M'Fadyean Stain (Polychrome Methylene Blue)

Intended Use
M'Fadyean Stain (Polychrome Methylene blue) is used for capsule staining of Bacillus anthracis.

Composition**

Ingredients
- Methylene blue: 0.300 gm
- 95% ethyl alcohol: 30,000 ml
- Potassium hydroxide: 0.010 gm
- Distilled water: 100,000 ml

**Formula adjusted, standardized to suit performance parameters

Directions
1. Make a thin smear by spreading a small drop of culture on a slide.
2. Air dry and fix by dipping in absolute alcohol for 30-60 seconds. Remove slide and allow the remaining solution to evaporate.
3. Place a large drop of Polychrome methylene blue stain on the smear, spreading with an inoculating loop to cover all parts of the smear. Leave for 30-60 seconds.
4. Wash the stain off with water, blot dry and observe under oil immersion (100X objective).

Principle And Interpretation
Anthrax is primarily a disease of domesticated and wild animals, particularly herbivorous animals, such as cattle, sheep, horses, mules and goats. Humans become infected incidentally when brought into contact with diseased animals, which includes their flesh, bones, hides, hair and excrement. Bacillus anthracis clearly owes its pathogenicity to two major determinants of virulence: the formation of a poly-D-glutamyl capsule, which mediates the invasive stage of the infection, and the production of the multicomponent anthrax toxin which mediates the toxigenic stage.

Polychrome methylene blue staining is the simplest and most reliable method of confirming the presence of capsulated B. anthracis. Polychrome methylene blue and substantial amounts of other homologs, primarily azure A and azure B, which are produced by oxidation (“ripening”) that takes place in methylene blue solution upon standing the oxidation of methylene blue forms a violet compound that gives the stain its polychromic property which stains the capsulated B.anthracis.

Type of specimen
Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, 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 (4, 5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1, 3).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. (2) 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 guidleines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.
Limitations
1. Slide should not be heat dried to avoid distortion of morphology of the capsule.
2. Smear should be made on a slide immediately after collection of blood or should be done within hours of collecting blood since vegetative cells disintegrate in blood held for much more than a day.
3. Environmental samples may contain substances which inhibit germination and growth of *B. anthracis*, and therefore appear negative on culture. Such conditions may result in false negative test.

Performance and Evaluation
Performace of the stain is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
- **Appearance**
  Deep violet coloured solution.
- **Clarity**
  Clear without any particles.
- **Microscopic Examination**
  Capsule staining is carried out using M'Fadyean stain and staining characteristic is observed under microscope using oil immersion lens.

Results
- *Bacillus anthracis* ATCC 14578: Pink capsule seen surrounding the deep bluish bacilli.
- *Bacillus cereus* ATCC 10876: Deep bluish bacilli seen.

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 (6,7).

Reference
6. Koch, R (1876) [Investigations into bacteria: V. The etiology of anthrax, based on the ontogenesis of Bacillus anthracis],Cohns.

Revision : 01 / 2019

Please refer disclaimer Overleaf.
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<th>IVD</th>
<th>In vitro diagnostic medical device</th>
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<td>CE</td>
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<tr>
<td>30°C</td>
<td>Storage temperature</td>
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