Bacteria Detection Techniques

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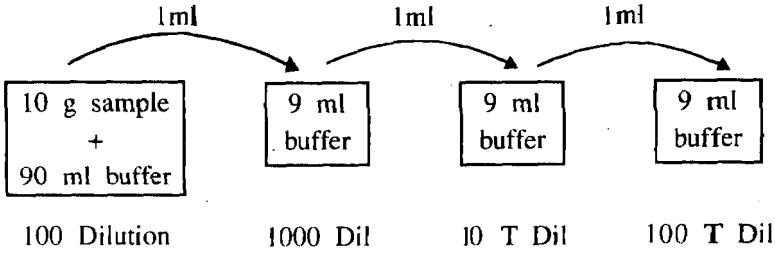
I. Total Bacterial Count

1. Aseptically collect 10 g. of sample in a sterile sample dish.
2. Transfer the sample into a sterile mortar or sterile stomacher bag.
3. Homogenise with 90 ml of sterile phosphate buffer.
4. Depending upon the products tested, the homogenised sample may be diluted as given below, so that the number of colonies in a petri dish is between 30-300.



5. 1 ml each from the required dilutions is transferred to separate sterile petri dishes.
5. Add to each petri dish 10-15 ml of sterile Tryptone Glucose Beef Extract Agar (cooled to 40°C); mix well and allow to solidify.
7. The plates are then incubated at 37°C for 48 hours and the colonies are counted.

$$\text{TPC per gm} = \frac{\text{Number of colonies x Dilution}}{\text{Weight of the sample}}$$

II. *E. coli*

10 g sample

+

90 ml Buffer

↓ 0.5 ml

Streak on to Tergitol 7 Agar

↓ Incubation at 37°C, 18-24 hrs.

Yellow, circular, flat, non-mucoid colonies

↓

Confirm by the following bio-chemical tests

(1) Eijkmans test (gas at 44.5°C, 48 hrs)	Positive
(2) Indole test	Positive
(3) Methyl red test	Positive
(4) Voges Proskauer test	Negative
(5) Citrate Utilization	Negative

Calculation

$$\textit{E. coli} \text{ per gram} = \frac{\text{Number of Positive Colonies} \times 2 \times 100}{\text{Weight of the sample}}$$

III. *Staphylococcus aureus*

10 g sample

+

90 ml Buffer

↓ 0.5 ml

Streak on to Baird-Parker Agar

↓ Incubation at 37°C, 36-48 hours

Black colonies surrounded by clear zone

↓

Nutrient Agar slants

↓ Incubation at 37°C, 18-24 hours

Confirm by plasma coagulase test

Calculation

$$\textit{Staphylococcus aureus}/\text{g} = \frac{\text{Number of Positive Colonies} \times 2 \times 100}{\text{Weight of sample}}$$

IV. *Salmonella*

1. Transfer 25 g of sample to 225 ml of Lactose broth and incubate at 37°C for 24±2 hrs.

2. Transfer 1 ml each from above to 10 ml Selenite cysteine broth and 10 ml Tetrathionate broth and incubate both the tubes at 37°C for 24±2 hrs.

3. Streak a 3 mm loopful from the incubated Selenite cysteine broth on Brilliant Green (BG) Agar, Bismuth Sulphite (BS) Agar, Salmonella Shigella (SS) Agar, Hektoen Enteric Agar (HEA) and Xylose Lysine Desoxycholate (XLD) Agar. Also repeat the streaking from the incubated tetrathionate broth to BG, BS, SS, HEA and XLD agars. Incubate the plates at 37°C for 24±2 hrs.

4. Examine the plates for the presence of suspicious Salmonella colonies. The characteristic colonies of Salmonella will appear as follows:-

   Brilliant Green Agar: The colonies of Salmonella are transparent pink with the surrounding medium turning red.

   Bismuth Sulphite Agar: Typical Salmonella colonies will appear as lustrous black to brownish, with silvery metallic sheen. If BS agar plates do not have typical or suspicious Salmonella colonies, incubate them for an additional 24 hours.

   Salmonella-Shigella Agar: Salmonella colonies appear as transparent-to-opaque, cream coloured or colourless colonies, with or without black centres.

   Hektoen Enteric Agar: Blue-green colonies with or without black centres.

   XLD Agar: Red colonies with black centres.

5. Select the Salmonella suspicious colonies from each selective agar and inoculate into Triple Sugar Iron (TSI) Agar and incubate at 37°C for 24±2 hrs. Typical Salmonella cultures will produce an alkaline

slant (Red) and an acid butt (Yellow) with the production of H_2S (blackening of agar) and gas. Normally, a maximum of 10 suspicious colonies will be selected from each of the presumptive plates. If only less than 10 colonies are available in a plate, all such colonies will be selected.

6. If the cultures give typical reaction of Salmonella on TSI, confirm by the following bio-chemical and serological tests:

a)	Fermentation of Lactose	No acid, No gas
b)	" Sucrose	" " "
c)	" Salicin	" " " "
d)	" Dulcitol	Acid and Gas
e)	Indole production	Negative
f)	Urease test	Negative (No red colour)
g)	Lysine Iron Agar	Alkaline (Purple) Slant and butt with production of H_2S.
h)	Agglutination with Salmonella Polyvalent '0' antiserum	Positive (Agglutination)

The following additional tests may also be done, if required:-

a)	KCN Broth	Negative (No growth)
b)	Malonate Broth	Negative
c)	V.P. Test	Negative (No red colour)
d)	Methyl Red Test	Positive (Red colour)

V. *Vibrio cholerae*

1. Transfer 25 g of sample to 225 ml alkaline peptone water and incubate for 18 hours at 37°C.

2. Streak one loopful from the surface growth of the above to TCBS Agar plates. Also transfer 1 ml to 9 ml alkaline peptone water and incubate for 6 hours at 37°C and then streak one loopful from the

second enrichment to another TCBS plate. Incubate both the TCBS plates for 18-24 hours at 37°C.

3. Transfer the suspected colonies (yellow flat smooth colonies with opaque centres and transparent peripheries, 2-3 mm diameter) from both TCBS plates to Kligler Iron Agar (KIA) slants and incubate at 37°C for 18 hours. *Vibrio cholerae* will give acid (yellow) butt and alkaline (pink) slant with no gas and no hydrogen sulphide (no black colour).

4. Strains giving the typical positive reaction in KIA are confirmed by slide agglutination with *V. cholerae* 01 polyvalent antisera and also by the following biochemical tests:-

Tests	Result
1. Oxidase test	Positive
2. Fermentation of glucose	Acid, No gas.
3. " sucrose	Acid, No gas
4. " mannitol	Acid, No gas
5. " inositol	No acid, no gas
6. " arabinose	No acid, no gas
7. Lysine decarboxylation	Positive
8. Ornithine decarboxylation	Positive
9. Arginine dehydrolation	Negative

The cultures with characteristic biochemical reactions of *V. cholerae* and agglutination with *V. cholerae* 01 polyvalent antisera are classified as *V. cholerae* 01 and the cultures with similar biochemical reactions but without agglutination with *V. cholerae* 01 polyvalent antisera are classified as *V. cholerae* non 01 (NAG vibrios).

VI. *Listeria monocytogenes*

Stage 1 : Enrichment

Inoculate 25 g sample in 225 ml Oxoid Listeria Enrichment Broth base (UVM) formulation; CM 863) containing Oxoid Listeria Primary Selective Enrichment Supplement (UVM; SR 142 E). Incubate for 24 hrs at room temperature.

Stage 2 : Selective media

Streak 1 loopful from stage 1 to plates of Oxoid Listeria Selective Agar Base (CM 856) containing Oxoid Listeria Selective Supplement (Oxford formulation; SR 140E) and incubate at room temperature for 24-48 hrs.

Listeria monocytogenes colonies will be brown, transparent and the surrounding media, brown.

Stage 3

Transfer (stab) typical colonies to SIM media and incubate at room temperature for 48 hrs.

L. monocytogenes exhibits "Umbrella motility"

Stage 4 : Biochemical tests of the suspicious

1)	Gram Reaction	-	+ve Rods
2)	Motility	-	Tumbling motility
3)	TSI	-	Acid Butt and Slant, No gas, no H_2S
4)	Urea hydrolysis	-	Negative
5)	MR	-	+ve
6)	VP	-	+ve
7)	Dextrose	-	+ve
8)	Maltose	-	+ve
9)	Esculin	-	+ve
10)	Mannitol	-	-ve
11)	Rhamnose	-	+ve
12)	Xylose	-	-ve
13)	Camp test	-	S+ve