How to Detect Microorganisms in Food: Methods and Techniques

Food serves as excellent substrate for the growth of different kinds of microorganisms. Microorganisms enter into food and grow as contaminants or intended additions. Growth of microorganisms in food may spoil food quality and consumption of such food creates hazardous health effects in human and animal.

Food is assessed for their quality in terms of physical, chemical, sensory and microbiological characteristics. Microbiological characteristics are assessed in terms of the microorganisms- moulds, yeast, bacteria, protozoa and virus- present in food, their characters, ability to change the quality, their influence on health of consumer.

It is necessary for food microbiologists to become acquainted with the microorganisms important in food at least to the extent that will enable them to identify the main types with their characteristics. Knowledge of general characters and primary identification methods is necessary for the people working with food science and technology.

Enumeration and Detection of Food Borne Organisms:

Detection of Bacteria- *E. coli*, *Salmonella*, *Clostridium botulinum* and *Vibrio cholerae*.

There are number of steps used for the detection of microbial flora, especially bacteria present in food. The most important methods for detecting the microorganisms from food are:

1. Microscopic studies— morphology and staining reactions

2. Motility studies

3. Cultural characteristics

4. Biochemical tests

5. Chemical and molecular biology techniques, and

6. Immunological techniques.

Culture, Microscopic and Sampling Methods:

The examination of food for the presence, types, and numbers of microorganisms and their products is the fundamental procedure in food microbiology. The detection of microorganisms mainly looks for the total count of a particular type of microorganisms in a particular gram weight of food. The most important standard microbiological methods used for the detection of total number of microorganisms in food are:

1. Direct Microscopic Count (DMC)

2. Aerobic Plate Counts (APC) or Standard Plate Counts (SPC)

3. Most Probable Numbers (MPN)

4. Microscope Colony Counts

5. Agar Droplets

6. Dry Film

7. Dye Reduction - MBRT Test

8. Roll Tubes.

1. Direct Microscopic Count (DMC):

This method is very simple and rapid method for the initial morphological identification and count of bacteria and moulds. Morphologically bacteria are classified into different groups.

A. Cocci: These are round shaped organism. The various types of cocci are:

(i) Diplococci: Cocci in pair e.g. Neisseria gonorrheae (causative organism of gonorrheae), Neisseria meningitidis (causative organism of meningitis).

(ii) Tetrad: Cocci arranged in a group of four cells, e.g. Micrococcus sps, Pediococcns sps.

(iii) Sarcinia: Cocci arranged in a group of eight cells, e.g Methanosarcina sps.

(iv) Cocci in Chain: Their plane of cell division is always in one directioin, e.g. Streptococcus faecalis.

(v) Cocci in Cluster: Because of their plane of cell division is more than one direction e.g. Staphylococcus areus.

B. Bacilli: These are rod shaped bacteria.

(i) Diplobaccili: Bacilli in pair, e.g. *Klebsiella pneumoniae*

(ii) Bacilli in Chain: Bacilli are arranged in long chains, e.g. *Bacillus anthracis*

C. Pleomorphic Bacteria: Bacteria do not have a definite shape they lack cell wall e.g. *Mycoplasma pneumoniae*

D. Spirillum (Spiral Shaped): They are thick, slunder, short spiral shaped bacteia, e.g. Azospirillum sps.

E. Spirochete: These are thin, long helical or spiral shaped bacteria, e.g. – *Treponema pallidum*, *Helicobacter pylori, Leptospira icterohaemorrhagiae*.

F. Vibrio: These are curved rods, e.g. Vibrio cholerae

The DMC consists of making smears of food specimens or cultures onto a microscope slide, staining with an appropriate dye, and viewing and counting cells with the aid of a microscope (oil immersion objective). Simple stains with any basic dyes are the simplest method for the detection and enumeration of microorganism especially bacteria and yeast.

But differential staining such as **gram stain**, **acid fast stains**, **capsule staining**, **cell wall staining**, etc., are more reliable method because it not only gives the microbial count but also gives types of the bacteria present in the food.

In the case of fungus, a **lactophenol cotton blue staining** is the simplest and suitable method for the detection and enumeration of moulds. DMCs are most widely used in the dairy industry for assessing the microbial quality of raw milk and other dairy products.

There are three main types of direct microscopic count methods:

1. Breed Count:

It is developed by R.S. Breed (Breed count). The sample is spread (about 0.01 ml) over 1 cm^2 of a microscope slide. Milk smear is dried and stained with **Newman Lampert stain**; **methylene blue milk smear stain**. This stain fixes the smear, dissolves fat globules and stains bacteria with methylene blue. Slide is then observed under several oil immersion microscopic fields.

The number of organisms in milk is determined based the following calculation, are made as follows:

Area of one microscope field = 0.02 mm^2.

Area over which milk sample is spread on the slide = 1 cm^2 or 100 mm^2

Then, number of fields possible under the lens = 100 mm^2/0.02 mm^2= 5000 fields

This number of fields is for 0.01 ml of milk. However, final count is expressed as bacterial number per ml of sample. Thus, factor is to be multiplied by 100, i.e. 5000 x 100 = 500,000

This is the microscope factor.

For example– 20 fields yielded an average of 0.4 bacteria per field. Then, the final bacterial count would be 0.4 x 500, 000 (microscope factor) = 200,000 bacteria per ml of milk sample.

2. Slide Method Using INT:

A slide method to detect and enumerate viable cells has been developed. The method employs the use of the **tetrazolium salt** (p-iodophenyl-3-p-nitrophenyl)-5-phenyl tetrazolium chloride (INT). In this method cells are exposed to filter-sterilized INT for 10 minutes at 37°C in water bath followed by filtration on 0.45-µm membranes. Following drying of membranes for 10 minutes at 50°C, the special membranes are mounted in cottonseed oil and viewed with cover slip in place. The method was found to be effective for pure cultures of bacteria and yeasts.

3. Howard Mould Counts:

This is a microscope slide method developed by B.J. Howard in 1911 primarily for the purpose of monitoring tomato products. This method involves the detection of fungi especially moulds. The method requires the use of a special chamber (slide) designed to enumerate mould mycelia. By this method we can identify almost all moulds which are responsible for the spoilage of fruits and vegetables.

Merits and Demerits of Direct Microscopic Count:

The advantages of DMC are it is rapid and simple, cell morphology can be assessed and it lends itself to fluorescent probes for improved efficiency.

DMS has got so many disadvantages also. It is a microscopic method and therefore fatiguing to the analyst, both viable and nonviable cells are enumerated, food particles are not always distinguishable from microorganisms, microbial cells are not uniformly distributed relative to single cells and clumps, some cells do not take the stain well and may not be counted and DMC counts are invariably higher than counts by SPC.

2. Aerobic Plate Counts (APC) or Standard Plate Counts (SPC):

In this method portions of food samples are blended or homogenized, serially diluted in appropriate diluents, plated in or onto a suitable agar medium and incubated at an appropriate temperature for a given time, after which all visible colonies are counted by use of a colony counter. The SPC is the

most important method for determining the number of viable cells or colony-forming units (CFU) in a food product.

SPC determines the following factors:

(i) The method of sampling and plating.

(ii) Nature and type, distribution of microorganisms in the food sample.

(iii) Nature and type of food material.

(iv) The pre-evaluation results of the food product.

(v) Nutritional contents of plating medium.

(vi) Temperature of incubation and time.

(vii) pH, water activity (aw), and oxidation-reduction potential (Eh) of the plating medium.

(viii) Type of diluents used for the preparation of food samples.

(ix) Relative number of organisms in food sample.

(x) Presence and competition between microorganisms in food.

SPC can be performed by two methods:

(i) Spread plate and

(ii) Pour plate

(i) Spread Plate Method:

In spread plate method pre poured and hardened agar plates with dry surfaces are used. The food samples are serially diluted and 0.1 mm inoculum is taken using sterile pipette and evenly distributed over the agar surface. The inoculum is distributed over the agar surface with the help of bent glass rod.

The dispersed cells develop into isolated colonies called Colony Forming Units (CFU). Because the number of colonies should equal the number of viable organisms in the food sample and spread plates can be used to count the microbial population in food sample.

Surface plating offers advantages in determining the number of heat- sensitive psychrotrophs in a food product because the organisms do not come in contact with melted agar. It is the method of choice when the features of a colony are important to its presumptive identification and for most selective media. Strict aerobes are obviously favored by surface plating, but microaerophilic organisms tend to grow slower rate.

The disadvantages of surface plating are the problem of spreaders (especially when the agar surface is not adequately dry prior to plating) and the crowding of colonies, which makes enumeration more difficult.

(ii) Pour Plate Method:

The original food sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. Then small volumes, around one ml of several diluted

samples are mixed with liquid agar that has been cooled to about 45°C, and the mixtures are poured immediately into sterile culture dishes.

After the agar has hardened, each cell is fixed in place and forms an individual colony (CFU). Plates containing between 30 and 300 colonies are counted. The total number of colonies equals the number of viable microorganisms in the diluted sample. Colonies growing on the surface also can be used to inoculate fresh medium and prepare pure cultures.

Preparation of Food Sample for SPC:

The primary steps of food sample preparation for the detection of microorganisms are homogenization.

The most important types of food homogenization equipment's are:

(i) Colwell Stomacher:

It was developed in England by Sharpe and Jackson and this device is one of the best methods for homogenizing food for plating and enumeration. The Stomacher is a simple device which homogenizes specimens in a special plastic bag by the vigorous pounding of two paddles. The pounding effects the shearing of food specimens releases microorganisms into the diluents.

(ii) High-Speed Blender:

It helps to homogenize the food material effectively by high speed-blender and the microorganisms are released from food.

(iii) Shaker:

It is the common method for homogenizing the food. Shaking of food samples releases the microorganisms in to diluents.

(iv) The Spiral Plater:

It is a mechanical device that distributes the liquid inoculum on the surface of a rotating plate containing a suitable poured and hardened agar medium. The enumeration of colonies on plates prepared with a spiral plater is achieved by use of a special counting grid. Depending on the relative density of colonies, colonies that appear in one or more specific areas of the superimposed grid are counted.

Membrane Filter Techniques:

Membranes with a pore size that will retain bacteria (generally 0.45 and μm) but allow water or diluent to pass are used. A given volume of food sample is filtered through the membrane and the membrane is placed on an agar plate or an absorbent pad saturated with the culture medium.

The membranes along with the plates are incubated and the colonies are enumerated. The organisms collected on the membrane are viewed and counted microscopically following appropriate staining, washing and treatment of the membrane to render it transparent. There are two types of membranes can be used polypore filters and millipore filters.

These methods are especially suited for samples that contain low numbers of bacteria. Although relatively large volumes of water can be passed through a membrane without clogging it, only small samples of dilute homogenates from certain foods can be used for a single membrane.

The overall efficiency of membrane filter methods for determining microbial numbers by the direct microscopic count has been improved by the introduction of fluorescent dyes. The use of fluorescent dyes and epifluorescent microscopes to enumerate bacteria in water introduced recently. The membrane is made up of nitrocellulose filters or polycarbonate nucleopore filters. The second one offers the advantage of retaining all bacteria on top of the filter. There are wide varieties of membrane filter techniques developed for enumeration of bacteria.

The most important types are explained below:

a. Direct Epifluorescent Filter Technique:

The Direct Epifluorescent Filter Technique (DEFT) employs fluorescent dyes and fluorescent microscopy and it is a rapid method to enumerate microorganisms in food. In this method, the food sample is first homogenized, diluted and filtered through a 5-µm nylon filter; the filtrate is then collected and treated with 2 ml of Triton X-100 and 0.5 ml of trypsin.

The trypsin will breake somatic cells and to prevent clogging of filters. The treated filtrate is passed through a 0.6-µm nucleopore polycarbonate membrane and the filter is stained with acridine orange. After drying, the stained cells are enumerated by epifluorescence microscopy and the number of cells per gram is calculated by multiplying the average number per field by the microscope factor.

b. Microcolony-DEFT:

Microcolony-DEFT is a variation of DEFT that allows one to determine viable cells only. The food homogenates are filtered through DEFT membranes, placed on the surface of appropriate culture media and incubated for microcolony development. Three-hour incubation can be used for gram-negative bacteria and 6-hour incubation for gram-positives. The microcolonies that develop must be viewed with a microscope.

In another variation, a micro colony epifluorescence microscopy method that combined DEFT with hydrophobic grid membrane filter (HGMF) was devised. By this method, nonenzyme detergent treated samples are filtered through nucleopore polycarbonate membranes, which are transferred to the surface of a selective agar medium and incubated for 3 or 6 hours for gram-negative or gram-positive bacteria as for microcolony-DEFT. The membranes are then stained with acridine orange and the microcolonies are enumerated by epifluorescence microscopy.

c. Hydrophobic Grid Membrane Filter (HGMF):

The Hydrophobic Grid Membrane Filter (HGMF) technique was developed by Sharpe and Michaud and it used to enumerate microorganisms from a variety of food products. The method employs a special type of filter that consists of 1600 wax grids on a single membrane filter that restricts growth and colony size to individual grids. Using one filter 10 to 9 x 104 cells can be enumerated by an MPN procedure and enumeration can be automated.

It can be used to enumerate all CFUs or specific groups such as indicator organisms, other types of bacteria and fungi. In a typical application, 1 ml of a 1:10 homogenized food sample is filtered through a membrane and the membrane is placed on suitable agar medium for overnight incubation for colony development. The grids that contain colonies are enumerated and the MPN is calculated. The method allows the filtering of up to 1 g of food per membrane.

3. Most Probable Numbers (MPN):

Monitoring and detection of indicator and disease-causing microorganisms are a major part of sanitary microbiology. A wide range of viral, bacterial and protozoan diseases result from the contamination of water with human fecal wastes. Although many of these pathogens can be detected directly, environmental microbiologists have generally used indicator organisms as an index of possible water contamination by human pathogen.

Coliforms are defined as gram-negative, non-sporing, facultatively anaerobic, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C.

The following are among the suggested criteria for indicator organisms:

(i) The indicator bacterium should be suitable for the analysis of all types of water – tap, river, ground, impounded, recreational, estuary, sea, and waste.

(ii) These organisms should be present wherever enteric pathogens are present.

(iii) The indicator bacterium should survive longer than the hardiest enteric pathogen.

(iv) The indicator bacterium should not reproduce in the contaminated water and produce an inflated value.

(v) The assay procedure for the indicator should have great specificity; in other words, other bacteria should not give positive results. In addition, the procedure should have high sensitivity and detect low levels of the indicator.

(vi) The testing method should be easy to perform.

(vii) The indicator should be harmless to humans.

(viii) The level of the indicator bacterium in contaminated water should have some direct relationship to the degree of fecal pollution.

MPN test is the most widely used method to find out the potability of water by testing the viable bacterial count. This method was introduced by McCrady in 1915.

The original test for coliforms that was used to meet this definition involved the presumptive, confirmed, and completed tests. The presumptive step is carried out by means of tubes inoculated with three different sample volumes to give an estimate of the most probable number (MPN) of coliforms in the water.

The complete process, including the confirmed and completed tests, requires at least 4 days of incubations and transfers. So, water to be tested to find out presence of fecal coliforms. These coliforms are derived from the intestine of warm-blooded animals, which can grow at the more restrictive temperature of 44.5°C.

Three serial aliquots or dilutions are then planted into 9 or 15 tubes of appropriate medium for the three- or five-tube method, respectively. Numbers of organisms in the original sample are determined by use of standard MPN tables. It is not a precise method of analysis; the 95% confidence intervals for a three-tube test range from 21 to 395.

4. Microscope Colony Counts:

It is a simple method to detect the microbial count in variety of food that involves the counting of colonies that developed over a microslide consists of a thin layer of culture medium. A 0.1 ml of milk-agar mixture is spreaded over a 4-cm2 area on a glass slide.

Following incubation, drying, and staining, microcolonies are counted with the aid of a microscope. In another method, 2 ml of melted agar are mixed with 2 ml of warmed milk and 0.1 ml of the inoculated agar is spread over a 4-cm2 area. The slide is then viewed with the 16-mm objective of a microscope after staining.

5. Agar Droplets:

The food homogenate is diluted in tubes of melted agar. For each food sample, three tubes of agar are used, the first tube being inoculated with 1 ml of food homogenate. After proper mixing transfer a line of 5 x 0.1-ml droplets to the bottom of an empty petri dish by sterile capillary pipette.

With the same capillary pipette, three drops (0.1 ml) from the first 9-ml tube are transferred to the second tube, after mixing; another line of 5 x 0.1-ml droplets is placed next to the first. This step is repeated for the third tube of agar. Petri plates containing the agar droplets are incubated for 24 hours and colonies are enumerated.

This method is reliable to enumerate microorganisms present in meats and vegetables compared other conventional plate counts; droplet counts from ground meat were slightly higher than plate counts. The method was about three times faster and 24-hour incubation gave counts equal to those obtained after 48 hours by the conventional plate count.

6. Dry Film:

A dry film method involves the use of two plastic films attached together on one side and coated with culture medium ingredients and a cold-water-soluble jelling agent to designated petrifilm. For use, 1ml of diluent is placed between the two films and spread over the nutrient area by pressing with a special flat-surface device. Following incubations, microcolonies appear red on the nonselective film because of the presence of a tetrazolium dye in the nutrient phase. Petrifilm methods exist for the detection and enumeration of specific groups, such as coliforms.

7. Dye Reduction – MBRT Test:

Dye reduction test is a common technique used to detect the microorganisms from food. Two dyes are commonly employed in this procedure to estimate the number of viable organisms in suitable products: **methylene blue and resazurin**. To conduct a dye-reduction test, the supernatant of food is prepared and added to standard solutions of either dye for reduction from blue to white for methylene blue; and from slate blue to pink or white for resazurin. The time for dye reduction to occur is inversely proportional to the number of organisms in the sample.

In a study of resazurin reduction as a rapid method for assessing ground beef spoilage, reduction to the colorless state, odor scores, and SPC. One of the problems of using dye reduction for some food is the existence of inherent reductive substances.

Dye-reduction tests have a long history of use in the dairy industry for assessing the overall microbial quality of raw milk. This test is called MBRT (Methylene Blue Reduction Test). In this test, we are detecting the number of bacteria responsible for dye reduction. If the sample contains more number of microorganisms. It will reduce the methylene blue very faster rate. The color of methylene blue changes from blue to white indicates positive result. If dye reduced very slowly which indicate the number of reducing microorganisms are less in such sample.

The advantages of this test are that they are simple, rapid, and inexpensive; and only viable cells actively reduce the dyes. Disadvantages are that not all organisms reduce the dyes equally, and they are not applicable to food specimens that contain reductive enzymes.

Interpretation of MBRT Test of Milk Quality Analysis:

Class 1 – Excellent, not decolorized in 8 hours.

Class 2 – Good, decolorized in less than 8 hours but not less than 6 hours.

Class 3 – Fair, decolorized in less than 6 hours but not less than 2 hours.

Class 4 – Poor, decolorized in less than 2 hours.

8. Roll Tubes:

Screw-capped tubes or bottles of varying sizes are used in this method. A known amount of the melted and inoculated agar is added to the tube and it is solidified as a thin layer inside the vessel. Following appropriate incubation, colonies are counted by rotating the vessel. It has been found to be an excellent method for enumerating fastidious anaerobes.

Chemical and Molecular Biology Techniques:

1. Radiometry:

The radiometric detection of microorganisms is based on the incorporation of a ^{14}C-labeled metabolite in a growth medium so that when the organisms utilize this metabolite, $^{14}CO_2$ is released and measured by use of a radioactivity.

2. Adenosine Triphosphate (ATP) Measurement:

Adenosine triphosphate (ATP) is the primary source of energy in all living cells. It disappears within 2 hours after cell death, and the amount per cell is generally constant, with values of 10 -18 to 10 -17 moles per bacterial cell. The complete extraction and accurate measurement of cellular ATP can be equated to individual groups of microorganisms in the same general way as endotoxins for gram-negative bacteria.

One of the simplest ways to measure ATP is by use of the firefly luciferin-luciferase system. In the presence of ATP, luciferase emits light, which is measured with a luminometer. The amount of light produced by firefly luciferase is directly proportional to the amount of ATP added.

3. DNA Amplification (PCR):

PCR has been used to detect enterotoxigenic E. coli, Vibrio, Clostridium, etc. and this method is an elegant technique to determine the pathogen by amplifying their DNA using specific primer.

4. Nucleic Acid (DNA) Probe:

A DNA probe consists of the DNA sequence of an organism of interest that can be used to detect homologous DNA or RNA sequence. The probe DNA must hybridize with that of the strain.

The probe must be labelled with radioisotopes or fluorescent dyes. In this method DNA fragment of unknown organisms are prepared by use of restriction enzymes. The fragments are separated by agarose gel electrophoresis and transferred to cellulose nitrate filter and hybridized to the

radiolabelled probe. After washing the presence of the radiolabelled DNA can be detected by autoradiography.

5. Immunologic Methods:

Serological reactions are effective method for detecting the pathogenic microorganisms or their toxin. The most commonly used serological methods are discussed below:

i. Fluorescent Antibody:

An antibody to a given antigen is made fluorescent by coupling it to a fluorescent compound and when the antibody reacts with its antigen, the antigen-antibody complex emits fluorescence and can be detected by the use of a fluorescence microscope. The fluorescent markers used are rhodamine B, fluorescein isocyanate, and fluorescein isothiocyanate with the last being the most widely used.

The fluorescent antibody (FA) technique can be carried out by use of either of two basic methods. The direct method employs antigen and specific antibody to which is coupled the fluorescent compound (antigen coated by specific antibody with fluorescent label).

With the indirect method, the homologous antibody is not coupled with the fluorescent label, but instead an antibody to this antibody is prepared and coupled in the indirect method, the labelled compound detects the presence of the homologous antibody; in the direct method, it detects the presence of the antigen.

ii. Enrichment Serology:

The use of Enrichment Serology (ES) is a more rapid method for recovering salmonellae from foods than the conventional culture method. It is carried out in four steps: pre-enrichment in a nonselective medium for 18 hours; selective enrichment in selenite-cystine and/or tetrathionate broth for 24 hours; elective enrichment in M broth for either 6-8 hours or 24 hours; and agglutination with polyvalent H antisera at 50°C for 1 hour.

The Oxoid Salmonella Rapid Test (OSRT) is a variation of ES. It consists of a culture vessel containing two tubes, each of which contains dehydrated enrichment media in the lower compartments and dehydrated selective media in the upper compartments.

The media are hydrated with sterile distilled water and a special salmonella elective medium is added to the culture vessel along with a novobiocin antibiotic disk, followed by 1ml of pre-enrichment culture of sample. Following incubation at 41°C for 24 hours, media in the upper compartment (selective media) of each tube are examined for color change, indicating the presence of salmonella.

iii. Salmonella 1-2 Test:

Salmonella 1-2 Test employs the use of a semisolid phase. The test is conducted in a specially designed plastic device that has two chambers, one for selective broth and the other for a nonselective motility medium. In addition to selective ingredients, the nonselective medium contains the amino acid L-Serine, which is selective for salmonellae.

Following inoculation of the selective medium chamber, the device is incubated, during which time motile salmonellae move into the nonselective medium chamber. The non-selective medium contains flagellar antibodies, and when the motile organisms enter the antibody area, an immunoband develops, indicating antigen-antibody reaction.

iv. Radioimmunoassay:

This technique consists of adding a radioactive label to an antigen, allowing the labeled antigen to react with its specific antibody, and measuring the amount of antigen that combined with the antibody by the use of a counter to measure radioactivity. Solid- phase radioimmunoassay (RIA) refers to methods that employ solid materials or surfaces onto which a monolayer of antibody molecules binds electrostatically.

The solid materials used include polypropylene, polystyrene and bromacetylcellulose. The ability of antibody-coated polymers to bind specifically with radioactive tracer antigens is essential to the basic principle of solid-phase RIA. When the free-labeled antigen is washed out, the radioactivity measurements are quantitative.

v. ELISA:

The enzyme-linked immunosorbent assay (ELISA, enzyme im-munoassay, or EIA) is an immunological method similar to RIA but employing an enzyme coupled to either an antigen or an antibody. A typical ELISA is performed with a solid-phase (polystyrene) coated with antigen and incubated with antiserum. Following incubation and washing, an enzyme-labeled prepa-ration of anti-immunoglobulin is added.

After gentle washing, the enzyme remaining in the tube or microtiter well is assayed to determine the amount of specific antibodies in the initial se-rum. A commonly used enzyme is horseradish peroxidase and its presence is measured by the addition of peroxidase substrate.

The amount of enzyme present is ascertained by the colorimetric determination of enzyme substrate. One variation of basic ELISA consist of a “sandwich” ELISA in which the antigen is required to have at least two binding sites. The antigen reacts first with excess solid-phase antibody, and following incubation and washing, the bound antigen is treated with excess labeled antibody. The ELISA technique is used widely to detect and quantify organisms and/ or their products in foods

vi. Gel Diffusion:

Gel diffusion methods have been widely used for the detection and quantitation of bacterial toxins and enterotoxins. The four most widely used methods are the single-diffusion tube (Oudin), microslide double diffusion, micro-ouchterlony slide and electroimmunodiffusion. They have been employed to measure enterotoxins of staphylococci and C. perfringens; and the toxins of C. botulinum.

vii. Hemagglutination:

Gel diffusion methods generally require at least 24 hours for results but this method yield results in 2-4 hours. There are two types of heme agglutination: hemagglutination-inhibition (HI) and reverse passive hemagglutination (RPH). Unlike the gel diffusion methods, antigens are not required to be in precipitable form for these two tests.

In the HI test, specific antibody is kept constant and enterotoxin (antigen) is diluted out. Following incubation for about 20 minutes, treated sheep red blood cells (SRBCs) are added. Hemagglutination (HA) occurs only when antibody is not bound by antigen. HA is prevented (inhibited) where toxin is present in optimal proportions with antibody. In

RPH antitoxin globulin is attached directly to SRBCs and used to detect toxin. When diluted toxin preparations are added, the test is read for HA after incubation for 2 hours. HA occurs only where optimal antigen antibody levels occur. No HA occurs if no toxin or enterotoxin is present.