TB DECOLORIZER (3% ACID ALCOHOL)

INTENDED USE
Remel TB Decolorizer is a reagent recommended for use with the Kinyoun or Ziehl-Neelsen carbolfuchsin staining procedure to differentiate acid-fast bacteria from nonacid-fast bacteria.

SUMMARY AND EXPLANATION
The microscopic acid-fast staining technique is one of the earliest methods used for detection of the tubercle bacillus. Because the acid-fast stain remains the most rapid method for detection of mycobacteria, it continues to be an invaluable adjunct to culture in clinical microbiology laboratories. The Ziehl-Neelsen carbolfuchsin technique, or hot acid-fast stain, uses heat to facilitate penetration of the dye into the cell wall of the microorganism. The Kinyoun carbolfuchsin method, or cold acid-fast stain, uses high concentrations of basic carbolfuchsin and phenol to facilitate penetration of the dye into the cell wall, obviating the need for heat. The carbolfuchsin stain is also used to detect partially acid-fast organisms such as Rhodococcus and Nocardia.

PRINCIPLE
The cell walls of acid-fast bacteria bind carbolfuchsin so that it is not destained with acid alcohol. Typical acid-fast bacteria stain purple to red. A counterstain of a contrasting color, such as methylene blue or gentian violet, is used to detect nonacid-fast organisms in the smear, red. A counterstain of a contrasting color, such as methylene blue or blue, depending on the counterstain used.

REAGENTS (CLASSICAL FORMULA)*
Hydrochloric Acid Conc. (CAS 7647-01-0).................................30.0 ml
Ethyl Alcohol 95% (CAS 64-17-5)............................................970.0 ml
*Adjusted as required to meet performance standards.

PRECAUTIONS
Warning! Causes severe eye irritation. Flammable liquid and vapor. Causes respiratory tract irritation. Harmful if swallowed, inhaled, or absorbed through the skin. This substance has caused adverse reproductive and fetal effects in humans. May cause central nervous system depression. May cause liver, kidney, and heart damage. Corrosive to metal. Causes moderate skin irritation.

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, test materials, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

STORAGE
This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature (20-25°C) until used.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed from a colorless liquid, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT
Specimens should be collected and handled following recommended guidelines.

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemen tal media, (5) TB Kinyoun Carbolfuchsin (REF R40104) or TB Ziehl-Neelsen Carbolfuchsin (REF R40102), (6) TB Methylene Blue (REF R40110) or TB Brilliant Green (REF R40100), (7) Microscope, glass slides, immersion oil, (8) Bunsen burner or slide warmer, (9) Quality control organisms.

PROCEDURE
Every specimen represents a potential source of infectious material and should be handled accordingly.4,6

Kinyoun Carbolfuchsin Stain:
1. Make a thin smear of the material for study and allow it to air dry. Heat fix by passing the slide through the flame of a Bunsen burner or use a slide warmer.
2. Flood the smear with TB Kinyoun Carbolfuchsin for 5 minutes. Rinse with water and drain.
3. Decolorize with TB Decolorizer for 3 minutes. Rinse with water and drain.
4. Repeat decolorization for 1-2 minutes or until no red stain flows from the smear. Rinse with water and drain.
5. Flood the smear with TB Methylene Blue or TB Brilliant Green and stain for 3-4 minutes. Rinse with water and allow to air dry.
6. Examine under oil immersion objective (100 X) for purple to red acid-fast bacilli.

Ziehl-Neelsen Carbolfuchsin Stain:
1. Make a thin smear of the material for study and allow it to air dry. Heat fix by passing the slide through the flame of a Bunsen burner or use a slide warmer.
2. Flood the smear with TB Ziehl-Neelsen Carbolfuchsin and steam the slides gently for 1 minute using a Bunsen burner flame below the rack or use a slide warmer. Do not allow the slides to boil or dry out.
3. Allow the stain to remain on the slide for an additional 4-5 minutes without heat. Rinse with water and drain.
4. Decolorize with TB Decolorizer for 3 minutes. Rinse with water and drain.
5. Flood slide with TB Methylene Blue or TB Brilliant Green for 1 minute. Rinse with water and allow to air dry.
6. Examine under oil immersion objective (100 X) for purple to red acid-fast bacilli.

INTERPRETATION
Positive Test - Mycobacteria stain purple to red and are small slightly curved rods, possibly beaded or banded, with tapered ends.

Negative Test - Nonacid-fast organisms stain blue or green depending on the counterstain used.

QUALITY CONTROL
All lot numbers of TB Decolorizer (3% Acid Alcohol) have been tested and have been found to yield acceptable stain results as listed in the Interpretation section. Quality control testing should be performed following procedures established by each laboratory according to applicable regulatory guidelines. If aberrant quality control results are noted, patient results should not be reported.

Acid-Fast Stain for Mycobacteria: Positive and negative control slides should be included every time the acid-fast stain is performed for detection of mycobacteria.

LIMITATIONS
1. Examine a minimum of 300 oil immersion fields before reporting as negative.
2. Nontuberculous strains of Mycobacterium (e.g., Mycobacterium avium complex) retain the basic dye and appear acid-fast; however, such strains are usually morphologically atypical (i.e., pleomorphic or coccoid). Positive acid-fast smear reports should be based only on typical forms, but atypical cells should be noted.
3. Atypical rods may represent other organisms such as Nocardia or Rhodococcus, which are partially acid-fast. A weaker acid or a shorter destaining period should be used to detect these organisms.
4. The sensitivity of the direct acid-fast smear examination for the diagnosis of mycobacterial infection is lower than that of culture methods. Cultures should be performed on all specimens.
BIBLIOGRAPHY

PACKAGING
REF R40106, TB Decolorizer (3% Acid Alcohol) .........................250 ml/Btl

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