GTP for Microbiological Analysis of Water

Media and reagents:

1. Plate count agar
2. Soyabean casein digest medium
3. MacConkey's Broth
4. Tetrathionate- bile- brilliant green broth
5. Bismuth sulfite agar
6. Brilliant green agar
7. Triple sugar iron agar
8. Cetrimide agar
9. Vogel johnson agar
10. Violet red bile glucose agar
11. Kovac's Reagent

Total Viable Aerobic Count:

Using petri dishes 9 to 10 cm in diameter, add 1ml from water sample to two sterile petridishes. Add about 15 ml of sterile Plate count agar medium cooled to 40-45°C to the dishes and one petridish without sample as control. Allow the medium to solidify. Invert and incubate the dishes at 30-35° C for 72 hrs..
Count the number of colony forming units (CFUs).

Calculate the Total microbial count as follows -
Average No. of colony forming units (CFUs) X 1 and express the results as CFU per ml.

Pathogens:

Preparation of Enrichment culture :
Filter 100 ml water sample through Sterilised0.45 micron cellulose nitrate membrane filtration assembly. After completion of filtration aseptically place the membrane filter in 100ml sterile soyabean casein digest broth medium, and incubate at 35°C to 37° C for 24 hours.

Escherichia coli:
PRIMARY TEST:
Transfer 1ml to 5 ml of MacConkey's Broth with durhams tube and incubate at 36-38°C for 48 hours.

SECONDARY TEST:
If acid or gas is present inoculate 0.1 ml to 5 ml of peptone water and 5 ml macconkey broth. Incubate in a water bath at 43.5 to 44.5° C for 24 hours. To test for Indole, add 0.5 mL Kovac's Reagent, shake well, and allow to stand for 1 min. If a red colour is produced in the reagent layer Indole is present, confirming the presence of E.coli.

***Salmonella*:**

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PRIMARY TEST:
Add 1 mL of the enrichment culture to 10 ml of Tetrathionate Bile brilliant green broth and incubate at 41 - 43° C for 24 hours. Subculture on three plates containing (i) Brilliant Green Agar (ii) Bismuth sulphite agar. Incubate the plates at 37° for 48 hours. If any characteristic colonies confirming to Salmonella are present carry out the secondary test.

SECONDARY TEST:
Subculture any characteristic colony on triple sugar iron agar medium, by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle and the same time inoculate into Urea Broth. Incubate at 37° C for 18 hours. The formation of acid and gas in the stab culture and the absence of acidity from the surface growth in Triple Sugar Iron agar together with absence of a Red colour in the Urea Broth, indicates the presence of Salmonella.

Pseudomonas aeruginosa:

PRIMARY TEST
Subculture from the enrichment culture to a plate of cetrimide agar and incubate at 35-37° C for 48 hours. If greenish colonies are present carry out the secondary test.

SECONDARY TEST
Smear the suspected colonies on oxidase discs. No development of purple colour within 60 secs indicates the absence of Pseudomonas aeruginosa.

Staphylococcus aureus:

PRIMARY TEST
Subculture on a plate of Vogel Johnson agar and incubate at 35-37° C for 48 hours. If black colonies surrounded yellow zone are present carry out the secondary test.

SECONDARY TEST
Transfer representative suspect colonies from the agar surface to tubes containing 0.5ml of mammalian plasma. Incubate in a water bath at 37° C examining the tubes at 3 hours up to 24 hours. If no coagulation occurs, Staphylococcus aureus is absent.

Enterobacteriacae:

Subculture on a plate of Violet red bile glucose agar and incubate at 35-37° C for 24 hours. If reddish colonies are present , *Enterobacteriacae is present*.