Shi Media Preparation

1. Preparation of stock solutions
    a. Hemin Solution – Combine in flask and heat to boiling. Store at -20°C and store in 5-10mL aliquots depending upon expected media preparation volumes.

50mg	Hemin	Sigma, 51280
1.74g	K_2HPO_4	Fisher, P288-500
100mL	ddH_2O	Fisher, BP2470-1

    b. NAM Solution – Dissolve NAM in ddH2O and filter sterilize; store at -20°C in 0.5-1mL aliquots.

100mg	N-Acetyl Muramic Acid	Sigma, A3008-100MG
10mL	ddH_2O	Fisher, BP2470-1

2. Combine the following in a clean, autoclavable bottle for 1L Shi Media:

10g	Proteose Peptone	Fisher, BP1420-500
5g	Trypticase Peptone	BD Bacto, 211705
5g	Yeast Extract	BD Bacto, 212750
2.5g	KCl	Fisher, P217-500
10mL	Hemin Solution	(see 1a)
0.06g	Urea	Fisher, U15-3
0.17g	Arginine	Fisher, BP370-100
2.5g	Mucin	Sigma, M1778-10G
990mL	ddH_2O	Fisher, BP2470-12

3. Autoclave at 121°C for 15min, remove from autoclave and let cool to 50°C or place in 50°C water bath and allow temperature to equalize.
4. Add the following to 1L autoclaved mixture

1mg	Vitamin K	Alpha Aesar, L10575
50mL	Sheep's Blood	Colorado Serum Co., CS1122
1mL	NAM Solution	(see 1b)

5. Let cool to room temperature to use immediately or store at 4°C.

Methods Section

SHI medium (1 L) was prepared as follows: 10 g protease peptone (Fisher), 5 g trypticase peptone(BD Bacto), 5 g yeast extract(BD Bacto), 2.5 g KCl, 10 ml hemin solution (from 50 mg hemin stock, Sigma), 0.06 g urea, 0.17 g arginine, 2.5 g mucin (type III, porcine, gastric, Sigma), 990 mL distilled water. This solution was autoclaved in 121°C for 15 min, cool to 50°C: 1 mg vitamin K (Alpha Aesar, dissolved in water and filter sterilized,) 50 mL sheep blood

(CS1122, Colorado serum company), I mL NAM solution was added after cooling. NAM stock solution is prepared as follows: 100 mg N-Acetyl Muramic Acid (Sigma) was dissolved in 10 mL distilled water. The stock solution was then filter sterilized and stored in -20°C.