



Methylene Blue(Loeffler's)

S022

Intended use

Methylene Blue (Loeffler's) is used for metachromatic staining and as a counter stain in Acid fast staining.

Composition**

Ingredients

Methylene blue	0.3 gm
Ethyl alcohol, 95%	30.0 ml
Distilled water	100.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a thin smear of *Corynebacterium diphtheriae*.
2. Allow it to air dry and fix with gentle heat.
3. Flood the smear with Methylene Blue (S022) for 1 minute.
4. Wash in the tap water.
5. Blot dry, then examine under oil immersion objective.

Principle And Interpretation

Methylene Blue (Loeffler's) is used in the presumptive identification of *Corynebacterium diphtheria*. Metachromasia, a condition characteristically seen in *Corynebacterium diphtheria*, is due to an accumulation of polymerized polyphosphate in high concentration inside the cell. This condition appears as polyphosphate granules stained deeply blue, surrounded by lighter blue stained cytoplasm, and are often called Babes Ernst bodies or metachromatic granules. The cell itself is characterized by a banded or beaded appearance. Methylene Blue, Loefflers is also a counter stain in Acid fast staining; where Non Acid Fast bacteria and background material is appaer as blue in colour.

Type of specimen

Any isolated colony on primary or subculture 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, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

Generally, the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Morphology of *C. diphtheriae* is most distinctive when grown on Loefflers medium, If *C. diphtheriae* is grown on other media, less satisfactory results may be seen.
2. Overstaining may reduce the contrast between the bacteria and background, or between the cytoplasm and granules.
3. Some strains of *Propionibacterium*, *Actinomyces*, and pleomorphic forms of streptococci may mimic the characteristic stained appearance of *C. diphtheriae*.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance** : Dark blue coloured solution.
- **Clarity** : Clear solution without any particles
- **Microscopic Examination** : Metachromatic staining is carried out where Methylene Blue is used as one of the stains and staining characteristic of organisms is observed under microscope by using oil immersion lens.
- **Results** : Deep blue Pale blue

Storage and Shelf Life

Store between 10 - 30 °C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

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Storage temperature



Do not use if package is damaged



In vitro diagnostic medical device



CE Marking



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