**TB METHYLENE BLUE**

**INTENDED USE**
Rremel TB Methylene Blue stain is recommended for use in qualitative procedures as a counterstain 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. 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 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. 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 Kinyoun carbolfuchsin is recommended for detection of Cryptosporidium in clinical specimens.

**PRINCIPLE**
Mycotic acids and waxes in the cell wall of the acid-fast organism complex with carbolfuchsin, a basic dye, which is retained in the cell wall after mild acid decolorization. Typical acid-fast organisms which retain the dye appear purple to red. TB Methylene Blue is used as a counterstain to detect nonacid-fast bacteria in the smear which stain blue. Cryptosporidium oocysts stain pink to red to purple with the modified Kinyoun carbolfuchsin stain.

**REAGENTS (CLASSICAL FORMULA)*

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene Blue (CAS 61-73-4)</td>
<td>3.0 g/1000.0 ml</td>
</tr>
<tr>
<td>Demineralized Water (CAS 7732-18-5)</td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

*Adjusted as required to meet performance standards.

**PRECAUTIONS**
Caution! May cause eye, skin, and respiratory tract irritation. This product is considered a low hazard for usual industrial handling. 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, and test materials 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 dark blue, (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**

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Methanol (REF R40121)</td>
<td></td>
</tr>
<tr>
<td>TB Kinyoun Carbolfuchsin (REF R40104)</td>
<td></td>
</tr>
<tr>
<td>Ethyl Alcohol 50% (REF R40124)</td>
<td></td>
</tr>
</tbody>
</table>

**PRODUCTION**
Every specimen represents a potential source of infectious material and should be handled accordingly.

**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 Carbolfuchsin and stain 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 smear with TB Methylene Blue and allow staining 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.

**Modified Acid-Fast Stain for Cryptosporidia**:  
**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.

1. Make a thin smear of the specimen on a slide and allow to air dry at (room temperature) 20-25°C.
2. Fix the smear with Absolute Methanol for 1 minute (dip slide).
3. Stain with TB Kinyoun Carbolfuchsin 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 smear. Rinse with water and drain.
6. Counterstain with TB Methylene Blue for 1 minute.
7. Slides may be mounted with mounting medium and a coverslip.
8. Observe with bright field microscopy (40 X objective). Use 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.

**Modified Acid-Fast Stain for Cryptosporidia:**
- **Positive Test** - Oocysts stain pink to red, 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.

**QUALITY CONTROL**
All lot numbers of TB Methylene Blue have been tested and found to be acceptable. Testing of control organisms should be performed in accordance with the quality control procedures established by each laboratory following applicable regulatory agencies. 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.

**Modified Acid-Fast Stain for Cryptosporidia:** A positive control slide should be included with each staining run. 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.
LIMITATIONS

1. Examine a minimum of 300 oil immersion fields before reporting as negative.

2. Some mycobacteria (e.g., *Mycobacterium avium* complex) are pleomorphic and usually coccoid. Positive acid-fast smear reports should be based only on typical forms, but atypical cells should be noted. 6, 7

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. 8

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. 7, 8

5. When staining 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 will usually stain uniformly blue. 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 (Ziehl-Neelsen) has been reported to maximize detection and identification of *Cryptosporidium* in formed stools. 5

BIBLIOGRAPHY


PACKAGING

REF R40110 TB Methylene Blue........................................ 250 ml/Btl

REF R40210 TB Methylene Blue........................................ 250 ml/Btl, 5/Pk