FAQs

1. What is food microbiology?Why is it important?

Food microbiology is the study of fundamental factors affecting the presence, activity, and control of microorganisms in food.

Food microbiology helps us to understand the crucial concepts required to meet the minimum standards for food safety. It includes study of food production, processing, detection of food spoilage andprevention of food borne diseases.

2. **Which are the intrinsic and extrinsic parameters affecting the growth of microorganisms in food?**

Intrinsic parameters

1. pH
2. Moisture content
3. Oxidation-reduction potential
4. Nutrient content
5. Antimicrobial constituents
6. Biological structures

Extrinsic parameters

1. Storage temperature
2. Relative humidity
3. Presence/concentration of gases
4. Presence/activities of other microorganisms

3. What is the significance of Microbiological criteria?

Microbiological criteria are used to differentiate between an acceptable and unacceptable product/ food processing and handling practices. Microbiological criteria ensures the safety and quality of food.It is used to assess; a) adherence to Good Manufacturing Practices (GMPs), b) the safety of food, (c) the maintenance of quality/ shelf life of certain perishable foods, and d) the suitability of food/ingredient for consumption.

4. **Describe the properties of indicator organisms used in food quality studies?**

The indicator organisms of food quality should possess the following properties.

1. Should be present and detectable in the food where quality is being assessed
2. The growth / numbers should share negative correlation with product quality
3. Detected and enumeration should be easy and within a short time

4. Clearly distinguishable from other organisms
5. Their growth should not be inhibited by other background microflora

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5. Distinguish between quantitative and qualitative methods of microbiological evaluation of food and food ingredients?

Quantitative methods	Qualitative methods
➢ Measure indirectly or directly the microbial load in the test material.	➢ Measure the presence of particular bacterial species in a food sample in a particular batchamong the total microbial population or not.
➢ Method of analysis include, Plate count method, Direct Microscopic counts (DMC), Most probable number (MPN), Dye reduction test.	➢ Method of analysis includes Isolation of pathogens and Test for toxins.

6. **What is Plate count method and which are its drawbacks.**

The standard plate count or aerobic plate count (APC) method is a commonly used method to enumerate the total number of microorganisms in food products by determining the colony forming units (CFU). Most commonly, aliquots from selected dilutions are either pour plated or surface plated on either nonselective/selective agar medium or nonselective/selective differential media.

Drawbacks of plate count method include (i) this method measures only live cells and therefore would not be of value to determine the quality of raw material used in heat processed food (ii) certain bacteria though present in low counts may be able to induce preponderant quality loss due to enhanced biochemical activity (iii) this method is of little value in evaluating the organoleptic quality of food.

7. Write a note on Direct Microscopic count method and explain its limitations?

Direct Microscopic count method is used to give an estimate of microbial cells in samples either counting stained cells under a bright field or live cells under a phase contrast microscope. The counts are expressed as microscopic counts per milliliter or gram food sample.

Limitations of DMC method are (i) the sample should contain relatively large number (> 10^5 CFU/ml) of microorganisms for effective use of this method, (ii)not recommended to differentiate between live and dead cells (requires the use of fluorescent dyes such as acridine orange) (iii) cannot effectively enumerate microorganisms in foods that have particles.

8. What is most probable number and how it is calculated?

The most probable number (MPN) method, is a method of deriving quantitative data on concentrations of discrete items from positive/negative (incidence) data. To calculate MPN aliquots from serially diluted samples are inoculated in broth (tubes) containing selective agents (one or more), and incubated at recommended temperature and time. The broth tubes in each dilution are then scored for the presence and absence of growth. The number of tubes displaying growth in each of the three successive dilutions is correlated with the number of viable cells of the specific microbial groups using the available statistically calculated table.

9. What is the principle behind dye reduction test?

Dye reduction test relies on the principle that some dyes (eg. Methylene blue) are coloured in oxidized states but become colourless in reduced state under the influence of microbial growth and metabolism. The assumption is that, the rate of reduction of the dye added to food is directly proportional to the initial microbial load of the sample.

10. Write a note on techniques developed by modification of conventional microbiological methods?

The different techniques developed by modification of conventional microbiological methods are:

a) Petrifilm test:

In this test disposable cardboard containing dehydrated media is used for the enumeration of total bacteria, specific bacterial species, or mold and yeasts. This test eliminates the requirement for preparing media and agar plates, and economizes in storage and incubation space.

b) Chromogenic and Fluorogenic substrates:

The introduction of chromogenic and fluorogenic substrates in special microbiological media provides a quick measure of specific enzyme activities which are characteristic

traits of certain bacteria or bacterial groups. Example; Chromogenic substrate like o-nitrophenyl-β-D-galactoside (ONPG) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) are incorporated in the culture media to measure the activity of β-galactosidase (GAL).

c) ATP Bioluminescence:

This technique measures (using luminometer) the light emitted by an enzymatic reaction between luciferin and luciferase that requires the presence of ATP. The amount of light emitted relates to the number of microorganisms.

11. Explain different types of Polymerase Chain Reaction (PCR) basedfood pathogen detection methods

Polymerase Chain Reaction (PCR) based methods are highly specific and accurate. Some of the assays include

Simple PCR Method:

In this method, double-stranded DNA is denatured into single strands, and specific primers are annealed to these DNA strands, followed by extension of the primers complementary to the single stranded DNA, with by *Taq*and other thermoresistant DNA polymerases. The quantity of the products of amplification can be visualized as a band on an ethidium-bromide-stained electrophoresis gel. SimplePCR methodsfor toxin detection have been developed for a range ofbacterial species, such as *B. cereus, V. cholera, S. aureus*and*E. coli.*

Multiplex PCR: The method of rapid detection of multiple microorganisms in a single reaction by simultaneous amplification of more than one locus is referred to as multiplex PCR (mPCR). Example, mPCR assay for the simultaneous detection of *Salmonella* sppetc

12. Which are the differentIsothermal Amplification techniques used in detection of food pathogens?

The different Isothermal Amplification techniques used in detection of food pathogens are i) nucleic acid sequence-based amplification (NASBA),ii) loop mediated isothermal amplification (LAMP), iii) strand displacement amplification (SDA) and iv) rolling circle amplification (RCA).

13. **What is Lateral Flow Immunoassay ?**

Lateral flow assays are a type of immunoassay where the test sample flows along the solid substrate via capillaryaction. After the sample is applied to the test, it encountersa colored reagent, which mixes with the sample andtransits the substrate, in the process encountering lines or zones that havebeen pretreated with an antigen or antibody. Depending on the type ofanalytes present in the sample, the colored reagent canbecome bound at the test line or zone. Lateral flow immunoassays such as immunochromatography, dipstick, and immunofiltration are garnering attention in the detection of pathogens and toxins in food Examples include, immunoassay-basedlateral flow dipstick for the rapid detection of aflatoxin B1 in pig feed.

14. **What is the principle behind Biosensor-Based Methods and mention its types.**

Biosensor is an analytical device that comprises of two main elements: a bio receptor and transducer. The bio receptor that recognizes the target analyte can either be a, biological material (antibodies, enzymes, cell receptor and nucleic acids and cell receptors) or biologically derived material (recombinant antibodies and aptamers) or biomimic (imprinted polymers and synthetic catalysts). The transducer which converts the biological interactions into a measurable electrical signal can be electrochemical, optical, thermometric, mass-based, micromechanical or magnetic.

It can be divided into

a) Optical biosensors:

b) Electrochemical biosensors:

c) Mass-based biosensors:

15. What are the limitations of rapid detection techniques

The rapid methods of pathogen/ toxin detection have the distinct advantages over conventional culture-based methods. However these techniques have limitations which include

1. Expensive investment in new lab equipment’s
2. May require additional technical expertise
3. AOAC International approved rapid methods are mostly designated for preliminary screening- negative results are regarded as definite but positive results are regarded as presumptive and need to be confirmed by standard microbiological methods

Potential for abuse- bioterrorism