

Spore staining

Dr Murtakab Y Al-Hejjaj

Bacterial Spores

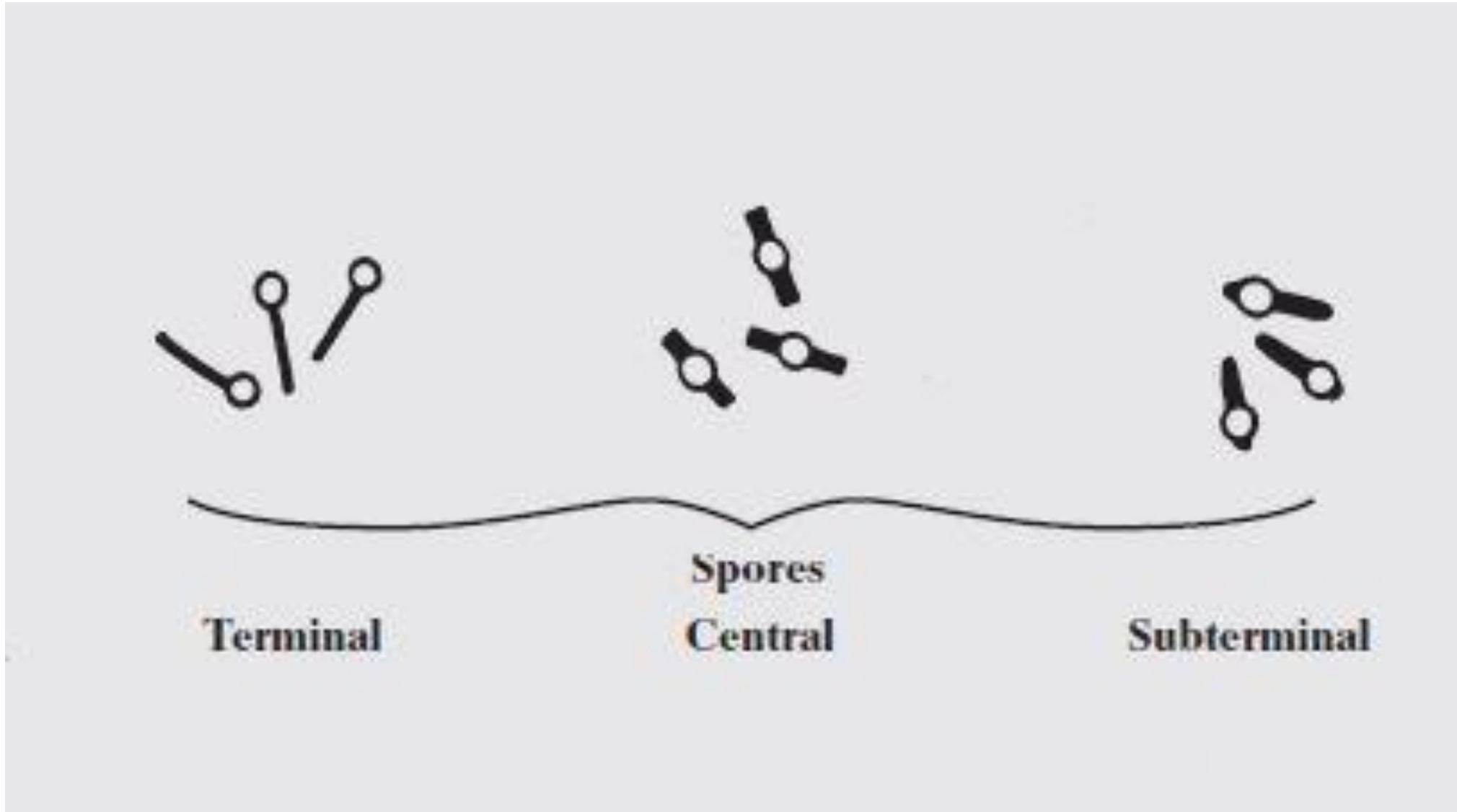
- Bacterial spore is a complex structure of protein, lipid and peptidoglycan serve largely as a resting or dormant stage in the bacterial life cycle. which help to preserve the bacterium through periods of unfavourable conditions. Spore production is particularly common among *Bacillus* and *Clostridium* bacteria. Many bacterial spores are highly durable and can germinate even after years of dormancy.

Types of bacterial spore

- **Endospore:**
 - It is produced within the bacterial cell.
 - E.g. *Bacillus*, *Clostridium*

- **Exospore:**
 - It is produced outside the cell
 - E.g. *Methylosinus*

Positions of endospores



Principles

- Endospores are spherical in shape and may be either smaller or larger than the parent bacterial cell.
- Because of their tough protein coats made of keratin, endospores do not stain easily. But once stained, they strongly resist decolourisation
- The endospores are stained with malachite green. Heat is used to provide stain penetration.
- Vegetative cells easily disrupted by heat. Thus the malachite green rinses easily from the vegetative cells.
- The rest of the cell is then decolourised and counterstained a light red with safranin.

Spore Staining



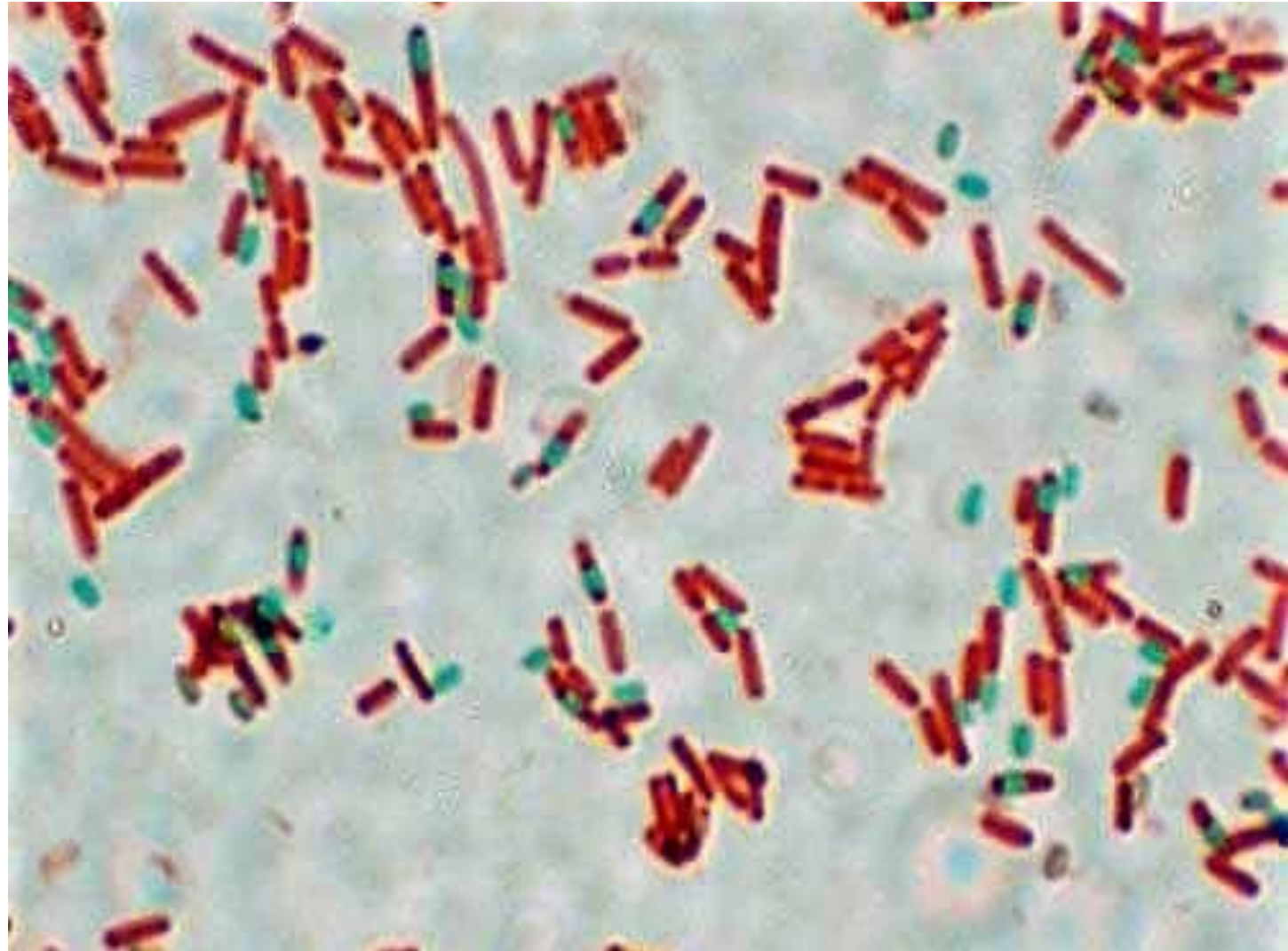
Schaeffer-Fulton

- Aseptically, make a smear of the bacterial sample with an inoculating loop, air dry and heat-fix the organism on a glass slide.
- Cover the smear with a square of blotting paper of appropriate size.
- Saturate the blotting paper with malachite green stain solution, gently heat over the Bunsen burner for 3 to 5 minutes, keeping the paper moist and adding more dye as required, as it evaporates.
- Alternatively, it can be done by placing the slide over a beaker of boiling water. Do not allow the slide to become dry.

- Remove the blotting paper, and rinse the slide gently with distilled water.
- Dispose of the used blotting paper in the trash as it may contain some spores.
- Counterstain with safranin for 60 to 90 seconds.
- Rinse the slide with water for 30 seconds.
- Dry the slide with absorbent paper or blotting paper and examine under oil immersion.

Microscopically

- The cells containing endospores appear as the red coloured rod-shaped along with an intracellular spherical green coloured structure.
- The spores, both endospores and free spores, stain green.
- The non-sporing bacterial (Vegetative cells) stain red.



References

- H Prescottt (2002). Laboratory exercise in microbiology. Fifth ed, The McGraw–Hill Companies.
- <https://paramedicsworld.com/microbiology-practicals/endospore-staining-principle-procedure-interpretation/medical-paramedical-studynotes>