Blood Agar

**PURPOSE** Blood agar is used for isolation and cultivation of many types of fastidious bacteria. It is also used to differentiate bacteria based on their hemolytic characteristics, especially within the genera *Streptococcus*, *Enterococcus*, and *Aerococcus*.

**PRINCIPLE** Several species of Gram-positive cocci produce exotoxins called hemolysins able to destroy red blood cells (RBCs) and hemoglobin. Blood agar, which is a mixture of tryptic soy agar and sheep blood, allows differentiation of bacteria based on their ability to hemolyze RBCs.

The three major types of hemolysis are β hemolysis, α hemolysis, and γ hemolysis. β hemolysis is the complete destruction of RBCs and hemoglobin, and results in a clearing of the medium around the colonies (Figure 6-10). α hemolysis is the partial destruction of RBCs and produces a greenish discoloration of the agar around the colonies (Figure 6-11). γ hemolysis is actually non-hemolysis and appears as simple growth with no change to the medium (Figure 6-12).

Hemolysins produced by streptococci are called streptolysins. Streptolysins come in two forms—type O and type S. Streptolysin O is oxygen labile and expresses maximal activity under anaerobic conditions. Streptolysin S is oxygen stable, but expresses itself optimally under anaerobic
The easiest way to provide an environment favorable for streptolysins on blood agar is to perform what is called the streak-stab technique. In this procedure the blood agar plate is streaked for isolation and then stabbed with a loop. The stabs encourage streptolysin activity due to the reduced oxygen concentration of the subsurface environment (Figure 6-13).

**Figure 6-10.** β-Hemolysis. *Streptococcus pyogenes* demonstrates β-hemolysis. The clearing around the growth is due to complete lysis of red blood cells. This photograph was taken with transmitted light.

**Figure 6-11.** α-Hemolysis. An unidentified throat culture isolate demonstrating α-hemolysis. The greenish zone around the colonies is due to incomplete lysis of red blood cells.

**Figure 6-12.** γ-Hemolysis. A streak plate of *Staphylococcus epidermidis* on a Sheep Blood Agar illustrates no hemolysis.

**Casease Test**

- **PURPOSE** The casease test is used to identify bacteria capable of hydrolyzing casein with the enzyme casease.

- **PRINCIPLE** Many bacteria, like other living organisms, require proteins as a source of amino acids and other components for synthetic processes. Some bacteria have the ability to produce and secrete enzymes into the environment that catalyze the break-down of large proteins to smaller peptides or individual amino acids, thus enabling their uptake across the membrane.

  **Casease** is an enzyme some bacteria produce to hydrolyze the milk protein *casein* (Figure 6-14). Casein is the molecule that gives milk its white color. When broken down to smaller fragments, the ordinarily white casein loses its opacity and becomes invisible to the naked eye.

  The presence of casease can easily be detected with the test medium, Milk Agar. Milk Agar is an undefined medium containing peptone, beef extract, and casein. When plated Milk Agar is inoculated with a casease-positive organism, secreted casease will diffuse into the medium around the colonies and create a zone of clearing where the casein has been hydrolyzed (Figure 6-15). Casease-negative organisms do not secrete casease and, thus, do not produce clear zones around the growth.
Blood agar

- α hemolysis - Streptococcus pyogenes (strept throat)
  - B hemolytic strep
  - Hemolysins -> RBC
  - Halo (greenish) - partial lysis of RBCs
  - Blood digested (clearing of RBCs)
- β hemolysis - complete lysis of RBCs
- γ hemolysis - no hemolysis of RBCs

bacterial growth:
- α = alpha
- β = beta
- γ = gamma