Identification of pneumococcal serotypes from nasopharynx using culture and PCR

NP swab collected →

1.0 ml STGG medium

→ vortex → Store at - 70°C

Quick thaw and vortex →

STGG

→ Return to store at - 70°C

200ul →

5.0 ml enrichment -Todd Hewitt w/ 0.5% yeast (THY) broth + 1.0 ml rabbit serum

4-6h/37°C
5%CO2

200ul →

DNA extraction

↓

DNA store at -70°C

↓

Optional: *-lytA* real time PCR detection

(or skip *lytA* detection step)

Ct ≤30

↓

Multiplex PCR: reactions 1– 8 for 40 serotypes

10ul loop →

Plate culture

↓

Colony isolation, identification (optochin, bile solubility)

↓

Store isolates at -70°C in 1.0ml STGG

↓

Isolates for: conventional serotyping, MIC, genotyping, etc