MAKING BLOOD PLATES

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BACKGROUND

S. pneumoniae naturally produces a high concentration of H_2O_2 during growth, thus culturing the bacteria on a plate requires a source of catalase to neutralize this compound. Red blood cells can be utilized as a source of catalase because *S. pneumoniae* are alpha-hemolytic and the enzyme is released into the agar plate as they burst the red blood cells.

NOTES

- If the tryptic soy agar mixture is cooled for too long, small chunks of solidified agar will appear. The mixture can be re-autoclaved or heated on a hot plate. **(Ensure cap of the bottle is loosened to prevent pressure from building up inside the bottle during this process)**

EQUIPMENT

- Autoclave
- Tryptic soy broth powder
- Agar
- Double-distilled water (ddH_20)
- Defibrillated Sheep's blood (Cederlane)
- Neomycin (Sigma-Aldrich)

PROTOCOL

1. Add 15 g of tryptic soy broth powder and 7.5 g of agar to 500 mL of ddH_2O. Autoclave the mixture. (**Important:** Make sure cap is slightly loosened when autoclaving to prevent pressure from building up inside the bottle)
2. Cool the tryptic soy agar mixture at room temperature for 45 minutes – 1 hour. The mixture should reach a temperature where it is comfortable to touch the bottle for 10 seconds with your hands in order to prevent the blood from burning.

3. Add 25 mL of defibrillated sheep's blood and 500 uL of neomycin [10 mg/mL] to the tryptic soy agar mixture. Mix well.
4. Quickly pour the blood plate mixture into 100mm x 15 mm petri dishes to prevent the blood from burning.
5. Cool the blood plates under a BSL 2 cabinet for 1 hour to allow for solidification and to prevent condensation on the plates.
6. Store at 4 °C until further use.