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QUALITY CONTROL METHOD - 12

Preparation of BG Sulfa Agar

Purpose:

This quality control procedure is designed to reproducibly prepare petri dishes or bulk quantities of BGS Agar. This procedure should be performed by a trained laboratory technician.

Ingredients:

BG Sulfa agar (Difco # 0717-17-5)
Deionized water 1 liter

Final pH 6.9 ± 0.2 at 25° C

Equipment:

Erlenmeyer flask with screw cap (double the volume of media beign prepared)
Heated stirrer plate
Screw cap test tubes
Autoclave
Rapid-Flo Milk filter (from Filter Fabrics Inc.) or Bellco silicone sponge stopper
Steri-Wrap II (green)

Procedure:

1. Weigh ingredients

2. Fill screw cap, Erlenmeyer Flask with half of the required volume of media being prepared.

3. Add the rest of the agar ingredients to the Erlenmeyer flask.

4. Add the balance of the deionized water, washing down the sides of the container.

5. Apply closure and sterilize for 15 minutes at 121°C or 15 lbs. pressure for 15 minutes.

5.1 A 250 ml flask closure consists of a Bellco silicone sponge stopper (or equivalent) covered with double layer of green Steri-Wrap II (or equivalent) and secured with rubber bands.

5.2 A 2 liter flask closure consists of a double layer of Steri-Wrap (or equivalent) with a single gauze milk filter (or equivalent) sandwiched in between secured with rubber bands

6. Place flask over heated stir plate, and heat gently with frequent agitation, until it reaches the boiling point. Boil as briefly as possible to obtain solution. Avoid excess boiling so as to minimize foaming and water evaporation. To avoid loss of flask contents, be prepared for foaming of the medium and immediately remove the flask from the heat source when this begins.

7. Dispense about 10 mls liquified agar into screw cap test tubes.

8. Sterilize agar in autoclave for 15 - 20 minutes at 121°C.

9. Remove media from autoclave as soon as possible after the pressure has fallen to zero. Hastening the opening of the autoclave before zero pressure is reached can result in the loss of tube contents due to boiling. Do not permit media to remain in the autoclave for any appreciable length of time after the sterilization period. Prolonged heating can cause destruction of agar, destroy its gelling properties and/or may increase acidity

10. Aseptically pour 15-20 ml agar per sterile, plastic disposable petri dish.

11. Allow agar to gel and air dry overnight. If plates are needed immediately, dry the agar surface by opening the lids slightly and allowing air from a clean bench to blow over the surface for about 30 minutes.

12. Store plates inverted and refrigeratred sealed in original plastic sleeve or equivalent for a maximum of one month.

13. Label the package of plates with preparation date, expiry date (one month from date of pouring) and type of medium.

14. Incubate one plate for several days at 35° C as a sterility check.

15. Discard the entire batch of agar if contaminants are present on the control plate.