Chapter 3

Methods for the microbiological examination of seafood

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Aerobic plate count

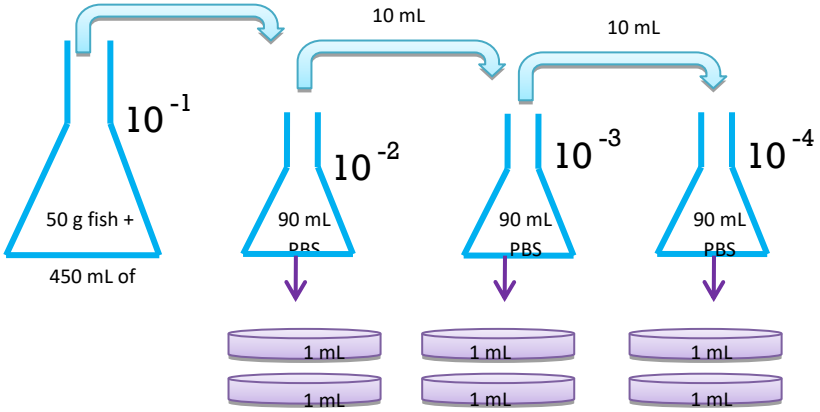
The Aerobic plate count method is used for examining frozen, chilled, pre-cooked, or prepared foods. It is also known as the total mesophilic count, total bacterial count, and viable aerobic bacteria.

- Take 50 g analytical unit fish to determine aerobic plate count
- Add 450 ml Butterfield's phosphate-buffered dilution water to a blender jar containing 50 g analytical unit and blend for 2 min (This results in a dilution of 10^{-1}). Using separate sterile pipets, prepare decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and further as appropriate.
- Shake all dilutions 25 times in 30 cm (1 ft) arc within 7 s. and reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into a petri dish.
- Pipette 1 ml of each dilution into separate, duplicate, appropriately marked Petri dishes.
- Add 12-15 ml plate count agar (cooled to 44 ± 1°C) to each plate within 15 min of original dilution.
- After solidification of agar, Invert the solidified Petri dishes and incubate promptly for 48 ± 2 h at 35°C. Do not stack plates when pouring agar or when agar is solidifying.
- Select plates with 25-250 colonies including those of pinpoint size

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2) \times (d)}$$

where: N = Number of colonies per ml or g of product;$\sum$ C = Sum of all colonies on all plates counted;n_1 = Number of plates in the first dilution counted; n_2 = Number of plates in the second dilution counted;d = Dilution from which the first counts were obtained

Aerobic Plate count (APC)



50 g of fish sample + 450 ml of sterile Butterfield'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)

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Incubate at 37° C for 48 hrs

Enumeration of Faecal Coliform and *Escherichia* (Most Probable Number technique - 3 tubes)

I. Presumptive test for coliforms, faecal coliforms and *E. coli*

- Weigh 50 g of food into a sterile high-speed blender jar (for frozen samples can be softened by storing for <18 h at 2-5°C, but do not thaw)
- Add 450 mL of Butterfield's phosphate-buffered water and blend for 2 min.

- Prepare decimal dilutions (1:10) with sterile Butterfield's phosphate diluents or equivalent in a test tube.
- Shake all suspensions 25 times in 30 cm arc or vortex mix for 7 s.
- Using at least 3 consecutive dilutions, inoculate 1 mL aliquots from each dilution into 3 LST/Lactose broth tubes for a 3 tube MPN analysis
- Incubate LST tubes at 35°C± 0.5°C.
- Examine tubes and record reactions at 24 ± 2 h for gas, i.e., displacement of the medium in fermentation vial or effervescence when tubes are gently agitated.
- Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48 ± 3 h. Perform a confirmed test on all presumptive positive (gas) tubes.

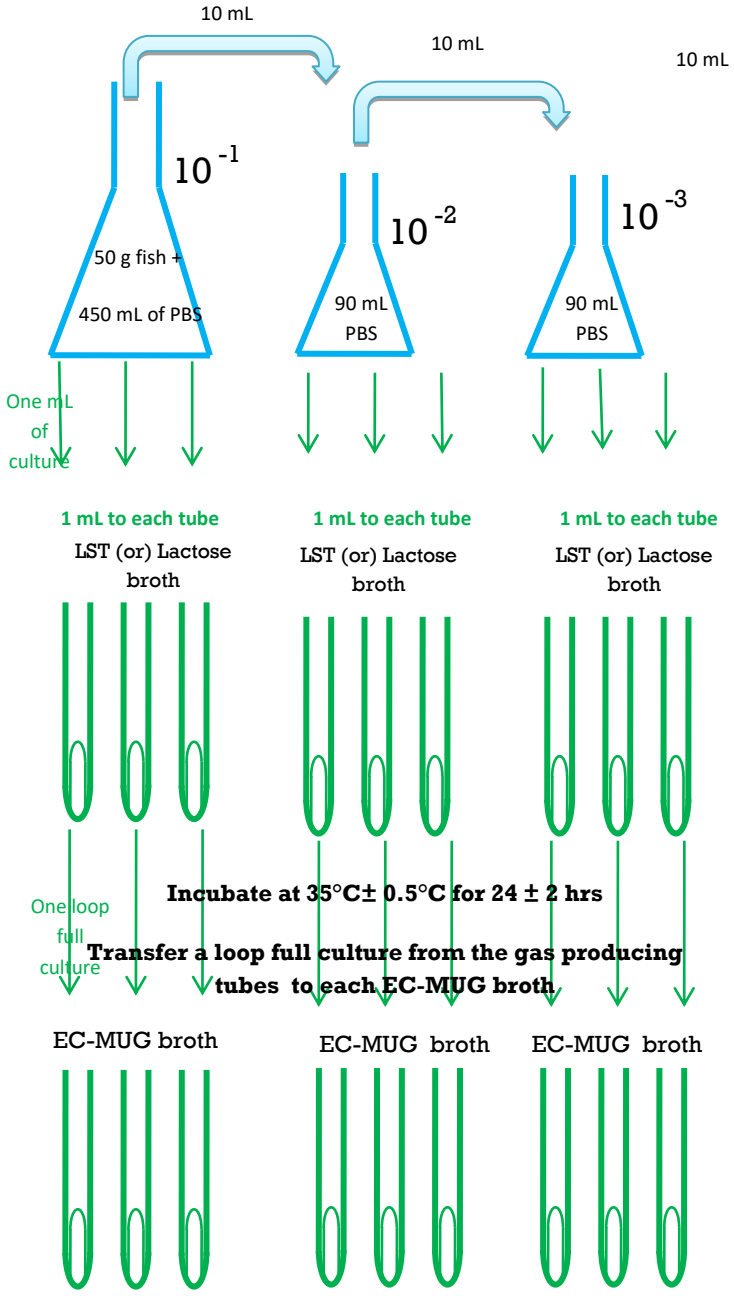
II. MPN - Confirmed test for Faecal Coliforms

- From each gassing LST or Lactose broth tube from the presumptive test, transfer a loopful of each suspension to a tube of EC-MUG broth
- Incubate EC-MUG tubes 24 ± 2 h at 45.5 °C and examine for gas production.
- If negative, re-incubate and examine again at 48 ± 2 h.
- Use the results of this test to calculate faecal coliform MPN.

II. MPN - Confirmed test for *E. coli*

- Only positive tubes (gas production) checked for the MUG positive in UV-light 356nm
- Bluish fluorescence in the test tube indicates the presence of *E. coli*
- Use the results of this test to calculate faecal coliform MPN

Enumeration of *E. coli* by MPN method



Incubate at 24 ± 2 h at 45.5 °C (in water bath) and examine for gas production in normal eye vision

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Only posive tubes (gas) check for the MUG utilization in UV-light (356nm)

MPN Table (3 tube method)

Table 21-3. Most probable number (MPN) index for 9-sample method.

Number of + tubes				Number of + tubes			
0.1	0.01	0.001	MPN/ 100 g	0.1	0.01	0.001	MPN/ 100 g
0	0	0	<3.0	2	2	0	21
0	0	1	3.0	2	2	1	28
0	1	0	3.0	2	2	2	35
0	1	1	6.1	2	3	0	29
0	2	0	6.2	2	3	1	36
0	3	0	9.4	3	0	0	23
1	0	0	3.6	3	0	1	38
1	0	1	7.2	3	0	2	64
1	0	2	11	3	1	0	43
1	1	0	7.4	3	1	1	75
1	1	1	11	3	1	2	120
1	2	0	11	3	1	3	160
1	2	1	15	3	2	0	93
1	3	0	16	3	2	1	150
2	0	0	9.2	3	2	2	210
2	0	1	14	3	2	3	290
2	0	2	20	3	3	0	240
2	1	0	15	3	3	1	460
2	1	1	20	3	3	2	1100
2	1	2	27	3	3	3	>1100

Source: Bacteriologic *Analytic Manual*, 8th ed. Maryland: Food and Drug Administration, 2010.

Enumeration of *S. aureus*

- Take 50 g analytical unit fish to determine aerobic plate count
- Add 450 ml Butterfield's phosphate-buffered dilution water to blender jar containing 50 g analytical unit and blend 2 min (This results in a dilution of 10^{-1}). Using separate sterile pipets, prepare decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and further as appropriate.
- For each dilution to be plated, aseptically transfer 1 ml sample suspension to 3 plates of Baird-Parker agar, distributing 1 ml of inoculum equitably to 3 plates (e.g., 0.4 ml, 0.3 ml, and 0.3 ml).
- Spread inoculum over surface of agar plate, using sterile bent glass streaking rod. Retain plates in upright position until inoculum is absorbed by agar (about 10 min on properly dried plates).
- Invert plates and incubate for 45-48 h at 35°C.

- Select plates containing 20-200 colonies, unless only plates at lower dilutions (>200 colonies) have colonies with typical appearance of *S. aureus*.
- Colonies of *S. aureus* are circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle.

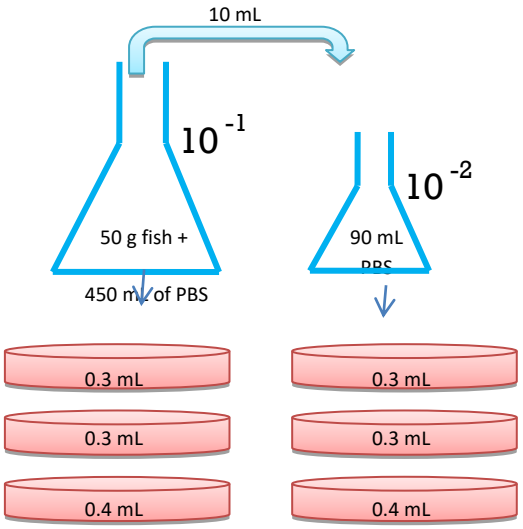
Note: Count and record colonies. If several types of colonies are observed which appear to be *S. aureus* on selected plates, count number of colonies of each type and record counts separately. When plates of the lowest dilution contain <20 colonies, these may be used.

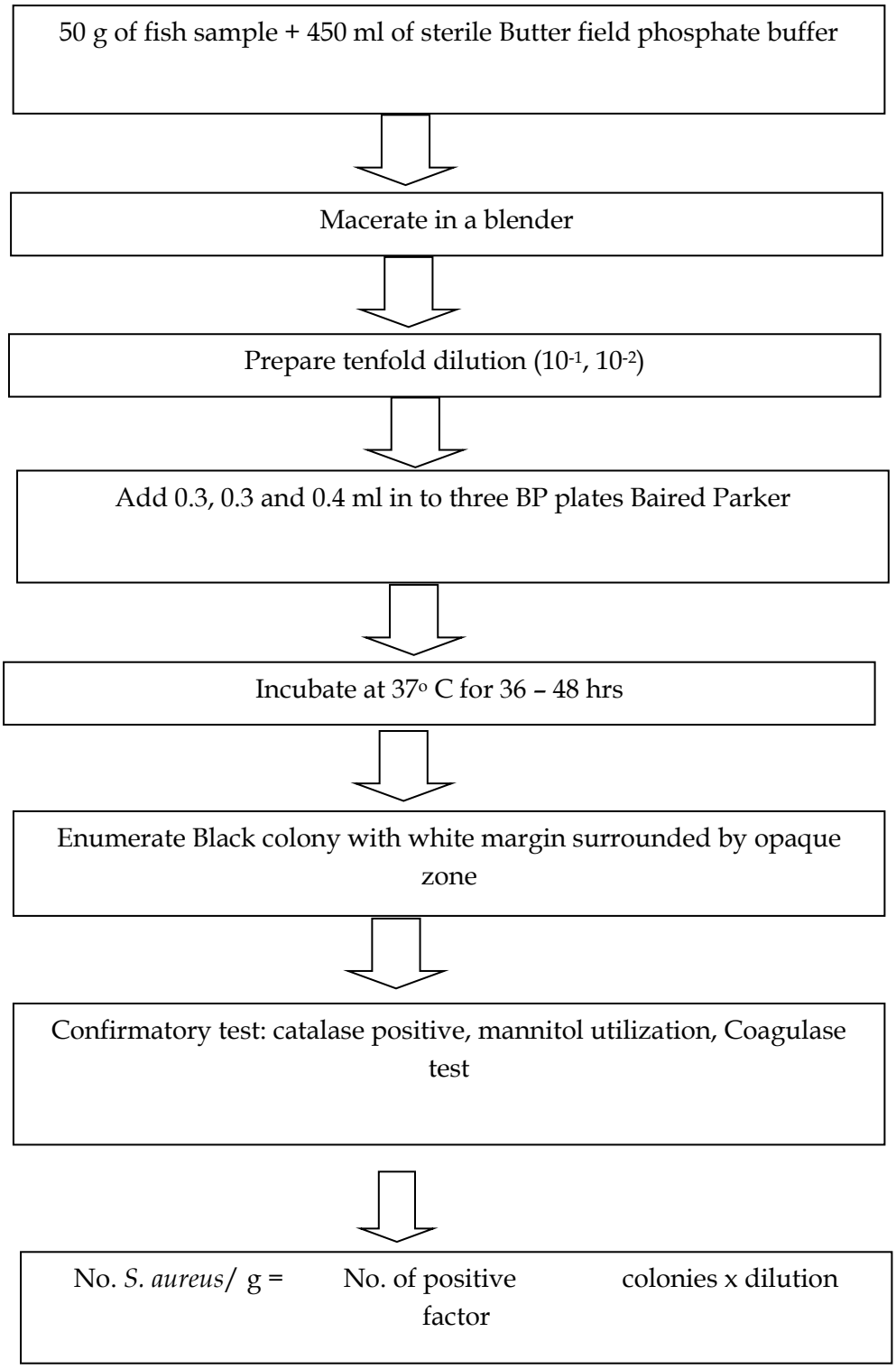
Test for confirmation of *S. aureus*

- Catalase test: Positive
- Grams stain: Gram-positive cocci
- Utilization of mannitol
- Coagulase test: Coagulase production for confirmation

TPC/g = No. of colonies x dilution factor CFU/Gram

Enumeration of *Staphylococcus aureus*




50 g of fish sample + 450 ml of sterile Butter field phosphate buffer
Macerate in a blender
Prepare tenfold dilution (10^{-1}, 10^{-2})
Add 0.3, 0.3 and 0.4 ml in to three BP plates Baired Parker
Incubate at 37° C for 36 – 48 hrs
Enumerate Black colony with white margin surrounded by opaque zone
Confirmatory test: catalase positive, mannitol utilization, Coagulase test
No. *S. aureus*/ g = No. of positive colonies x dilution factor