Blood agar (BAP) is a common medium used to culture bacteria because 1) it is a great enrichment medium for fastidious bacteria, and 2) hemolysis of blood cells can be very useful as an identification test. Blood agar is made with 5% sheep blood. This You have actually used a variation of blood agar before, in CNA agar: the only difference is that CNA has an antibiotic that inhibits gram – bacteria.

Hemolysis is the breakdown of red blood cells: hemolysins are enzymes produced by some bacteria and are released into the medium around the bacterial colony. It can be a complete breakdown of the cells, with the release of hemoglobin and a clearing of the red from the surrounding medium around the colony. Or the hemolysis can be a partial breakdown, resulting in a greenish or green-yellow zone around the colony.

You will find a variety of bacteria in the throat: it is normal. This is true also of the ear and nose, but there is less diversity. In this lab exercise, different people on the table will use different specimens. In addition to your identification of different hemolytic reactions, you are also going to isolate a Staphylococcus species to be used in a later exercise---one on the Kirby-Bauer test for antibiotic sensitivity. The candle jar will produce a high carbon dioxide/low oxygen environment which is advantageous for microaerophilic bacteria. As the oxygen is burned off to less than 10%, the carbon dioxide level rises to around 10% as a result of the combustion process.

OBJECTIVES:

Differentiate among various species from a clinical specimen.
Isolate a species of Staphylococcus for antibiotic susceptibility testing.
Identify the 3 hemolytic types on blood agar.

MATERIALS NEEDED:

sterile swabs
1 blood agar plate/person
tongue depressers
candle jar for the lab section

NEXT period: hydrogen peroxide for catalase test
sample plates of various hemolyses (Streptococcus and Staphylococcus)

THE PROCEDURE:
1. **Each person will prepare a culture:** different people on the lab table can pick different specimen types—ear, nose, throat, and a KISS plate. *At the very least*, the ear and nose specimens need to be performed.

2. **THROAT CULTURE:** Take a sterile swab and place it in the orifice of your choice. If performing a throat swab, be sure to hold the tongue down with the tongue depresser while going to the BACK of the throat with the swab.

3. **THROAT, NOSE, EAR CULTURES:** Prepare a streak plate (see earlier procedure if you need a reminder). However, you will use the swab for the first section of the plate, rolling it around to get as many bacteria off as possible. Switch over to your inoculating loop and continue on as you would a regular streak plate.

4. **KISS PLATE:** The agar plate is inoculated by kissing it—*gently*, because the agar medium is not very strong. You also want to slightly touch the nose while kissing, so you can see the various populations in different areas.

5. Place the plates upside down in the candle jar. When all plates are in the jars, the candle will be lit, and the jars incubated at 37 °C.

**INTERPRETATION:**

After incubation you will check the plates for:

- **different hemolytic reactions**
  - complete hemolysis = beta hemolysins produce a clear, yellow zone
  - partial helomysis = alpha hemolysins produce an opaque green/green-yellow zone
  - no hemolysis = gamma, no hemolysins, no zone

![Hemolytic Reactions](image)

- **different gram reactions** Gram stain a couple of different species. In particular, you are looking for a G+ coccus in clusters (potentially *Staph*).
- **a *Staph* isolate**
  1. Find a colony that is white, off white, or cream pigmented on the BAP. Gram stain. You will confirm that it is a *Staph* not only with a gram stain, but with a catalase test.
  2. Once the colony is confirmed as a *Staph* species, subculture the colony onto a TSA plate, streak for isolation.
  3. Incubate at 37 °C and then check for purity of the culture.
  4. You will run the antibiotic susceptibility test on your own species of *Staph* in the next week or after.
  5. **One period** before the day we are to perform the antibiotic sensitivity test, you need to subculture your *Staph* isolate into an TSB broth.

**CATALASE TEST:**

Place the inoculum from the BAP onto a microscope slide (this is not a smear, no water!).
Add one drop of \( \text{H}_2\text{O}_2 \) and watch for immediate bubbles (\( \text{O}_2 \) released from the hydrogen peroxide).

QUESTIONS:

1. Which type of hemolysis is often associated with pathogenicity?

2. What are the distinguishing features of \textit{Staph}?

3. What does the environment of the candle jar tell you about the environment of the throat and mouth?

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