

CULTURE OF STREPTOCOCCUS PNEUMONIAE

Name: Dessi Loukov

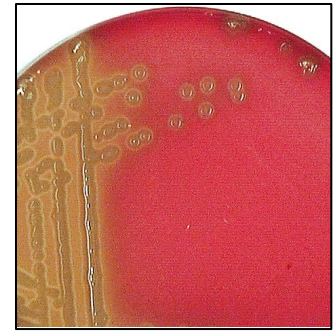
Date: February 11, 2014

Bowdish Lab, McMaster University
Hamilton, ON, Canada

www.bowdish.ca

BACKGROUND

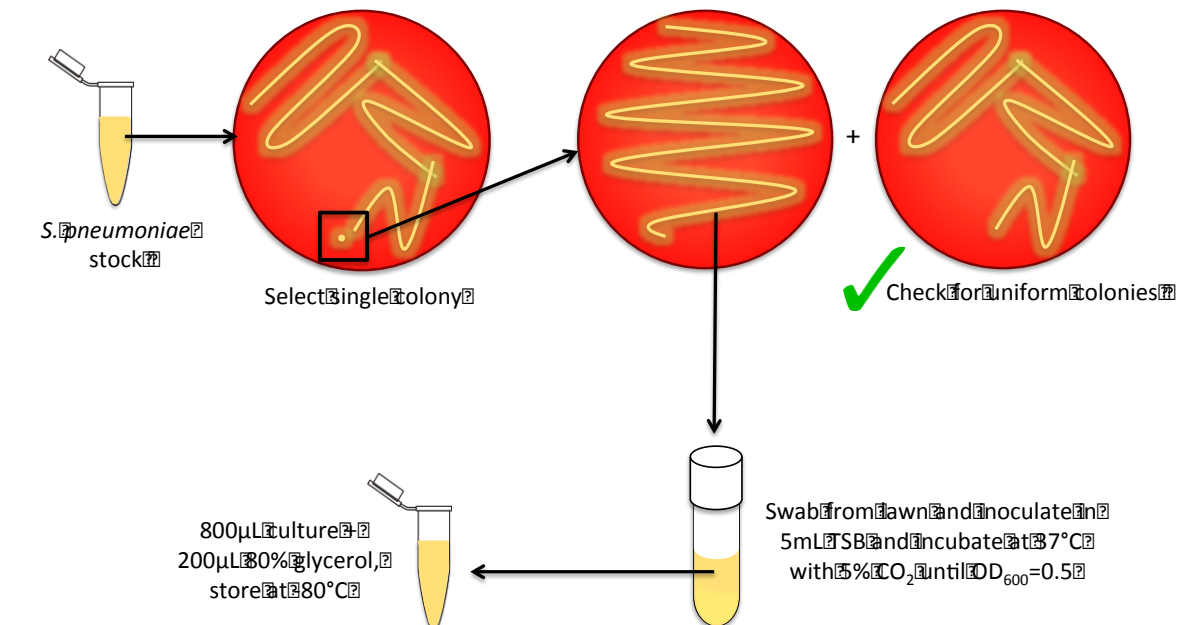
Streptococcus pneumoniae is an anaerobic, gram-positive bacterium that is cultured at 35-37°C with 5% CO₂. *S. pneumoniae* produces H₂O₂ through a flavoenzyme system and grows better in the presence of a catalase source (i.e. red blood cells). The bacterium is alpha-hemolytic and when grown on blood agar colonies appear small, grey and produce a green zone of hemolysis. When cultured for longer than 24 hours, the centres of the colonies are depressed.



MATERIALS

- Tryptic Soy Agar, 5% Sheep's Blood, 10ug/mL Neomycin plates
- Tryptic Soy Broth (TSB)
- 80% Glycerol

PROTOCOL



1. Using a sterile cotton swab, streak isolate of *S. pneumoniae* on a blood plate and incubate at 37°C with 5% CO₂ overnight.
2. Re-streak a single colony on two plates: 1) across the entirety of a fresh plate and 2) using the three-phase streak method. Incubate plates at 37°C with 5% CO₂ overnight.
3. If all colonies appear to be uniform on the three-phase plate, use a sterile cotton swab and inoculate bacterium from the lawn of the other plate in 5mL of TSB.
4. When culture reaches an OD₆₀₀=0.5, 800μL culture can be frozen down with 200μL 80% glycerol and stored at -80°C.