



## TB KINYOUN CARBOLFUCHSIN

### INTENDED USE

Remel TB Kinyoun Carbofuchsin 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.<sup>1-3</sup> 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.<sup>4</sup> The Kinyoun carbofuchsin method, or cold acid-fast stain, uses high concentrations of basic carbofuchsin and phenol to facilitate penetration of the dye into the cell wall, obviating the need for heat. In addition to detecting *Mycobacterium*, Kinyoun carbofuchsin is also used to detect partially acid-fast organisms such as *Rhodococcus* and *Nocardia*. A modification of the Kinyoun carbofuchsin staining method is recommended for the detection of *Cryptosporidium* oocysts.<sup>5,6</sup>

### PRINCIPLE

Mycolic acids and waxes in the cell wall of the acid-fast organism complex with carbofuchsin, a basic dye, which is retained in the cell wall after mild acid decolorization. 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 bacteria in the smear which do not retain the carbofuchsin. Nonacid-fast bacteria stain green or blue, depending on the counterstain used. *Cryptosporidium* oocysts stain pink to red to purple with the modified Kinyoun carbofuchsin stain.

### REAGENTS (CLASSICAL FORMULA)\*

Basic Fuchsin (CAS 569-61-9).....	40.0 g
Ethyl Alcohol 95% (CAS 64-17-5).....	200.0 ml
Phenol (CAS 108-95-2).....	80.0 ml
Demineralized Water (CAS 7732-18-5).....	1000.0 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 red-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.<sup>7,8</sup>

### MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, (3) Glass slides, coverslips, mounting medium, (4) Slide staining rack, (5) Microscope, immersion oil, (6) Quality control organisms

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

**Modified Acid-Fast Stain for Cryptosporidia:** (1) Absolute Methanol (REF R40121), (2) Ethyl Alcohol 50%, (3) Sulfuric Acid 1% (REF R40124), (4) TB Methylene Blue (REF R40110) or Light Green (REF R40123).

### PROCEDURE

Every specimen represents a potential source of infectious material and should be handled accordingly.<sup>7,8</sup>

#### 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 Kinyoun Carbofuchsin 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 appears. Rinse with water and drain.
5. Flood 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 smears under oil immersion objective (100 X) for purple to red acid-fast bacilli.

#### Modified Acid-Fast Stain for Cryptosporidia<sup>5</sup>:

**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.<sup>7,8</sup>

1. Make a thin smear of the specimen on a slide and allow it to air dry at room temperature.
2. Fix the smear with Absolute Methanol for 1 minute (dip slide).
3. Stain with TB Kinyoun Carbofuchsin for 5 minutes at room temperature.
4. Wash slides with Ethyl Alcohol 50% (3-5 seconds) followed by an immediate wash with water.
5. Decolorize with Sulfuric Acid 1% for 2 minutes or until no color runs from the slide. Rinse with water and drain.
6. Counterstain with TB Methylene Blue or Light Green for 1 minute. Rinse with water and drain.
7. Slides may be mounted with mounting medium and a coverslip.
8. 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. One to four sporozoites may be visible within the oocyst.
- Negative Test - Yeast cells and background material stain blue or green (depending on the counterstain used).

## QUALITY CONTROL

All lot numbers of TB Kinyoun Carbofuchsin 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.<sup>7</sup>

**Modified Acid-Fast Stain for Cryptosporidia:** A positive control slide should be included with each staining run.<sup>5</sup> 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.<sup>7</sup>

## LIMITATIONS

1. Examine a minimum of 300 oil immersion fields before reporting as negative.<sup>6</sup>
2. Nontuberculous *Mycobacterium* strains (e.g., *M. 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.<sup>7</sup>
3. Atypical rods may represent partially acid-fast organisms, such as *Nocardia* or *Rhodococcus*. 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.<sup>4,6</sup>
5. When using the stain for *Cryptosporidium*, oocysts stain pink to red depending on the stain 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.<sup>4</sup>
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 (Ziehl-Neelsen) has been reported to maximize detection and identification of *Cryptosporidium* in formed stools.<sup>5</sup>
7. Carbofuchsin 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.<sup>9</sup>




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## PACKAGING

REF R40104, TB Kinyoun Carbofuchsin .....250 ml/Btl  
REF R40204, TB Kinyoun Carbofuchsin ..... 250 ml/Btl, 5/Pk

## Symbol Legend

<b>REF</b>	Catalog Number
<b>IVD</b>	In Vitro Diagnostic Medical Device
<b>LAB</b>	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
<b>LOT</b>	Batch Code (Lot Number)
	Use By (Expiration Date)

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

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