SOP: M011.3
Updated 01/13/22 DRM

Preparation of Diffco Nutrient agar protocol

Materials and Reagents:

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. Diffco Nutrient Agar (VWR 90000-476)
6. Graduated cylinder, 1 liter
7. Autoclave
8. Serological pipet, 50 ml, sterile
9. Electric pipettor
10. Sterile plates, 15 x 150 mm or 15 x 100 mm
11. Sharpie marker
12. Ziploc bag, one gallon

Protocol:

1._____ Pour 1L of Milli-Q water into a 1 liter beaker.

2._____ Add magnetic stir bar to beaker and place on magnetic stir plate.

3._____ Add 23.0 g of dehydrated Diffco Nutrient Agar.

4._____ Make sure powder is completely in solution.

5._____ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes (Note 1).

6._____ Let cool on counter.

7._____ Turn on and clean BioSafety Cabinet (Note 2).

8._____ Pour agar into plates using the electric pipettor (Note 3).

9._____ Remove any bubbles on plates using an electric pipettor.

10._____ Allow plates to cool and solidify.

11._____ Label plates and store at 4°C in a Ziploc bag.

Notes:

1. Placed the bottle in an autoclave bin with a small amount of water in it to reduce boil over during the exhaust cycle.
2. See SOP SP041.
3. One batch of Nutrient Agar will make approximate nine 15 x 150 mm plates or twenty 15 x 100 mm plates. Plates should be poured thickly to ensure they do not completely dry out when used for culturing of *M. tuberculosis*.