BSC 242-section 009-Review

Review:

- **Culture**: A medium that contains living microbes
- **Pure culture**: A culture contains a single species
- **Sterile**: free from bacteria or other living microorganisms
- **Aseptic process**: process by which a sterile product is packaged in a sterile container in a way that maintains sterility.
- **Broth**: it is used to grow microbes when fresh cultures or large numbers of cells are required
- **Agar Slant**: it is used to grow stock cultures that can be refrigerated after incubation (development) and maintained for a while
- **Plate Media**: are used for obtaining isolation of species, differential testing, and quantifying bacterial densities.
- **Inoculating loop or Inoculating Needle**: transfer instruments (Transfer from a broth culture to a sterile broth, Transfer from Agar slant culture to sterile agar slant, transfer from plate culture to sterile broth)

Aseptic technique examples:

- Broth culture *(E.coli or B.meg)* → Sterile broth (loop)
- Broth culture (*E.coli or B. meg*) → Sterile slant (loop)
- Slant culture (*Proteus*) → Sterile deep (needle)

Endospore Stain: *A differential stain used to detect the presence and location of spores in bacterial cells*

I. An endospore is a dormant form of the bacterium that allows it to survive poor environmental conditions. Spores are resistant to heat and chemicals because of a tough outer coving made of the protein **Keratin**.

The **Schaeffer-Fulton method**:

a) Cells and spores prior to staining are transparent
b) After staining with Malachite Green, cells and spores are green. Heat (steaming) is used to force the stain into spores
c) Decolorization with water removes stain from cells, but not spores
d) Safranin is used to counterstain cells (spores (spore producer) are green while vegetative and spore mother cells (spore nonproducer) are red)

Reagents:

- **Primary stain**: malachite green (water soluble and has a low affinity for cellular material)
- **Decolorized by water**
- **Counterstain:** safranin

Spores location & shape:

- Central – in the middle of the cell
- Terminal – at the end of the cell
- Subterminal – between the end and middle of the cell
- Different shapes – spherical and elliptical (oval)

Examples (pg. 188):

a) Central elliptical endospore – *Bacillus megaterium*
b) Subterminal spores - *Clostridium botulinum*
c) Elliptical terminal endospore – *Clostridium tetani* (by using Carbolfuchsin)

Capsule Stain: A differential stain used to detect cells capable of producing an extracellular capsule.

II. Capsules are composed of mucoid polysaccharides or polypeptides that repel most stains. Its production increases virulence in some microbes by making them less vulnerable to phagocytosis.

Technique: Bacterial capsules are non-ionic, so neither acidic nor basic stains will adhere to there surfaces. Therefore, the best way to visualize them is **to stain the background using an acidic stain (congo red) and to stain the cell itself using a basic stain (Maneval's stain)**. It leaves the capsule as a tiny white halo surrounding the cell.

***Air dry! Heat-fixing causes the cells to shrink, leaving an artifactual white halo around them might be interpreted as a capsule.**

Example (pg. 183): *Streptococcus pneumonia (capsule producer); Bacillus anthracis*

Acid-Fast Stain: A differential stain used to identify acid-fast organisms

III. Mycolic acid is a waxy substance that gives acid-fast cells a higher affinity to the primary stain and resistance to decolorized by an acid alcohol solution. Acid-fast organisms are highly resistant to disinfectants and dry conditions.

Methods:

a) Cells prior to satin are transparent
b) After staining with carbolfuchsin, cells are reddish-purple. In the ZN stain, steam heat enhances carbolfusin the cell. In K stain, a higher concentration of carbolfuchsin is used to promote entry into the cells.
c) Decolorization with acid alcohol removes stain from acid-fast negative cells
d) Methylene blue or brilliant green is used to counterstain acid-fast negative cells

***Steam-heating purpose: to melt the waxy cell wall and allow the stain (carbolfuchsin) to move into the cell.**

Examples:

a) The **Ziehl-Neelson** method (the phenolic compound Carbolfuchsin): *Mycobacterium phlei* (AF+) – RODS (reddish-purple); *Staphylococcus epidermidis* (AF-) – GRAPE-LIKE CLUSTERS (blue)
b) The **Kinyoun** method (a slightly more lipid-soluble and concentrated Carbolfuchsin that allows the stain to penetrate the acid-fast walls without the use of heat): *Mycobacterium smegmatis* (AF+); *Staphylococcus epidermidis* (AF-) - green

Simple Stain: Staining with only one stain/dye