
Capsule Stain Solution (M'Fadyean Method)

C1510420

Storage Room temperature.
Shipping Regular transportation.

Introduction

Anthrax is an acute zoonotic infectious disease caused by *Bacillus anthracis*. It mainly occurs in livestock, with herbivores such as cattle, sheep and horses being the most susceptible. Humans are mainly infected by contact with anthrax-affected animals, contaminated animal products or the polluted environment. The main clinical type is cutaneous anthrax, with a few cases of pulmonary anthrax and intestinal anthrax, which can be complicated by sepsis and meningitis. The mortality rate of cutaneous anthrax is relatively low, while that of other anthrax types is considerably higher.

Bacillus anthracis is the largest species of Gram-positive bacilli, with a size of 1–1.5×3–5 μm. It has truncated ends, arranges in chains, and presents a bamboo joint-like morphology under the microscope. The length of the chains varies depending on the bacterial strain and the surrounding environment. In the human or animal body, or under specific environmental conditions (cultured on carbonate agar medium with 5%–25% CO₂), the bacterium can form a capsule. A transparent ring around the bacilli can be observed by India ink staining, and the capsule appears red by methylene blue staining. The bacterium can produce spores in the external environment, with the non-free spores located in the center of the bacterial cells. This bacterium is non-flagellated and non-motile.

The presence of spores, as well as the shape and location of spores, are important characteristics of bacteria. The spore wall has a more complex, compact structure and lower permeability than the cell wall of vegetative cells, making it more difficult to stain and decolorize than vegetative cells. Spores have strong resistance to heat and chemical substances. When stained with basic dyes under heating or with extended staining time, both the bacterial cells and spores can be colored. Then, the color of the bacterial cells can be removed by rinsing with distilled water, while the color of the spores can be retained, allowing for clear morphological differentiation between spores and vegetative cells under a microscope.

Aladdin Capsule Stain Solution (M'Fadyean Method) is mainly composed of methylene blue staining solution and hypochlorous acid solution. With this solution, spores are stained red and bacterial cells are stained blue. This product can be used for the etiological staining of *Bacillus anthracis*, featuring simple operation and more reliable result interpretation. However, it should be noted that the observation of spores should be carried out in a biosafety level 2 (BSL-2) laboratory. This reagent is for research use only and is not suitable for clinical diagnosis or other purposes.

Product Components and Storage Conditions

C1510420	Component	2×50mL	Storage
C1510420A	Methylene Blue Staining Solution	50mL	RT
C1510420B	Hypochlorous Acid Solution	50mL	RT

Self-prepared Materials:

1. Alcohol lamp, glass slides, inoculation loops, microscopes.
2. Distilled water, test samples (smears or cultures prepared from specimens such as exudates from skin ulcers, excreta, blood, pleural effusion, ascites and cerebrospinal fluid).

Operating Procedures (For Reference Only):

1. Place 1 drop of distilled water on a clean, grease-free glass slide.
2. Pick a small amount of the test sample with an inoculation loop, mix it thoroughly with the distilled water, prepare a smear, and let it air-dry naturally.
3. Fix the smear with anhydrous methanol or ethanol for 2–5 minutes.
4. Add methylene blue staining solution to cover the smear area, stain for 3–5 minutes (if the capsule staining is not clear, the staining time can be appropriately extended).
5. Rinse the glass slide gently with water.
6. Immerse the glass slide in hypochlorous acid solution for decolorization and disinfection for 2 minutes, then rinse it with water.
7. After drying, observe the slide under the microscope to check the shape, size, and position of spores in the bacterial cells, as well as whether the spores cause swelling of the bacterial cells.

Staining Results:

Bacillus anthracis has truncated ends, appears in a bamboo joint-like morphology and long chains arrangement; the capsule stains pink and the bacterial cells stain blue.

Precautions:

1. The glass slides should be clean and free of oil stains.
2. The reproductive capacity and resistance of *Bacillus anthracis* are similar to those of ordinary bacteria, but its spores have strong resistance and high hazard. The experiment should be carried out in a biosafety level 2 (BSL-2) laboratory.
3. Operate carefully during the staining process to prevent the bacteria from detaching from the slide.
4. Avoid high-temperature flame fixation during the fixation step; room-temperature fixation is sufficient.
5. The wastewater after rinsing in the experiment and the absorbent paper should be autoclaved or treated with sodium hypochlorite solution overnight.
6. For your safety and health, please wear a lab coat and disposable gloves during the operation.