SOP: M009.3
Updated 01/11/23 DRM

Preparation of Middlebrook 7H11 agar protocol

Materials and Reagents:

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. Middlebrook 7H11 agar (VWR 90004-942)
6. Glycerol (VWR IC800689)
7. Graduated cylinder, 1 liter
8. Autoclave
9. Water bath, 55ºC
10. OADC solution (VWR 90000-614)
11. Serological pipet, 50 ml, sterile
12. Electric pipettor
13. Sterile plates, 15 x 150 mm or 15 x 100 mm.
14. Serological pipet, 10 ml, sterile
15. Sharpie marker
16. Ziploc bag, one gallon

Protocol:

1._____ Pour 895 ml of Milli-Q water into a 1liter beaker.

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

3._____ Add 21.0 g of Middlebrook 7H11 dehydrated agar.

4._____ Make sure all components are completely in solution.

5._____ Add 5 ml of glycerol.

6._____ Make sure the glycerol is fully dispersed.

7._____ Autoclave on liquid cycle (slow exhaust) at 121ºC for 45 minutes.

8._____ Place sterile medium in 55ºC water bath for 30 minutes (Note 1).

9._____ Turn on and clean Biosafety Cabinet (Note 2).

10._____ Inside the BSC, add 100 ml of sterile OADC solution to agar solution once it has cooled. (Note 3)

11._____ Pour agar into plates (Note 4).

12._____ Remove any bubbles on plates by pipetting with a 10 ml pipet and electric pipettor.

13._____ Allow plates to cool and solidify (Note 5).

14._____ Label plates and store at 4ºC in a Ziploc bag.

Notes:

1. If you are immediately making the agar, let it cool until it is safe to touch with gloves on and use a 50mL pipette to remix the agar inside (instead of placing in a water bath).
2. See SOP SP041.

3. If the OADC is added to the sterile medium while the medium is too hot, the components of the OADC will degrade.
4. One liter of 7H11 agar with 10% OADC will make approximate seven 15 x 150 mm plates or fifteen 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*.
5. Make sure the plates fully cool before moving them, any slight touch to an uncooled plate can cause undesirable movement of the agar.

Reference:

Diffco manual, 10th edition. 1984 Difco Lab, Inc. Detroit, MI 48232.