

Staining

- Staining
 - **Staining** = coloring key structures of a microbe using a dye
 - **Smear** = thin film containing a solution of microbes fixed and attached to a slide
 - Fixing the microbes usually kills them ☹️
- Staining Basics
 - Simple Stain
 - Uses one basic stain, so the cells are all the same color
 - Increased contrast between cells and background
 - Steps for a Simple Stain
 - Smear -> air dry -> fixing (using heat) -> stain -> rinse -> dry -> examine
 - Basic Dyes
 - Positive charge
 - Bonds to negatively charged parts of the cell
 - Acidic Dyes
 - Negative charge
 - Bonds to positively charged parts of the cell
 - Mordant
 - Maintains the integrity of the stain
 - Enlarges specimen
- Shapes and Arrangements of Bacteria
 - Shape #1: **Cocci (Coccus)**
 - Can be singular, pairs, chains, tetrads, and clusters
 - Spherical
 - Example: Streptococcus (chain of spherical-shaped bacteria)
 - Shape #2: **Bacilli (Bacillus)**
 - Rod-shaped
 - Can also be coccoid shaped
 - Curved rods = vibrios
 - Singular or short chains
 - Example: Bacillus anthracis
 - Shape #3: **Spirilla (Spirillum)**
 - Spiral-shaped (like a corkscrew pasta noodle ☺️)
 - Flexible helix = spirochete
 - Example: Helicobacter pylori
 - Arrangements
 - One plane of division = pairs and chains
 - Pairs = diplo (e.g. diplococci)
 - Chains = strepto (e.g. streptococci)
 - Two-three planes of division = tetrads and cubical packets
 - Random planes of division = clusters
 - Clusters = staphylo (e.g. staphylococcus)

- Gram Stain
 - o Differential stain
 - o Gram positive bacteria stains purple
 - Bacillus, Streptococcus, Staphylococcus, Mycobacterium
 - o Gram negative bacteria stains pink/red
 - Escherichia, Helicobacter, Pseudomonas, Salmonella
 - o Differences in color is a result of differences in the structure of the cell walls
- Gram Stain Process
 - o Step 1: Primary Stain
 - Crystal violet
 - Stains both positive and negative bacteria
 - Gram Positive Bacteria**
 - Gram Negative Bacteria**
 - o Step 2: Mordant
 - Gram's Iodine
 - Forms large crystals of dye
 - Gram Positive Bacteria**
 - Gram Negative Bacteria**
 - o Step 3: Decolorizer
 - Alcohol/alcohol acetone
 - Removes crystal violet from Gram negative bacteria
 - Gram Positive Bacteria**
 - Gram Negative Bacteria**
 - o Step 4: Counterstain
 - Safranin (red)
 - Stains Gram negative bacteria
 - Gram Positive Bacteria**
 - Gram Negative Bacteria**