Page 1 of 21 Pages in this Full pdf

MVPI-001: FOOD MICROBIOLOGY
GUESS PAPER-1

Q1. Enumerate various types of food poisoning with suitable example for each.

Ans. Types of food poisoning include the following:

(1) **Intoxications:** Intoxications involve food poisoning in which the organism grows in the food and releases a toxin from the cells. When the toxin is ingested along with the food, the toxin gives rise to the food poisoning syndrome (signs and symptoms that indicate a particular disease). The presence of the organism in the food is irrelevant to disease production. It is the toxin that gives rise to the disease. Bacterial toxins that produce intoxications are exotoxins that are either enterotoxins affecting the gut, as in the disease caused by Staphylococcus aureus, or neurotoxins, as in the disease caused by Clostridium botulinum, the toxin in this case affecting the nervous system. Mycotoxicosis (diseases produced by the ingestion of food containing mycotoxin produced by moulds) and the diseases produced by algal toxins that find their way into shellfish can also be considered intoxications. Generally, intoxications have short incubation periods (time from ingestion of the food to the appearance of symptoms).

(2) **Infections:** Infections involve food poisoning caused by the ingestion of live organisms when, typically, the organisms grow in the gastrointestinal tract to produce the disease. Most food poisoning caused by micro-organisms falls into this category, for example, food poisoning caused by Salmonella spp (salmonellosis). Enteritis associated with food poisoning infections is due to the production of exotoxins or endotoxins that act as enterotoxins.

(3) **Toxi:** Infections In some types of food poisoning, e.g. Clostridium perfringens, live cells need to be ingested for the disease to occur but the organism does not grow and reproduce in the gut. Vegetative cells sporulate after ingestion, and an enterotoxin is released when the spore mother cells break down releasing the toxin. Because living cells also need to be ingested to cause this type of food poisoning, it can be considered as a food-borne toxi-infection.

Type of disease	Causative microorganism	Microbial group	Major symptom(s) type
Intoxication			
Staph. poisoning	*Staphylococcus aureus* strains	Bacteria, Gm+*	Gastric
Botulism	*Clostridium botulinum* strains	Bacteria, Gm+	Non gastric
Mycotoxin poisoning	Mycotoxins producing mold strains, e.g., *Aspergillus flavus*	Molds	Non gastric
Infection			
Salmonellosis	Over 2000 Salmonella serovars (except *S. typhi and S. paratyphi*)	Bacteria, Gm - *	Gastric
Campylobacter enteritis	*Campylobacter jejuni* and *C. coli* strains	Bacteria, Gm -	Gastric
Yersiniosis	Pathogenic strains of *Yersinia enterocolitica*	Bacteria, Gm -	Gastric
Intoxication			
Enterohemorrhagic *E. coli* colitis	*Escherichia coli* O157:H7	Bacteria, Gm –	Gastric and nongastric
Nonhaemorrhegic *E. coli* colitis	Shiga-like toxin (verotoxin) producing *E. coli* strains like *E. coli* 026:H11	Bacteria, Gm –	Gastric

Page 2 of 21 Pages in this Full pdf

Listeriosis	*Listeria monocytogenes* (Pathogenic strains)	Bacteria, Gm +	Gastric and nongastric
Shigellosis	Four Shigella species e.g., *S. dysenteriæ*	Bacteria, Gm -	Gastric
Vibrio parahaemolyticus gastroenteritis	Pathogenic strains of *V. parahaemolyticus*	Bacteria, Gm -	Gastric
Vibrio vulnificus infection	*Vibrio vulnificus* strains	Bacteria, Gm -	Gastric and nongastric
Brucellosis	*Brucella abortus*	Bacteria, Gm -	Gastric and nongastric
Viral infections	*Pathogenic enteric viruses, e.g., Hepatitis A. virus*	Viruses	Gastric and nongastric
Toxi infection			
Clostridium perfringens gastroenteritis	*Clostridium perfringens* strains	Bacteria, Gm+	Gastric
Bacillus cereus gastroenteritis	*Bacillus cereus* strains	Bacteria, Gm +	Gastric

Q2. Describe the methods for detection of B. cereus.

Ans. The traditional method of B. cereus detection is based on the bacterial culturing onto selective agars and cells enumeration. In addition, molecular and chemical methods are proposed for toxin gene profiling, toxin quantification and strain screening for defined virulence factors. Food poisoning caused by B. cereus can be confirmed by isolation of this bacterium in food, stool or vomit samples. Different media are available to culture this pathogen. Commercial kits are also available to detect the diarrhoeal enterotoxin, but this is not the case for emetic toxin

Q3. Enumerate various methods used for detecting viruses extracted from food.

Ans. A general outline can be given for virus detection protocols for fresh fruit and vegetables. However, due to the large variations in steps and parameters and the shortage of comparison studies, it is impossible to compose an optimal protocol from the literature. The overall method to extract and detect viruses in foods could be divided into three different steps:

- Virus elution and clarification from substrates,
- Concentration of the viruses, and
- RNA extraction and purification, amplification, detection of amplified products, and confirmation of the results. Different strategies have been proposed and used to perform these different steps.

To come to a standardized horizontal virus detection method for fruit and vegetables, a standard protocol will have to be established for each of the three consecutive steps to form an overall standard method. Given the large differences in composition between soft fruit and hard surface vegetables, it will probably be very difficult, if possible, to select one elution and clarification method of viruses that can be used in all kinds of fruits and vegetables. Several very different methods are used for concentration of viruses eluted from fruit and vegetable matrices. To select a high-yield standard virus concentration method for horizontal use, accurate comparison studies will have to be performed with different fruit and vegetable matrices. The virus concentration methods described in this paper could be the methods of choice in such comparison studies.

Q4. What is probiotic? Enumerate health benefits of probiotics.

Ans. The term Probiotic means "for life", is derived from the Greek language. It is now defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". The most common probiotic organisms clinically useful include members of the bacteria such as Lactobacillus and Bifidobacteria species and some selected strains of Streptococcus, Lactococcus and members of yeast and molds such as Saccharomyces, Aspergillus, Acanthosis and Candida species.

Page 3 of 21 Pages in this Full pdf

Health Benefits of Probiotics: Few well designed, well conducted human clinical trials of probiotics have been conducted over the past 30 years. Only in recent years, has the importance of choosing probiotic strains of demonstrated efficacy been recognized. With respect to health benefits of probiotics, research studies indicate the following:

- Improved intestinal health.
- Modulation of the immune response and immune function.
- Reduced risk of cancer.
- Reduced risk of heart disease.
- Improved tolerance to milk.
- Reduce food allergies.
- Reduce ulcers by decreasing the growth of ulcer-inducing bacteria (i.e., Helicobacter pylori).

Q5. Give the procedure for detection and enumeration of thermoduric organism.

Ans. (1) Weigh 25 g of sample in a sterile bag and add 100 ml Boulton broth to it. Shake it gently for 5 min.

(2) Filter the contents of bag through a sterile cheese cloth in a sterile bag or flask. Adjust the pH of broth obtained after rinsing to 7.4 by 2N NaOH.

(3) Keep the bag for pre-enrichment at 37oC for 4h under micro-aerophilic conditions (Campy gas generating system).

(4) Further incubate the samples for enrichment, at 42oC for 24-48 h, using Campy gas envelope.

(5) For isolation streak the enriched broth on AHB agar and incubate at 42oC for 24-48 h, anaerobically. Observe for typical colonies. Campylobacter produce thick translucent white growth to spreading, film-like transparent growth.

(6) Check for motility as described in section 3.5.2. Cells picked from agar often demonstrate only "wiggly" motility, whereas those from broth swim rapidly in corkscrew motion.

(7) If organism appear typical then re-streak on AHB agar without antibiotics and incubate at 42oC for 24-48 h.

(8) Perform catalase test as described below (Campylobacter are positive for catalase): The enzyme catalase acts upon hydrogen peroxide and converts it to water and molecular oxygen. Hydrogen peroxide is toxic to bacterial cells and catalase serves as a mechanism for removal of this toxic compound.

- Take a drop of H2O2 on a clean dry slide.
- Transfer growth from the TSA slant to the drop of H2O2.
- Observe the slide for the production of gas bubbles.
- Camylobacter is positive for catalase test.

Q6. What are the methods of detection of salmonella?

Ans. Plate Count

(1) Sample preparation is carried out by dissolving a representative quantity of food product in the medium for pre-enrichment/enrichment purposes. The exact procedure differs for the different kinds of foods, but overall it involves adding 25 g of food product to the enrichment broth so as to have a ratio of 1:9. This step is followed by the homogenization of food sample and incubation at 35oC for 24 h. The enrichment medium and its components depend on the type of food from which the isolation of Salmonella is desired. Not only the media for enrichment varies but the procedure for sample preparation also varies e.g. cellulose is added during the sample preparation of guar gum while for other foods surfactants such as triton X100 may be added.

(2) Gently shake the samples after incubation.

(3) Add 1ml of prepared sample to SC broth (10ml) and TT broth (10 ml). This step is to be taken for foods where the presence of S. typhi is suspected.

(4) For all other foods add 0.1 ml of sample to RV medium (10 ml) and 1 ml to TT broth (10 ml).

(5) Incubate the tubes of RV medium at 42oC for 24 h and tubes of TT broth at 43oC. This step is to be taken for foods with high microbial load.

(6) For foods with low microbial load incubate RV tubes as in step 5 while incubate TT at 35oC for 24 h.

(7) For guar gum and foods suspected for the presence of S. typhi incubate the SC and the TT tubes at 35oC for 24 h.

(8) Vortex the tubes and streak 10 µl of the culture from all the tubes on BS agar, XLD agar and HE agar. Incubate the plates at 35oC for 24 h.

(9) Examine the plates for the colonies that may be Salmonella i.e., yellow colonies with or without black centers. Biochemical tests

(10) Pick at least two suspected Salmonella colonies from each agar and carry out Triple sugar iron (TSI) agar and Lysine iron (LI) agar tests.

Page 4 of 21 Pages in this Full pdf

(11) Inoculate the TSI slant by stab and streak method. Without heating the loop stab the butt twice and streak the slant of LI agar.
(12) Incubate the tubes at 35oC for 24 h.
(13) Examine the results obtained in the TSI and LI tests. Salmonella produces an acidic butt (yellow) and alkaline slant (red). The blackening of butt may or may not occur depending on the H2S production. In LI test Salmonella produce an alkaline butt (purple) with H2S production. All the cultures giving alkaline reaction (purple butt) for LI agar are used for confirmation. Cultures giving an acidic reaction (yellow butt) in LI agar but an acidic butt and alkaline slant in TSI are also used.
(14) Examine a minimum of 6 TSI cultures for 25 g of sample, for identification of Salmonella.
(15) Inoculate urea broth with the presumptive Salmonella isolates and incubate the tubes at 35oC for 24 h. Keep an uninoculated tube as a control. This broth contains the pH indicator phenol red. The phenol red is peach color at a pH of 6.8 and turns a hot pink at a pH of 8.4. Discard the cultures if the colour of medium changes to purple red indicating a positive test for urease production because Salmonella is known to be negative for urease test.
(16) Carry out the serological polyvalent flagellar (H) test with the urease negative cultures. Inoculate BHI broth with the cultures from TSI slant and incubate at 35oC until visible growth occurs. Add 2.5 ml of formalinized physiological saline solution to 5 ml of BHI culture. Add 0.5 ml of polyvalent flagellar (H) antisera to 10X75 mm serological test tube. To this add 0.5 ml of formalinized broth. Prepare control by adding 0.5 ml of formalinized physiological saline to the formalinized antigen. Incubate the tubes at 48 – 50oC for 1 hr. Observe for agglutination. The test is positive if agglutination is observed and negative if there is no agglutination. In some cases the test may be nonspecific where agglutination is observed both in the test mixture and in control. Repeat this test with Spicer-Edwards flagellar (H) antisera for the cultures giving a negative result.
(17) Perform the additional biochemical tests on the urease negative isolates by inoculating them in Phenol red dulcitol broth in Durhams tube. Development of yellow color and gas production indicates positive reaction. Salmonella is positive for this test.
(18) Inoculate the growth from TSI slants into tryptone broth tube and incubate at 35oC for 24 h. Further inoculate potassium cyanide broth and malonate broth from the tube containing tryptone broth. Keep these tubes at 35oC for 48 h. Observe for growth in potassium cyanide broth. Salmonella is not able to grow in this broth. Salmonella also give a negative test for malonate which is indicated by the green or unchanged color of the broth.
(19) Transfer 5 ml of tryptone broth culture to an empty test tube and add 0.3 ml of Kovacs reagent. Development of deep red color is positive test. Salmonella is negative for this test.
(20) Perform the polyvalent somatic (O) test for Salmonella by preparing an emulsion of growth obtained from TSI slant in 2 ml of 0.85% saline. Take a drop of this suspension on a clean slide and add a drop of polyvalent somatic (O) antisera. Mix them and tilt the slide back and forth for 1 min. For control add a drop of saline to the drop of culture suspension and mix as described above. Observe for agglutination. If agglutination is positive for test and negative for culture the result is taken as positive for Salmonella. If both are negative the test is also negative for Salmonella and if agglutination is positive then the test is nonspecific.
(21) Perform additional biochemical tests including lactose fermentation, sucrose fermentation, MR-VP and citrate utilization.
(22) Compare the results obtained in the above biochemical and serological tests with the standard reactions exhibited by Salmonella.

Q7. Define Impedance.

Ans. Impedance can be simply defined as the resistance to flow of an alternating current as it passes through a conducting material. To elaborate it further, when two metal electrodes are immersed in a conductive medium the test system behaves as a resistor and capacitor in series. Considering the case where the system is treated as a series combination then application of an alternating sinusoidal potential will produce a resultant current which is dependent on the impedance of the system which in turn is a function of its resistance, capacitance and applied frequency.

When micro-organisms grow in culture media, they metabolize substrates of low conductivity into products of higher conductivity and thereby decrease the impedance of the media. Simple examples include the conversion of glucose from a non ionized substrate to two molecules of lactic acid with a corresponding increase in conductivity. Further metabolism will take the lactic acid and three oxygen molecules to produce carbonic acid resulting in three ion- pairs, including the smaller, more mobile bicarbonate ion which is a more effective electrical conductor than the lactate ion. The impedance of a system is a function of its resistance, capacitance and the applied frequency. It is usually the

Page 5 of 21 Pages in this Full pdf

resistive element which is measured and this is most frequently recorded as changes in conductance. These changes occur in the bulk electrolyte solution (culture medium) due to metabolism of uncharged or weakly charged substrates which are converted to highly charged end products e.g. proteins to amino acids. Capacitance is related to the behaviour of ions at the surface of the electrode and can be monitored separately from conductance. Several instruments which monitor changes in impedance are available and include the Bactometer 123 (Bactomatic Ltd.), Malthus 2000 (Malthus Instruments Ltd) and the Rapid Automated Bacterial Impedance Technique (RABIT) (Don Whitley Scientific Ltd). Detection of bacteria can be by direct conductimetry which is achieved by monitoring changes in the growth medium or by indirect conductimetry which monitors changes due to evolution of CO2 produced by the metabolism of substrates in the culture medium. When the impedance of broth cultures is measured, the curves are reproducible for species and strains, and mixed cultures can be identified by the use of specific growth inhibitors. The technique has been shown capable of detecting as few as 10 to 100 cells and cell populations of 105-106/ml can be detected in 3-5 h and 104-105/ml in 5-7 h. Impedance has been evaluated by a large number of investigators as a means of monitoring the overall microbial quality of various foods.

The redox potential is one of the most complex indicators of the physiological state of microbial cultures and its measurement could be a useful tool for the qualitative and quantitative determination of the microbial contamination. During the bacterial growth, the redox potential of the medium decreases. The shape of the redox potential curve is characteristic of the type of micro- organism, and the rate of the change (dE/dt) is proportional to the living cell concentration. The time required to reach a significant change in redox potential is defined as Time to Detection (TTD). Similarly to the impedimetric measurements, a strict linear correlation could be established between the TTD and the logarithm of the initial concentration of micro-organisms. On the basis of this calibration curve, the determination of living cell concentration could be simplified.

The applications of this technique remains to be fully exploited though it has already been shown to be a powerful tool for working with strains of Aerobic mesophilic micro-organisms, Psychrotrophic micro-organisms, Thermophilic micro-organisms, gram negative bacteria, Enterobacteria, Enterococci, Lactobacilli, Coliforms, E. coli, Salmonella, Listeria monocytogenes, Staphylococcus aureus, Beer spoilers, Clostridia, Aerobic spore formers, Bacillus cereus, Yeasts and Molds.

Q8. What are Immunological Methods?

Ans. Immunological methods of rapid detection are based on the highly specific antigen-antibody reactions and involve the use of monoclonal antibodies for detection of bacteria in the food samples. These methods comprise the largest group of methods used for detection purposes. The versatility and specificity of these reactions have prompted the production of immunological kits in various formats such as:

(1) **Latex Agglutination Assay:** This involves the antibodies tagged with the colored latex beads or colloidal gold for the detection purposes e.g. Campyslide, Microscreen for the detection of Campylobacter, RIM, Prolex, Ecolex O157, Wellcolex O157 for E. coli O157:H7, Microscreen, Listeria Latex for Listeria.

(2) **Immuno-diffusion Format:** This involves the diffusion of antigen through a gel impregnated with antibody. The appearance of precipitation line indicates the presence of specific antigen and is considered a positive test. An example of the kit based on the immunodiffusion is 1-2 test for the detection of Salmonella.

(3) **Enzyme Linked Immunosorbent Assay (ELISA):** This is the most popular rapid method for the detection of food borne pathogens. This is also based on the specificity of antigen-antibody reaction. In most of the immunological kits, sandwich format for ELISA is used. In this format antibody is coated on the surface of microtitre well. This antibody is known as primary antibody. Bacterial antigen obtained from the food sample is added to the antibody coated wells. Antigen-antibody reaction is allowed to occur followed by washing the wells to remove the unbound antigen. After this, a secondary antibody tagged with an enzyme is added to the microtitre plate. This antibody is also specific for the antigen to be detected but it recognizes a different epitope on the surface of antigen. This step is again followed by washing to remove the unbound antibody, followed by the addition of substrate for the enzyme. This substrate is usually colourless and a coloured product is formed by the action of antibody bound enzyme. The colour development is taken as a positive reaction and even the intensity of the colour can be measured using ELISA reader. This intensity when compared with standard controls can be used for quantification of the antigen or the results may be reported as positive and negative depending on the instructions given with the specific kit. If specific antigen is not present in food then the primary antibody will not bind to the added antigen, which will be removed during washing step. In the absence of specific antigen the secondary antibody will also not bind and will be washed from the well. The absence of enzyme conjugated secondary antibody from the well will effect the formation of coloured product from the substrate, thus giving a negative signal. Several enzymes such as horseradish peroxidase, alkaline phosphatase and p-nitrophenyl phosphatase have been used to tag

IGNOU MOTHER PUBLICATION - ASSIGNMENTS & GUESS PAPERS & PYQ SOLVED PDF

INDIA'S NUMBER 1 STUDY MATERIAL PROVIDER

यह केवल एक सैंपल पीडीएफ है।

यह केवल Sample PDF है। पूरी Solved Assignment या Guess Paper PDF खरीदने के लिए हमारी वेबसाइट पर जाएँ

या

WhatsApp पर मैसेज करें।

WHATSAPP ONLY: +91 9485943709

WHATSAPP ONLY:: +91 9992290905

1. Solved Assignment Visit -:: https://motherpublication.in/

2. Pyq Solved Guess paper Visit:-: https://mothernotes.in/

Mother Publication • IGNOU Study Resources