

Cytology and morphology of bacteria

2. practice

Structures of cell (inclusion and capsule staining)



Volutin staining



- 1. Drop of water on microsopic slide mix with small ammount of culture
- 2. Spread the drop a little bit and allowed to air dry
- 3. Fix dry microscopic slide by the flame
- 4. Drop of Loefflers methylene blue and rinse with distilled water after 5 min
- 5. Drop of 1% H₂SO₄ (rinse with distilled water after few seconds)
- 6. Observe with immersion oil



Lipids (fat) staining



- 1. Drop of water on microsopic slide mix with small ammount of culture
- 2. Spread the drop a little bit and allowed to air dry
- 3. Fix dry microscopic slide by the flame
- 4. Drop of formaldehyde for 5 min (rinse with distilled water)
- 5. Drop of methylene blue (1:40) for 10 min (rinse with distilled water)
- 6. Drop of Sudan III. (afer 10-15 min rinse with distilled water)
- 7. Observe with immersion oil



Glycogen staining



- 1. Drop of water on microsopic slide mix with small ammount of culture
- 2. Mix it a little bit with drop of Lugol solution
- 3. Cover with cover glass and observe firstly 40x and after 100x magnification



Negative staining of capsules

○ Nigrosin

- 1. Drop of nigrosin + small ammount of culture
- 2. Spread over the slide with another slide and allowed to air dry
- 3. Cover the smear with methylene blue solution for 5 min at least
- 4. Rinse slide with distilled water and observe with immersion oil

Kongo red

- 1. Drop of Kongo red + small ammount of culture
- 2. Spread over the slide with another slide and allowed to air dry
- 3. Rinse the smear with HCl for several second (change to blue color) pour off the acid, do not wash it with distilled water
- 4. Cover the smear with methylene blue solution for 5 min at least
- 5. Rinse slide with distilled water and observe with immersion oil
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What to observe ?



• Volution staining (1)

• Lipids (fat) staining (1)

○ Glycogen staining

• Negative staining (1)

Nigrosin or Kongo red

<u>Bacilli:</u>

Bacillus cereus Bacillus megaterium

Cocci:

"Azotobacter vinelandii"

Leuconostoc

Eukaryota:

Saccharomyces cerevisiae