



remel

## TB Malachite Green

### INTENDED USE

Remel TB Malachite Green is a stain recommended for use in qualitative procedures as a counterstain to differentiate acid-fast from nonacid-fast bacteria.

### SUMMARY AND EXPLANATION

One of the earliest methods devised for the detection of tubercle bacilli is the microscopic staining technique. This method is valuable for presumptive diagnosis of mycobacterial infections. Mycobacteria possess cell walls that contain mycolic acid which complex with basic dyes resulting in the characteristic known as "acid-fastness." Species of *Nocardia* and *Rhodococcus* are also partially acid-fast. Three types of staining procedures are used for the detection of acid-fast organisms: fluorochrome, Ziehl-Neelsen (hot stain), and Kinyoun (cold stain).<sup>1,2,3</sup>

### PRINCIPLE

Mycolic acids and waxes in the mycobacterial cell wall complex with the Kinyoun carbolfuchsin (basic dye), which then fails to wash out with mild acid decolorization. Typical acid-fast bacteria, which retain the dye, appear purple to red. A counterstain of a contrasting color, such as Malachite Green, is used to detect nonacid-fast bacteria present in the smear, which do not retain the red dye after being treated with acid and alcohol.

### REAGENTS (CLASSICAL FORMULA)\*

Malachite Green (CAS 2437-29-8) .....	3.0	g
Demineralized Water (CAS 7732-18-5) .....	1000.0	ml

\*Adjusted as required to meet performance standards.

### PRECAUTIONS

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 media after use. Directions should be read and followed carefully.

### STORAGE

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

### PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from green, (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>4</sup>

### MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) QC-Slide™ AFB Stain Control (REF 40144) or quality control organisms, (6) TB Kinyoun Carbolfuchsin (REF 40104), (7) TB Decolorizer (REF 40106), (8) Demineralized water, (9) Glass slides, (10) Bunsen burner or slide warmer, (11) Microscope, (12) Immersion oil.

### PROCEDURE

1. Make a thin smear of the material for study and 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.
3. Rinse with demineralized water and drain.
4. Decolorize with TB Decolorizer for 3 minutes.
5. Rinse with demineralized water and drain.
6. Repeat decolorization for 1-2 minutes or until no red stain appears.
7. Rinse with demineralized water and drain.
8. Flood smear for 3-4 minutes with TB Malachite Green.
9. Rinse with demineralized water and allow to air dry.
10. Examine microscopically under oil immersion (100X objective) for presence of acid-fast bacilli.

### INTERPRETATION

Positive Test - Acid-fast positive organisms stain purple to red, mycobacteria appear red, small, slightly curved, possibly beaded or banded, with tapered ends.

Negative Test - Nonacid-fast organisms stain green.

### QUALITY CONTROL

All lot numbers of TB Malachite Green have been tested using the QC-Slide™ AFB Stain Control and have been found to yield acceptable stain results as listed in the INTERPRETATION section. Appropriate testing should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

### LIMITATIONS

1. Examine a minimum of 300 fields (magnification X 1000) before reporting as negative.<sup>4</sup>
2. Some nontuberculous species, such as *Mycobacterium avium* complex, appear pleomorphic, usually coccoid. Positive findings should be based only on typical forms but atypical cells should be noted.<sup>5</sup>
3. Atypical rods may represent other partially acid-fast organisms, such as species of *Nocardia* or *Rhodococcus*. A weaker acid or a shorter destaining period is 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. Therefore, cultures should be performed on all specimens.<sup>4</sup>




**BIBLIOGRAPHY**

1. Bennedson, J. and S.O. Larsen. 1966. Scand. J. Respir. Dis. 47:114-120.
2. Bishop, P.J. and G. Neumann. 1970. Tubercle. 51:196-206.
3. Kinyoun, J.J. 1915. Am. J. Public Health. 5:867-870.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenenbaum. 2003. Manual of Clinical Microbiology, 8<sup>th</sup> ed. ASM, Washington, D.C.
5. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's Diagnostic Microbiology, 11<sup>th</sup> ed. Mosby, Inc., St. Louis, MO.

**PACKAGING**

REF 40108, 250 ml/Btl.....Each  
 REF 40208, 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)

QC-Slide™ is a trademark of Remel Inc.  
 CAS (Chemical Abstracts Service Registry No.)  
 Manufactured for Remel Inc.

IFU 40108, Revised November 11, 2004

Printed in the U.S.A.

12076 Santa Fe Drive, Lenexa, KS 66215, USA  
 General Information: (800) 255-6730    Technical Services: (800) 447-3641    Order Entry: (800) 447-3635  
 Local/International Phone: (913) 888-0939    International Fax: (913) 895-4128  
 Website: www.remel.com    Email: remel@remel.com