25.ISOLATION AND IDENTIFICATION OF SEAFOOD PATHOGENS BY ISO METHOD

Abhay Kumar,Megha G., Tulsiram Waghmare, L. Narasimha Murthy

Mumbai Research Centre of ICAR-Central Institute of Fisheries Technology, Navi Mumbai

*kumarabhay275@gmail.com

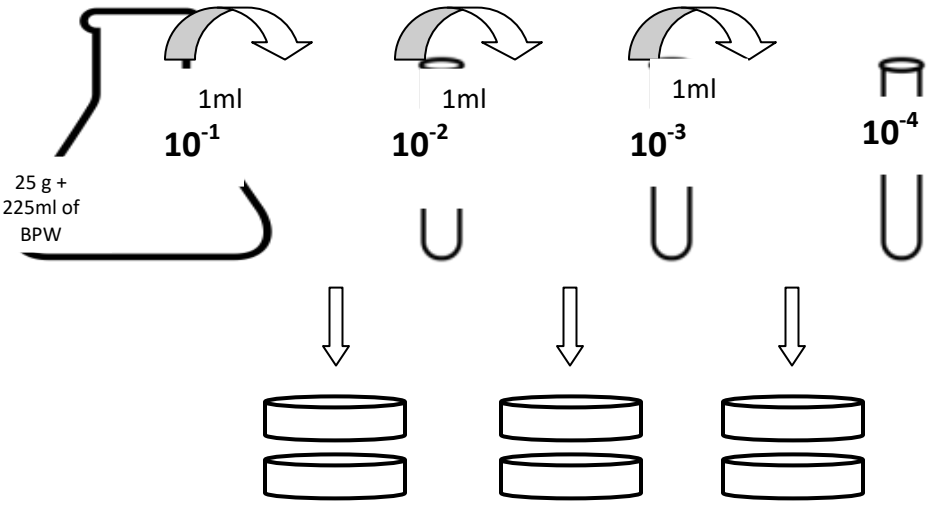
Introduction

Raw ready-to-eat seafood products can be easily contaminated with various bacteria from aquatic environments and human reservoirs, which subsequently bring about a risk in food poisoning.Seafood-associated infections are caused by a variety of bacteria, viruses, and parasites; this diverse group of pathogens results in a wide variety of clinical syndromes, each with its own epidemiology. Prevention of seafood infections require mechanisms of infections that are amendable to control.Coordinated efforts are necessary to further reduce the risk of seafood-associated illnesses. Continued surveillance will be important to assess the effectiveness of current and future prevention strategies.

Microbiological testing also has some limitations as a control option. These are constraints of time, as results are not available until several days after testing as well as difficulties related to sampling, analytical methods and the use of indicator organisms.

ISO standards are reference methods for food microbiological regulations and widely used for food microbiological analysis. In 2017 several methods reviews were published, including new protocols and validation data. ISO methods are also required to demonstrate that new, rapid alternative methods are valid, provide equivalent results, and follow the ISO 16140‑2:2016 requirements for validation. ISO standards incorporate some new technologies when classical technologies have limitations in providing accurate results. For example, qPCR is applied for detecting enteric viruses and HPLC for bacterial toxins. New standard methods also provide validation data for method performance, which is useful for method implementation in quality control labs and to understand a method’s limitations.

Total Plate Count



25 g of fish sample +225ml of BPW and Macerate in blender

⇩

Prepare tenfold dilution (10^{-1}, 10^{-2},10^{-3},10^{-4}) in BPW tubes (9ml)

⇩

Add 1ml of sample of each dilution into sterile petri dishes (in duplicates) and Pour 15-20ml of Plate Count Agar (PCA) into each petridish

⇩

Invert the plates and incubate at 30°C for 72 hrs

⇩

$$\text{TPC} = \frac{\sum C}{V(n1+0.1n2)\times d}$$

If count fewer than 300 colonies on the plate. Use formula for calculation

∑C= Sum of all colonies counted on the two dishes retained from two successive dilutions, at least one of which contains a minimum of 10 colonies

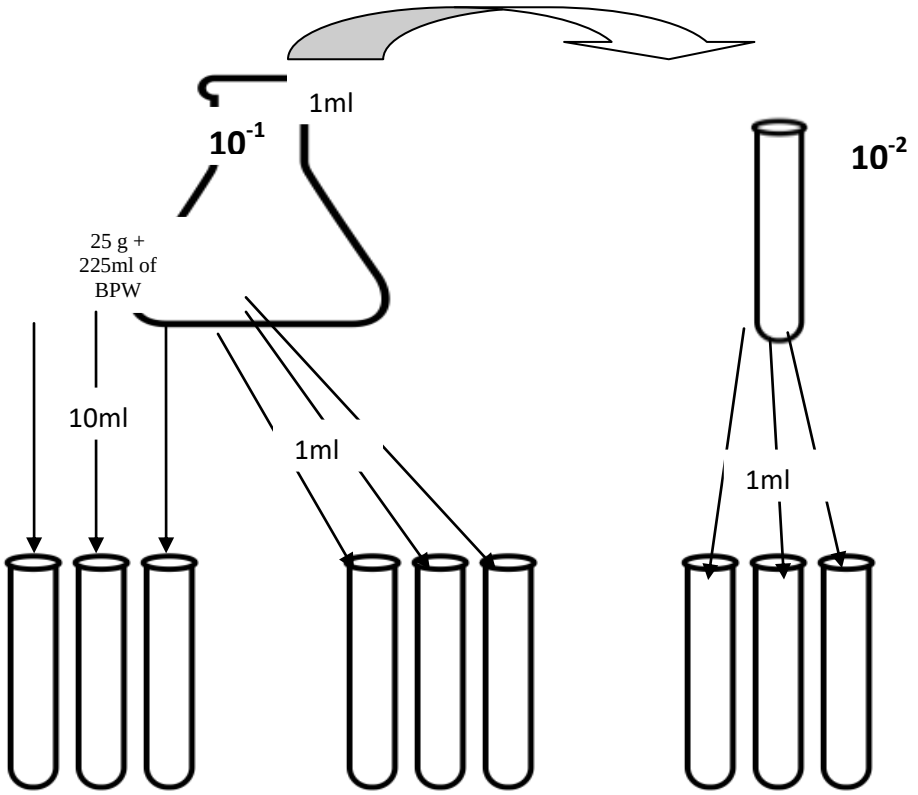
n1= No of plates retain in first dilution

n2= No of plates retain in second dilution

d= dilution corresponding to the first dilution retained (d=1 when the undiluted liquid product is retained).

V= Volume of inoculums placed in each plate in ml.

Detection of *Escherichia coli*



10ml double strength MMGM broth

10ml single strength MMGM broth

10ml single strength MMGM

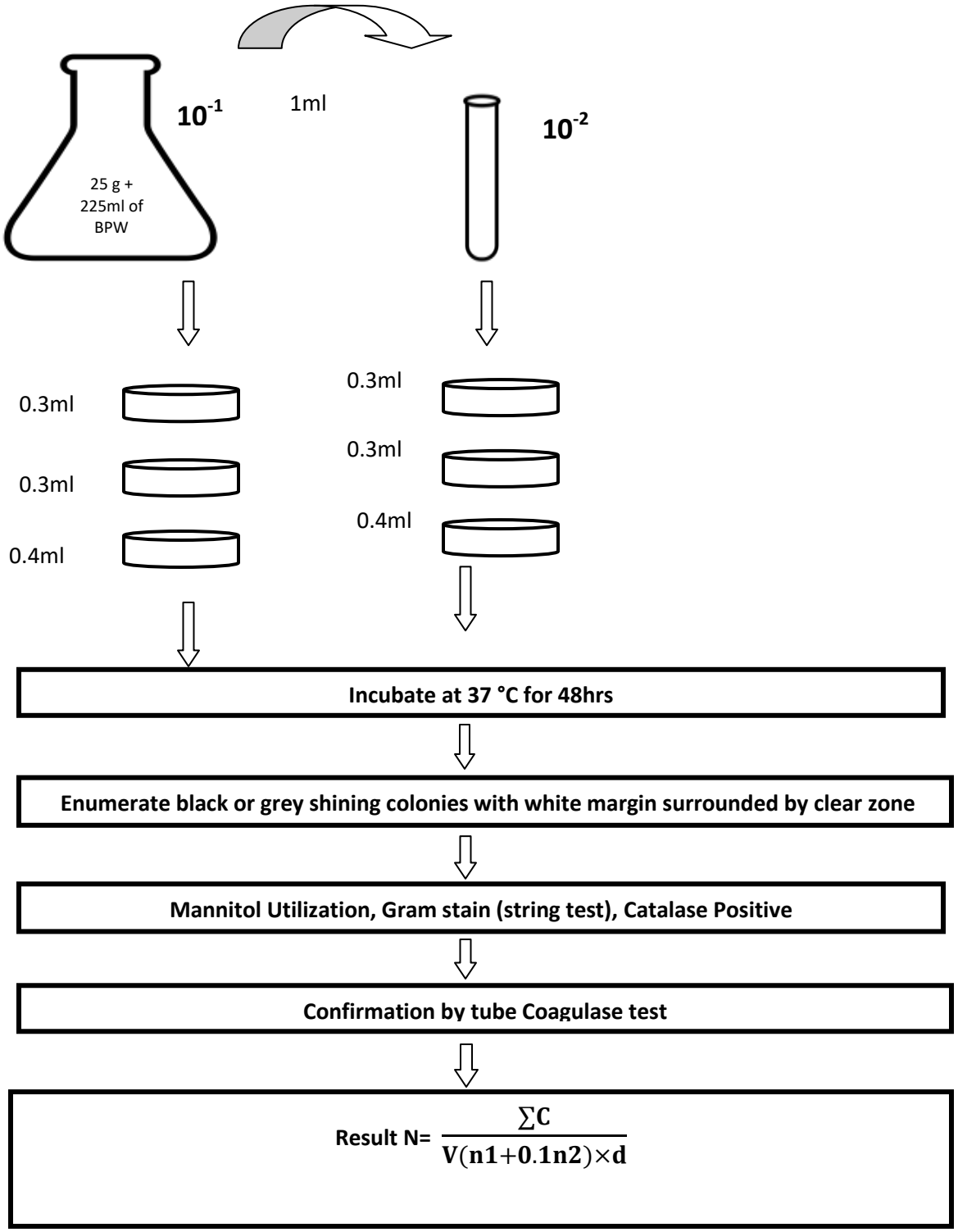
Incubate tubes at 37 °C for 24hrs and observe for yellow colouration

For each tube showing colour change take a loop, streak on tryptone bile glucuronide agar (TBX) and incubate the plate at 44°C for 24hrs

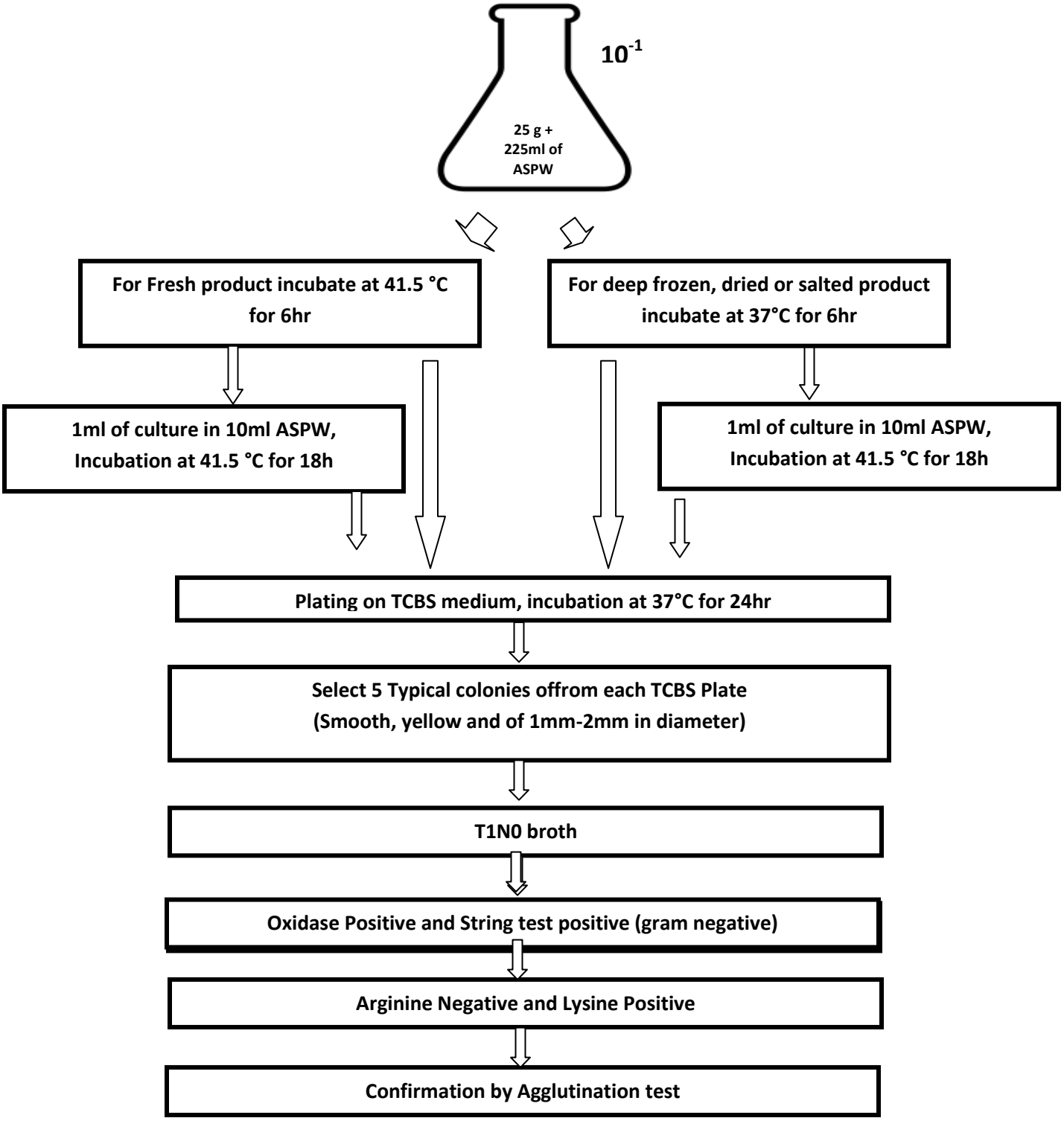
Blue green colonies

Refer 3 tube MPN table
Result ----MPN/gram

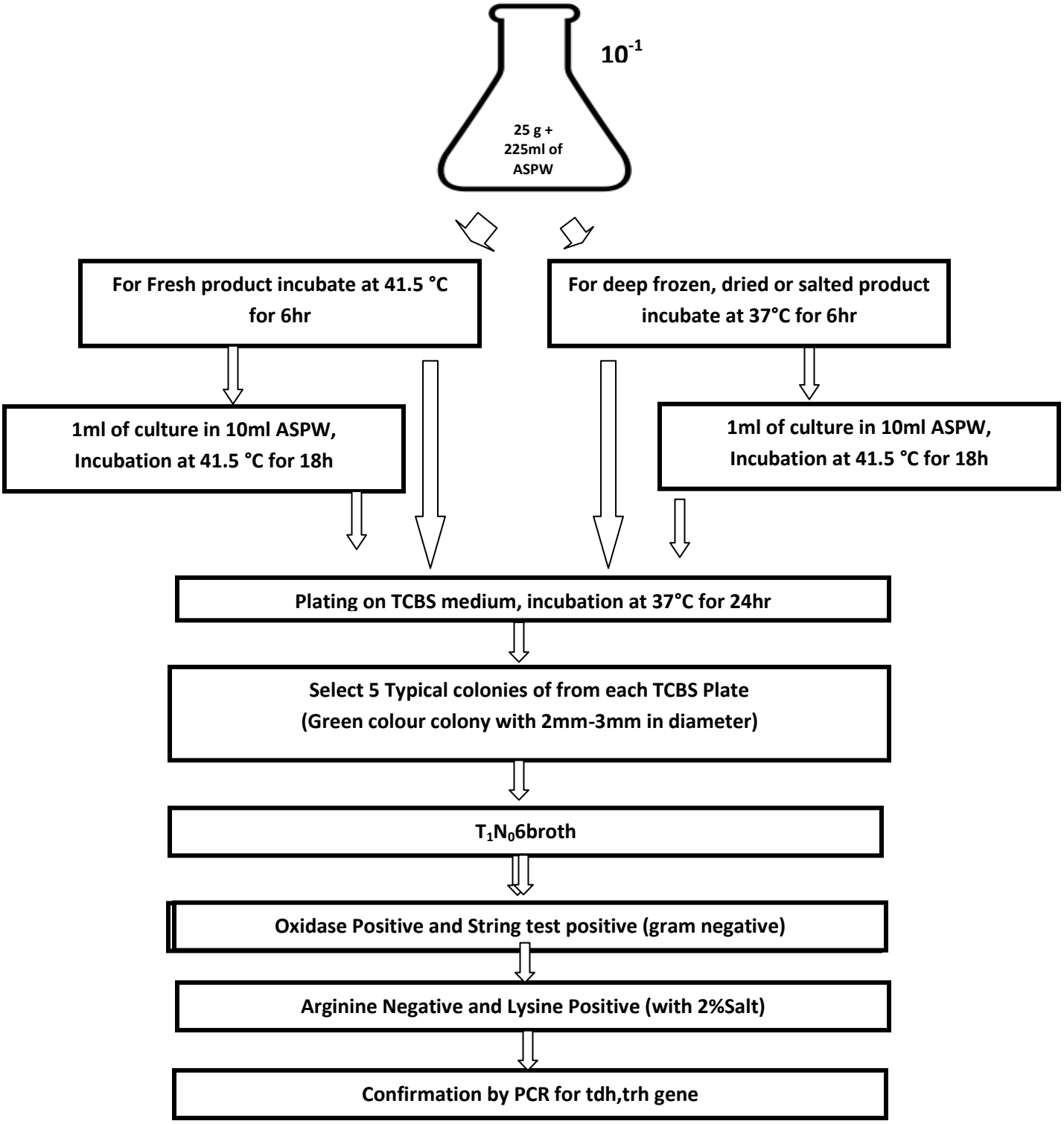
Enumeration of *Staphylococcus aureus*



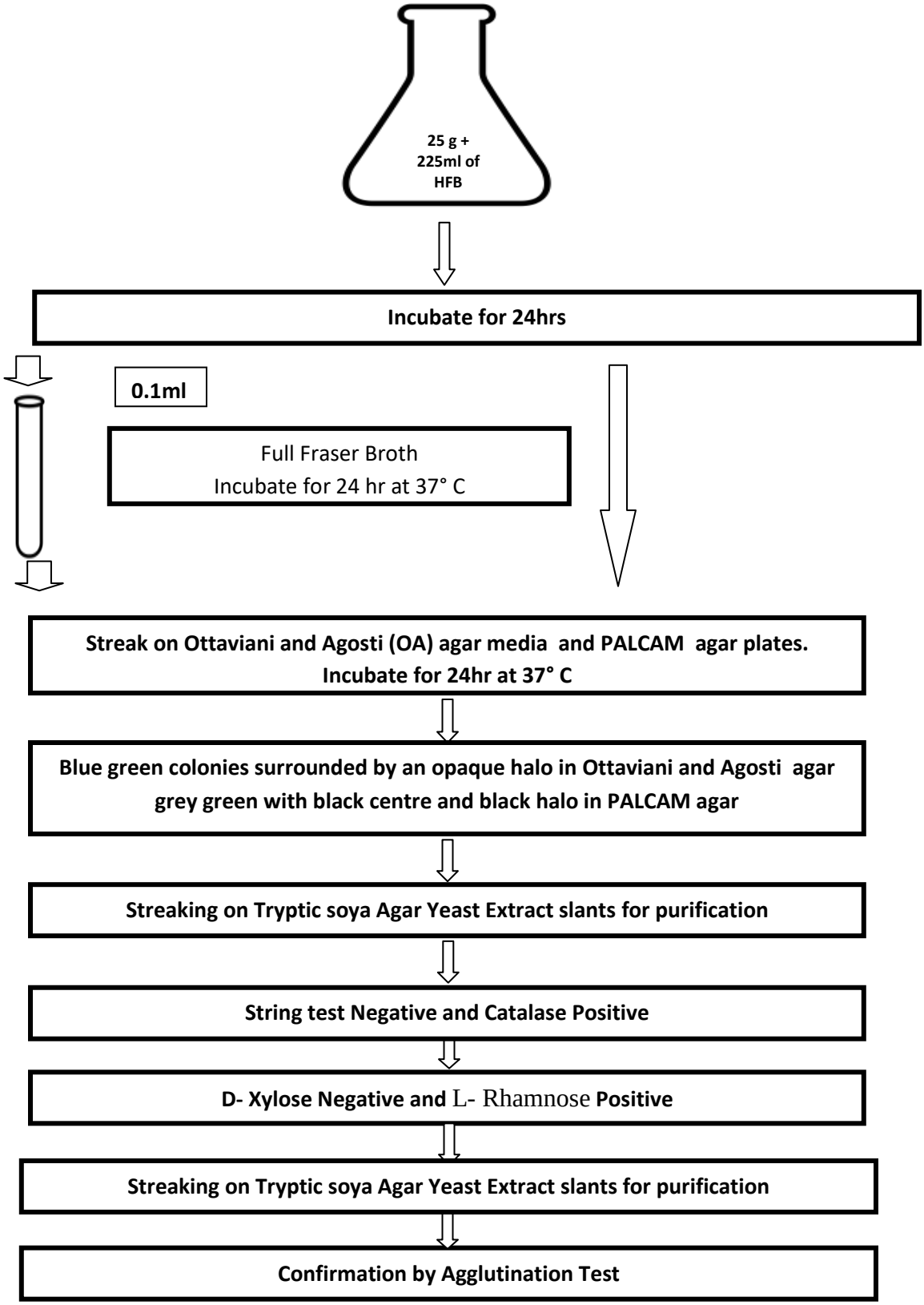
Detection of *Vibrio cholerae*



Detection of *Vibrio parahaemolyticus*



Detection of *Listeria monocytogenes*



Detection of *Salmonella*