remel

TB Decolorizer (Truant-Moore)

INTENDED USE
Remel TB Decolorizer is a reagent recommended for use in qualitative procedures in the fluorescent microscopic detection of mycobacteria.

SUMMARY AND EXPLANATION
One of the earliest methods devised for the detection of tubercle bacilli is the microscopic staining technique.[1] Mycobacteria possess cell walls that contain mycolic acid which complex with dyes resulting in the characteristic known as "acid-fastness." Acid-fast microscopy is the most rapid, initial step in diagnosis and in providing information about the number of acid-fast bacilli present. The use of fluorescent dyes for the detection of acid-fast bacilli in clinical specimens was described by Hagemann in 1937.[2] In 1962, Truant, Brett, and Thomas evaluated the usefulness of the fluorescent staining technique for screening clinical specimens suspected to contain acid-fast bacilli and found it to yield a larger number of positive smears than the conventional fuchsin-stained method.[3] They used auramine and rhodamine separately and in combination and found the latter to be the most satisfactory. In 1966, Bennedson and Larsen also reported a higher yield of positive smears and a substantially reduced time requirement needed for examining smears when using the fluorescent technique rather than carbolfuchsin stain.[4]

PRINCIPLE
The fluorochrome dyes, Auramine O or Auramine-Rhodamine, used in fluorescent staining will complex with mycolic acids found in the acid-fast cell wall of organisms and are refractory to rinsing by acid-alcohol (TB Decolorizer). The counterstain, TB Potassium Permanganate, renders tissue and its debris non-fluorescent, thus reducing the possibility of artifacts. The cells visualized under ultraviolet light appear bright yellow-green or reddish orange.

REAGENTS (CLASSICAL FORMULA)*

Hydrochloric Acid (CAS 7647-01-0)	5.0 ml
Ethyl Alcohol 70% (CAS 64-17-5)	1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
WARNING! Flammable, keep away from heat, sparks and flame. Avoid breathing vapor and eye/skin contact.

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. Refer to Material Safety Data Sheet for additional information.

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 a clear 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.[5]

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 40146) or quality control organisms, (6) TB Auramine O (REF 40086) or TB Auramine-Rhodamine (REF 40090), (7) TB Potassium Permanganate (REF 40092), (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 Auramine O or TB Auramine-Rhodamine for 15 minutes at room temperature or 37°C.
3. Rinse with demineralized water and drain.
4. Decolorize with TB Decolorizer for 2-3 minutes
5. Rinse with demineralized water and drain.
6. Flood smear with TB Potassium Permanganate counter-stain for no longer than 2-4 minutes.
7. Rinse with demineralized water and allow to air dry.
8. Examine microscopically under low power (25X objective) using a fluorescent microscope; confirm under oil immersion (400-630X magnification).
9. A positive fluorescent smear may be restained by the conventional Ziehl-Neelsen or Kinyoun procedure.

INTERPRETATION
Positive Test - Acid-fast positive organisms fluoresce bright yellow-green with Auramine O and reddish-orange with Auramine-Rhodamine against a dark background.

Negative Test - Nonacid-fast organisms will not fluoresce or may appear a pale yellow, quite distinct from the bright acid-fast organisms.

QUALITY CONTROL
All lot numbers of TB Decolorizer (Truant-Moore) 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. A positive staining reaction provides presumptive evidence of the presence of mycobacteria. A negative staining reaction does not indicate that the specimen will be culturally negative. Therefore, cultural methods must be employed.

2. Most strains of rapid growers may not appear fluorescent. It is recommended that all negative fluorescent smears be confirmed with Ziehl-Neelsen stain; at least 100 fields should be examined before being reported as negative.[6]
3. Excessive exposure to the counterstain may result in a loss of brilliance of the fluorescing organism.
4. Stained smears should be observed within 24 hours of staining because of the possibility of the fluorescence fading.[7]

BIBLIOGRAPHY

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2. Hagemann, P.K.H. 1937. Zbl. Bakt. 140:184.
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5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASM, Washington, D.C.
6. Baron, E.J., L.R. Peterson, and S.M. Finegold. 1994. Bailey and Scott's Diagnostic Microbiology, 9th ed. Mosby, St. Louis, MO.
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PACKAGING

REF 40107, 250 ml/Btl ..... Each
REF 40207, 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)

QC-Slide™ is a trademark of Remel Inc.
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 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