Part 1: Eosin Methylene Blue Agar, Mannitol Salt Agar, and Blood Agar

Theory:

Eosin Methylene Blue (**EMB**) **Agar** is a type of media that falls under three categories:

Complex — This means part of the media (the **peptone**) is "**chemically undefined**". The peptone comes from animal **milk** or **meat** which is digested by proteolytic enzymes into various small peptides, but can also include various fats, metals, salts, and vitamins which were in the original biologic starting material.

Selective — It favors the growth of **enteric** bacteria (also known as "**coliforms**" as they come from your colon). **Lactose** is added to encourage the growth of *Escherichia coli* and **Sucrose** is added to support the species of *Proteus* and *Salmonella*.

— Another part of EMB agar that makes it selective are the dyes **Eosin Y** and **Methylene Blue** which inhibit the growth of many Gram-positive bacteria.

Differential — The two dyes, Eosin Y and Methylene Blue react with the bacteria that can ferment the sugar Lactose. The acidic conditions resulting from Lactose fermentation will result in a color change in the colony.

— The **vigorous lactose fermentation** by ***Escherichia coli*** results in a much lower pH and thus a colony with a shiny **Dark Green** to **Purple** to **Black** color.

— **Less vigorous lactose fermenters** such as species of ***Enterobacter*** or ***Klebsiella*** results in a pH that does not drop as low which produces a colony with a color range of **pink** to **dark purple**.

— **Non-fermenters** of Lactose and any which are Sucrose fermenters will typically hold on to their normal color or take on the coloration of the EMB's reddish-colored media.

Mannitol Salt Agar (MSA) also falls under the same three categories, the last two for different reasons:

Complex — MSA also contains **peptone** as well as **beef extract**. Any media which contains animal, plant or yeast derived components (usually enzymatically digested) is labeled as "complex".

Selective — The added Sodium Chloride (NaCl) is at a high enough concentration to dehydrate and kill most bacteria. Nutrient agar has a salt concentration of 0.5%, while MSA has a salt concentration of 7.5% (15 times more concentrated!). Only bacteria which are **halophiles** such as *Staphylococcus aureus* and *Staphylococcus epidermidis* (which can easily grow on your sweaty skin) can survive and grow on MSA agar. **Halophilic** bacteria either need the salt to grow or they can tolerate its presence.

Differential — Mannitol is a sugar alcohol that can be fermented by *Staphylococcus aureus* but not *Staphylococcus epidermidis*. The fermentation process lowers the pH and the **Phenol Red dye** added to MSA will turn yellow once the pH drops below 6.8. This results in a colony that is yellow or has a yellow halo around it.

Blood Agar is Tryptic (Trypticase) Soy Agar (TSA) containing 5% sheep blood. Because we will be culturing the pharynx (throat) it is important to consider that the organisms growing here are used to a higher carbon dioxide content—especially if the sample is obtained near the tonsils where they have deep pits. To grow well, these organisms not only need an enriched media, but also a **microaerophilic** atmosphere (one low in oxygen). After inoculation, the Blood Agar plates will be placed in a **Candle Jar** which (instead of a candle) will have a special packet that absorbs the oxygen once the jar is sealed. Blood Agar is a type of media that also falls into many categories:

Complex — The TSA portion contains enzymatically digested soybean meal, casein and yeast extracts. The other obvious ingredient with unknown components is the sheep blood.

Enriched
- — While Nutrient Agar is used for growing non-fastidious organisms and for easily seeing a colony's natural pigment color, Blood Agar is able to support fastidious organisms (i.e., "picky eaters").
- — The **TSA portion** will support many "**semi-fastidious**" bacteria such as species of *Brucella*, *Listeria*, *Corynebacterium*, *Neisseria*, and *Vibrio*.
- — The **5% Sheep Blood** will support additional "**highly-fastidious**" bacterial species such as various species of *Streptococcus*, which are commonly found in the mouth and throat. *Don't drink, rinse, or gargle any fluid before swabbing the throat as those activities can greatly reduce their numbers before you obtain the sample.*

Differential
- — Once the fastidious bacteria are growing on the Blood Agar, any hemolytic abilities they possess can now be seen. There are three types of hemolytic (blood–digesting) categories:
- — **Beta–Hemolytic** bacteria such as *Streptococcus pyogenes*, and *S. agalactiae*, will **completely lyse** the red blood cells resulting in a **clear zone around and under the colony**. ***S. pyogenes*** is also known as **Group A strep** is NOT part of the normal flora of the mouth and throat and is a common cause of pharyngitis and impetigo. ***S. agalactiae*** is also known as **Group B strep** and while part of the normal flora of the GI tract and the vagina, if it is present in the vagina when the water breaks, it can transfer to the infant and cause pneumonia and meningitis.
- — **Alpha–Hemolytic** bacteria such as *Streptococcus mitis* or *S. viridans*, will **partially lyse** the red blood cells resulting in a **green zone around and under the colony**. *S. viridans* is a common microbial culprit in cavity formation.
- — **Gamma–Hemolytic** bacteria are also called **Non–Hemolytic** bacteria as they do NOT have the enzymes to lyse open (i.e., digest) red blood cells. If it is not Beta or Alpha, then the organism is Gamma–Hemolytic by default.

Part 2: Sampling Normal Flora Microorganisms

Lab Materials:

Amount per Student	Material
1	MSA plate
1	Blood Agar plate
1	EMB plate
1 (as needed)	tubes of sterile saline (as needed, if your skin is really dry)
3	sterile cotton swabs
1	tongue depressor
1 (at your station)	Bunsen Burner
1 (at your station)	Inoculation Loop

Lab Procedure:

Gastrointestinal Culture:

1. Wet the sterile swab in sterile saline solution and return it to the packaging.
2. Go to the bathroom and swab your rectum and return the swab to the packaging for transport back into the lab. Be sure to wash your hands before returning to lab. As long as the swab is in the packaging, you should be fine to rest it on the counter in the restroom while you wash your hands, but as a courtesy, rest it on a dry paper towel.
3. Swab 1/2 of the **EMB plate** with the rectal sample and perform a streak isolation on the rest of the plate using an inoculation loop in the usual fashion.
4. Label your plate, invert it and place it in the 37 ºC incubator. I will remove the plates in 24 hours to room temperature. *The heat accelerates the growth, but it is not required for the microbes to grow.*
5. When you examine the plates next week, the presence of any colonies of *E. coli* or species of *Citrobacter* will produce a green metallic sheen. If *E. coli* is refrigerated, or cools off too much, it will lose its metallic sheen and its colonies will appear pink.

Throat Culture:

1. Wet the sterile swab in sterile saline solution and have another person in lab swab your throat. To do this, use a tongue depressor and be sure to NOT hit the tongue, but rather hit the back of the oropharynx where the uvula is. One or two seconds of swabbing is enough.
2. Swab 1/2 of the **Blood Agar plate** with the throat sample and perform a streak isolation on the rest of the plate using an inoculation loop in the usual fashion.
3. Label your plate, invert it and place it in the candle jar. Once all the plates are in the candle jar, your instructor will insert a tear-open packet which will remove the oxygen and the jar will be incubated at 37 ºC for 24 hours. *The heat accelerates the growth, but it is not required for the microbes to grow.*
4. Observe the plates during the next lab for any colonies of Beta or Alpha hemolysis bacteria.

Skin Culture:

1. Wet the sterile swab in sterile saline solution and swab a sweaty location of your skin. The best areas are behind your ears, along your neck line and between your shoulder blades. Avoid your armpit as deodorant will affect your results.
2. Swab 1/2 of the **MSA plate** with the sweaty skin sample and perform a streak isolation on the rest of the plate using an inoculation loop in the usual fashion.
3. Label your plate, invert it and place it in the 37 ºC incubator. I will remove the plates in 24 hours to room temperature. *The heat accelerates the growth, but it is not required for the microbes to grow.*
4. Examine your plates at the next lab for the presence of mannitol fermentation which will appear as yellow pigmented halos around the colonies of *S. aureus*, which is also a bacterial colony that appears yellow while *S. epidermidis* will appear as a white colony with no yellow halo.

Lab 5 Questions (Due at the end of lab)

Name: _____ Grade: _____ of 10 points

1. When the rectal sample is obtained, you will very likely get a mixture of skin normal flora (such as *Staphylococcus aureus*, and *Staphylococcus epidermidis*) and colonic bacteria (such as *Escherichia coli*, and other coliforms like *Enterobacter*). All of these bacteria are on the cotton swab and all are applied to the EMB agar.
   a. (1 point) What **ingredient/property** of EMB agar prevents the skin microbes from growing well?

   b. (2 points) What **ingredient/property** of EMB agar allows you to distinguish among multiple colonic bacterial species? **Explain** what you would look for and **why** it would look that way.

2. If you wanted to, you could have used the same rectal swab and applied it to the MSA plate. Remember it will have a mixture of Gram-negative normal colonic flora and Gram-positive normal skin flora.
   a. (1 point) What **ingredient/property** of MSA agar prevents the colon microbes from growing well?

   b. (2 points) What **ingredient/property** of MSA agar allows you to distinguish among the multiple skin bacterial species? **Explain** what you would look for and **why** it would look that way.

3. All microbes can be applied to blood agar to see if they can grow on it and which of the three types of hemolysis would result (alpha, beta, or gamma). Human tonsil tissue has an incomplete capsule and deep pit (called tonsillar crypts) where food and bacteria (such as *Streptococcus pyogenes*) can get trapped and occasionally cause a case of tonsillitis. It is possible to culture *S. pyogenes* on Blood agar (in addition to other normal flora microbes).
   a. (2 points) What **physical features of the tonsil** described above, and what **enrichment ingredient** make it the tonsil a more likely location for a *S. pyogenes* infection than the areas just next to the tonsils (such as your cheeks or palate)?

   b. (1 point) If you think you have a case of tonsillitis, then **what should you NOT do** before you go and visit the doctor to have the best chance of the doctor finding the bacteria? Provide a short explanation of **why**.

4. (1 point) If you wanted to catch people *not washing their hands after pooping* and all you were allowed to do was to swab their hands as they exited the bathroom, then **which of today's media** (EMB, MSA, or Blood Agar) would be the most reliable one to use in order to prove their bad habit and **why**?