Research Article

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Annex Text 1: Preparation and procedure of microbiological media used for isolation and identification of Salmonella from slaughtered goats.

Buffered Peptone Water

Preparation

Add 15 grams of the components in the 1000 ml of distilled water, mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test. Sterilize by autoclaving for 15 min in the autoclave set at 121 °C.

Procedure

Suspend the sample in Buffered Peptone Water to make dilutions as required. For pre-enrichment, add sample to Buffered Peptone Water at a ratio of 1:10 or 1:9 depending on the method being used. Incubate at 37°C for 16-20 hours before transferring to selective enrichment media.

Rappaport -Vassiliadis (RV) Soya Broth

Preparation

Weigh 30 g (the equivalent weight of dehydrated medium per Litre) and add 1 Litre of distilled water. Heat gently until completely dissolved. Dispense 10 ml volumes into screw capped bottles or tubes and sterilize by autoclaving at 115°C for 15 minutes.

2.2. Procedure

1. Prepare RVS Broth as instructed.
2. Add 25g or 25ml of the test sample to 225ml of Buffered Peptone Water and incubate at 37°C for 16-20 hours.
3. Transfer 0.1ml of the pre-enrichment peptone water culture to 10ml of RVS Broth and incubate at 42°C for 24 hours.
4. Subculture the enrichment broth by streaking onto plates of XLD Agar and Brilliant Green Agar.
5. Incubate at 37°C for 18-24 hours. Colonies suspected as salmonellae should be confirmed by biochemical methods.

Xylose Lysine Deoxycholate Agar (XLD Agar)

Preparation

Suspend 56.68gm in 1000 (1 Litre) of distilled water by heating, with frequent agitation, until the medium starts to boil. Avoid overheating. Adjust the pH, if necessary, so that after sterilization it is 7.4 at 25 °C. Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat. Transfer immediately to a water bath at 50 °C, agitate and pour into plates. Allow to solidify. Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven set between 37 °C and 55 °C until the surface of the agar is dry. It is advisable not to prepare large volumes which will require prolonged heating.

Procedure

Inoculate the plates by spread method. Incubate aerobically at 37 °C for up to 48 hours. After incubation observe the color of the colonies and interpret the results as indicated in the media.

Nutrient Agar

Preparation

Dissolve 28g of the components or the dehydrated complete medium in 1000ml of distilled water, by heating if necessary. Sterilize for 15 min in the autoclave set at 121 °C. Transfer about 15 ml of the melted medium to sterile small Petri dishes and proceed.

Procedure

Liquefy the agar if prepared tubes are used, cool to 45-50°C and pour into Petri dishes. Allow to solidify for at least 30 minutes. Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 37°C for 18-24 hours and 42-48 hours, if necessary. Tubed slants are used primarily for the cultivation and maintenance of pure cultures. They should be inoculated with an inoculating loop and incubated under the same conditions as the plated medium.

Triple sugar/Iron Agar (TSI agar)

Preparation

Suspend 65 grams in one Litre of distilled water and bring to the boil to dissolve completely. Sterilize in the autoclave set at 121 °C for 15 minutes. Dispense the medium into test tubes or dishes in quantities of 10 ml Allow to set in a slopped form to give a butt of depth 2. 5 cm to about 5 cm.

Procedure

1. With a sterilized straight inoculation needle touch the top of a well-isolated colony
2. Inoculate TSI agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
3. Leave the cap on loosely and incubate the tube at 35°C in ambient air for 18 to 24 hours.

Tryptophan Soya Broth for Indole Test and Motility Tests

Preparation

Dissolve 30gm of the components of tryptone broth in one-liter distilled water. Dispense 3 to 5 ml of the medium into each tube. Sterilize for 15 min in the autoclave set at 121 °C.

Simmons Citrate Agar for Simmon Citrate Tests

Preparation

1. In a beaker, 24.28 grams of dehydrated powder or lab-prepared media is added to 1000 milliliters of pure distilled or deionized water.
2. The solution is then heated to bring it to a boil in order to dissolve the medium completely.
3. The dissolved medium is then dispensed into tubes and sterilized in an autoclave at 15 lbs pressure (121°C) for 15 minutes.
4. Once the autoclaving process is complete, the tubes are taken out and cooled at a slanted position to a temperature of about 40-45°C. The position should be maintained in order to obtain butts of 1.5-2.0 cm depth.

Procedure

1. Streak the slant back and forth with a light inoculum picked from the center of a well-isolated colony.
2. Incubate aerobically at 35 to 37 °C for up to 4-7 days.
3. Observe a color change from green to blue along the slant.

MR-VP Medium (Glucose Phosphate Broth) used for MR Test and VP Test

Preparation

Suspend 17.0 grams in 1000 ml of purified / distilled water. Heat if necessary, to dissolve the medium completely. Distribute in test tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Procedure

1. Obtain one tube of MR-VP broth.
2. Using an inoculating loop, swish some of your assigned organism in the broth
3. Incubate the tube for at least 48 hours.
4. After the incubation period, vortex the tube and pour half of the broth into another clean test tube.
5. Complete the tests on the two individual tubes as follows:

- MR: to one tube add a drop or two of the pH indicator methyl red.
- VP: TO the other tube add three drops of (5% alpha-naphthol solution) and a drop of (40% potassium hydroxide solution).

Then after one hour examine the color.