Polychrome Methylene Blue & Fixative

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: PL.7061, PL.7061/5, PL.7066, PL.7066/5

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INTENDED USE
For use in detecting capsulated *Bacillus anthracis* in clinical specimens.

SUMMARY AND EXPLANATION
The Polychrome Methylene Blue staining procedure for blood or tissue smears taken from dead animals (M'Fadyean's reaction) was established in 1930, and remains accepted as a highly reliable, rapid diagnostic test for anthrax. *Bacillus anthracis* is a Hazard Group 3 organism. If suspected clinically, refer specimens directly to the appropriate Reference Laboratory without doing any further work/manipulations. All laboratory procedures such as staining should be performed by experienced scientists in a Containment Level 3 facility using a Class 1 protective safety cabinet.

PRINCIPLE OF THE TEST
Polychromatic Methylene Blue is a 'ripened' methylene blue solution, which is still recognised as the most simple and reliable method for the identification of capsulated *B. anthracis*. When Methylene Blue solution is left to stand, oxidation takes place which produces homologs such as Azure. These homologs stain the capsule surrounding *B. anthracis*, allowing its visualisation in blood/tissue smears.

MATERIALS PROVIDED

-	PL.7061	Polychrome Methylene Blue	250 ml
-	PL.7061/5	Polychrome Methylene Blue	500ml
-	PL.7066	Polychrome Methylene Blue Fixative	250ml
-	PL.7066/5	Polychrome Methylene Blue Fixative	500ml

Per 100ml solution:
- Polychrome Methylene Blue contains 0.37g of Methylene Blue powder.

MATERIALS REQUIRED BUT NOT PROVIDED
- Glass slides
- Inoculating loop
- Immersion oil PL.396
- Microscope

STABILITY AND STORAGE
Polychrome Methylene Blue and Polychrome Methylene Blue Fixative should be stored at 15-25ºC in their original containers. 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.
- Samples should be processed 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.
2. Cover smear with Polychrome Methylene Blue Fixative for 3 minutes and allow to air dry.
3. Flood the slide with Polychrome Methylene Blue for 30-45 seconds.
4. Wash the slide gently with water or as a safety precaution, wash the slide using a 10% hypochlorite solution.
5. Examine using a microscope.

QUALITY CONTROL PROCEDURE
Internal quality control of the Polychrome Methylene Blue and Polychrome Methylene Blue Fixative must be performed regularly on known reference material.

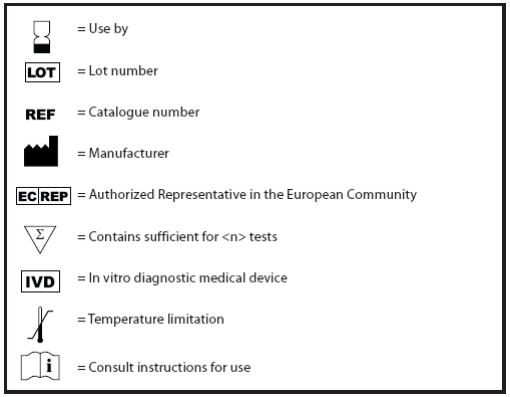
Recommended Quality Control:
Positive – a proven positive
Negative – a proven negative

INTERPRETATION OF RESULTS
Virulent *B.anthracis* rods will be surrounded by a clearly demarcated zone giving the appearance of a reddish-pink capsule.
If *B.anthracis* is suspected, all washings, blotting materials, and slides must be properly autoclaved.

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.
- Many species of bacilli may also be encapsulated. Any positives must be confirmed at a Reference Laboratory.

REFERENCES

- Balzevic, D.J. and Edrer, G.M. (1975). Principles of Biochemical Tests in Diagnostic Microbiology. *John Wiley & sons*, New York, NY.
- Koch, R. (1876) [Investigations into bacteria: V. The etiology of anthrax, based on the ontogenesis of Bacillus anthracis], *Cohns*.
- Lowrance, B.L., Reich, P. and Traub, W.H. (1969). *Journal of Applied Microbiology* 17:923-924.
- M'Fadyean, J. (1903a). A peculiar staining reaction of the blood of animal's dead of anthrax. *Journal of Comparative Pathology*. 16:35-41.
- 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.

WARNING	**PL.7061 PL.7061/5**	**H226, H319** **P210, P233, P337+P313, P363, P370+P378, P403+P235**
DANGER	**PL.7066 PL.7066/5**	**H225, H302, H311, H319, H331, H371** **P210, P260, P280, P321, P370+P378, P403+P233**

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Revision: 2023 12