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QUALITY CONTROL METHOD -13

Preparation of Lysine Iron Agar

Purpose:

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

Ingredients:

LSI agar (Difco # 0849-01-4)	17.25 g
OR	
BBL #11363	16.5 g
Deionized water	500 ml

OR make according to FDA recipe:

Peptone water	2.5 g
Yeast extract	1.5 g
Glucose (dextrose)	0.5 g
L-lysine hydrochloride	5 g (or prepare L-lysine by dissolving 5 g L-lysine in 2 ml 1N HCL)
Ferric ammonium citrate	0.25 g
Sodium thiosulfate (anhydrous)	0.02 g
Bromcresol purple	0.01
Agar	7.5 g
Distilled water	500 ml

Final pH 6.7 ± 0.2 at 25° C

Equipment:

Erlenmeyer flask (1000 ml or double the volume of media being prepared)
Heated stirrer plate
Balance accurate to 0.001 g
Screw cap test tubes
Autoclave
Steri-Wrap II (green)
4-ply Mirasorb gauze sponges (for test tubes)

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. 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.

6. Dispense about 4 ml liquified agar into 13 x 100 screw-cap test tubes.

7. Wrap test tube caps in Steri-Wrap and place in autoclave with filled test tubes.

8. Apply closure and sterilize for 12 minutes at 121°C or 12 lbs. pressure for 15 minutes.

    8.1 Test tube closure consists of a 4 ply Mirasorb gauze sponge between a double layer of green Steri-Wrap II (or equivalent) and secured with rubber bands.

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

10. 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

11. Allow agar to solidify in slanted position to form 4 cm butts and 2.5 cm slants and air dry overnight.

12. Aseptically replace closure with sterile test tube caps.

13. Store tightly covered test tubes in the refrigerator for a maximum of one month before use..

14. Label the package of test tubes with preparation date, expiry date (one month from date of pouring) and type of medium.

15. Incubate one tube for several days at 35° C as a sterility check.

16. Discard the entire batch of agar if contaminants are present on the control tube.

17. Inoculate LIA slant by touching the colony to be tested once with a sterile inoculating needle and double stabbing the butt and streaking the slant.

18. Incubate overnight at 35°C, with their caps loose (or if performed soon after sterilization of media - keep the original closures to allow air flow and minimize hydrogen sulfide production).

Interpreting Results:

1. If testing for presence of *Salmonella,* follow instructions in QC-3. Media intially has a purplish color. Lysine deamination produces a dark red slant (top of the tube). Purple slant is negative.

2. Lysine decarboxylation (anaerobic alkaline reaction - over-neutralizing the acid formed by glucose fermentation) produces a red butt. A yellow butt is negative for lysine decarboxylation, but positive for glucose utilization.

3. A purple butt is negative both for glucose utilization and for lysine decarboxylation.

4. Black precipitate represents hydrogen sulfide, but LIA is not as accurate as TSI (QC-14) for this detection.