus* should be partially to completely inhibited after 24 hours of incubation.

Note: The elevated pH is the inhibitory factor in TB Base Digestant, therefore, some pH tolerant organisms may break through (e.g. *S. aureus* ATCC® 25923), especially during the QC procedure when the process sample is plated on non-selective media.

PHYSICAL APPEARANCE

- TB Base Digestant (X45) should appear clear, and colorless.
- NALC Powder (Z160A) should appear free-flowing, and white in color.

TB Prep Kit™ (Cat. no. Z160).

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

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.

IFU-10779[A]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207F [D]