

#### Intended use

Malachite Green 1% is used as a staining solution in bacterial endospore staining and simple staining.

# **Summary**

Malachite Green is used for bacterial spore staining by Schaeffer and Fulton's method. It can also be used as a simple stain for bacterial cells and in place of methyl-green in Pappenheim stain, when combined with Gram stain.

# **Principle**

A spore is a dormant form of the bacterium that allows it to survive in drastic environmental conditions. Spores have a tough outer covering made of the protein keratin and are resistant to heat and chemicals. The keratin also resists staining, so extreme measures must be taken to stain the spore. In the Schaeffer-Fulton's method, a primary stain malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolourized with water. Vegetative cells are then counterstained with safranin. Spores may be located in the middle of the cell, at the end of the cell, or between the end and middle of the cell. Spores shape may also be of diagnostic use. Spores may be spherical or elliptical. Members of the genus Corynebacterium may exhibit club-shaped swellings that might be confused with spores. Spore staining distinguishes between true spores and these structures.

# **Reagent / Contents**

Malachite green 1.0 g
Distilled water 100 mL

# **Appearance**

Dark green coloured solution.

# **Storage and Stability**

Store at 15°C-25°C in tightly closed container and away from bright light. Stability of Malachite Green 1% is as per the expiry date mentioned on the label.

# Materials required but not provided

Clean grease-free glass slide, loops, Bunsen's burner, water-bath, staining rack, blotting paper, immersion oil , microscope.

### **Type of Specimen**

Endospore / capsule forming bacteria isolated colony on primary or sub-cultured plates can be isolated from following specimens.

Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc.

From environment: Air, water, soil, sludge, wastewater, food, dairy samples etc.

#### **Procedure**

- 1. Prepare a smear on a clean, dry glass slide.
- 2. Allow it to air dry and fix it with gentle heat.
- 3. Flood the slide with 1% w/v Malachite Green.
- 4. Heat this preparation in the water-bath for 3-6 minutes till the stain starts steaming.
- 5. Allow stain to be in contact with the smear for 2-3 minutes, & then allow to cool.
- 6. Wash in slow-running tap water.
- 7. Counterstain with 0.5% aqu. Safranin/ Schaeffer & Fulton's Spore stain B for 30 seconds.
- 8. Wash with water, blot dry and examine under oil immersion objective.

#### **Interpretation of Results**

Spores are observed as green spherules in red stained rods or red stained debris.









# **Warranty**

This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

#### Reference

1. Data on file: UltraCare Diagnostics .

#### Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.





