Techniques for Detection and Enumeration of Microorganisms in Seafood

Nidhi Dhansukhbhai Patel[1], Kishan Kishorchandra Kalaria[1], A. M. Parmar[1], and Bhavika Tandel[1]

[1]*Assistant Professor, College of Fisheries Science, Kamdhenu University, Navsari*

Corresponding Author
Nidhi Dhansukhbhai Patel
Email: nidhi25.np@gmail.com

OPEN ACCESS

Keywords

Microbiology, Seafood, Enumeration Tests

How to cite this article:

Patel, N. D., Kalaria, K. K., Parmar, A. M. and Tandel, B. 2025. Techniques for Detection and Enumeration of Microorganisms in Seafood. *Vigyan Varta* 6 (11): 53-59.

ABSTRACT

Fish and seafood are key sources of animal protein with growing consumption due to health benefits over red meats. However, seafood-related infections are caused by diverse pathogens, including bacteria, viruses, and parasites, resulting in various illnesses. Risk levels vary by seafood type, influenced by factors like harvesting environment, feeding habits, season, and preparation methods. Preventing these infections requires understanding the causative agents, associated seafood types, and contamination routes that can be controlled. Effective control and monitoring strategies are essential to ensure the safety of seafood products.

INTRODUCTION

Various different methods are available for the enumeration of microorganisms from food. These can be broadly divided in to direct methods or indirect enumeration methods.

I. Direct enumeration methods

1. Direct counting methods

- Direct microscopic count (DMC)
- Direct counting on membrane filters

2. Culture based methods Plate count method

- Pour plate method
- Spread plate method

E-ISSN: 2582-9467
Popular Article
Patel et al. (2025)

- Most probable number (MPN) technique

II. Indirect enumeration methods

a. Alternative Methods (chemical and physical methods)

- Dye reduction test
- Electrical methods
- ATP determination

b. Rapid Methods

- Immunological methods
- DNA/RNA methodology

Direct counting methods

In direct counting methods, microorganisms in food are quantified by either observing them directly under a microscope from the food sample or by first filtering the sample to trap the microorganisms on a filter paper and then examining them microscopically.

A. Direct microscopic count (DMC)

The Direct Microscopic Count (DMC) is a straightforward method used to detect microorganisms in food by microscopic examination. In this technique, a smear of the food sample or culture is prepared on a slide, stained with a suitable dye, and observed under an oil immersion lens to count all cells. This approach is reliable only when microbial numbers are high, approximately 106106 cells per milliliter, and is commonly employed in the dairy industry to assess the microbial quality of raw milk and dairy products. (Kator and Rhodes, 2003).

Advantages	Disadvantages
• Quick and convenient method for counting microorganisms • Applicable to various types of foods • Easy to carry out • Allows observation of cell shape and structure	• Microscopic counting is tedious and causes fatigue. • Counts both live and dead cells. • Food debris can be mistaken for microbes. • Cells may be unevenly distributed. • Some cells do not stain and go unnoticed. • DMC results are higher than plate counts. • Requires sample dilution

B. Direct counting on membrane filters

Membrane filters with pore sizes smaller than 0.45 µm trap bacteria, and the captured cells can then be counted under a microscope.

Procedure involved

- Bacteria are collected on polycarbonate filters from a measured sample volume.
- The retained bacteria are stained and counted.
- The membrane is placed on nutrient agar or a media-soaked pad and incubated.
- After incubation, colonies are counted.

Advantages
• Suitable for samples with low bacterial counts. • Allows concentration of bacteria by filtering large sample volumes. • Only a small food sample can be tested per membrane. • Efficiency improves with fluorescent

staining (e.g., acridine orange) and epifluorescence microscopy.

- Useful for counting microorganisms in various foods like fish, meat, and water samples.

Culture based methods

Culture methods are used to examine microorganisms in food by encouraging their growth in liquid or solid media. When grown on solid agar, bacteria produce visible colonies, and counting these viable colonies provides an estimate of the microbial load in the food. Enumeration of microorganisms using culture-based techniques can be performed through either the plate count method or the Most Probable Number (MPN) method.

A wide range of culture media with different compositions is available to support the growth and cultivation of various microorganisms.

- Media composition depends on the microorganism group or type being studied.
- It varies according to the study's purpose.
- Can be tailored to grow a broad range or specific types of microorganisms.
- May support recovery of damaged but viable cells.
- Depends on the diagnostic information needed

Example:

✓ General-purpose medium: Plate count agar

✓ Lactose broth: Used for isolating *Escherichia coli*

✓ Selective medium: Baird Parker agar for detecting *Staphylococcus*

✓ Bismuth sulphite agar: Used for identifying *Salmonella*

✓ TCBS agar: Suitable for isolating *Vibrio* species

A. Plate count method

The Standard Plate Count (SPC), also commonly known as the Total Plate Count (TPC) or Aerobic Plate Count (APC), is a widely used conventional technique for quantifying viable cells or colony-forming units (CFU) in food samples. In SPC, the food sample is first blended or homogenized, followed by serial dilution in an appropriate diluent. The diluted sample is then plated—either in agar (pour plate method) or on the agar surface (spread plate method)—and incubated at a suitable temperature for a defined period. After incubation, visible colonies are counted as CFU.

The principle of SPC is that each viable bacterial cell multiplies and develops into a visible colony. Therefore, counting these colonies provides an estimate of the number of bacterial cells present in the sample. For accuracy, results are calculated by averaging replicate plates that contain between 30 and 300 colonies (Feng et al., 2020)

A. Pour plate method

Appropriate dilution of the sample (1 ml) is mixed with agar medium, allowed to set, incubated at appropriate temperature and colonies developed are counted. Here colonies develop both on surface and subsurface of agar plate. Proper mixing of sample with agar medium is necessary so as to get isolated colonies which can be done by 2 ways (Boziaris and Parlapani, 2014).

- Add 1 ml of the suitable sample dilution to a petri dish, then pour approximately 15 ml of agar medium and mix by rotating the dish in both clockwise and counterclockwise directions.

- Alternatively, place 1 ml of the appropriate dilution into a test tube with about 15 ml of molten agar, mix by rolling the tube between your palms, pour the mixture into petri dishes, let it solidify, and then incubate.

B. Spread plate method

A 0.1 ml portion of the diluted sample is spread over the surface of a pre-poured, solidified agar plate using a glass rod, then incubated at the suitable temperature, and the surface colonies are counted (Boziaris and Parlapani, 2014).

Advantages	Disadvantage
✓Suitable for heat-sensitive psychrotrophs ✓Helps identify colonies on selective media ✓Supports aerobes; microaerophiles grow slowly	✓Problem of spreaders and colony crowding makes the enumeration difficult.

B. Most probable number (MPN) technique

The Most Probable Number (MPN) method is useful for estimating low levels of microorganisms in food samples. It involves inoculating sets of liquid media tubes (usually 3 or 5 tubes) with three different sample volumes or dilutions and incubating them at a suitable temperature. After incubation, the presence or absence of microbial growth is recorded, and the MPN table is referenced to determine the estimated number of organisms in the sample. Typically, MPN values tend to be higher than those obtained from Standard Plate Counts (SPC) (Kator and Rhodes, 2003).

Advantages	Disadvantages
✓Simple and easy to perform. ✓Gives comparable results across laboratories. ✓Uses specific media to detect particular organisms. ✓Effective for detecting low numbers of microorganisms	✓Requires use of large number of glassware and large volume of sample ✓Can not observe colony morphology ✓Lack of precision

Study of microorganisms by alternate and rapid methods

The types and number of microorganisms present in food sample can be determined either by measuring the metabolites released by the microorganisms and constituents of microbial cells employing physical /chemical methods, or by using rapid methods such as enzyme linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR).

Alternative methods (physical and chemical methods)

1. Dye reduction test (DRT)

The Dye Reduction Test (DRT), used in the dairy industry to assess raw milk quality, measures viable bacteria by their ability to reduce redox dyes. These dyes change color when oxidized and become colorless or differently colored when reduced. Examples include methylene blue (blue to colorless), resazurin (blue to pink/white), and triphenyltetrazolium salt (colorless to red/maroon). In the test, food supernatant is mixed with dye, incubated briefly, and observed for color change. Faster reduction indicates higher bacterial counts.

Advantages	Disadvantages
✓ Simple, rapid and inexpensive method. ✓ Suitable for assessing quality of raw material at farm or dairy.	✓ Not all organisms are able to reduce dye equally. ✓ Not suitable for food that contain reductive enzymes.

2. Electrical methods

The electrical method is a physical technique widely used for microbiological analysis of foods. It works on the principle that microbial growth alters the chemical composition and electrical properties of a liquid medium. These changes, measured as variations in electrical impedance, indicate microbial load. As microorganisms grow, they convert low-conductivity compounds into high-conductivity products, reducing impedance. The method provides consistent results across species and can detect as few as 10–100 cells. Populations of 10^5–10^6 cells/ml are detected in 3–5 hours, while 10^4–10^5cells/ml are detected in 5–7 hours (Boziaris and Parlapani, 2014).

Application

- Effective for assessing vegetable quality, showing 90–95% agreement with total plate count (TPC); analysis takes about 5 hours and suits ground meat and similar foods.
- In pasteurized milk, an impedance detection time of 7 hours or less indicates a bacterial load equivalent to a TPC of 10^4 cells/ml or higher.

3. ATP measurement

ATP is the main energy source in all living cells and links energy release from catabolism to energy use in anabolism. It disappears within about 2 hours after cell death, and its amount per bacterial cell is fairly constant, allowing ATP measurement to estimate microbial numbers. In growing bacteria, ATP content is 2–6 nanomoles per mg dry weight. Measurement uses the firefly luciferin–luciferase system, where ATP triggers light emission that is proportional to its amount, with luminometers detecting the photons produced during ATP hydrolysis.

Applications:

- ATP assay helps assess microbial quality in foods.
- Requires removing non-microbial ATP (e.g., centrifugation, resin, filtration).
- Rapid and sensitive but not used for routine microbial testing.
- Suitable for hygiene checks in food plants.
- Surfaces are swabbed, and relative light units (RLU) measured with a luminometer.
- High ATP from any source signals poor hygiene.

4. Thermostable nuclease test

Thermostable nuclease assay is a chemical method used to detect *Staphylococcus aureus* in foods by identifying its heat-stable nuclease enzyme. *S. aureus* causes food poisoning through enterotoxin production, which correlates with nuclease and coagulase enzyme activity. Since coagulase is heat-sensitive, nuclease is preferred. Higher bacterial counts increase detectable thermonuclease levels; 0.34 enzyme units correspond to about 9.5×10^{-3} µg of enterotoxin. The assay is as effective as the coagulase test, with nuclease detectable at 10^5–10^6/ml and enterotoxin appearing above 10^6/ml.

Advantages

- Thermostable nuclease remains in food after bacteria are destroyed.
- Detectable within 3 hours, faster than enterotoxin.
- Toxigenic strains produce nuclease before enterotoxin.
- Nuclease detection does not need culture concentration; enterotoxin detection does.

- Nucleases are heat stable, similar to enterotoxins.

5. Limulus lysate test

The Limulus lysate test detects endotoxins from pathogenic Gram-negative bacteria, which contain lipopolysaccharides (LPS) and lipid A in their cell walls. LPS is pyrogenic and linked to infection symptoms. The test uses lysate proteins from horseshoe crab amoebocytes, highly sensitive to endotoxin. Food suspension samples are mixed with lysate and incubated at 37∘C for 1 hour; endotoxin presence causes gel formation. With a chromogenic substrate, the endotoxin-activated enzyme releases p-nitroaniline, measurable at 405 nm, proportional to endotoxin levels. Knowing endotoxin content per bacterial cell allows estimation of total bacterial load. This rapid method is ideal for detecting Gram-negative bacteria spoilage in refrigerated foods like fish and meat (Boziaris and Parlapani, 2014).

Advantages

- Detects both viable and non-viable Gram-negative bacteria.
- Suitable for testing milk, milk products, and raw fish.
- Provides quick results; high LAL values need further testing.
- Low LAL titres indicate low risk of Gram-negative contamination.

Enumeration of microorganisms by rapid methods

Traditional culture-based techniques often struggle to detect microorganisms in food because of their low concentrations or loss of viability caused by cell damage, and they also require lengthy laboratory processing times. These limitations can be addressed using culture-independent methods that identify bacterial components or metabolites through immunological and molecular approaches. Among the rapid detection techniques commonly used in food microbiology are the Enzyme-Linked Immunosorbent Assay (ELISA) and the Polymerase Chain Reaction (PCR).

1. Enzyme Linked Immunosorbent Assay (ELISA)

ELISA (Enzyme-Linked Immunosorbent Assay) in food microbiology is used to identify specific pathogens or their toxins. It relies on a highly specific antigen-antibody reaction. When the antigen (such as bacterial cells or toxins) binds to its antibody, an enzyme catalyzes a reaction with a chromogenic substrate, producing a color. The color intensity corresponds to the amount of antigen present. Common enzymes used include horseradish peroxidase and alkaline phosphatase, which release a colored dye upon substrate interaction. This makes ELISA a sensitive method to detect low levels of microbial antigens or toxins effectively.

Procedure

- Antigen is placed in a tube or microtitre plate with antiserum and incubated.
- Excess antiserum is washed away, then enzyme-labeled anti-immunoglobulin is added.
- After washing, the remaining enzyme is measured to find antibody levels.
- A substrate is added that reacts with the enzyme, producing a color measured colorimetrically.

Polymerase chain reaction: (PCR)

Polymerase Chain Reaction (PCR) is a molecular biology method used in food microbiology to detect specific microorganisms by targeting their unique gene sequences, especially when bacteria are present in very low numbers or are injured and cannot be cultured conventionally. PCR commonly targets virulence genes of pathogens like cholera toxin gene in Vibrio cholerae, hemolysin gene in V. parahaemolyticus, and toxin genes in E. coli and Salmonella. The technique amplifies these target DNA sequences using specific primers and polymerase enzyme through repeated heating and cooling cycles, producing millions of DNA copies rapidly. The amplified DNA is then detected by gel electrophoresis and visualized under UV light after staining. PCR enables rapid, sensitive, and specific microbial detection in foods (Zammit et al., 2008).

Advantages

1. PCR is highly specific, amplifying the target DNA from the pathogen even among other DNA.
2. It is very sensitive, detecting DNA from very few microbial cells.
3. PCR provides rapid results within a few hours.
4. It can use food samples directly or after enrichment for DNA extraction and amplification.
5. These features make PCR a fast, accurate, and sensitive method for detecting microorganisms in food

REFERENCES:

Boziaris, I. S., & Parlapani, F. F. (2014). Microbiological examination of seafood. *Seafood Processing: Technology, Quality and Safety*, 387-418

Feng, P., Weagant, S. D., Grant, M. A., & Burkhardt, W. (2020). Aerobic Plate Count. In *Bacteriological Analytical Manual (BAM), Chapter 3*. U.S. Food & Drug Administration.

Kator, H., & Rhodes, M. (2003). Detection, enumeration and identification of environmental microorganisms of public health significance. *Handbook of Water and Wastewater Microbiology*, 113-144

Zammit, C. M., Mutch, L. A., Watling, H. R., & Watkin, E. L. J. (2008). Evaluation of quantitative real-time polymerase chain reaction for enumeration of biomining microorganisms in culture. *Hydrometallurgy*, *94*(1-4), 185-189.