

HEMOLYSIS

Principle and Purpose

A number of pathogenic bacteria are capable of lysing red blood cells (RBCs). When grown on blood agar (BA) plates, three different types of RBC lysis can be discerned: alpha (α), beta (β), or gamma (γ) (Fig. 1). Bacteria that are α -hemolytic only partially lyse RBCs and generate a zone of greenish coloration around colonies. The greenish tint is the result of the oxidation of iron released by the lysed RBCs. In contrast, β -hemolytic bacteria completely lyse RBCs forming a clear zone surrounding colonies. Strains that produce no clearing of any type around bacterial colonies on BA are termed γ -hemolytic.

Among the cocci, all three types of hemolysis can be observed. Within the streptococci, hemolysis patterns can help differentiate the various groups and narrow the possible identification of an isolate. Species of *Staphylococcus* and *Enterococcus* may also exhibit different patterns of hemolysis, so additional tests beyond the detection of RBC lysis must be conducted to provide a definitive identification. Nonetheless, RBC hemolysis is often used in the clinical lab as a starting point in identifying pathogenic cocci.

The following simple exercise shall present students with definitive examples of α , β , and γ hemolysis.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand how hemolysis can be used to differentiate among different bacterial strains;
- Properly conduct the hemolysis test; and
- Accurately interpret the results of this test.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms - The following organisms should be provided as 24-48 hour-old TSA slants/plates or TSB cultures:

- *Streptococcus pyogenes* (ATCC 19615) [abbreviated at *S. pyogenes*]
- *Streptococcus pneumoniae* (ATCC 49619) [abbreviated at *S. pneumoniae*]
- *Staphylococcus epidermidis* (ATCC 12228) [abbreviated at *S. epidermidis*]

Materials

- Blood agar (BA) plates

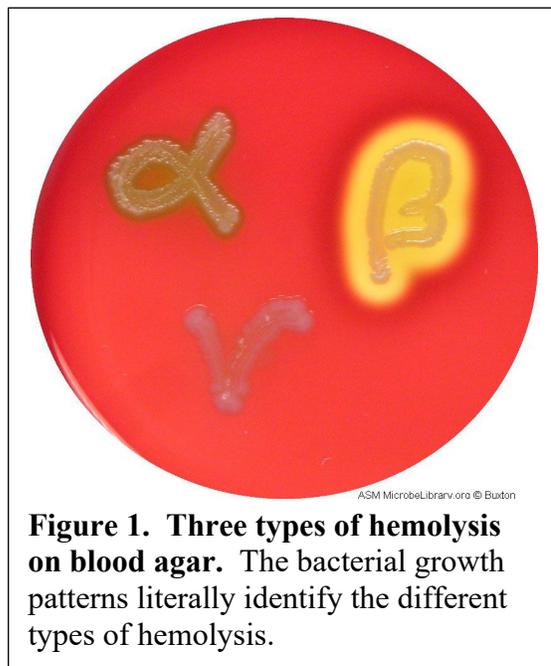


Figure 1. Three types of hemolysis on blood agar. The bacterial growth patterns literally identify the different types of hemolysis.

Procedure

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

- 1) Obtain three BA plates. Label one on the bottom (agar-containing half) as *Streptococcus pyogenes*, another on the bottom as *Streptococcus pneumoniae*, and the remaining plate on the bottom as *Staphylococcus epidermidis*. Include other relevant information as well, e.g., name, date, etc.
- 2) Aseptically remove some growth from the stock culture plate/slant or broth provided and streak it on the BA plate labeled for *S. pyogenes*. Similarly, streak the BA plates labeled for *S. pneumoniae* and *S. epidermidis* using growth from the appropriate stock cultures provided.
- 3) Incubate all three streaked BA plates at 37°C for 18-24 hours.
- 4) Remove the plates and observe any hemolytic patterns expressed by each species.

Record any observations on the data report sheet attached to this document.

Note: BA plates must be read within 24 hours. Incubation of these plates for longer periods of time may affect the ability to correctly assess the results.

- 5) Properly dispose all three plates in the appropriate waste bin.

Interpretation of Results: Figure 1 depicts the three patterns of RBC hemolysis to which the results of the present exercise can be compared.

Student Name: _____

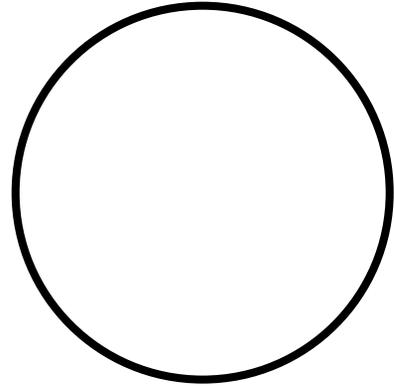
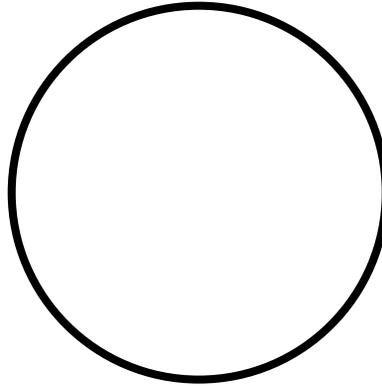
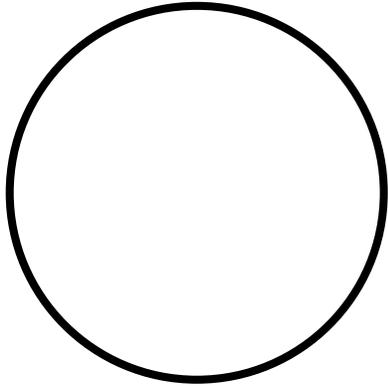
DRAWINGS OF HEMOLYSIS PATTERNS OF VARIOUS COCCI

Within the circles below (representing Petri dishes of blood agar), draw the growth patterns of *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. Be sure to identify the hemolysis pattern of each species.

Streptococcus pneumoniae

Streptococcus pyogenes

Staphylococcus epidermidis



Hemolysis Type _____

Hemolysis Type _____

Hemolysis Type _____

Discussion Question

What is the molecular basis for RBC hemolysis, i.e., what exactly causing the cells to lyse? This question may require additional research. Be sure to cite your source(s).