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QUALITY CONTROL METHOD -14

Preparation of Triple Sugar Agar Slants (TSI)

Description:

This quality control procedure is designed to reproducibly prepare slants of sterile TSI agar. TSI agar is a medium used in the identification of Gram-negative enteric rods. The medium measures a bacterium's ability to utilize three sugars, glucose, sucrose, and lactose, the concentrations of which are 0.1%, 1.0%, and 1.0% respectively. TSI slants are used in QC-3 *Salmonella/Shigella* test procedure. This procedure should be performed by a trained laboratory technician.

Equipment:

Erlenmeyer flask (double the volume of media being prepared)
Heated stirrer plate
Screw-cap test tubes
Autoclave
Rapid-Flo gauze milk filter
Mirasorb gauze sponges
Steri-Wrap II (green)
Bacterial cell spreading loop-needle (sterile blue disposable 1 µl)

Ingredients:

TSI (Difco #0265-01-9)	6.5 mg or multiple thereof
Deionized water	100 ml or multiple thereof

Final pH 7.4 ± 0.2 at 25°C

Preparation Procedure:

1. Weigh ingredients

2. Fill Erlenmeyer Flask with half of the required volume of deionized water.

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. Insert stirbar in flask and apply closure to the flask.

   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 1 - 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 until it reaches the boiling point. Boil as for one minute. Avoid excess boiling so as to minimize foaming and water evaporation. To avoid loss of flask contents, be prepared for possible 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. Apply closure and sterilize for 15 minutes at 121°C or 12 lbs. pressure for 15 minutes.

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

9. Wrap tube caps in Steri-Wrap to sterilize them too.

10. Sterilize agar in autoclave for 15 - 20 minutes at 121°C (15 lbs psi)

11. 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.

12. Allow tubes to cool in a slant ract at 20° C. Incline so that a generous butt is formed. Aseptically replace Steri-Wrap closure with cap and tighten the cap and **store at room temperature in the dark** for a maximum of 3 months.

13. Label tubes with contents, preparation date, expiration date (3 months).

Testing Procedure

1. Use a bacterial cell spreading loop (blue sterile 1 µl) to spread bacteria across the surface of the agar.

2. Dip the reverse end of the loop (sterile needle) into a bacterial culture colony. Insert this needle (stab) into the bottom (butt) of the tube.

3. Incubate slants at approximately 35° C with their **caps loose** or with original closure from sterilizing (to prevent excess H_2S production) for 18-24 hours.

Interpreting Results:

A pH indicator included in the medium can detect acid production from fermentation of these carbohydrates. A yellow color change indicates acid in the medium, while no color change indicates an alkaline surrounding. Carbohydrate utilization can be determined through analysis of the extent of acid production.

1. Acid production **limited to only the butt of the tube** (yellow butt) is indicative of **glucose** utilization. This is because the concentration of glucose is lower than that of the other sugars, thus the acid production is not very extensive.
2. Acid production (yellow) in the slant and butt indicates **sucrose or lactose** fermentation because of the releatively high concentrations of these sugars, thus leading to extensive acid production.
3. TSI agar can also detect reduction of sodium thiosulfate to hydrogen sulfide. **Hydrogen sulfide production will turn parts of the agar black**. Production of other gases is marked by cracks in the agar as well as an air gap at the bottom of the test tube. Record results in the appropriate QC database log.

Results (slant/butt)	**Symbol**	**Interpretation**
Red/yellow	K/A	Glucose fermentation only, Peptone catabolized
Yellow/yellow	A/A	Glucose and lactose and/or sucrose fermentation
Red/red	K/K	No fermentation, Peptone catabolized
Red/no color change	K/NC	No fermentation, Peptone used aerobically
Yellow/yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation, Gas produced
Red/yellow with bubbles	K/A,G	Glucose fermented only, Gas produced
Red/yellow with bubbles and black precipitate	K/A,G, H_2S	Glucose fermentation only, Gas produced, H_2S produced
Red/yellow with black precipitate	K/A, H_2S	Glucose fermentation only, H_2S produced
Yellow/yellow with black precipitate	A/A, H_2S	Glucose and lactose and/or sucrose fermentation, H_2S produced
No change/no change	NC/NC	No fermentation

A=acid production; K=alkaline reaction; G=gas production,
H_2S=sulfur reduction