Bacteria	Shape	Gram +	Gram -	Acid Fast
Escherichia coli (*E. coli*)	Bacillus (Rod)		X	Non Acid Fast
Staphylococcus aureus	Coccus	X		Non Acid Fast
Bacillus subtilis	Bacillus	X		Non Acid Fast
Micrococcus luteus	Coccus	X		Non Acid Fast
Serratia marcescens	Bacillus		X	Non Acid Fast
Mycobaterium smegmatis	Bacillus	X		Acid Fast
Clostridium sporangenes	Bacillus	X		Non Acid Fast
Proteus vulgaris	Bacillus		x	Non Acid Fast
Staphylococcus epidermidis	Coccus	x		Non Acid Fast
Enterococcus (streptococcus) faecalis	Coccus (in chain)	x		Non Acid Fast

Aseptic Technique

1) Aseptic: without contamination
2) Inoculation: transferring microbe from one medium to the next
3) Inoculum: sample(microbe) being transferred
4) Instruments for transferring inoculum: (1) Inoculating Loop (2) Inoculating needle
5) Mediums: Liquid Culture (broth), Slant, and Agar Plate

Streak Plate

1) Used to isolate individual bacteria's in a mixture.
2) After creating a streak plate, you will notice that colonies of individual bacteria begin to form.
3) These colonies should all be the same bacteria.
    a. Bacteria doesn't have a specific color, therefore colonization helps you individualize and identify

Gram Staining

1) Gram staining is based on structure of cell wall
    a. Gram + has thick layer of peptidoglycan
    b. Gram – has thin layer of peptidoglycan surrounded by outer membrane containing lipopolysaccharide

c. Differential Stain: Uses more than 2 dyes to differentiate the bacteria

2) Method for gram staining:
    a. **PRIMARY STAIN=CRYSTAL VIOLET**: Used to stain all of the cells the color purple. (Stain for 1 minute)
    b. Rinse with water then smear is covered in **MORDANT**.
    c. **MORDANT=GRAM IODINE**: Mordant is used to fix the color into the cell and intensify the color.
    d. **DECOLORIZATION=ACETONE-ALCOHOL:** Slide is rinsed with AceA. It creates large holes in the Gram – 's outer lipopolysaccharide wall (Alcohol dissolves lipids). AceA washes out the iodine-crystal violet complex. Since the Gram + has such a thick layer, it retains some of the Iodine-CV complex. At this point, Gram – is colorless & Gram + is purple.
    e. **COUNTERSTAIN=SAFRANIN:** Safranin is applied for 1 minute. After rinsing with water, Gram + will be purple & Gram – will be pink.

Acid Fast Stain (Ziehl-Neelsen Method)

1) Staining technique used to differentiate between acid fast and non acid fast bacteria.
    a. Acid Fast genera include Mycobacterium and Nocardia. Pathogenic Mycobacterium species causes TB and leprosy.
    b. Acid fast cells have a waxy cell wall. **MYCOLIC ACID** in the cell walls make them resistant to desiccation (drying out) and disinfectants.
    c. Zeihl-Neelsen method uses a steaming water bath to help stain the cells
        i. Heating increases the fluidity of the waxy cell layer, and allows primary stain to penetrate the wall of acid fast cells.
    d. As slide cools, the waxy cell wall begins to firm up and traps the stains.
2) Ziehl-Neelsen Method
    a. After heat fixing smear, slide is placed over boiling water bath.
    b. Small piece of paper is used to cover the slide so that we can apply the primary stain. Paper also used to prevent color from evaporating.
    c. **PRIMARY STAIN=CARBOL FUCHSIN:** Is used to soak the piece of paper while over the steam bath. Keep adding more Carbol fuchsin as it evaporates. Heating allows stain to penetrate the cell. Steam for at least 5 minutes.
    d. Remove the slide off of the bath, let it cool, and rinse with distilled water
    e. **DECOLORIZER=ACID ALCOHOL**
    f. **COUNTERSTAIN=METHYLENE BLUE:** Counterstain for 3 minutes then view under microscope after rinsing off
        i. ACID FAST WILL BE REDDISH PINK
        ii. NON ACID FAST WILL BE BLUE (due to counterstain)

Endospore Stain (Schaeffer-Fulton Method)

1) Bacteria are subjective to their environment
    a. Bacteria must adapt to changing weather conditions or die.
    b. Two genera have found a way to adapt when exposed to adverse environment conditions, nutrient limitation, and waste accumulation

i. Bacillus- aerobe
    1. Bacillus Anthracis
ii. Clostridium-anaerobe
    1. Clostridium tetani - Tetanus
    2. Clostridium perfringens - Gas gagreme
    3. Clostridium botulinum - Botuslism

c. Vegetative cells (actively metabolizing) of these genera begin sporulation, and eventually form endospores (very difficult to destroy)

2) Endospore formation
    a. When vegetative cells are stressed, process of sporulation begins
    b. Bacterial chromosome begins to replicate and is separated within cytoplasm
    c. Septum is formed within the cell. One side holds the **FORESPORE** (mature into endospore). Other side is called the **SPORANGIUM**
        i. Sporangium is metabolically active and synthesizes compounds necessary for the spore coat
        ii. Spore coat contains peptidoglycan, keratin, calcium, and dipicolinic acid
    d. Sporangium engulfs the forespore and begins to create spore layers
    e. Cortex (outer layer structure) and coat layers are deposited.
    f. Endospore has matured, and free spore is released with the loss of sporangium
    g. Once conditions are favorable, germination occurs and releases a new vegetative cell
3) Endospore Staining
    a. Prepare a smear, place small piece of paper over smear, and place the slide over boiling water bath.
    b. **PRIMARY STAIN=MALCHITE GREEN:** Stain for 5 min without letting the dye evaporate.
        i. Malachite green is able to penetrate and dye the spore coat
    c. Remove slide slide after 5 minutes and let it cool.
        i. Cooling allows dye to be trapped inside of the coat
    d. **DECOLORIZER=WATER ONLY**
    e. **COUNTERSTAIN=SAFRANIN:** Counterstain for 1 min.
        i. Safranin helps colorize the vegetative cells

<u>Types of Media</u>

1) Two different types of media to help us distinguish bacteria
    a. **SELECTIVE**: Allows only certain bacteria to grow
    b. **DIFFERENTIAL:** Allows us to distinguish one colony from another
2) **Blood Agar [5-10% Sheep Blood]**
    a. Differential media used to distinguish between pathogenic bacteria's.
    b. Streptococcus and Staphylococcus both make toxin called **HEMOLYSINS**, which has the ability to break down red blood cells.
        i. Alpha hemolysis – Causes partial lysis of RBCs. Hemoglobin breakdown causes bacteria to present with green halo

ii. Beta hemolysis – Complete breakdown of RBC and hemoglobin. Should be able to see clearing of blood through the agar.
iii. Gamma hemolysis – Bacteria can be seen growing, but no visible damage to the blood

3) **Mannitol Salt Agar**
    a. Media that is both selective and differential
    b. Contains **HIGH CONCENTRAION OF SALT (7.5% NaCl)**, which makes the media selective by allowing halophilic (salt loving) staphylococci to grow and inhibiting growth of most bacteria
    c. Also contains **SUGAR: MANNITOL & pH INDICATOR: PHENOL RED**
        i. Bacteria that ferments (breakdown of sugar) mannitol release acid into the medium. This causes the pH indicator in the medium to turn yellow around the colony.
        ii. Non-Disease causing bacteria grow, but does not effect the media color
        iii. Mannitol sugar + phenol red allows us to differentiate between pathogenic and non pathogenic staphylococci
4) **MacConkey Agar**
    a. Media that is both selective and differential
    b. Contains **BILES SALTS AND CRYSTAL VIOLET (makes media selective)**, which inhibit the growth of G+ bacteria and allows G- bacteria to grow.
    c. Also contains **SUGAR: LACTOSE & pH INDICATOR: NEUTRAL RED (allows for differentiation between non lactose and lactose fermenters)**
        i. Lactose is fermented, and acid is released into medium. This lowers the pH and changes media from pink to red color.
            1. Lactose fermenters will be pink
            2. Non-lactose fermenters will be clear/uncolored
5) **Phenylethyl Alcohol Agar (PEA)**
    a. Selective media
    b. Inhibits growth of G- bacteria by interfering with DNA synthesis,
    c. Allows G+ bacteria to grow