Processing Winter School on Advances in microbiological and biotechnological methods for the detection of pathogenic microorganisms and their toxins in fishery environments,organized by CIFT at Cochin,5-25 November:72-73,Yr.2001

P1: Salmonella Detection -USFDA Method

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1. Macerate 25g of sample with 225 ml of Buffered Peptone Water (Nutrient Broth or Lactose Broth can also be used). Incubate at 37°C for 18-24h
2. Transfer 1ml of the pre-enrichment culture to one tube each of Selenite Cystine broth and Tetrathionate Broth. Incubate at 37°C for 18-24h.
3. Mix well and streak one loopful of the culture each from Selenite Cystine Broth and Tetrathionate Broth on pre-dried paltes of Brilliant Green Agar, Hektoen Enteric Agar, Xylose Lysine Desoxycholate Agar and Bismuth Sulphite Agar. Incubate at 37°C for 18-24h.
4. Examine plates for colonies that may be Salmonella.

   Typical Colony characteristics

   Brilliant Green Agar: Smooth, low, convex, moist pink colonies; surrounding medium bright red.

   Hektoen Enteric Agar: Blue-green to blue colonies with or without black centers.

   Xylose Lysine Desoxycholate Agar: Pink colonies with or without black centers.

   Bismuth Sulphite Agar: Brown, gray or black colonies, sometimes with metallic sheen, surrounding medium brown, may turn black on prolonged incubation.
5. Pick two or more typical colonies from the plates giving typical colonies. Lightly touch the center of the colony to be picked with sterile inoculating needle and inoculate Triple Sugar Iron Agar slant by streaking slant and stabbing the butt. Without flaming the needle, inoculate Lysine Iron Agar medium by stabbing butt twice and streaking slant. Since Lysine decarboxylation is a strictly anaerobic reaction the LIA slants must have deep butt. Also streak on Urea Agar slant. Incubate TSI, LIA and urea Agar at 37°C for 24 h.
6. Observe the reactions. Typically, Salmonella produces alkaline (red) slant and acid (yellow) butt, with or without production of Hydrogen sulphide (blackening of agar) in TSI. In LIA, salmonella produces alkaline (purple) reaction in butt of the tube. Most salmonella cultures produce hydrogen sulphide in LIA. Consider distinct yellow in the butt of tube as acid reaction. On urea agar, salmonella produces no colour change. Pink colour indicates Urease positive reaction. Salmonella is Urease negative.
7. Retain all cultures giving positive reactions on TSI and LIA and negative reaction in Urea agar. All cultures giving an alkaline butt in LIA, regardless of TSI reaction, should be retained as potential salmonella isolates. Cultures that give an acid butt in LIA and an alkaline slant and acid butt in TSI should also be retained.
8. Streak the suspected cultures on MacConkey agar for purification. Typical colonies appear transparent and colourless, sometimes with pink colour. Any other selective media also can be used for purification.
9. Pick the typical, well isolated colony from the purification plate and transfer to Nutrient Agar slants and incubate at 37°C.

10. Perform the following tests with the isolated suspected cultures.
    a) Gram stain and motility
    b) Glucose fermentation
    c) Lactose fermentation
    d) Sucrose fermentation
    e) Dulcitol fermentation
    f) Salicin fermentation
    g) Indole production
    h) Methyl Red test
    i) VP test
    j) Citrate utilization
    k) Malonate utilization

Typical characteristics of Salmonella

1.	Gram stain	Negative
2.	Motility	Motile
3.	TSI medium	Acid butt, alkaline slant, H_2S and gas
4.	LIA medium	Alkaline butt and slant with H_2S in the butt
5.	Urea agar	Negative
6.	Indole	Negative
7.	Glucose	Acid and gas
8.	Lactose	No acid and no gas
9.	Sucrose	No acid and no gas
10.	Dulcitol	Acid and gas
11.	Salicin	No acid no gas
12.	Citrate medium	Positive
13.	Methyl red	Positive
14.	VP test	Negative
15.	Malonate	Negative

Serology:

All cultures giving typical reactions are confirmed by agglutination test with Salmonella polyvalent O antiserum.

Mark two sections about 1x2 cm each on a glass slide. Emulsify a loopful of the isolated culture with 2ml of 0.85% saline in a test tube. Add one drop of culture suspension to both the marked sections on the slide. Add one drop of saline solution to one section only. Add one drop of the Salmonella Polyvalent O antiserum to the other section only. Using a loop mix culture suspension with saline solution in one section and with the antiserum on the other section. Tilt mixture in back and forth motion for 1 min and observe against dark background in good illumination. Consider any degree of agglutination a positive reaction.

Positive : agglutination in test mixture; no agglutination in saline control

Negative : no agglutination in test mixture; no agglutination in saline control.

Nonspecific : agglutination in test and in control mixtures.

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