

# $\beta$ HEMOLYTIC STREPTOCOCCUS DETECTION BY THROAT CULTURE

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[http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Strep\\_Detection/Strep\\_Test.htm](http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Strep_Detection/Strep_Test.htm)

Streptococci cause more disease than any other single genus of bacteria. They are aerotolerant, but do not use O<sub>2</sub> and lack catalase. Most streptococci are non-pathogenic, including lactic acid fermenters found in fermented milk products such as yogurt, buttermilk and sour cream.

*Pathogenic* strains of strep are characterized by two traits:

1. **Hemolysis:** pathogenic strains produce the exotoxin streptolysin which causes the complete lysis of red blood cells. When these strains are grown on Blood Agar, their colonies are surrounded by a halo of complete clearing with a yellowish cast in the bright red agar, called **beta hemolysis**.

Some strains produce partial hemolysis on Blood Agar, and produce greenish turbid halos around the colonies, termed **alpha hemolysis**. Those strains which produce no lysis are termed (for some reason) gamma hemolytic.

2. **Antigenicity:** The M protein is part of the Gram + cell wall, and functions to mediate attachment and to resist phagocytosis. These M proteins have been serologically classified by Rebecca Lancefield into groups A through O. Pathogenic strains of strep are limited to only those possessing the group A antigen.

Thus, pathogenic strep belongs to **Group A, beta hemolytic**. *Streptococcus pyogenes* is the most common member of this group. It causes a wide variety of diseases including:

**Strep throat:** beefy red pharynx, fever, sore throat (80% Strep infections are asymptomatic)

**Puerperal fever:** infection of the uterus following contamination during childbirth.

**Rheumatic fever** (as a sequella) occurs in 3% of untreated strep-infected children, and is thought to be an autoimmune manifestation. One to five weeks following a strep infection the sequelae may also include rheumatoid arthritis, endocarditis, and/or pyelonephritis.

We will learn to perform a diagnostic test for beta hemolysis in which a Blood Agar plate is inoculated with a throat swab (of the oropharynx and palatine tonsils) and incubated at 35°C. Colonies with the  $\beta$  hemolytic reaction on the plate strongly suggests strep throat.

Illustrate the structure of the oropharynx, and to review the subject in an anatomy text.

<b>EQUIPMENT</b>	Tongue depressors	Blood Agar Plates
<b>&amp; SUPPLIES:</b>	Sterile swabs, 15 cm long	Incubator, 35°C
	well-focused light	fresh culture of <i>Streptococcus pyogenes</i>

1. **SELECT A BLOOD AGAR PLATE:** Mark the plate: divide in half, write "throat" on one side, "*S. pyo*" the other, and enter initials, date, and seat number.
2. **SETUP FOR RIGHT HANDED PERSONS:** Position subject at the left seat of the desk, you sit in the isle around the corner at the left end of desk. Place the light to your left and adjust the subject's height so that the *light projects on rear of throat*.
3. Have subject comfortably open mouth, relax tongue. Insert depressor all the way to the rear of tongue and have subject say "Aaaah", depressing the entire tongue to hold out of way.
4. **TAKE SPECIMEN:** Immediately and *GENTLY* swab across the rear of the oropharynx and if present, across the tonsils (suspended on either side of the oropharynx).
5. **APPLY TO BLOOD AGAR PLATE:** Swab the specimen across the surface of the marked Blood Agar Plate (marked "throat"), rolling the swab as you sweep it across, then stab almost to the bottom of the agar several times at the origin of the streak. On the half marked *S.pyo*, streak a known culture of *Streptococcus pyogenes* as a positive control, also stab three times.
6. **INCUBATE:** Place the plate agar side up at 35°C for 24 hrs.
7. **SCORE THE PLATE:** Examine the plate for hemolysis, record and illustrate results. Handle with careful attention to aseptic technique, since known pathogens are growing on the plates.