INDIAN STANDARDS FOR ANALYZING MICROBIOLOGICAL SAFETY OF FOOD PRODUCTS

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Introduction to National Standardization

Standards contribute in making the development, manufacturing, supply and trade of products and services efficient and safe. They provide government with a technical base for health, safety and environmental legislation.

Bureau of Indian Standards is the National Standards body of India and was constituted under the Bureau of Indian Standards Act, 1986 taking over the assets, liabilities of the Indian Standards Institution. BIS Act 2016 came into force on 12 October 2017 superseding BIS Act 1986. BIS Act 2016 provides for the establishment of a national standards body for the harmonious development of the activities of standardization, conformity assessment and quality assurance of goods, articles, processes, systems and services and for matters connected therewith or incidental thereto.

Indian Standards formulated by Bureau of Indian Standards (BIS) are the national standards of the country, formulated by subject specific technical committees comprising of all stakeholders – producers, consumers, technologists, government bodies, research organizations and testing laboratories. Indian Standards are formulated by Technical Committees through the principles of consensus.

BIS Stays in tune with the latest developments in its fields. Standards are periodically reviewed by the technical committees so as to keep pace with technical growth. Each standard is subject to a review every five years for updating or reaffirmation.

Adoption of Indian Standards is generally voluntary in nature and their implementation depends on adoption by concerned parties. An Indian Standard becomes binding if it is in a contract referred to in legislation or made mandatory by specific orders by the Central or State Governments.

The Food and Agriculture Department (FAD) under the BIS is entrusted with the task of formulation of national standards in the field of food and agriculture under the aegis of the Food and Agriculture Division Council (FADC) which oversees and supervises its work. The scope pf FADC is as under:

‘Standardization in the field of food, feed and agriculture produce covering food and feed chain from primary production to consumption. This also includes soil management, agricultural inputs agricultural machinery, farm management, animal keeping and husbandry, fisheries and aquaculture, food processing, food and feed safety management, biotechnology for food and agriculture, drinking water and AYUSH.’

2. Aspects of Standardization - The standards are formulated covering different aspects of a subject which include:

a) **Nomenclature or Terminology**
b) **Product Specification**
c) **Test Methods**
d) **Code of Practice/Guidelines**
e) **Basic Standards**

The food standards are aimed at protecting the health of the consumers and ensuring fair practices in the food trade. A total of 1851 standards have been published under FAD. The aspect wise distribution of the standards is as under:

Product specification	-	1298
Test methods	-	546
Codes of practice	-	198
Terminology	-	56
Dimensions	-	6
System Standard	-	12
Safety Standard	-	5
Others	-	31

3. Indian Standards for Analyzing Microbiological Safety of Food products

Microorganisms pose a great challenge to the food safety as there are more than 200 foodborne diseases caused by bacteria, viruses, protozoa and fungi. Thousands of people die every year due to foodborne illnesses. Foodborne illnesses are primarily classified as food infection and food intoxication. While in the first case, live microorganism enters the body, multiplies and damages host tissue, the later can cause damage by the production of toxin in food, despite being absent in the body.

Therefore, microbial food safety is very important in the supply chain to reduce the potential for contamination. Microbial contamination may take place at pre-farming, farming or post-farming stages of the food supply chain. *Salmonella, Listeria monocytogenes, Escherichia coli, Clostridium spp*. etc. are the most common pathogenic bacteria associated with food safety issues in the food supply chain. Efficient process controls and effective food safety management systems are vital elements to reduce microbial contamination and improve food security.

Bureau of Indian Standards through its Food Hygiene, Safety Management and Other Systems Sectional Committee, FAD 15 involves in the formulation of Indian Standards in the field of -

a) Microbiological methods of tests and specifications for ingredients used in media for microbiological work;

b) Food hygiene including codes of hygienic practices applicable for food products in general (except subject/ product specific codes covered under other Sectional Committees of FAD);

c) Food safety management systems; and

d) Food Labelling.

The standards on **Microbiological methods of tests** developed under FAD 15 Committee may be categorized into various sub-categories, such as, Indian Standards on:

i) Horizontal methods for enumeration of microorganisms by pour plate and surface plate methods.

ii) Methods for detection and enumeration of various microorganisms in food chain and in animal feeding stuff, like, *Escherichia coli, Staphylococcus aureus, Salmonella, Vibrio cholrea, Vibrio parahaemolyticus etc.*

iii) Preparation of test samples, initial suspension and decimal dilutions for microbiological examination;

iv) Polymerase chain reaction (PCR & qRT-PCR) based method for the detection of food borne pathogens;

v) Sampling Techniques (Primary Production Stage) for Microbiological Analysis

vi) Method Validation (of Microbiological Methods)

vii) Specific Requirements and Guidance for Proficiency Testing by Interlaboratory Comparison

viii) Preparation, Production, Storage and Performance Testing of Culture Media

3.1 Indian Standards on Horizontal Methods for Enumeration of Microorganisms by Pour Plate and Surface Plate Methods

The microorganisms exist in nature in the form of mixture of many other cell types. Microbial population are not segregated by species. In order to identify, isolate and enumerate organisms of particular genus or species, these microbial populations are required to be separated into pure culture containing only one type of organisms suitable for the study of their cultural, morphological, and biochemical properties. To accomplish this, the serial dilution agar plate technique is used which involves serial dilution of a bacterial suspension in sterile water blanks, which serve as a diluent of known volume. Once diluted, the suspensions are placed on suitable nutrient media. The pour plate or surface plate technique are the procedures usually employed for placing the suspensions.

The Indian Standards formulated by the Sectional Committee, FAD 15, on these techniques are mentioned as under:

1.	IS 5402 (Part 1) : 2021	Microbiology of the food chain — Horizontal method for the enumeration of microorganisms Part 1 Colony count at 30°C by the Pour Plate Technique
2.	IS 5402 (Part 2) : 2021	Microbiology of the food chain — Horizontal method for the enumeration of microorganisms Part 2 Colony count at 30°C by the Surface Plating Technique

The table below gives a comparison of both the methods as prescribed in IS 5402 (Part 1) :

2021 (Pour Plate Technique) and IS 5402 (Part 2) : 2021 (Surface Plate Technique):

S. No.	IS 5402 - Microbiology of the Food Chain — Horizontal Method for the Enumeration of Microorganisms	
	Part 1 Pour Plate Technique	**Part 2 Surface Plate Technique**
1.	These standards specify a horizontal method for enumeration of microorganisms that are able to grow and form colonies in a solid medium after aerobic incubation at 30°C. The method is applicable to: a) products intended for human consumption and for animal feed; b) environmental samples in the area of food and feed production and handling.	
2.	This part of IS 5402 is applicable to: i) products that require a reliable count when a low limit of detection is specified (below 10^2/g or 10^2/ml for liquid samples or below 10^3/g for solid samples); ii) products expected to contain spreading colonies that obscure colonies of other organisms, e.g. milk and milk products likely to contain spreading *Bacillus* spp.	This part of IS 5402 is applicable to: i) products containing heat-sensitive organisms that are likely to form a significant proportion of the total flora (e.g. psychrotrophic organisms in chilled and frozen foods, dried foods, other foods that may contain heat-sensitive organisms); ii) products containing obligately aerobic bacteria that are likely to form a significant proportion of the total flora (e.g. *Pseudomonas* spp.); iii) products that contain small particles that can prove difficult to distinguish from colonies in a pour plate; iv) products whose intense colour prevents the recognition of colonies in a pour plate; v) products for which distinction between different types of colony is required as part of the assessment of food quality.
2.	A specified quantity of the liquid test sample, or a specified quantity of an initial suspension in the case of other products, is dispensed into an empty Petri dish and mixed with a specified molten agar culture medium to form a poured plate.	A specified quantity of the test sample, or a specified quantity of an initial suspension in the case of other products, is surface plated on a solid agar culture medium contained in Petri dishes.

3.2 Indian Standards on Methods for Detection and Enumeration of Microorganisms in Food Chain Responsible for Food Poisoning

Foodborne illness, more commonly referred to as food poisoning is the result of eating contaminated, spoiled, or toxic food. Foodborne illnesses are usually infectious or toxic in nature and caused by bacteria, viruses, parasites or chemical substances entering the body through contaminated food or water.

Generally, the term "food poisoning," as applied to diseases caused by microorganisms, is used to include both the illnesses caused by the ingestion of toxins released by the organisms and those resulting from infection of the host through the intestinal tract. Thus, food-borne diseases may be classified into 'poisonings' and 'infections'.

a) Food poisonings can be the result of either chemical poisoning or the ingestion of a toxicant (intoxication). The toxicant might be found naturally in certain plants or animals or it may be a toxic metabolic product excreted by a microorganism. A bacterial food intoxication, therefore, refers to food-borne illnesses caused by the presence of a bacterial toxin formed in the food.

There are two chief kinds of food intoxications caused by bacteria:

1) Botulism, caused by the presence of toxin (Neurotoxin) produced by *Clostridium botulinum* in food; and

2) Staphylococcal intoxication, caused by a toxin (Enterotoxin) from *Staphylococcus aureus* in the food.

b) Food infection by bacteria refers to foodborne illnesses caused by the entrance of bacteria into the body through ingestion of contaminated foods and the reaction of the body to their presence or to their metabolites.

Food infections can further be divided into two types:

1) Those in which the food does not ordinarily support growth of the pathogens but merely carries them. For example, the pathogens causing tuberculosis, diphtheria, the dysenteries, typhoid fever, brucellosis, cholera, infectious hepatitis, Q fever, etc.; and

(2) Those in which the food can serve as a culture medium for growth of the pathogens to numbers that will increase the likelihood of infection of the consumer of the food. For example, *Salmonella spp., Vibrio parahaemolyticus*, and enteropathogenic *Escherichia coli*.

Outbreaks of food infections of the second type are likely to be more explosive than outbreaks caused by other intestinal pathogens.

Food Hygiene, Safety Management and Other Systems Sectional Committee, FAD 15 has formulated standards on methods for detection and enumeration of various organisms responsible for food poisoning and infection. Brief of these standards is given as under:

3.2.1 Indian Standards on Method of Tests for Coliforms

Coliforms are short rods, aerobic and facultative anaerobic, Gram-negative, non-spore-forming bacteria which ferment lactose with gas formation containing the enzyme β-galactosidase. The leading species of coliform bacteria are *Escherichia coli* and *Enterobacter aerogenes*. They are commonly used as indicator of sanitary quality of foods and water. Coliforms normally do not cause serious illness, however, their presence indicate that other pathogenic organisms of fecal origin may be present in the sample.

The two standards formulated by BIS for detection and enumeration of coliforms are mentioned as under:

1. IS 5401 (Part 1) : 2012 — Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms: Part 1 Colony Count Technique
2. IS 5401 (Part 2) : 2012 — Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms: Part 2 Most Probable Number Technique

These standards are applicable to products intended for human consumption and for the feeding of animals, and environmental samples in the area of food production and food handling.

The table below gives a brief account of comparison of the **Colony Count technique** [as per IS 5401 (Part-1) : 2012] and **Most Probable Number Technique** [as per IS 5401 (Part-2) : 2012]:

S. No.	**IS 5401 - Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection and Enumeration of Coliforms**	
	Part 1 Colony Count Technique	**Part 2 Most Probable Number Technique**
1.	This standard gives general guidelines for the enumeration of coliforms by means of the technique of counting colonies after incubation on a solid medium at 30°C or at 37°C.	This standard gives general guidelines for the detection and the enumeration of coliforms. Enumeration is carried out by calculation of the most probable number (MPN) after incubation in a liquid medium at 30 °C or 37 °C.
2.	This technique is recommended when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample	This enumeration method is applicable when the number sought is expected to be in the range 1 to 100 per millilitre or per gram of test sample.
3.	The standard suggests to prepare poured plates using a solid selective culture medium, i.e. **Crystal violet neutral red bile lactose (VRBL) agar**, inoculated with a specified quantity of the test sample followed by incubation at 30°C or at 37°C	The **MPN analysis** is a statistical method based on the random dispersion of microorganisms per volume in a given sample. This method is used to estimate the concentration of viable

	for 24 hours ± 2 h. The characteristic colonies (purplish red colonies with a diameter of at least 0.5 mm, sometimes surrounded by a reddish zone of precipitated bile) are counted and, if required, a number of colonies are confirmed by fermentation of lactose in brilliant green lactose bile broth. The number of coliforms per millilitre or per gram of sample is calculated from the number of characteristic colonies obtained in the plates chosen.	microorganisms in a sample by means of a replicate liquid broth growth in ten-fold dilutions. In this method, samples are serially diluted to the point of extinction, that is, to a point where no more viable microorganisms may present. To detect the end point, multiple serial dilutions are inoculated into a suitable growth medium, and the development of opacity, cloudiness or gaseous emission is used to indicate growth (the presence of at least one viable microorganism in the diluted sample). In this standard, **Lauryl sulphate tryptose broth** has been used as a Selective enrichment medium and **Brilliant green lactose bile broth** as Confirmation medium.

3.2.2 Indian Standards on Method of Tests for *Escherichia coli*

Escherichia coli is a facultatively anaerobic, gram-negative, rod shaped bacterium that is primarily present in the gastrointestinal tract of humans and warm-blooded animals. It is one of the 'coliform groups'. Although most of the strains of *E. coli* are harmless, many are pathogenic and cause a variety of diseases in humans and animals. The genus *Escherichia* is divided into many biotypes and serotypes, some of which can be pathogenic to humans. Specific virulence attributes that have been acquired by such strains enable them to cause three principal types of infections in humans including intestinal gastroenteritis, urinary tract infections, and neonatal sepsis/meningitis.

A total of five Indian Standards on methods for detection and enumeration of *Escherichia coli* have been formulated by BIS which are given as under:

1. IS 5887 (Part I) : 1976 — Methods for detection of bacteria responsible for food poisoning part 1 isolation, identification and enumeration of *Escherichia coli*

2. IS 16067 (Part 1) : 2020 — Microbiology of the food chain — Horizontal method for the enumeration of Beta-Glucuronidase-Positive *Escherichia coli* Part 1 Colony-count technique at 44°C using membranes and 5-Bromo-4-Chloro-3-Indolyl Beta-D-Glucuronide

3. IS 16424 : 2016 — Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *Escherichia coli* — Most Probable Number Technique

4. IS 16426 : 2017 — Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157

5. IS 16987 : 2018 Microbiology of food and animal feed - Real Time Polymerase Chain Reaction (PCR) based method for the detection of food borne pathogens - Horizontal method for the detection of Shiga Toxin producing *Escherichia Coli* (STEC) and the determination of O157, O111, O26, O103 and O145 Serogroups

3.2.2.1 IS 5887 (Part-1) : 1976 - Methods for Detection of Bacteria Responsible for Food Poisoning Part 1 Isolation, Identification and Enumeration of *Escherichia coli*

The method given in this standard is a basic method for detection, isolation and enumeration of *E. coli*. The standard prescribes the **general characteristics** of the organisms, their growth characteristics on nutrient agar as well as selective media and biochemical test properties. A list of all such media has also been provided in the standard along with their composition which are required for isolation, identification and enumeration of *E. coli*.

The standard also defines procedure of **isolation** of organism. It starts with the sample preparation by homogenizing it in the sterile blender and then diluting it using peptone solution followed by inoculation on MacConkey broth, MacConkey medium, Eosin methylene blue lactose agar and Tergitol-7 agar (optional) and then incubation at 37ºC for overnight. The inoculated samples are further used for the identification of *E. coli*.

For **identification,** the standard prescribes to test the suspected colonies showing characteristic properties (as defined in the standard) for Gram's Stain and motility test followed by biochemical tests for H_2S production, indole, urease, methyl red, Voges-Proskauer reaction, citrate utilization, fermentation of carbohydrates and test for growth with acid and gas production in MacConkey broth.

This standard recommends both 'Plate Count' and Most Probable Number (MPN) method for the **enumeration** of *E.coli*, if all the prescribed identification tests give positive results.

3.2.2.2 IS 16067 (Part 1) : 2020 - Microbiology of the Food Chain — Horizontal Method for the Enumeration of Beta-Glucuronidase-Positive *Escherichia coli*: Part 1 - Colony-Count Technique at 44°C Using Membranes and 5-Bromo-4-Chloro-3-Indolyl Beta-D-Glucuronide

In 1976, Kilian and Bulow described the association of beta-glucuronidase with the genus *Escherichia* (97% positive) and suggested that a beta-glucuronidase would be useful in identification of this organism. β-glucuronidase is an enzyme of glycosyl hydrolase group (characterized with hydrolyzing the glycosidic bond in carbohydrates or its derivatives) which hydrolyses β-glucuronic acid residues from the non-reducing termini of glycosaminoglycans (GAGs). IS 16067 (Part 1) : 2020 uses this property of the enzyme for detection and enumeration of *E. coil*.

The standard specifies a horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* by colony-count technique. The first step involves **reviving the organism** (resuscitation stage) present in the sample by inoculating it onto membranes overlaid on minerals-modified glutamate agar (MMGA) and then incubate it at 37 °C for 4 h. **For isolation**, the membranes from the resuscitation stage on the MMGA are transferred to tryptone bile X-glucuronide agar (TBX) which contains 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (BCIG) as an ingredient. The sample is then incubated at 44 °C for 20 h to 24 h.

For **enumeration**, the number of colony-forming units (cfu) of β-glucuronidase-positive *Escherichia coli* per gram or per millilitre of sample is calculated from the number of typical blue or blue-green colonies per plate (colour to the colonies is imparted by 5-bromo-4-chloro-3-indolyl- ß-D-glucuronic acid in TBX media).

3.2.2.3 IS 16424 : 2016 - Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Detection and Enumeration of Presumptive Escherichia coli — Most Probable Number Technique

The standard defines that **presumptive *Escherichia coli*** are bacteria which at 44°C ferment lactose with the production of gas, and produce indole from tryptophan.

The **enumeration** of presumptive *Escherichia coli* in the standard is prescribed by MPN method by calculating most probable number of *E. coli* per millilitre or per gram of the test sample. In this standard, Lauryl sulphate broth has been used as a Selective enrichment medium and EC broth as Selective medium.

3.2.2.4 IS 16426 : 2017 - Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection of *Escherichia coli* O157

E. coli O157 is a major foodborne pathogen causing severe life-threatening illness in humans. It has low infective dose. Skilled laboratory personnel having experience to handle pathogens perform tests to detect and enumerate this bacterium.

This standard specifies a horizontal method for the detection of *Escherichia coli* serogroup O157 in four stages. The first stage involves **enrichment** of the test sample by homogenizing it in modified soya broth containing novobiocin followed by incubation at 41.5 °C ±1 °C for 6 hours and subsequently for a further 12 hours to 18 hours. The second stage is **separation and concentration** of microorganisms by means of immunomagnetic particles coated with antibodies to *E. coli* O157. Third stage comprises of **isolation** by subculture of the immunomagnetic particles with adhering bacteria onto cefixime tellurite sorbitol MacConkey agar (CT-SMAC) and a second selective isolation agar (any selective media for *E. coli*). The last and fourth stage provides **confirmation** of sorbitol-negative colonies from CT-SMAC and colonies typical of *E. coli* O157 on the second isolation agar, by indole production and agglutination with *E. coli* O157 antiserum.

3.2.2.5 IS 16987 : 2018 - Microbiology of Food and Animal Feed - Real Time Polymerase Chain Reaction (PCR) Based Method for the Detection of Food Borne Pathogens - Horizontal Method for the Detection of Shiga Toxin Producing *Escherichia coli* (STEC) and the Determination of O157, O111, O26, O103 and O145 Serogroups

Shiga toxin-producing *Escherichia coli* (STEC) are pathogenic *E. coli*, which can cause diarrhoea as well as more severe diseases in humans such as haemorrhagic colitis and haemolytic uremic syndrome (HUS). *E. coli* isolates can be serologically or genetically differentiated based on three major surface antigens or their encoding genes, which enable serotyping: the “O” (somatic), “H” (flagella), and “K” (capsule) antigens. Of these three, O and the H antigens are considered for serotyping the strains of *E. coli* associated with diarrheal disease. Serogroups O157, O111, O26, O103, and O145 fall under these categories.

The main virulence genes for STEC are *stx* gene which encodes the 'Shiga toxins' and the *eae* gene which encoded the 'intimin' (a 90 kDa protein) involved in the attaching and effacing mechanism of adhesion, a typical feature of the STEC strains causing severe disease. The genes responsible for coding given serotypes are *rfbE (for O157), wbdl (for O111), wzx (for O26), ihp1 (for O145)* and *wzx (for O103)* genes.

This bacterium and its associated serotypes can be detected in a non-selective liquid nutrient medium i,e modified tryptone-soy broth (MTSB) using Real-Time Polymerase Chain Reaction (PCR) based method. The standard specifies the method in following sequential steps:

a) Microbial enrichment – Here, the number of STEC cells to be detected is increased by incubating the test sample in a non-selective liquid nutrient medium given in the standard.

b) Nucleic acid extraction – The purified nucleic acid extraction for gram negative bacterium is performed by any standardized method for the detection of target genes responsible to code for their respective serotypes, i.e., *rfbE(O157), wbdl(O111), wzx(O26), ihp1(O145)* and *wzx(O103).*

c) Detection of virulence and serogroup-associated genes - It is performed by the PCR amplification approach based on Real Time PCR method. The detection of PCR product was based on light emission captured by the Real Time PCR apparatus during the amplification. Suitable primers and detection probes for virulence and serogroup associated genes are used for conducting Real Time PCR and the result is interpreted by the software linked with the apparatus.

d) Isolation from positive samples - The isolation is attempted based on serogroups specified detection using serogroup-specific enrichment media, i.e., tryptone–bile–glucuronic agar (TBX) or a specific selective medium for the organism in order to facilitate the isolation of the STEC.

3.2.3 Indian Standards on Method of Tests for *Enterobacteriaceae*

Enterobacteriaceae family contains a large number of genera that are biochemically and genetically related to one another, for example, *Escherichia, Shigella, Salmonella, Enterobacter, Proteus, Yersinia* etc. Members of the Enterobacteriaceae can be referred to as enterobacteria or "enteric bacteria" as several members live in the intestines of animals. These are gram-negative, short rods, non-sporulating, facultative anaerobes. Motility if present is by means of peritrichous (lateral) **flagella,** except ***Shigella*** and ***Klebsiella*** which are **non-motile**.

Two Indian Standards on methods for detection and enumeration of Enterobacteriaceae formulated by BIS are as follows:

1. IS 17112 (Part 1) : 2019 — Microbiology of the Food Chain —Horizontal Method for the Detection and Enumeration of Enterobacteriaceae Part 1 Detection of *Enterobacteriaceae*

2. IS 17112 (Part 2) : 2019 — Microbiology of the Food Chain — Horizontal Method for the Detection and Enumeration of *Enterobacteriaceae* Part 2 Colony-Count Technique

The method given in these standards are applicable to products intended for human consumption and the feeding of animals, and environmental samples in the area of primary production, food production and food handling. As per the standards, *Enterobacteriaceae* are those microorganisms that forms characteristic colonies on violet red bile glucose agar (VRBG) and that ferment glucose and show a negative oxidase reaction.

The table below gives a brief account of comparison of the **detection of Enterobacteriaceae** [as per IS 17112 (Part-1) : 2019] and **enumeration by Colony Count technique** [as per IS 17112 (Part-2) : 2019]

S. No.	**IS 17112 - Microbiology of the Food Chain - Horizontal Method for the Detection and Enumeration of *Enterobacteriaceae***	
	Part 1 Detection of Enterobacteriaceae	**Part 2 Colony-Count Technique**
1.	This standard specifies a method, with enrichment, for the detection of *Enterobacteriaceae*	This standard specifies a method for the enumeration of *Enterobacteriaceae*
2.	This method is applicable when the microorganisms sought are expected to need resuscitation (revival) by enrichment, and when the number sought is expected to be below 100 per millilitre or per gram of test sample.	This technique is intended to be used when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample.
3.	The standard involves **Enrichment of the organism** in non-selective medium, i.e, in Buffered peptone water (BPW) by inoculation of the test sample and then incubation at 37°C (or 30°C) for 18 hours. The standard provides conditions of using incubation at temperature 37°C or 30°C for the sample	An **initial suspension and decimal dilutions** are prepared from the test sample.
4.	Enrichment is followed by **isolation and selection** for confirmation**.** VRBG agar is inoculated with the culture obtained after enrichment in BPW, then incubated at 37°C. It is examined after 24 hours to detect the presence of typical colonies of Presumptive *Enterobacteriaceae*.	**Isolation and selection involve** inoculation with a specified quantity of the test sample in Violet red bile glucose (VRBG) agar followed by incubation at 37 °C (or 30 °C) for 24 hours.
5.	Typical colonies of presumptive *Enterobacteriaceae* are subcultured onto non-selective medium, and **confirmed** by means of tests for the fermentation of glucose and the presence of oxidase.	Colonies of presumptive *Enterobacteriaceae* are subcultured onto non-selective medium, and **confirmed** by means of tests for the fermentation of glucose and the presence of oxidase. The number of *Enterobacteriaceae* per millilitre or gram of the test sample is calculated

		from the number of confirmed typical colonies per dish.

3.2.4 Indian Standards on Method of Tests for *Staphylococcus aureus*

Staphylococci are a group of small, spherical, aerobic, Gram-positive cocci in clusters, usually, but not always. *Staphylococcus aureus* is a very important member of this group. It produces a golden yellow-coloured colonies on nutrient agar and blood agar and shiny black colonies with or without narrow grey-white margin when grown in Baird-Parker medium. Suspect colonies must show coagulase activity. This bacterium produces **Staphylococcal Enterotoxins** which are virulence factors responsible to cause two common human diseases, i.e., toxic shock syndrome (TSS) and staphylococcal food poisoning (SFP).

The Indian Standards formulated by BIS on methods for detection and enumeration of *Staphylococcus aureus* are given as under:

1.	IS 5887 (Part 2) : 1976	Methods for detection of bacteria responsible for food poisoning: Part 2 Isolation, identification and enumeration of *Staphylococcus aureus* and faecal streptococci
2.	IS 5887 (Part 8/Sec 1) : 2002	Methods for detection of bacteria responsible for food poisoning: Part 8 Horizontal method for enumeration of coagulase - Positive Staphylococci (*Staphylococcus aureus* and other species): Sec 1 Technique using Baird - Parker agar medium
3.	IS 5887 (Part 8/Sec 2) : 2002	Methods for detection of bacteria responsible for food poisoning: Part 8 Horizontal method for enumeration of coagulase - Positive (*Staphylococcus aureus* and other species): Sec 2 Technique using rabbit plasma fibrinogen agar medium
4.	IS 5887 (Part 8/Sec 3) : 2016	Methods for Detection of Bacteria Responsible for Food Poisoning Part 8 Horizontal Method for Enumeration of Coagulase-Positive Staphylococci (*Staphylococcus aureus* and other species) Section 3 Detection and MPN technique for low numbers

3.2.4.1 IS 5887 (Part 2) : 1976 - Methods for Detection of Bacteria Responsible for Food Poisoning: Part 2 Isolation, Identification and Enumeration of *Staphylococcus aureus* and Faecal Streptococci

This standard specifies basic method for the isolation, identification and enumeration of Faecal Streptococci along with *Staphylococcus aureus.* The characteristic properties of *S. aureus,* as defined in the standard which are mentioned at 3.1.4 above.

Faecal Streptococci are aerobic, Gram-positive cocci in pairs or short chains, producing small

pink colonies on MacConkey agar and colonies which are dark red or pink centres when grown on ethyl violet azide dextrose agar. Growth is obtained also at 44^{o}C.

The standard gives a list of selective media required for **isolation** of organisms based on the characteristic properties defined in the standard. For **identification** of the *S. aureus,* Gram's stain and colonial characteristics on selective media along with coagulase test are prescribed. However, identification of faecal streptococci is recommended based on the results of Gram's staining and colonial characteristics on selective media.

The standard prescribes both 'Plate Count' and Most Probable Number (MPN) method for the **enumeration** of *S. aureus and E* faecal streptococci, if all the prescribed identification tests give positive results.

3.2.4.2 IS 5887 (Part 8/Sec 1, 2 and 3) - Methods for detection of bacteria responsible for food poisoning: Part 8 Horizontal method for enumeration of coagulase - Positive Staphylococci (*Staphylococcus aureus* and other species)

IS 5887 (Part 8) has been published in three sections, i.e., 1, 2 and 3. These specify horizontal method for the enumeration of coagulase-positive staphylococci in products intended for human consumption or feeding of animals, by counting of colonies obtained on a different media after aerobic incubation at 35^{o}C or 37^{o}C or using MPN method.

The methods given in these three sections can be compared and understood briefly by the details given in the table as under:

IS 5887 (Part 8) - Methods for detection of bacteria responsible for food poisoning: Part 8 Horizontal method for enumeration of coagulase - Positive Staphylococci (*Staphylococcus aureus* and other species)		
Sec 1: Technique Using Baird - Parker Agar Medium	**Sec 2: Technique Using Rabbit Plasma Fibrinogen Agar Medium**	**Section 3: Detection and MPN Technique for Low Numbers**
Detection: **i) Inoculation** of the sample onto the **Baird-Parker agar medium**, using duplicate plates. **ii)** Aerobic **incubation** of the plates at 35^{o}C or 37^{o}C and examination after both 24 hours and 48 hours.	**Detection:** **i)** Preparation of duplicate poured plates of the **rabbit plasma fibrinogen agar medium** and **inoculation** with a specified quantity of the test sample. **ii) Incubation** of the plates at 35^{o}C or 37^{o}C for 18 hours to 24 hours, and a further 24 hours, if necessary.	**Detection:** **i) Modified Giolitti and Cantoni broth** is inoculated with a specified quantity of the test sample followed by incubation at 37 °C, anaerobically, for 24 hours and 48 hours. The presence of presumptive coagulase-positive staphylococci is indicated by the reduction of potassium tellurite. **ii)** The surface of solid selective Baird-Parker

		medium is inoculated from presumptive positive tubes after 24 hours, and all the remaining tubes after 48 hours followed by incubation at 37 °C for 24 hours and 48 hours. The presence of presumptive coagulase-positive staphylococci is indicated by the reduction of potassium tellurite and an egg yolk reaction. **iii)** Typical and/or atypical colonies are confirmed by a coagulase reaction.
Enumeration: **Calculation** of the number of coagulase-positive staphylococci per millilitre, or per gram, of sample from the number of typical and/or atypical colonies obtained on plates at dilution levels chosen so as to give a significant result, and confirmed **by a positive coagulase test** result.	**Enumeration:** From the number of typical colonies per Petri dish, **calculation** of the number of **coagulase-positive staphylococci** per millilitre or per gram of test sample.	**Enumeration:** **i)** Serial dilutions of product are inoculated into liquid selective culture medium. **ii)** The most probable number of coagulase-positive staphylococci per gram or per millilitre of sample is calculated by reference to most probable number tables for confirmed dilutions

3.2.5 Indian Standards on Method of Tests for *Salmonella spp.*

Salmonella spp. are facultatively anaerobic, Gram negative, rod-shaped, non-spore forming, oxidase negative bacteria belonging to the family *Enterobacteriaceae*. Although members of this genus are motile by peritrichous flagella, non-flagellated variants and nonmotile strains resulting from dysfunctional flagella do occur. Salmonellae are chemo-organotrophic with an ability to metabolize nutrients by both respiratory and fermentative pathways.

Three Indian Standards formulated by BIS on methods for detection and enumeration of *Salmonella spp.* are given as under:

1. IS 5887 (Part 3 / Sec 1) : 2020 — Methods for detection of bacteria responsible for food poisoning Part 3 Horizontal method for the detection, enumeration and serotyping of *Salmonella* Section 1 Detection of *Salmonella spp.*

2. IS 5887 (Part 3 /Sec 2) : 2021 — Methods for detection of bacteria responsible for food poisoning Part 3 Horizontal method for the detection, enumeration and serotyping of *Salmonella* Section 2 Enumeration by a miniaturized most probable number technique

3. IS 5887 (Part 3 /Sec 3) : 2021 — Methods for detection of bacteria responsible for food poisoning Part 3 Horizontal method for the detection, enumeration and serotyping of *Salmonella* Section 3 Guidelines for serotyping of *Salmonella spp.*

3.2.5.1 IS 5887 (Part 3 / Sec 1) : 2020 is applicable on the products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling samples from the primary production stage such as animal faeces, dust, and swabs. Detection of *Salmonella* in the standard has been described in four successive stages, i.e. pre-enrichment, selective enrichment, plating out and confirmation.

3.2.5.2 IS 5887 (Part 3 / Sec 2) : 2021 gives a method for the enumeration of *Salmonella* spp. present in products intended for human consumption and for the feeding of animals, environmental samples in the area of food production and food handling, animal faeces and environmental samples from the primary production stage by calculation of the most probable number (MPN).

3.2.5.3 IS 5887 (Part 3 / Sec 3) : 2021 gives guidance on the procedure for serotyping *Salmonella* serovars and is applicable to the serotyping of pure cultures of *Salmonella* spp., independent of the source from which they are isolated.

3.2.6 Indian Standards on Method of Tests for *Clostridium spp.*

The organisms belong to genus *Clostridium* are anaerobic to microaerophilic, rod shaped and endospore bearing usually at the end or middle of the rods. All species are catalase-negative. Many species actively ferment carbohydrates with the production of acids (usually including butyric) and gases (usually carbon dioxide and hydrogen). Different species may be mesophilic or thermophilic and proteolytic or nonproteolytic. The soil is the primary source of Clostridium spp., although they also may come from bad silage, feeds, and manure.

BIS has formulated following two Indian Standards on methods for detection and enumeration of *Clostridium spp.* are given as under:

1. IS 5887 (Part 4) : 1999 — Methods for detection of bacteria responsible for food poisoning: Part 4 isolation and identification of *Clostridium perfringens* (*Clostridium welchii*) and *Clostridium botulinum* and enumeration of *Clostridium perfringens*

2. IS 16425 : 2016 — Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Clostridium perfringens* - Colony Count technique

The standard, IS 5887 (Part 4) gives the basic method for the isolation and identification of *C. perfringens* and *C. botulinum* in food product along with enumeration of *C. perfringens* only. However, IS 16425 provides method for enumeration, including detection and identification of

C. perfringens for a range of commodities (food items, feeding stuffs and environmental samples).

3.2.7 Indian Standards on Method of Tests for *Vibrio spp.*

The organisms belonging to genus *Vibrio* are widely distributed in fresh and salt water, in soil, and in the alimentary canal of humans and animals. Some are moderately halophilic. Some species are pathogenic to humans. Majority of the human vibrio infections are associated with the seafood consumption.

Indian Standard formulated by FAD 15 Sectional Committee of BIS is given below which is in the process of revision to include detection of *V. vulnificus* also.

IS 5887 (Part 5) : 1976	Methods for detection of bacteria responsible for food poisoning: Part 5 isolation, identification and enumeration of *Vibrio cholerae* and *Vibrio parahaemolyticus*

The standard prescribes in detail, the method for the isolation, identification and enumeration of *V. cholerae* and *V. parahaemolyticus* using selective media, i.e., thiosulphate citrate bile salts sucrose agar (TCBS), glucose salt teepol broth and bile salt agar.

3.2.8 Indian Standards on Method of Tests for *Bacillus cereus*

Bacillus cereus is Gram positive, rod shaped, facultative anaerobic bacteria. They form endospores which do not swell the rods in which they are formed. The broad spectrum of B. cereus toxicity ranges from avirulent strains used as probiotics for humans to highly toxic strains responsible for food-related fatalities. *B. cereus* can cause two different types of foodborne illness: the diarrheal type and the emetic type.

A total of two Indian Standards have been formulated by BIS on *B. cereus* which are given as under:

1.	IS 5887 (Part 6) : 2012	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus*: Part 6 colony - Count technique at 30°C
2.	IS 16434 : 2018	Microbiology of food and animal feeding stuffs - Horizontal method for the determination of low numbers of presumptive *Bacillus cereus* - Most probable number technique and detection method

Both the Indian Standards prescribe methods for detection, identification and enumeration of *B. cereus* from a broad range of commodities, including food, animal feeding stuff and environmental samples. IS 5887 (Part 6) defines ‘colony count method’ whereas IS 16343 prescribes MPN method for *B. cereus*.

3.2.9 Indian Standards on Method of Tests for *Shigella*

Shigella is a gram-negative, nonsporulating, nonmotile, facultative anaerobic, rod-shaped bacterium. The genus *Shigella* has been subdivided on the basis of biochemical characters and serological testing into four subgroups, subgroup A *(Sh. dysenteriae,* containing 10 serotypes),

subgroup B (*Sh. flexneri,* containing 6 serotypes), subgroup C (*Sh. Boydii,* containing 15 serotypes*),* and subgroup D (*Sh. Sonnei*, containing 2 forms*).*

The Sectional Committee, FAD 15 of BIS has laid down two standards on *Shigella* as given below:

1. IS 5887 (Part 7) : 1999 — Methods for detection of bacteria responsible for food poisoning : Part 7 General guidance on methods for isolation and identification of *Shigella*

2. IS 16429 : 2018 — Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella* spp.

IS 5887 (Part 7) prescribes method for detection, identification and enumeration of *Shigella* in the food stuff, however, the method given in IS 16429 is a horizontal method applicable for detection of *Shigella* in a broad range of commodities, including food, animal feeding stuff and environmental samples.

3.2.10 Indian Standards on Method of Tests for *Listeria monocytogenes*

Genus *Listeria* consists of bacteria that are gram-positive, non-spore-forming rods with tumbling motility. *L. monocytogenes* can cause a major food-borne illness, called, listeriosis. The organism has been found responsible for various food-borne outbreaks reported in the Ready to Eat Foods.

1. IS 14988 (Part 1) : 2020 — Microbiology of the food chain — Horizontal method for detection and enumeration of *Listeria monocytogenes* and of *Listeria spp.* Part 1 Detection Method

2. IS 14988 (Part 2) : 2020 — Microbiology of the food chain — Horizontal method for detection and enumeration of *Listeria monocytogenes* and of *Listeria spp.* Part 2 Enumeration Method

Both the above mentioned Indian Standards provide method for the detection and enumeration of L monocytogenes and L spp. in a broad range of commodities. Part 1 of IS 14988 prescribes detection method, however, part 2 defines method for the enumeration of the organisms.

3.2.11 Indian Standards on Method of Tests for Yeast and Moulds

Yeasts can be differentiated from bacteria by their larger cell size and their oval, elongate, elliptical, or spherical cell shapes. Typical yeast cells range from 5 to 8 µm in diameter, with some being even larger. Older yeast cultures tend to have smaller cells. Most of those divide by budding or fission.

Moulds are filamentous fungi that grow in the form of a tangled mass that spreads rapidly and may cover several inches of area in 2 to 3 days. The total of the mass or any large portion of it is referred to as mycelium.

The two Indian Standards formulated by BIS for enumeration of yeast and moulds are given as under:

1. IS 16069 (Part 1) : 2013 — Microbiology of food and animal feeding stuffs — horizontal method for the enumeration of yeasts and moulds part 1 colony count technique in products with water activity greater than 0.95

2. IS 16069 (Part 2) : 2013 — Microbiology of food and animal feeding stuffs — horizontal method for the enumeration of yeasts and moulds part 2 colony count technique in products with water activity less than or equal to 0.95

Both the parts of IS 16069 specify a horizontal method for the enumeration of viable yeasts and moulds in products intended for human consumption or feeding of animals by means of the colony count technique at 25 °C ± 1 °C. **Part 1** of this standard is applicable on products that have a water activity greater than 0.95 [eggs, meat, dairy products (except milk powder), fruits, vegetables, fresh pastes, etc.], however, **Part 2** is applicable on the products having a water activity less than or equal to 0.95 (dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereal products, flours, nuts, spices and condiments, etc.) but more that 0.60.

3.2.12 Indian Standards on Method of Tests for mesophilic Lactic Acid Bacteria

The lactic acid bacteria used in milk fermentations fall into two general categories, the mesophilic organisms with optimum growth temperatures of less than 30°C and the thermophilic ones with growth optima at greater than 37 ° C. There are many bacterial genera which fall into these categories, such as, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus* etc. These bacteria produce lactic acid as the major metabolic end product of carbohydrate fermentation, giving them the common name **lactic acid bacteria**.

BIS has formulated following Indian Standard on the subject:

IS 16068 : 2013 — Microbiology of food and animal feeding stuffs — horizontal method for the enumeration of mesophilic lactic acid bacteria — colony-count technique at 30°C

3.3 Indian Standards on Preparation of Test Samples, Initial Suspension and Decimal Dilutions for Microbiological Examination

The microbiological examination of any food sample requires preparation of samples before taking it up for the testing. There may be varying degree of distribution of microorganisms in any food sample which may not give proper results when sample is subjected to testing as per devised method. Hence, to ensure even distribution of microorganisms, it is very important to prepare the sample following the laid down procedure.

The Food Hygiene, Safety Management and Other Systems Sectional Committee, FAD 15 of BIS has formulated Six Indian Standards on the subject which are given as under:

1. IS 10232 : 2020 — Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — General rules for the preparation of initial suspension and decimal dilutions

2. IS 16980 : 2018 — Microbiology of food and animal feed - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -

		Specific rules for the preparation of samples taken at the primary production stage
3.	IS 15990 : 2012	Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Specific rules for the preparation of meat and meat products
4.	IS 17448 : 2020	Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Specific rules for the preparation of fish and fishery products
5.	IS 17779 : 2021	Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Specific rules for the preparation of milk and milk products
6.	IS 17447 : 2020	Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Specific rules for the preparation of miscellaneous products

3.3.1 IS 10232 : 2020 - General Rules for the Preparation of Initial Suspension and Decimal Dilutions

This standard defines the general rules for the preparation of samples, initial suspensions and subsequent dilutions for microbiological examination. For a number of products, it is necessary to take special precautions, especially when preparing the initial suspension, because of the physical state of the product (such as dry products, highly viscous products) or the presence of inhibitory substances (such as spices, high salt content) or the acidity, etc. These are covered in general terms in this document. The standard also gives the definitions of ‘test samples’, ‘initial suspension’ and ‘decimal dilution’ to understand and follow the method appropriately.

3.3.2 IS 16980 : 2018 - Specific Rules for the Preparation of Samples Taken at the Primary Production Stage

This standard specifies rules for the preparation of samples taken at all stages from the farm to the slaughterhouse and their suspension for microbiological examination when the samples require different preparation (from the general rules) .The sample preparation rules are applicable to various samples taken from the hatchery, the farm, from the vehicle or the animals during transportation, or from animals or their carcasses in the slaughterhouse, to indicate the microbiological status of the animals in relation to zoonotic agents.

3.3.3 IS 15990 : 2012 - Specific Rules for the Preparation of Meat and Meat Products

This standard specifies rules for the preparation of meat and meat product samples and their suspension for microbiological examination when the samples require a different preparation from the general rules. The standard describes methods of preparation that are applicable to several microorganisms simultaneously. The method of sample preparation given in this standard is applicable to the fresh, raw and processed meats and poultry and their products, such as, refrigerated or frozen, cured or fermented, minced or comminuted, delicatessen meats, pre-cooked meals or poultry based meals, dried and smoked meats at various degrees of dehydration and concentrated meat extracts.

3.3.4 IS 17448 : 2020 - Specific Rules for the Preparation of Fish and Fishery Products

This standard specifies rules for the preparation of fish and fishery product samples and their suspension for microbiological examination. The standard includes special procedures for sampling raw molluscs, tunicates and echinoderms from primary production areas. Sampling of raw molluscs, tunicates and echinoderms from primary production areas is also included in this document. The method given is applicable to the raw, processed or frozen fish and shellfish and their products. The purpose of examinations performed on these samples can be either hygiene testing or quality control. However, the sampling techniques described in this standard relate mainly to hygiene testing (on muscle tissues).

3.3.5 IS 17779 : 2021 - Specific Rules for the Preparation of Milk and Milk Products

This standard specifies rules for the preparation of samples of milk and milk products and their suspensions for microbiological examination. The standard is applicable to milk and liquid milk products, dehydrated milk products, cheese and cheese products, casein and caseinates, butter, milk-based ice-cream, milk-based custard, desserts and sweet cream, fermented milks, yogurt, probiotics milk products and sour cream, and dehydrated milk-based infant foods, with or without probiotics.

3.3.6 IS 17447 : 2020 - Specific Rules for the Preparation of Miscellaneous Products

This standard specifies rules for the preparation of samples and dilutions for the microbiological examination of specific food products not covered in other standards mentioned above. This standard covers a wide range of miscellaneous products and is applicable to the products, such as acidic (low pH) products, hard and dry products, dehydrated, freeze-dried and other low aw (water activity) products (including those with inhibitory properties), flours, whole cereal grains, cereal by-products, animal feed, cattle cake, kibbles and pet chews, gelatine (powdered and leaf), margarines, spreads and non-dairy products with added water, eggs and egg products, bakery goods, pastries and cakes, fresh fruit and vegetables, fermented products and other products containing viable microorganisms, alcoholic and non-alcoholic beverages and alternative protein products.

3.4 Indian Standards on Polymerase Chain Reaction (PCR & quantitative Real Time - PCR) Based Method for the Detection of Food Borne Pathogens

Polymerase Chain Reaction (PCR) is a technique that takes specific sequences of nucleic acid (DNA) in a small amount and amplifies it for further testing. This is a technique to make many copies of a specific DNA region in vitro (in a test tube rather than an organism). PCR is based on three simple steps required for any DNA synthesis reaction: 1) denaturation of the template into single strands; 2) annealing of primers to each original strand for new strand synthesis; and 3) extension of the new DNA strands from the primers. PCR allows the detection of a single bacterial pathogen that is present in food by detecting its specific target DNA sequence.

Real-Time PCR also called quantitative real-time PCR, is a technique used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification. RT–PCR is a variation of PCR which consists of same process as that of PCR with an added step of reverse transcription for conversion of RNA to DNA followed by its amplification. For

the robust detection and quantification of gene expression from small amounts of RNA, the amplification of the gene transcript is always necessary. This measurement is made after each amplification cycle, and this is the reason why this method is called real-time PCR.

A total of five Indian Standards have been formulated by BIS on PCR based method for detection of food borne pathogens which are given as under:

1. IS 16427 : 2016 Microbiology of Food and Animal Feeding Stuffs — Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — Requirements for Sample Preparation for Qualitative Detection

2. IS 16428 : 2016 Microbiology of Food and Animal Feeding Stuffs — Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — Requirements for Amplification and Detection for Qualitative Methods

3. IS 16431 : 2018 Microbiology of food and animal feeding stuffs - Polymerase chain reaction (PCR) for the detection and quantification of food - Borne pathogens - Performance characteristics

4. IS 16432 : 2016 Microbiology of Food and Animal Feeding Stuffs — Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — General Requirements and Definitions

5. IS 16433 : 2016 Microbiology of Food and Animal Feeding Stuffs — Real-Time Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — General Requirements and Definitions

3.4.1 IS 16427:2016 - Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — Requirements for Sample Preparation for Qualitative Detection

This standard provides criteria and examples for sample preparation in order to obtain PCR compatible samples or nucleic acids of suitable quality and quantity for PCR. This standard describes the general principles involved in PCR-based detection of Food-borne pathogens and requirements of sample preparation for PCR. The objective of the sample preparation methods described is to obtain samples or nucleic acids of appropriate quality and quantity to perform PCR. The quality of nucleic acids depends for example on the chemical purity, the average length of the molecules, and the structural integrity of the extracted nucleic acid molecules.

3.4.2 IS 16428:2016 - Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens —Requirements for Amplification and Detection for Qualitative Methods

This standard provides the overall framework for qualitative methods for the detection of foodborne pathogens using the polymerase chain reaction (PCR). It covers the general requirements for the specific amplification of target nucleic acid sequences and the detection and confirmation of the identity of the amplified nucleic acid sequence. Guidelines, minimum requirements, and performance characteristics described in this standard are intended to ensure that comparable and reproducible results are obtained in different laboratories. This standard

has been established for food-borne pathogens in or isolated from food and feed matrices, but can also be applied to other matrices, for example, environmental samples, or to the detection of other microorganisms under investigation. As per the standard, the analysis consists of –

a) amplification by PCR of specific target sequences,
b) detection of the PCR product,
c) confirmation of the identity of the PCR product, and/or
d) confirmation by a standardized microbiological cultural method

3.4.3 IS 16431:2018 - Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — Performance Characteristics

This standard explains the minimum requirements of performance characteristics for the detection of nucleic acid sequences (DNA or RNA) by molecular methods. This standard specifies the detection of food-borne pathogens in foodstuffs and isolates obtained from them using molecular detection methods based on the polymerase chain reaction (PCR). The standard also includes specific information on the multi- or single-laboratory trial, including relevant information obtained during pre-validation of the method (e.g. variation of parameters, reagents). This standard clearly explains a defined test system for bacteria, viruses, and fungi. As per the standard, food-borne pathogens that require qualitative testing should be detected at levels of 1 cell to 10 cells per assay for bacteria or parasites, and 10 particles to 100 particles or genome equivalents for viruses, in a defined amount of the food matrix under investigation.

3.4.4 IS 16432:2016 - Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — General Requirements and Definitions

This standard gives the general requirements for the in vitro amplification of nucleic acid sequences (DNA or RNA). It applies to the testing of foodstuffs and isolates obtained from foodstuffs for food-borne pathogens using the polymerase chain reaction (PCR). The minimum requirements laid down in this standard are intended to ensure that comparable and reproducible results are obtained in different laboratories. The standard explains the general requirements and definitions of PCR components, process steps, and their different sub-types.

3.4.5 IS 16433:2016 - Real -Time Polymerase Chain Reaction (PCR) for the Detection of Food-Borne Pathogens — General Requirements and Definitions

This standard defines terms for the detection of food-borne pathogens in foodstuffs, and isolates obtained from them, using the Real-time polymerase chain reaction (Quantitative Real-time PCR). This standard also specifies requirements for the amplification and detection of nucleic acid sequences (DNA or RNA after reverse transcription) by real-time PCR. The minimum requirements laid down in this International Standard provide the basis for comparable and reproducible results within individual and between different laboratories. This standard explains the general requirements and definitions of Real-time PCR components, process steps, and their different sub-types

3.5 Indian Standards on Sampling Techniques (Primary Production Stage) for Microbiological Analysis

Sampling is an important quality control activity to ensure product quality through testing. The accuracy of results of a microbiological analysis is largely depends on the adequacy and

condition of the sample received. Since, only a relatively small sample of a lot is tested from a large consignment of food, it is very important to establish uniform sampling procedure for the correct interpretations of results. Also, it becomes essential to get a representative sample when the microorganisms are sparsely distributed within the food.

The Sectional Committee, FAD 15 has formulated following Indian Standard on the subject which specifies sampling techniques within the primary food-animal production stage, for detection or enumeration of viable microorganisms with particular reference to food-borne pathogens. However, this standard is not intended for use in diagnosis of animal disease.

IS 16965 : 2018 Microbiology of Food and Animal Feed - Primary Production Stage - Sampling Techniques

3.6 Indian Standards on Method Validation (of Microbiological Methods)

Method validation is an essential part of the process of ensuring that results of the analysis reported are correct and 'fit for purpose'. Any method validation study will require the laboratory to investigate several performance characteristics. Today, many alternative, mostly proprietary, methods exist that are used to assess the microbiological quality of raw materials and finished products and the microbiological status of manufacturing procedures. These methods are often faster and easier to perform than the corresponding standardized method. The developers, end users, and authorities need a reliable common protocol for the validation of such alternative methods. The data generated will also provide potential end users with performance data for a given method, thus, enabling them to make an informed choice on the adoption of a particular method.

The FAD 15 Sectional Committee has formulated following two Indian Standards on method validations for microbiological methods:

1. IS 17113 (Part 1) : 2019 Microbiology of the food chain — Method validation Part 1 Vocabulary

2. IS 17113 (Part 2) : 2019 Microbiology of the food chain — Method validation Part 2 Protocol for the validation of alternative (proprietary) methods against a reference method

Part 1 of IS 17113 : 2019 specifies general terms and definitions relating to method validation of microbiology in the food chain. **Part 2** of IS 17113 : 2019 provides a specific protocol and guidelines for the validation of proprietary methods intended to be used as a rapid and/or easier method to perform than the corresponding reference method and can also be used for the validation of other non-proprietary methods that are used instead of the reference method.

3.7 Indian Standards on Specific Requirements and Guidance for Proficiency Testing by Interlaboratory Comparison

Interlaboratory comparison is defined as the process of organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions. **Proficiency testing** is the process of evaluation of participant laboratory's performance against pre-established criteria by means of interlaboratory comparisons.

The Indian Standard formulated by BIS on the subject is given as under:

IS 17385 : 2020 Microbiology of the Food Chain — Specific Requirements and Guidance for Proficiency Testing by Interlaboratory Comparison

This standard specifies requirements and gives guidelines for the organization of proficiency testing (PT) schemes for microbiological examinations of foods and beverages, feeding animals, environmental samples from food and feed production and handling, and primary production stages. This standard relates to the technical organization and implementation of PT schemes, as well as the statistical treatment of results of microbiological examinations. This standard deals only with areas where specific or additional details are necessary for PT schemes dealing with microbiological examinations.

3.8 Indian Standards on Preparation, Production, Storage and Performance Testing of Culture Media

The main objectives of a microbiological laboratories are to maintain, resuscitate, grow, detect and/or enumerate a wide variety of microorganisms. A wide variety of culture media are used in all traditional microbiological culture techniques. Thus, it is important that the culture media used in the microbiological tests and procedures are capable of providing consistent and reproducible results. Culture media meeting established performance criteria are therefore a pre-requisite for any reliable microbiological work. Therefore, it is important to carry out sufficient testing to demonstrate -
a) the acceptability of each batch of medium,
b) that the medium is “fit for purpose”, and
c) that the medium can produce consistent results.

These three criteria are an essential part of internal quality control procedures and, with appropriate documentation, will permit effective monitoring of culture media and contribute to the production of both accurate and reliable data.

Indian Standard on the subject has been formulated which is given as under:

IS 17383 : 2020 Microbiology of Food, Animal Feed and Water — Preparation, Production, Storage and Performance Testing of Culture Media

The standard defines terms related to quality assurance of culture media and specifies the requirements for the preparation of culture media intended for the microbiological analysis of food, animal feed, and samples from the food or feed production environment. These requirements are applicable to all categories of culture media prepared for use in laboratories performing microbiological analyses. The standard also sets criteria and describes methods for the performance testing of culture media. This standard applies to producers such as

- commercial bodies producing and/or distributing ready-to-use or semi-finished reconstituted or dehydrated media;
- non-commercial bodies supplying media to third parties;
- microbiological laboratories preparing culture media for their own use.