Loeffler's Methylene Blue

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: PL.7050, PL.7051

INTENDED USE

For use in the identification of *Corynebacterium diphtheriae* in clinical specimens. It can also be used as a counterstain in acid-fast staining.

SUMMARY AND EXPLANATION

Loeffler's Methylene Blue is recognised as a simple stain for determining bacterial morphology; primarily, however, it is used in the presumptive identification of *Corynebacterium diphtheriae*. Loeffler's methylene blue is a metachromatic stain, which means that certain biological materials stain a different colour to that of the stain itself. In the case of *C. diphtheriae*, the polyphosphate granules stain a deep blue-black colour against the pale blue cytoplasm. The stain can also be used as a counterstain in the Kinyoun or ZN CF acid-fast methods.

PRINCIPLE OF THE TEST

Loeffler's Methylene Blue is a cationic dye which stains the cell blue. The presence of negatively charged polyphosphate molecules in the cell causes the staining phenomenon of metachromasia, as the positively charged dye is attracted to negatively charged particles such as DNA and RNA.

MATERIALS PROVIDED

-	PL.7050	Loeffler's Methylene Blue	500 ml
-	PL.7051	Loeffler's Methylene Blue	1000 ml

Per 100ml solution:

- Loeffler's Methylene Blue contains 0.39g of Methylene Blue powder.

MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Inoculating loop
- Immersion oil PL.396
- Microscope

STABILITY AND STORAGE

Loeffler's Methylene Blue should be stored at 15-25ºC in its original container. Product stored under these conditions will be stable until the expiry date shown on the product label.

PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Microbial contamination may decrease the accuracy of the staining.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Process slides in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.
- Any serious incident that occurs in relation to the device should be reported to the manufacturer and the competent authority of the member state in which the incident occurred.

TEST PROCEDURE

1. Prepare a smear on a clean glass slide and allow to air dry. Heat fix.
2. Flood the slide with Loeffler's Methylene Blue for 1 minute.
3. Wash the slide gently with tap water and blot dry.
4. Examine using a microscope.

QUALITY CONTROL PROCEDURE

Internal quality control of the Loeffler's Methylene Blue stain must be performed regularly on known reference material.

Recommended Quality Control:
Positive – *Corynebacterium diphtheriae* NCTC® 11397/ATCC® 27010*
Negative – a proven negative

INTERPRETATION OF RESULTS

Positive- *Corynebacterium diphtheriae* appear as banded blue rods arranged at angles to each other (resembling letter 'L' or 'V'), along with blue-black metachromatic granules at the poles.
Negative- short nonpathogenic diphtheroids or other organisms will lack the blue-black granules.

LIMITATIONS OF THE PROCEDURE

- Only experienced personnel should carry out the interpretation of stained slides
- Read prepared slides as soon as possible after staining. Failure to do so may affect the results.
- Some strains of *Propionibacterium*, *Actinomyces*, and pleomorphic forms of *Streptococci* may mimic the characteristic stained appearance of *C. diphtheriae*.

REFERENCES

- Albert, H. Modification of stain for diphtheria bacilli. *The Journal of the American Medical Association.* 1921 76:240.
- Crookham, J. and Dapson, R. Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 199, *Anatech*.
- Gurr, E. A. Practical Manual of Medical and Biological Staining Technique. 1953
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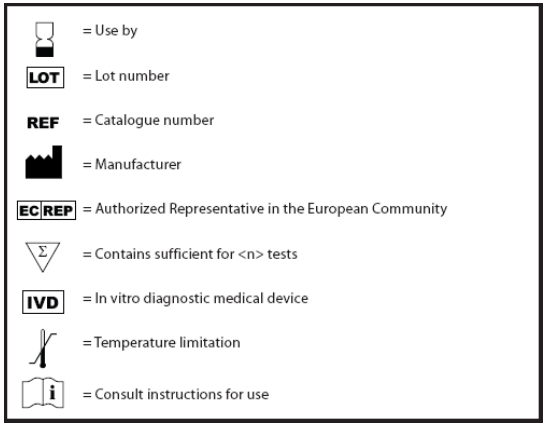
- Sutter, V.L. and Carter, W.T. (1972). *American Journal of Clinical Pathology* 58:335-338



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HAZARDS IDENTIFICATION

Please refer to Safety Data sheets for full text for all hazard and precautionary statements.

DANGER	**PL.7050 PL.7051**	**H226, H319** **P210, P233, P337+P313, P363, P370+P378, P403+P235**

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