remel

TB ZIEHL-NEELSEN CARBOLFUCHSIN

INTENDED USE

Remel TB Ziehl-Neelsen Carbolfuchsin stain is recommended for use in qualitative procedures to differentiate acid-fast bacteria from nonacid-fast bacteria and to detect *Cryptosporidium* oocysts in clinical specimens.

SUMMARY AND EXPLANATION

The microscopic acid-fast staining technique is one of the earliest methods used for detection of the tubercle bacillus.[1-3] 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.[4] 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. In addition to detecting *Mycobacterium*, Kinyoun carbolfuchsin is also used to detect partially acid-fast organisms such as *Rhodococcus* and *Nocardia*. A modification of the Ziehl-Neelsen carbolfuchsin stain is recommended for detection of *Cryptosporidium* oocysts in clinical specimens.[5]

PRINCIPLE

Mycolic acids and waxes in the cell wall of an acid-fast organism complex with carbolfuchsin, a basic dye, which is retained after mild acid decolorization. Heat is required to enhance penetration of the carbolfuchsin into the cell wall. Typical acid-fast organisms stain purple to red. A counterstain of a contrasting color, such as brilliant green or methylene blue, is used to detect nonacid-fast organisms in the smear and to stain background material. Nonacid-fast organisms stain green or blue, depending on the counterstain used. Oocysts of *Cryptosporidium* stain pink to red to purple with the modified Ziehl-Neelson carbolfuchsin stain.

REAGENTS (CLASSICAL FORMULA)*

Phenol Crystals (CAS 108-95-2)	5.0 g
Basic Fuchsin (CAS 569-61-9)	3.0 g
Alcohol 95% (64-17-5)	10.0 ml
Demineralized Water (CAS 7732-18-5)	990.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

Warning! Possible cancer hazard. May cause cancer based on animal data. Causes burns by all exposure routes. Flammable liquid and vapor. 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 and kidney damage. The toxicological properties of this material have not been fully investigated.

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 dark purple 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.[7,8]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, inoculating loop, swabs, (2) Collection containers, (3) Glass slides, coverslips, mounting medium, (4) Slide staining rack, forceps, (5) Microscope, immersion oil, (6) Bunsen burner or slide warmer, (7) Quality control slides.

Acid-Fast Stain for Mycobacteria: (1) TB Decolorizer (R40106), (2) TB Methylene Blue (R40110) or TB Brilliant Green (R40100).

Modified Acid-Fast Stain: (1) Sulfuric Acid 5% (R40125), (2) TB Methylene Blue (R40110).

PROCEDURE

Every specimen represents a potential source of infectious material and should be handled accordingly.[7,8]

Acid-Fast Stain for Mycobacteria:

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 slide warmer or a Bunsen burner flame below the slide rack. 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 smears under oil immersion objective (100 X) for purple to red acid-fast bacilli.

Modified Acid-Fast Stain for Cryptosporidia:[5]

Note: Use concentrated sediment of fresh or formalin-preserved stools. Consult appropriate references for detailed instructions regarding the selection and preparation of other clinical specimens.[7,8]

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 of use a slide warmer.
2. Flood the smear with TB Ziehl-Neelsen Carbolfuchsin and steam the slides gently for 1 minute using a slide warmer or a Bunsen burner flame below the slide rack. Do not allow the slides to boil of dry out.
3. Allow the stain to remain on the slide for and additional 5 minutes without heat. If the slide begins to dry out, add more stain. Rinse with water and drain.
4. Decolorize with Sulfuric Acid 5% for 30 seconds. Rinse with water and drain.
5. Flood slide with TB Methylene Blue for 1 minute. Rinse with water and drain. Allow the slide to air dry.
6. Slides may be mounted with mounting medium and a coverslip.
7. Examine smear with bright field microscopy under the high power objective (40 X). Use the oil immersion objective (100 X) to examine internal morphology.

INTERPRETATION

Acid-Fast Stain for Mycobacteria:

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).

Modified Acid-Fast Stain for Cryptosporidia:

Positive Test - Oocysts stain pink to red to purple, are 4-6 µm in diameter, and round or oval in shape; 1-4 sporozoites may be visible within the oocysts.

Negative Test - Background stains blue.

QUALITY CONTROL

All lot numbers of TB Ziehl-Neelsen Carbolfuchsin have been tested and 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.[7]

Modified Acid-Fast Stain for Cryptosporidia: A positive control slide should be included with each staining run.[5] Control slides can be prepared from 10% formalin-preserved stool specimens containing *Cryptosporidium*. If positive specimens are not available, use smears made from stool specimens containing leukocytes or epithelial cells to verify stain results.[7]

LIMITATIONS

1. Examine a minimum of 300 oil immersion fields before reporting as negative.[6]
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.[7]
3. Atypical rods may represent partially acid-fast bacteria, such as *Nocardia* or *Rhodococcus.* A weaker acid or shorter destaining period should be used to detect these organisms.[7]
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.[6,7]
5. When staining for *Cryptosporidium*, oocysts stain pink to red, depending on the stain penetration (heat increases penetration), the thickness of the smear, and the age of the specimen (length of time in fixative). The background usually stains uniformly blue or green, depending on the counterstain used.[5]
6. *Cryptosporidium* oocysts are more difficult to detect in formed stools than in liquid specimens. When testing formed stools, increase the staining time to allow the oocysts to stand out from the background material. The hot acid-fast stain has been reported to maximize detection and identification of *Cryptosporidium* in formed stools.[5]
7. Carbolfuchsin stain tends to precipitate upon standing. This will not affect the stain quality, but may make examination difficult when precipitates stick to the smear and are confused with microorganisms. If precipitates are observed on the smear the stain should be filtered before use.[9]

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PACKAGING

REF R40102, TB Ziehl-Neelsen Carbolfuchsin ..... 250 ml/Btl
REF R40202, TB Ziehl-Neelsen Carbolfuchsin ..... 250 ml/Btl, 5/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)

CAS (Chemical Abstracts Service Registry No.)
Manufactured for Remel Inc.

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