2.0 PURPOSE

This document describes the method for preparation of Asparagine Broth.

3.0 SCOPE

Asparagine Broth is a medium used in the confirmation step for Pseudomonas aeruginosa.

4.0 RESPONSIBILITY \ BUSINESS RULES

All microbiology staff in the laboratory.

5.0 PROCEDURE

5.1 Materials and Reagents required

5.1.1 Plastic spoon
5.1.2 Measuring Cylinder
5.1.3 RO Water & RO water squeeze bottle
5.1.4 3 Glass beakers
5.1.5 Funnel
5.1.6 Flat medical bottles / Schott bottles
5.1.7 Automatic dispenser
5.1.8 DL-Asparagine Monohydrate
5.1.9 Di Potassium Hydrogen Phosphate $K_2HPO_4$
5.1.10 Magnesium Sulphate Heptahydrate $MgSO_4\cdot7H_2O$
5.1.11 pH meter
5.1.12 1N NaOH and 1N HCl

5.2 Method

5.2.1 Weigh out 3.0g per litre of Asparagine DL.
5.2.2 Weigh out 1.0g per litre of Di Potassium Hydrogen Phosphate $K_2HPO_4$.
5.2.3 Weigh out 0.5g per litre of Magnesium Sulphate Heptahydrate $MgSO_4\cdot7H_2O$
5.2.4 Dissolve the above ingredients in approximately 10mL of RO water.
5.2.5 Combine all 3 ingredients. Use the squeeze bottle to wash out all particles.
5.2.6 Make up to required volume with RO water.
5.2.7 Adjust pH to 7.1 using 1N NaOH or 1N HCl.
5.2.8 Dispense 95mL volumes into flat medical bottles / schott bottles.
5.2.9 Label all bottles with medium name, batch number and date of preparation.
5.2.10 Sterilise by autoclaving at 121°C for 15 minutes.
5.2.11 Final pH after sterilisation should be 7.1 ± 0.2. If not within range, notify the microbiologist and discard the batch.
5.2.12 Record all details of media preparation on SF150712.

6.0 **DEFINITIONS / ACRONYMS**

NA