SOP-M0021

Making Agar for halophilic (salt-requiring) *Arcobacter* Growth Rectangle Plates

Objective: To prepare Agar for growing bacteria for the Bardot Project

Procedure: Use the Use the Brain Heart Infusion Agar Catalog #241830 BD (500g)
Plates: VWR cat#62409-600 Omni Tray w/lid, Sterile cs60 (Mfr #242811)

1. Weigh out 52gm of the powder and suspend it in to 970L of Millipore Water.

2. Weigh out 5gm of Difco agar Noble (Catalog 214230) and add to above mixture.

3. Weigh out 15gm of laboratory-grade NaCl and add to above mixture.

4. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve. Do not overheat.

5. Then Autoclave.

6. Prepare the Ferrous Sulfate solution. Dissolve 0.25 gm $FeSO_4$ in approximately 25ml distilled water and adjust to pH4 with Hydrochloric acid; this will help dissolve the ferrous sulfate more readily. Agitate until dissolved. Add distilled water to adjust the solution to a volume of 30 ml then filter sterilize.

7. Once autoclaved, place the bottles in a pre-warmed water bath, set at 60ºC. The agar should be around 55ºC before pouring. This usually takes approximately 10-15 minutes.

8. Then remove from the water bath and place the bottles on the flat surface. Place the sterile petri dishes on the flat surface to be filled with the agar.

9. Add the Ferrous sulfate solution to the agar and gently swirl to mix.

10. **Pipette 35ml** (draw up 37 but use only 35ml) of the agar into the petri dishes being careful not to create bubbles. If bubbles do occur, just remove them with the pipette. Let cool and then place them with the agar side up carefully in the plastic sleeve they came in. Seal the sleeve with tape and label each stack with date and type of agar. The next day, place all plates in the 37° incubator to check for sterility overnight. If no contamination, replace plates back in the sleeve and label "sterility check-good". Store in the cold room until needed.

Created by: Stephen On

Verified by:_____Date:____10/17/2019_____   _____

                        Print Name   Sign Name