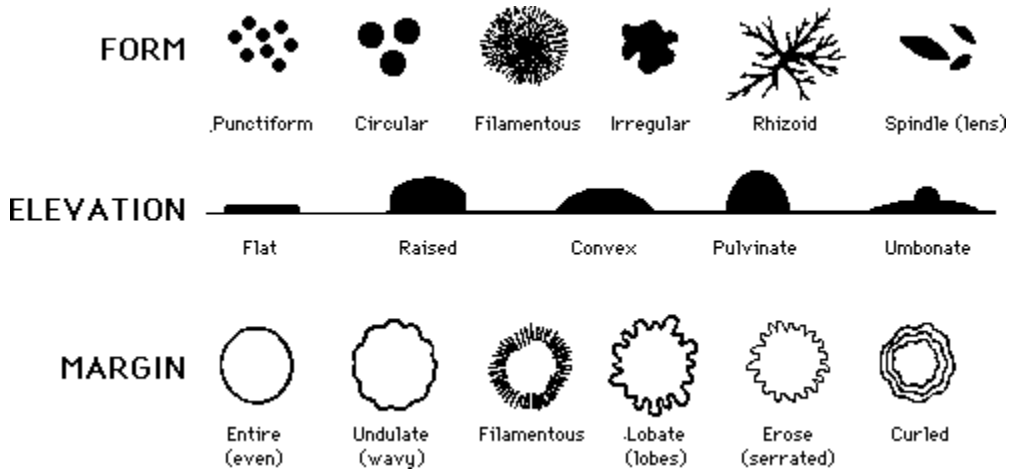
Macro-morphology of Bacterial Colonies

Background:

Bacterial species rarely exist by themselves. They usually live in a community with other bacteria. The samples a clinical lab receives contain a mix of organisms, including the normal flora inhabiting the collection site and hopefully the pathogenic organism causing the disease. The first step in isolating the bacteria is to streak for individual colonies. Next, a microbiologist will examine the visible appearance, or **macro-morphology**, of the isolated colonies in order to try and recognize different species. Some bacterial colonies are visually very different.

Microbiologists use a standard set of terms when describing the macro-morphology of bacterial colonies. They are listed and illustrated below.



http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayarticle&art_id=119

SIZE: **pinpoint, small, medium, large**

COLOR: **non-pigmented, white, creamy, tan**

TEXTURE: **moist, mucoid, dry**

OPTICAL QUALITY: **opaque, translucent, dull, shiny**

HEMOLYSIS: **Beta, alpha, alpha prime, gamma**

- ***Beta hemolysis*** is indicated by a clear colorless zone surrounding the colonies. There has been total lysis of the red blood cells.
- ***Alpha hemolysis*** is indicated by a small zone of greenish to brownish discoloration of the media. This is caused by the reduction of hemoglobin to methemoglobin and its subsequent diffusion into the surrounding medium.
- ***Alpha prime hemolysis*** is indicated by a zone of complete hemolysis, surrounded by a zone of partial hemolysis, a pink halo. This pattern can be easier to see if you scrape off the colony.
- ***Gamma hemolysis*** is indicated by no change in the media.

The purpose of this lab is to introduce you to most of the qualities described above (Form, Margin, Size, Color, Texture, Optical Quality, and Hemolysis) so that you can begin to "see" like a microbiologist and utilize this appropriate terminology in future laboratory exercises. To do this, your group will begin with one broth culture containing 3 different bacterial species. You will then streak appropriately for isolation and/or plate out serial dilutions in order to isolate the separate bacterial species as individual colonies. Your instructor may then have you subculture and Gram stain your isolates.

Materials:

1. **Form:** TSB broth culture containing *Staphlococcus aureus*, *Staphlococcus saprophyticus*, and *Bacillus cereus mycoides*
2. **Margin:** TSB broth culture containing *Staphlococcus epidermidis*, *Staphlococcus saprophyticus*, *Bacillus cereus mycoides*
3. **Size:** TSB broth culture containing *Escherichia coli*, *Staphlococcus epidermidis*, and *Bacillus cereus*
4. **Color:** TSB broth culture containing *Escherichia coli*, *Staphylococcus haemolyticus* and *Staphlococcus xylosis*
5. **Texture:** TSB broth culture containing *Klebsiella pneumoniae*, *Bacilllus cereus*, and *Escherichia coli*
6. **Optical Quality:** TSB broth culture containing *Bacillus cereus*, *Enterobacter aerogenes*, and *Staphlococcus aureus*

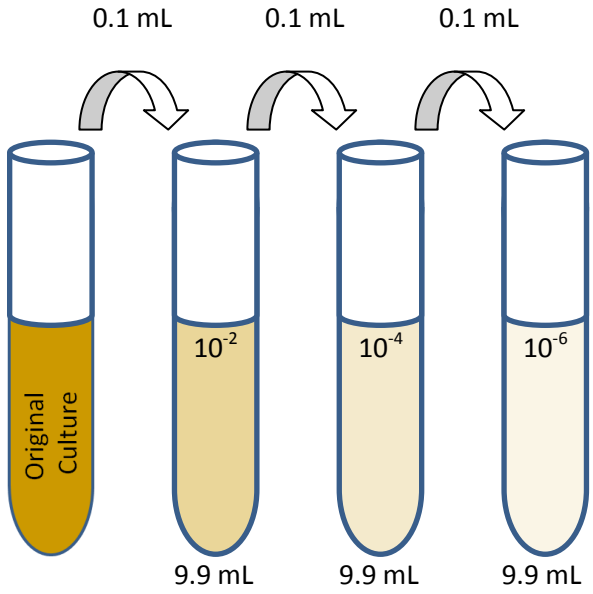
- TSA plates – 8 plates per group
- Sterile test tubes – 3 per bench
- Sterile water bottles – 1 per bench
- 1mL sterile pipettes with matching blue pipette aid
- 10mL sterile pipettes with matching green pipette aid
- 100µL micropipettes with matching 1-200µL micropipette tips
- Sterile L-Shaped Spreaders
- Hemolysis Plates (Day 2)

Procedures:

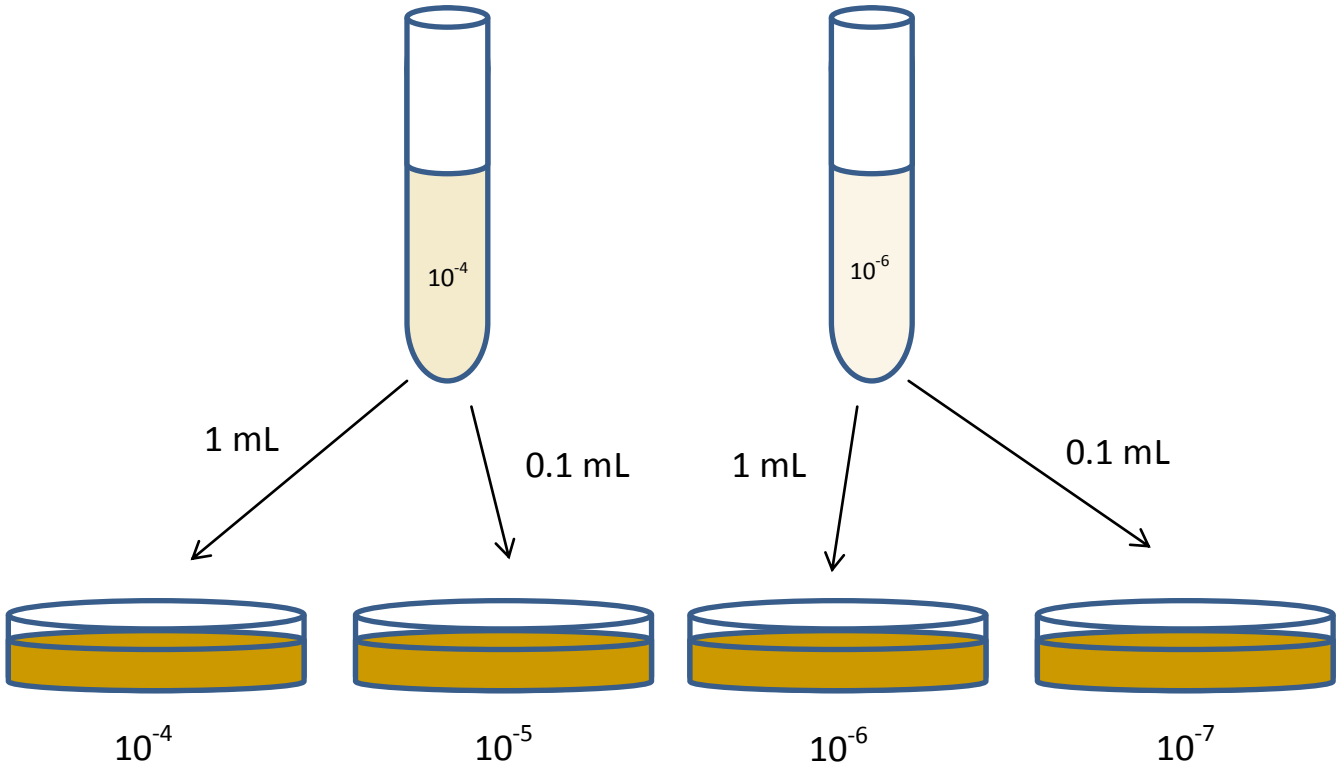
Day 1: Streak for Isolation and Small Volume Serial Dilutions

Each group will receive one of the six TSB broth cultures mentioned above: Form, Margin, Size, Color, Texture, or Optical Quality. Each group member will be responsible for streaking their own isolation plate from this broth culture. Each group will perform a serial dilution and share their results with the class.

1. ***Streak for Isolation:*** Assign broth cultures and then streak for isolation on TSA plates according to the Bacterial Isolation protocol outlined on the lab website and demonstrated by the IA.



2. ***Small Volume Serial Dilutions:*** Beginning with the same broth culture, make 10^{-2}, 10^{-4}, and 10^{-6} serial dilutions by adding 0.1 mL to 9.9 mL sterile water. Thoroughly mix samples before transferring to the next dilution. Your IA will demonstrate how to do this.
3. ***Plating:*** Plate 0.1 mL and 1.0 mL each of the 10^{-4} and 10^{-6} culture dilutions onto TSA plates. This will amount to 10^{-4}-10^{-7} dilutions being present on the agar plates.



Day 2: Macro-morphology Observations, Colony Counts, and Sub-culturing

1. ***Streak for Isolation:*** Carefully observe your isolation streaks and note whether you have successful isolations. If you successfully isolated your bacterial species, you should observe the following:
    a. **Size:** *B. cereus* will appear as large colonies, *E. coli* as medium colonies, and *S. epidermidis* as pinpoint colonies.
    b. **Color:** *E. coli* will appear as tan colonies, *S. xylosus* as yellow-orange colonies, and *S. haemolyticus* as white colonies.
    c. **Texture:** *K. pneumoniae* will appear as mucoid colonies, *B. cereus* as rough/dry colonies, and *E. coli* as moist colonies.
    d. **Optical Quality:** *B. cereus* will appear as dull colonies, *S. aureus* as opaque colonies, and *E. aerogenes* as shiny colonies.
    e. **Form:** *S. aureus* will appear circular, *S. saprophyticus* as irregular colonies, and *B cereus mycoides* as rhizoid colonies.
    f. **Margin:** *S. epidermidis* will appear as entire, *S. saprophyticus* as undulate or lobate colonies, and *B cereus mycoides* as filamentous colonies.

2. ***Hemolysis:*** There is one set of Hemolysis blood plates for all lab sections to share. Carefully observe the blood plates for the following:
    a. ***Beta Hemolysis:*** *S. pyogenes* will have clear colorless zones surrounding the colonies.
    b. ***Alpha Prime Hemolysis:*** *S. aureus* will have a zone of complete hemolysis (clear) surrounded by a zone of partial hemolysis (pink halo)
    c. ***Alpha Hemolysis:*** *S. xylosus* will have a small zone of greenish to brownish discoloration or the blood agar surrounding the colonies.
    d. ***Gamma Hemolysis:*** *S. epidermidis* will have no hemolysis.

3. ***Calculate the original CFU/mL:*** Observe your serial dilution plates. Count the total number of colonies obtained, regardless of species, from your 10^{-4}, 10^{-5}, 10^{-6}, or 10^{-7} dilutions and calculate the colony forming units per mL (CFU/mL) as indicated below. Express your answers in scientific notation with 2 significant figures.

$$\text{CFU/mL} = \frac{\text{\# colonies}}{\text{mL plated}} \times \text{dilution factor}$$

For example, if you counted 150 colonies on the 10^{-7} plate with an inoculum of 0.1 mL taken from your 10^{-6} dilution, then:

$$\text{CFU/mL} = \frac{150}{0.1\ \text{mL}} \times 10^{6} = 1.5 \times 10^{9}\ \text{CFU/mL}$$

4. ***Subculture:*** Streak an isolated colony onto a new agar plate as demonstrated by the IA. This is called **subculturing**, and the purpose is to create an agar plate with a "pure culture" of only one bacterial species on it. Subculture <u>one</u> of the species isolates per original broth culture.
    - If your isolation was unsuccessful, repeat the streak for isolation and/or serial dilution procedures from your original broth culture.
    - Record all observations as drawings of colonies followed by verbal descriptions in the Results section of your lab notebooks. Also, describe any difficulties you encountered or errors in technique in your Discussion section.

Day 3: Continue Macro-morphology Observations and Gram Stain Subcultures

1. ***Gram Stain:*** Observe your subcultures. Note whether you were successfully able to grow just one bacterial species on your agar plate and Gram stain each to ensure culture purity.
    - Record all observations as drawings of colonies and gram results followed by verbal descriptions in the **Results** section of your lab notebooks. Also, describe any difficulties you encountered or errors in technique in your **Discussion** section.