

7. ISOLATION AND ENUMERATION OF MICROBES FROM SEAFOOD

S. Visnuvinayagam*, Abhay Kumar, L. Narasimha Murthy, A. Jeyakumari,

* MFB Division, CIFT, Cochin-29

Mumbai Research Centre of CIFT, Vashi, Navi Mumbai - 400703

Introduction

Microbiology is the study of microorganisms like microscopic or barely visible single-celled life-forms such as bacteria, archaea, protozoans. Enumeration in microbiology is an estimation or determination of number of bacterial cells in a given sample. Enumeration of sea food has gained importance due to increased attention being paid to quality aspects of final product. The International Commission on Microbiological Specifications for Foods (ICMSF) established in 1962 to the need for internationally acceptable and authoritative decisions on microbiological limits for foods appropriate with public health safety, and particularly for foods in international commerce.

Methods to enumerate microbes can be divided into two categories.

- a) Total cell counts include dead and inactive cells.
- b) Viable methods only count cells that are metabolically active,

Direct Microscopic count/ Total cell count

Direct microscopic counts measures number of cells in a population of a given sample under a microscope. This can be possible for liquid samples using special slides known as counting chambers, consisting of a ruled slide and a cover slip. It is constructed in such a manner that the cover slip, slide, and ruled lines delimit a known volume. The number of bacteria in a small known volume is directly counted microscopically and the number of bacteria in the larger original sample is determined by extrapolation. Bacteria can be counted easily and accurately with the petroff-Hausser counting chamber. This is a special slide accurately ruled into squares that are $1/400 \text{ mm}^2$ in area; a glass cover slip rests $1/50 \text{ mm}$ above the slide, so that the volume over a square is $1/20,000 \text{ mm}^3$ i.e. $1/20,000,000 \text{ cm}^3$. If for example, an average of five bacteria is present in each ruled square, there is $5 \times 20,000,000$ or 108, bacteria per milliliter.

Advantages:

- a) It is quick way of estimating microbial cell number
- b) Morphology of the bacteria can be observed as they counted.

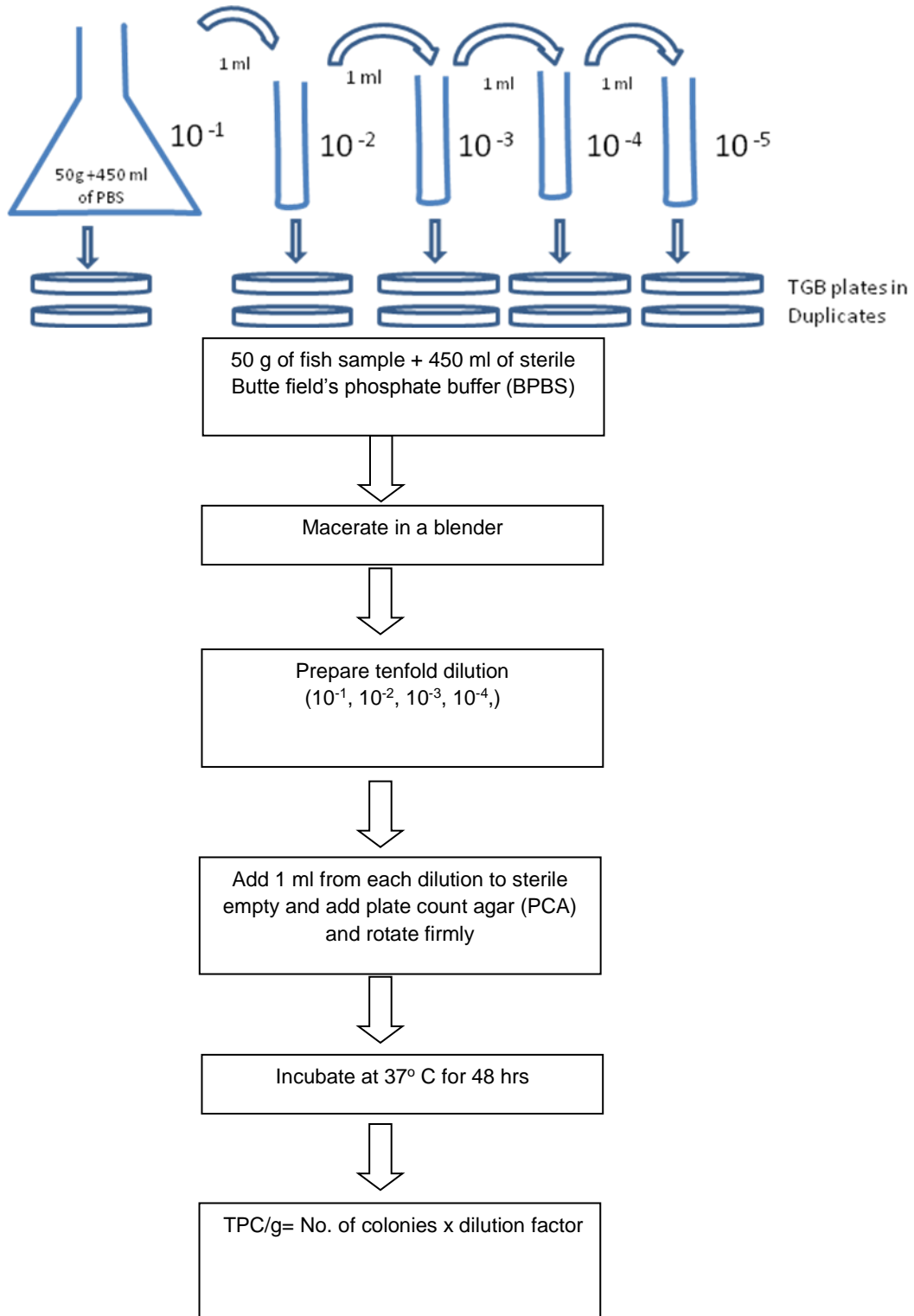
Limitations:

- a) Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted
- b) Difficulty in to count small cells
- c) Precision is difficult to achieve
- d) Require a phase- contrast microscope if sample is not stained.

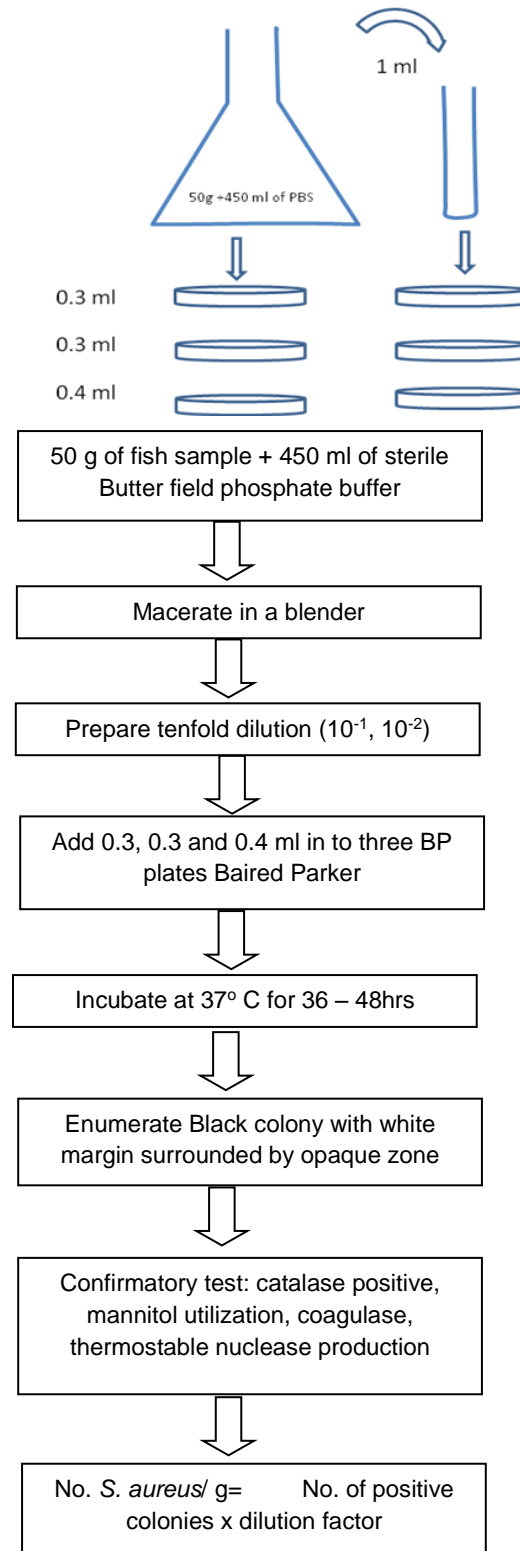
Standard Plate Count (Viable Counts): Any cell which has a capacity to divide and form a population or colony is defined as a viable cell. Viable count is also called as plate count or colony count. A viable cell count is usually done by diluting the original sample, plating aliquots of the dilutions on to an appropriate culture medium, then incubating the plates under suitable conditions for the colonies to be grown. Colonies are counted and, from a particular dilution used, the original number of viable cells can be calculated. For accurate determination of the total number of viable cells, it is critical that each colony comes from only one cell, so chains and clumps of cells must be broken apart. However, since one is never sure that all such groups have been broken apart, the total number of viable cells is usually reported as colony-forming units (CFUs) rather than cell numbers. This method of enumeration is relatively easy to perform but major disadvantage is the time necessary for dilutions, plantings and incubation. There are two ways to perform a plate count a) pour plate technique b) spread plate technique. Plating techniques are discussed detail in chapter no.5. Enumeration protocols of significant seafood borne pathogens are given below in flow chart.

Enumeration protocols of significant seafood borne pathogens

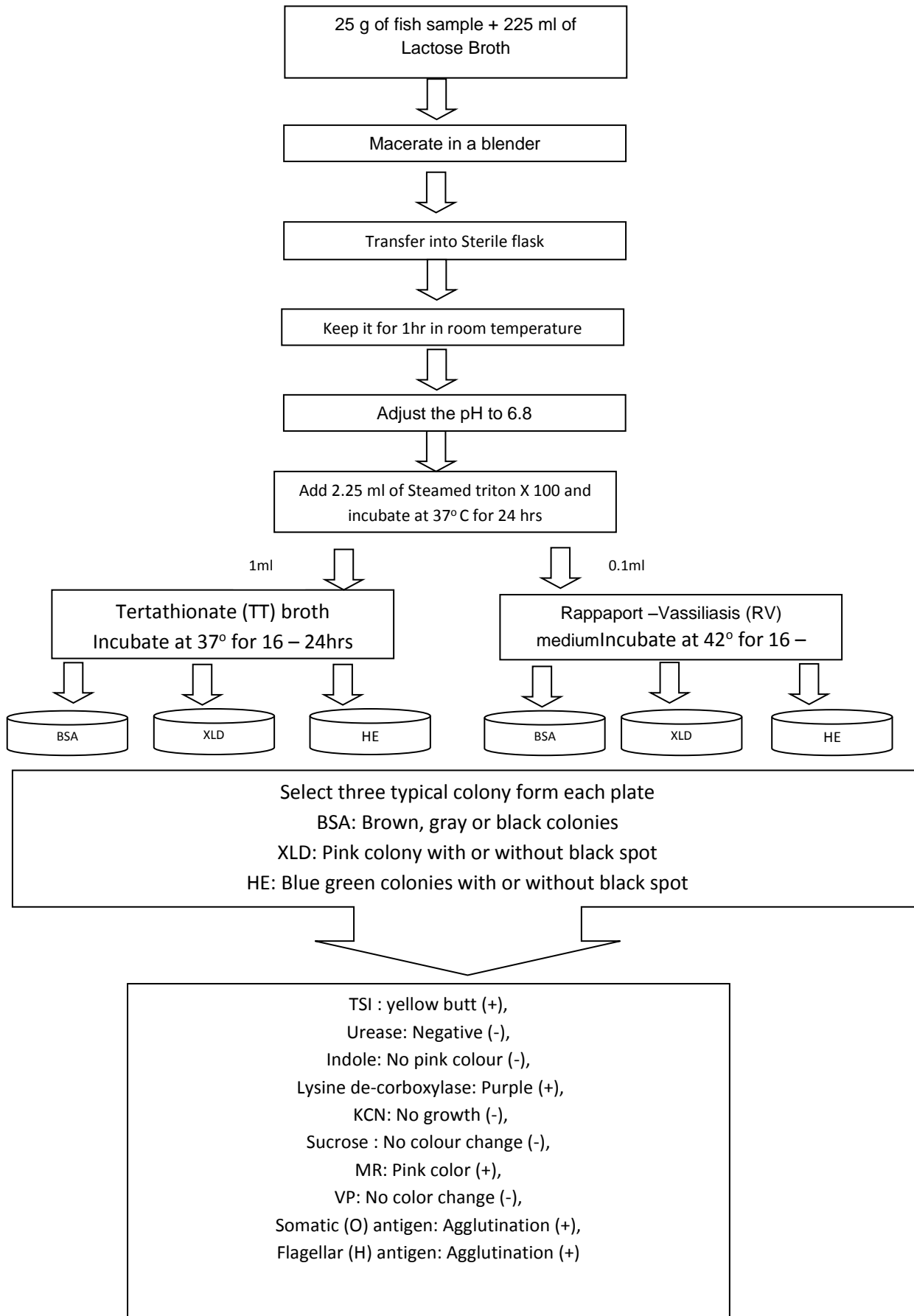
1. Aerobic plate count (APC)



2. Enumeration of *Staphylococcus aureus*



3. Detection of *Salmonella*



4. Detection of *Listeria monocytogenes*

