24. ENUMERATION PROTOCOLS OF SIGNIFICANT SEAFOOD BORNE PATHOGENS BY BAM METHOD

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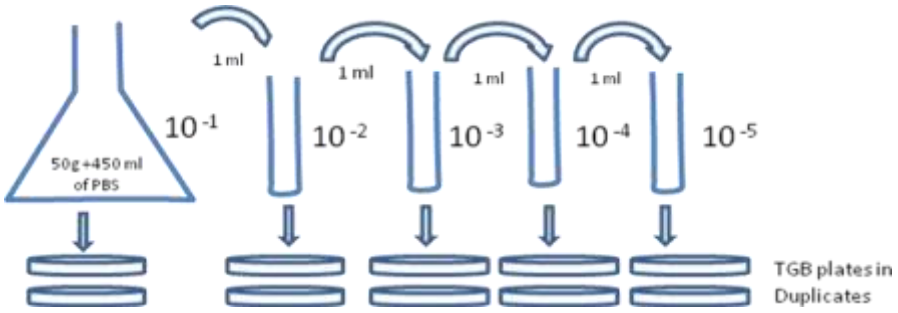
Aerobic plate count(APC)



50 g of fish sample + 450 ml of sterile Butter field's phosphate buffer (BPBS)

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Macerate in a blender

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Prepare tenfold dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4},)

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Add 1 ml from each dilution to sterile empty and add plate count agar (PCA) and rotate firmly

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Incubate at 37^{o} C for 48 hrs

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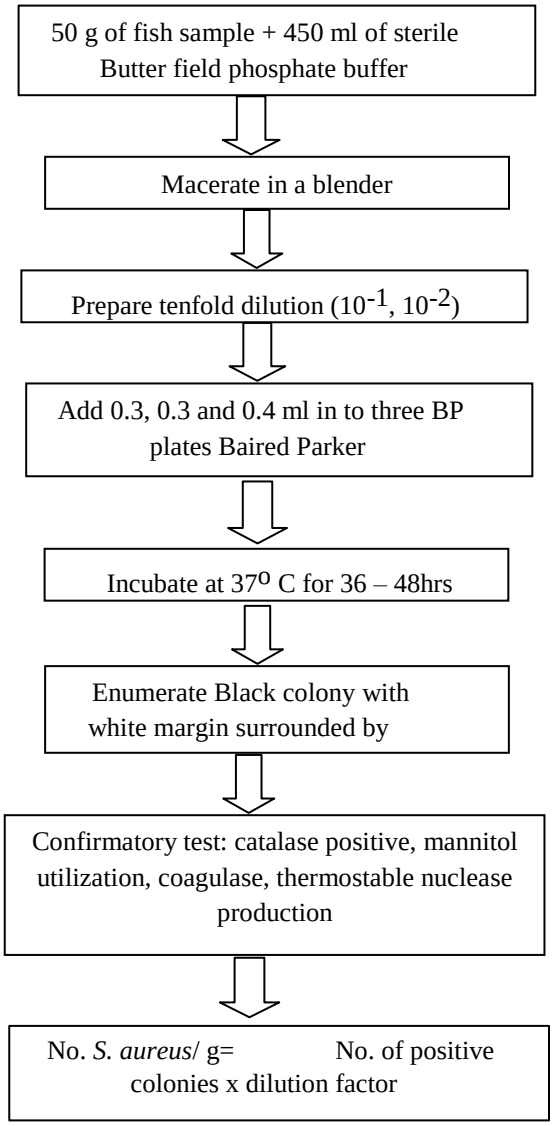
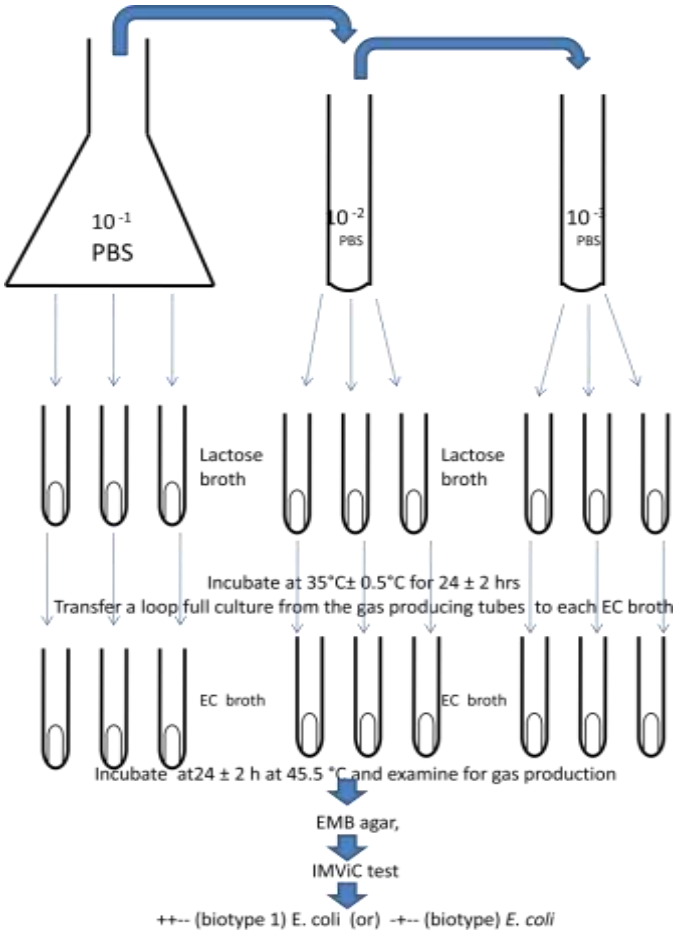
TPC/g= No. of colonies x dilution factor

Enumeration of *Staphylococcusaureus*



Enumeration of E. coli by MPN method



Preparation of the Medium

Prepare the medium (Mac Conkey or Lactose broth) in single and double strength concentration. Dispense the double strength medium and single strength medium either 5 ml or 10 ml (5 tubes for solid/ semi solid samples and 10 tubes for water and ice) in each tube and put durham tube in inverted position without air bubbles. Sterilize the medium by autoclaving at 15 lbs pressure (121°C) for 15minutes.

MPN Testing of samples

1. Take 5 tubes of double strength and 10 tubes of singlestrength.
2. Add 10 ml of the samples to 5 tubes containing 10 ml double strengthmedium.
3. Add 1ml of sample to 5tubes containing 10ml doublestrengthmediumand 0.1 ml water to

remaining 5 tubes containing 10 ml double strength medium.

4. Incubate all the tubes at 37°C for 24hrs.
5. Observe at 24 hrs, If no tubes shows positive for growth and gas production, re-incubate up to 48hrs.
6. Note the number of tubes for positives from each sets and compare the number of tubes givingpositivereaction to the 5tubes MPN standard chart and recordit.
7. The result is the total number of bacteria present in the sample as MPNvalues.
8. Forexample:5–4–3(5×10mlpositive,4×1mlpositive,3×0.1mlpositive)=theMPN valueis280.Sosamplecontainsanestimated280coliformsper100gram

StepII - (For confirmed total coliforms)

Requirements: BGLB 2% broth.

Inoculate one loopful of culture from the +ive tubes of step I to BGLB 2% broth. Incubate at 370C for 24 hrs. Note growth and gas production. Results are noted as +ives if there are growth and gas production. Compare with 3 tube MPN table.

Step III -(For faecal coliforms and *E.coli*)

From the +ive tubes of StepII, inoculate one loopful each to EC broth and Tryptone broth. (indole medium). Incubate at 44.50 °C for 24 hrs.

EC broth: Growth and gas production.

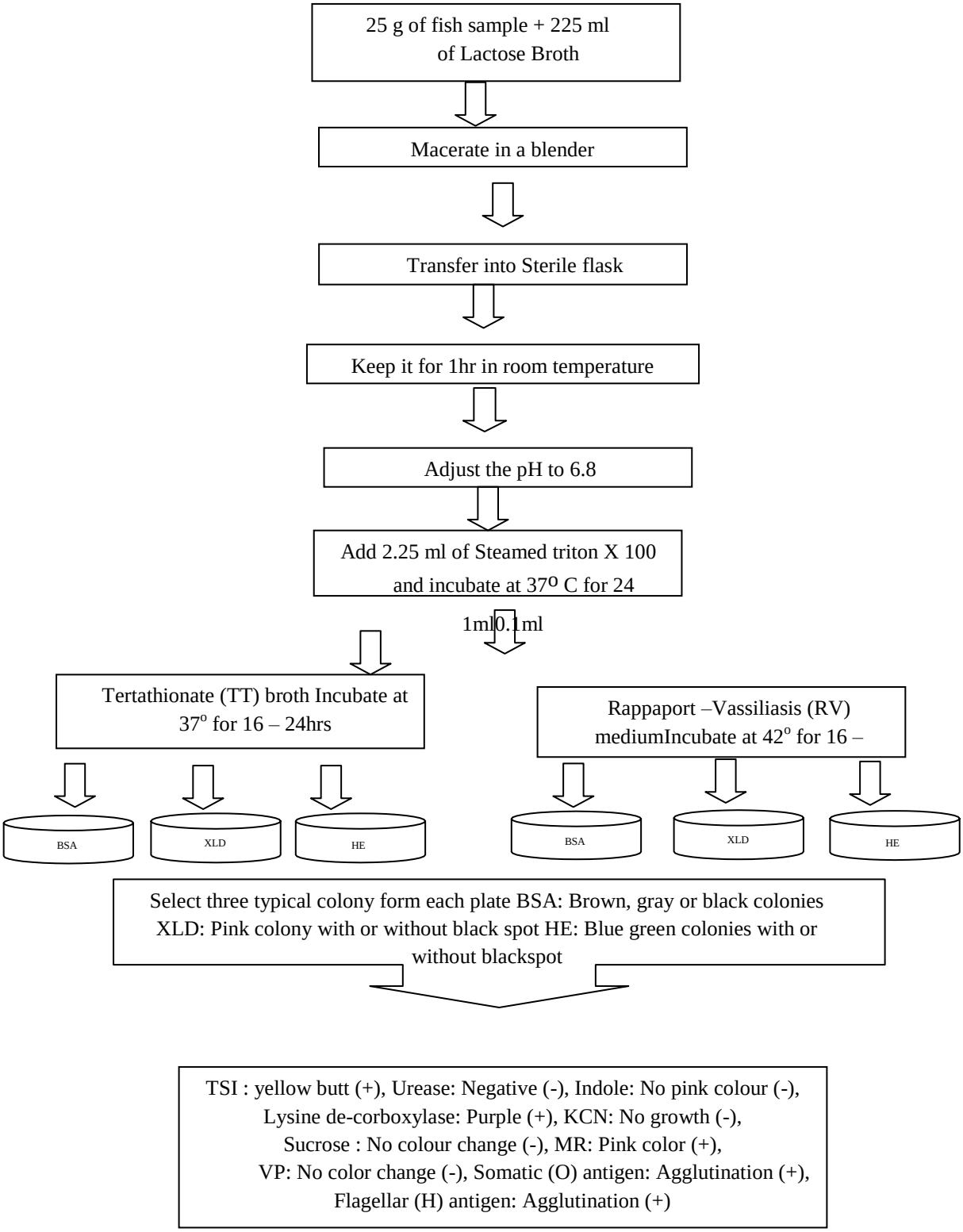
Tryptonebroth: Testforindoleproducesbyadding4dropsofKovac'sindolereagent.Apink or red color at the top layer indicates a +ive test forindole.

Coliforms bacteria which products gas in EC broth and indole in tryptone broth of 44.50 °C are*E.coli.*

A loopful of sample from each tube showing positive test *(color change with gas)* is streaked ontotwoselectivemediumlikeEosinMethyleneBlueagarorEndo'smedium.Oneplateeachis incubated at 37°C and another at 44.5± 0.2°C for 24hours.

High temperature incubation (44.5 ±0.2) is for detection of thermo tolerant *E.coli*

Detection of*Salmonella*



Protocol for the isolation of *V. cholerae* from fish

25 g of Sample

⇩

(Surface tissue, gills, gut-pooled sample)

Mix 25 g of pooled sample with 225 ml of APW, macerate in a stomacher blender

⇩

Incubate APW at 35 □ 2^0 C for 6 to 8 hours and transfer a loopful from the surface pellicle of APW culture to TCBS plate

⇩

Incubate APW overnight at 35 □ 2^0 C

⇩

Again transfer a transfer a loopful from the surface pellicle of APW culture to TCBS plate

⇩

Incubate overnight at 35 □ 2^0 C

⇩

*cholerae*appearsaslarge2-3mm,smoothyellowandslightlyflattenedwithopaquecentreand translucent peripheries onTCBS

⇩

Pick typical colonies on to TSA slants with 2% Nacl

⇩

Proceed for biochemical tests.

Biochemical confirmation

- Oxidasepositive
- String testpostive
- Arginine decarboxylase-Negative
- Lysine, Ornithine Decarboxylase-postive
- Sucrose positive; Growth in 0% salt, no growth in 6%salt

Protocol for the isolation of *V. Parahaemolyticus* from seafood

Allthemediausedforthebiochemicalidentificationof*Vibrioparahamolyticus*shouldcontain2or3% Nacl.

25 g of Sample

⇩

(Surface tissue, gills, gut-pooled sample)

Mix 25 g of pooled sample with 225 ml of APW with 3% salt and macerate in a stomacher blender

⇩

Incubate APW overnight at 35 □ 2^0 C

⇩

Streak a loopful from APW onto a TCBS plate with 3% Nacl.

Incubate APW overnight at 35 □ 2^0 C

⇩

*V. Parahaemolyticus*appearsasround,opaque,greenorbluishcolonies2-3mmindiameteronTCBS

Pick typical colonies on to TSA slants with 3% Nacl

⇩

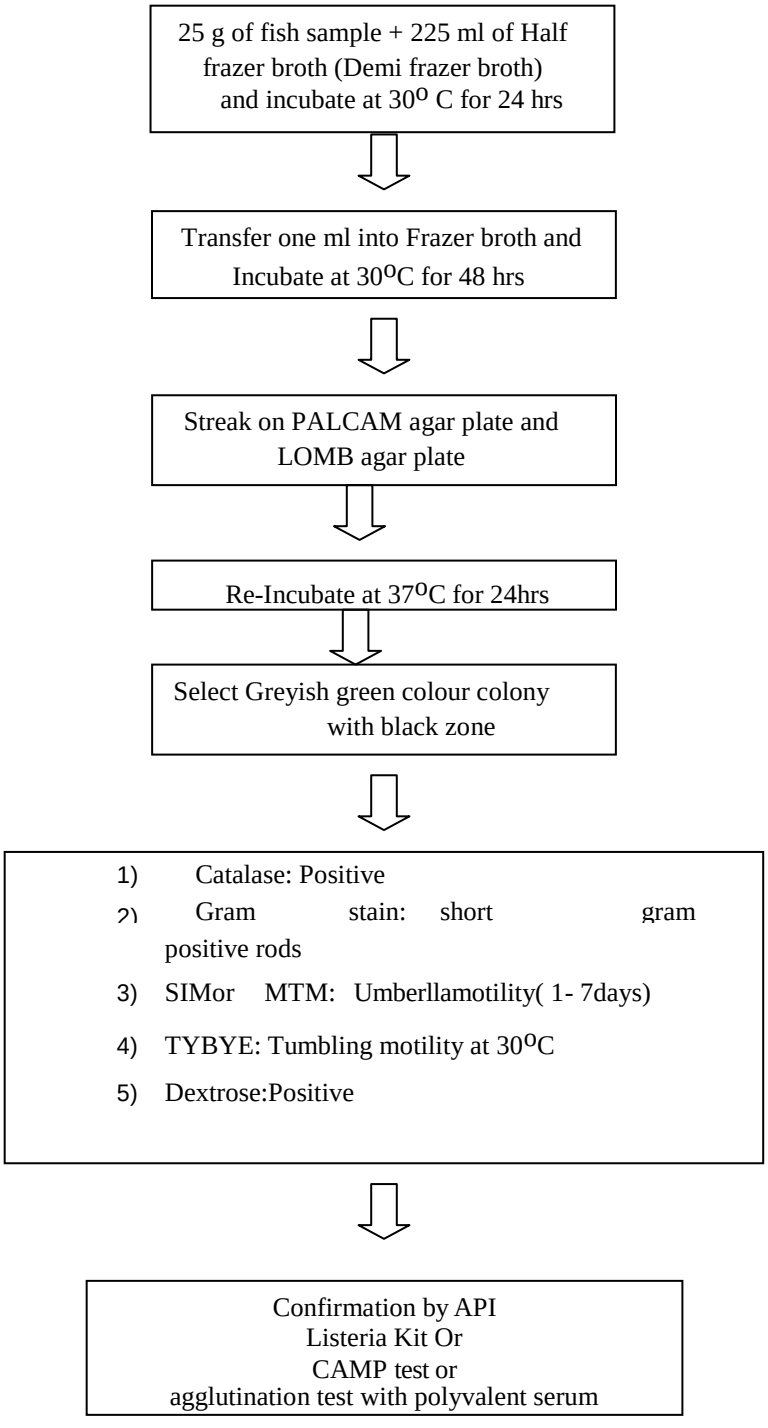
⇩

Proceed for biochemical tests.

Biochemical confirmation:

- Oxidase positive
- Gram negative, straight/ curvedrods
- Non H_2Sproducer
- Growth in 3 %, 6%, 8% Nacl, No growth in 0 %Nacl
- *V.Parahaemolyticus*can be differentiated from other Vibrios by ONPG, Salt tolerance and lactose reactions; Resistance to 10 □ g of O/129, sensitive to 150 □ g ofO/129.

Detection of *Listeriamonocytogenes*



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