

**Food Safety and Standards  
Practical Manual  
Course Code: EC-AGP 605**



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## Practical No. 1

### Water quality analysis physico-chemical and microbiological (Part 1)

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#### Objective

Physico-chemical analysis of given water sample

#### 1. Determination of turbidity (Nephelometric Method)

#### Requirements

Nephelo turbidity meter with sample cells, volumetric flask and amber glass bottle etc.

#### Reagents

(a) Solution I- Dissolve 1.0 gm hydrazine sulphate,  $(\text{NH}_2)_2$ ,  $\text{H}_2\text{SO}_4$  in distilled water and dilute to 100 ml in a volumetric flask.

(b) Solution II- Dissolve 10 gm hexamethylenetetramine,  $(\text{CH}_2)_6\text{N}_4$ , in distilled water and dilute to 100 ml in a volumetric flask.

(c) 4000 NTU suspension- In a flask mix 5.0 ml of solution I and 5 ml of solution II. Let stand for 24 hours at  $25 \pm 3$  °C. This result in a 4000 NTU suspension. Store in an amber glass bottle. The suspension is stable for up to 1 year.

(d) Dilute 4000 NTU stock solution with distilled water to prepare dilute standards just before use and discard after use.

#### Procedure

Calibrate the Nephelo turbidity meter with the 100 NTU suspensions and verify the setting with a reading on the 10 NTU suspensions. Then thoroughly shake sample, wait until air bubbles disappear and pour sample into turbidity meter tube. Read turbidity directly from instrument display. In case the turbidity is going beyond the scale dilute the sample and measure the turbidity. The dilution factor should be taken into account for the final calculation of the result.

Calculation:

Turbidity (NTU) = NTU measured/proportion of sample in dilution

#### Observation table

S. No.	Sample Description	Turbidity (NTU)	Average turbidity (NTU)
1.			
2.			
3			

## Result

The turbidity of given water sample was observed----- NTU (Nephelo Turbidity Unit).

## Precautions

1. Calibrate the instrument properly.
2. Prepare the standard solution carefully.
3. Note the reading carefully.

## 2. Determination of pH

### Requirements

pH meter, beaker, volumetric flask, buffer tablets (4.0, 7.0 and 9.2 pH), tissue paper, distilled water and wash bottle.

### Principle

pH is a negative logarithm of  $H^+$  concentration of a solution.

### Procedure

Switch on the pH meter and leave for 10 min to warm up. Rinse the electrode with distilled water and wipe off with tissue paper. Dip the electrode in standard buffer solution of pH 7 for calibration, wait until the displayed reading establish at 7.00. Rinse the electrode with distilled water and wipe off. Dip the electrode in standard buffer solution of pH 4 and wait to establish reading at  $4 \pm 0.1$  or adjust with calibration knob. Rinse the electrode and dip in the sample, wait until reading establish and note the reading.

### Observation table

S. No.	Sample description	pH value	Average pH
1.			
2.			
3.			

## Result

The pH of given water sample was observed -----.

## Precautions

1. All the glassware should be clean.
2. Calibrate the instruments properly.
3. Note the reading carefully.

### 3. Determination of total dissolved solid (TDS) (Gravimetric method)

#### Requirements

Filter paper (Whatman no - 41), beaker, hot plate, physical balance, funnel, measuring cylinder, desiccator, oven etc.

#### Procedure

Dry the beaker in oven (105 °C) and cool in desiccator then weight it. Then take 300 ml filtered water sample in this beaker and evaporate up to dryness on a hot plate, cool in a desiccator and again weigh it. Calculate the TDS using following formula-

$$\text{Formula} = (W_2 - W_1) \times (1000/V)$$

Where,

$W_1$  = Initial weight of beaker

$W_2$  = final weight of beaker

V = Volume of sample

#### Observation table

S. No.	Sample description	Initial weight of beaker (gm)	Final weight of beaker (gm)	TDS (mg/l)
1.				
2.				
3.				

#### Result

The total dissolved solids in given water sample was observed ----- mg/l.

#### Precautions

1. Glassware should be clean.
2. Weigh the beaker carefully.
3. Note the weight carefully.

### 4. Determination of chloride (Argentometric method)

#### Requirements

Burettes, pipette, volumetric flask, conical flask and beaker.

#### Reagents

(a) Potassium chromate indicator solution- 50 gm  $K_2Cr_2O_4$  is dissolved in a small quantity of distilled water  $AgNO_3$  solution is added drop by drop until a definite red precipitate is formed.

Let stand for 12 hours; filter and dilute to one liter with distilled water.

(b) Standard silver nitrate solution 0.0141N- 2.395 AgNO<sub>3</sub> is dissolved in distilled water and dilute to one liter standardized with standard sodium chloride (NaCl). Store in brown bottle.

### Procedure

Take 100 ml sample in a conical flask and adjust the pH in the range of 7-10. Add 1 ml K<sub>2</sub>Cr<sub>2</sub>O<sub>4</sub> indicator, titrate with AgNO<sub>3</sub>. The end point will be pinkish yellow. Note the reading and repeat the titration with distilled water blank.

### Calculation

$$\text{Cl}^- (\text{mg/l}) = (A-B) \times N \times 35.45 \times 1000 / \text{volume of sample}$$

Where,

A = ml AgNO<sub>3</sub> used for sample

B = ml AgNO<sub>3</sub> used for blank

N = Normality of AgNO<sub>3</sub>

### Observation table

S. No.	Sample description	Normality of AgNO <sub>3</sub>	AgNO <sub>3</sub> used for sample (ml) A	AgNO <sub>3</sub> used for blank (ml) B	Chloride (mg/l)
1.					
2.					

### Result

The chloride in given water sample was observed----- mg/l.

### Precautions

1. Glassware should be clean.
2. Prepare the standard solution carefully.
3. Note the end point reading carefully

### References

1. [Determination of Turbidity | Nephelometric Turbiditymeter| Calibration of digital Turbidity meter| - YouTube](#)
2. [Digital pH Meter = Calibration and Working Demonstration \(English\) By Solution Pharmacy - YouTube](#)
3. [Determination of TDS and TSS of water - YouTube](#)
4. [Determination of chloride in given water sample - YouTube](#)

## Practical No. 2

### Water quality analysis physico-chemical and microbiological (Part 2)

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#### Objective

Microbiological analysis (enumeration of coliforms) of given water sample using Most Probable Number Technique

#### Introduction

Serial dilution tests measure the concentration of a target microbe in a sample with an estimate called the most probable number (MPN). The MPN is particularly useful for low concentrations of organisms (<100/g), especially in milk and water, and for those foods whose particulate matter may interfere with accurate colony counts.

Only viable organisms are enumerated by the MPN determination.

The essence of the MPN method is to dilute the sample to such a degree that inoculum in the tubes will sometimes but not always contain viable organisms. The "outcome", *i.e.*, the number of tubes and the number of tubes with growth at each dilution, will simply an estimate of the original, undiluted concentration of bacteria in the sample.

#### Requirements

Laminar flow, autoclave, spirit lamp, Durham's tubes, test tubes, cotton bundle, test tube stand, pipette, incubator etc.

#### Table: Chemicals for preparation of culture medium

Constituents	Quantity required for 1 L medium
Tryptose	20.0 g
Lactose	05.0 g
Dipotassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	02.75 g
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	02.75 g
NaCl	5.0 g
Sodium lauryl sulphate	0.1 g
Distilled water	1 L

For preparation of 1 liter medium dissolve the quantity of all above chemicals by heating do not boil.

#### Procedure

Multiple tube fermentation (read the procedure carefully before analysis)

1. Prepare medium as per the procedure given above.
2. Fill 9 ml medium in washed test tubes carefully keep the tubes in test tube basket.
3. Fill the Durham tube with medium and put it in the test tube filled with medium.
4. Cap the test tube after putting Durham's tube by cotton plug.
5. Cover test tube basket with aluminum foil (food grade) and also put required number of 10 ml pipettes for sterilization in the autoclave.
6. Tighten autoclave cover put on autoclave when pressure is reached at 15 psi, keep on for about 20 min at 15 psi keep the pressure at 15 psi by releasing steam (if required) by steam release valve.
7. Put off autoclave after complete sterilization and open steam valve for releasing pressure.
8. After 30 min open autoclave and take out carefully the sterilized test tubes filled with medium and other glass ware like pipettes.
9. Now test tubes when at normal temp keep in the test tube stand keeping 15 test tubes for one sample in three groups of 5 test tubes each in the test tube stand with dilution tubes.
10. Arrange test tubes so that for each sample 15 test tubes in 3 groups of 5 test tubes and label the test tube stands as per sample codes.
11. Now take spirit lamps two numbers and put on the lamps at the table where test is being performed if laminar air flow is available use that. Then there is no need for spirit lamps.
12. Dilution of sample- There are different type of samples, which have different ranges of coli forms. Dilution for some samples are given below-
13. After making proper dilution keep the sample tubes in bacteriological incubator for 24 h  $35\pm 0.5^{\circ}$  C.
14. After 24 h check the tubes if there is air in the Durham's tube also the color of the media gets changed light red if coliform is present due to fermentation of the media and production of CO<sub>2</sub> gas which gets filled in the Durham's tube. These called positive tubes. Count and note the number of positive tubes.



S. No.	Category (Source)	Dilutions		
		I	II	III
1.	Ground water	1%	0.1%	0.01 %
2.	River	1%	0.1%	0.01 %
3.	Sewage treatment plant influent effluent	0.0001%	0.00001%	0.000001%
4.	Bathing Ghats	0.0001%	0.00001%	0.000001%
5.	Recreation at waters	1%	0.1%	0.01 %
6.	Swimming pool	1%	0.1%	0.01 %

Fig. Dilution of samples with different ranges of coliforms

**Reference Table-**

Combination of (+) tubes dilution % <b>10-1-0.1</b>	MPN index per 100 ml	Combination of (+) tubes dilution % <b>10-1-0.1</b>	MPN index per 100 ml
0-0-0	≤ 2	3-0-0	8
0-0-1	2	3-0-1	11
0-1-0	2	3-1-0	11
0-2-0	4	3-1-1	14
1-0-0	2	3-2-0	14
1-0-1	4	3-2-1	17
1-1-0	4	4-0-0	13
1-1-1	6	4-0-1	17
1-2-0	6	4-1-0	17
2-0-0	4	4-1-1	21
2-0-1	7	4-1-2	26
2-1-0	7	4-2-0	22
2-1-1	9	4-2-1	26
2-2-0	9	4-3-0	27
2-3-0	12	4-3-1	33
		4-4-0	34

### Reference Table:

Combination of (+) tubes dilution 10% 1% 0.1%	MPN index per 100 ml
5-0-0	23
5-0-1	30
5-0-2	40
5-1-0	30
5-1-1	50
5-1-2	60
5-2-0	50
5-2-1	70
5-2-2	90
5-3-0	80
5-3-1	110
5-3-2	140
5-3-3	170
5-4-0	130
5-4-1	170
5-4-2	220
5-4-3	280
5-4-4	350
5-5-0	240
5-5-1	300
5-5-2	500
5-5-3	900
5-5-4	1600
5-5-5	≥1600

### Result

The total coliform was found----- MPN/100ml in given water sample.

### Precautions

1. Sample should be taken carefully.
2. Sterilize carefully.

### Reference

1. [MPN test for Water in detail by IES Vishnu Maurya - YouTube](#)

## **Practical No. 3**

### **Preparation of different types of media (Part 1)**

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#### **Objective**

Preparation of different types of culture media

#### **Introduction**

For the identification of bacteria, it is essential to obtain a culture by growing the organisms on artificial media. The different types of media that are commonly used for identification of bacteria are

- a) Mac-Conkey's Agar media
- b) Mueller- Hinton Agar media
- c) Blood Agar
- d) Luria Broth

#### **a) Preparation of Mac-Conkey's Agar media**

##### **Principle**

Mac-Conkey agar is a differential medium used to distinguish lactose-fermenting from the non-lactose-fermenting bacteria.

##### **Requirements**

MacConkey agar, Autoclave, Water bath, Digital balance, Refrigerator, Petri-dishes, Conical/Erlenmeyer flask (500 ml), distilled water

##### **Procedure**

By following the manufacturer's instructions, the media was prepared:

1. Weight 51.53 g of MacConkey agar on a digital balance and allow to suspend in the demineralized H<sub>2</sub>O.
2. The powder dissolved completely by heating in a water bath sterilize by autoclaving for 15 min at 121° C.
3. Allow to pour into petri-dishes, about 16-18 ml in each.
4. Allow to solidify for leaving it at room temperature.
5. The shelf life is about one month.
6. Allow to store at 2-8° C.

## **b) Preparation of Mueller-Hinton Agar**

### **Principle**

This medium was originally formulated for the isolation of pathogenic *Neisseria* species. However, nowadays it is used in antimicrobial susceptibility testing.

### **Requirements**

Mueller- Hinton Agar, Autoclave, Conical flask (1 L), water bath, petri-dishes, refrigerator, distilled water, digital balance

### **Procedure**

By following the manufacturer's instructions, the media was prepared:

1. Weight and dissolve 30.0 g in 1 L of purified water.
2. Heat in boiling water and agitate frequently until completely dissolved.
3. Autoclave for 15 min at 121° C
4. It was then poured into petri-dishes, about 16-18 ml in each.
5. Allow to solidify for leaving it at room temperature.
6. The shelf life is about one month.
7. It was stored at 2-8° C.

### **Precautions**

1. Handle the glassware carefully.
2. Maintain utmost hygiene at the workplace.
3. Handle the autoclave carefully.

### **Reference**

1. [Preparation of Macconkey Agar Media | How to Prepare Macconkey Agar Media - YouTube](#)
2. [Preparation of Mueller Hinton Agar || Full preparation Procedure Bangla - YouTube](#)

## **Practical No. 4**

### **Preparation of different types of culture media (Part 2)**

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#### **Objective**

To prepare the different types of culture media

#### **a) Preparation of Blood Agar media**

##### **Principle**

Blood agar is an enriched medium. It can also be made selective by adding some antibiotics like Kanamycin. When blood agar is heated, the red cells lyse and the media becomes brown, called Chocolate agar.

##### **Requirements**

Blood agar, digital balance, distilled water, conical flask (1L), water bath, autoclave, petri-dishes, refrigerator

##### **Procedure**

1. Weigh and dissolve 40.0 g of Blood Agar in 1 L of purified water.
2. Heat in boiling water and agitate frequently until completely dissolved.
3. Autoclave for 15 min at 121°C Allow to pour into petri-dishes, about 16-18 ml in each.
4. Allow to solidify for leaving it at room temperature.
5. The shelf life is about one month.
6. Allow to store at 2-8 °C.

#### **b) Preparation of Luria Broth**

##### **Principle**

Luria Broth (LB) is a nutritionally rich medium due to the presence of casein enzymatic hydrolysate and yeast extract.

##### **Requirements**

Luria Broth, digital balance, distilled water, conical flask (1L), water bath, autoclave, petri-dishes, refrigerator, falcon tubes, inoculating loop, shaking incubator

##### **Procedure**

1. 15 g of LB was weighed and mixed in 600 ml of distilled water in 1000ml flask.
2. The flask was placed over the heat and stir for 10 min.
3. After complete shaking the flask was sealed with aluminum foil.

4. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 min.
5. Allowed to cool at room temperature.
6. At least 5 ml of LB was poured in each falcon tube.
7. With the help of sterilized inoculating loop the bacterial colony was mixed in the LB in each falcon tube.
8. Incubate bacterial culture at 37 °C for 12-18 h. in a shaking incubator
9. After incubation, check for growth, which is characterized by a cloudy haze in the media
10. For long term storage of the bacteria, it was stored at -20 °C

### **Precautions**

1. Handle the glassware carefully.
2. Maintain utmost hygiene at the workplace.
3. Handle the autoclave carefully.

### **References**

1. [Blood agar media preparation in Hindi | culture media microbiology | microbial growth microbiology - YouTube](#)
2. [LB Plate Preparation - YouTube](#)

## **Practical No. 5**

### **Microbiological examination of different food samples (Part 1)**

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#### **Objective**

Microbiological examination of cereal-based products (Bread)

#### **Introduction**

Cereal-based products usually do not support growth of bacterial but cream and other fillings of cakes are highly favorable for multiplication of bacteria, very rarely species of bacillus may cause defect in bread known as ropiness due to production of capsular material.

The material in microbiological examination of cake filling depends upon nature of constituents and are given in the sections appropriate to constituent. Microbiological control of bakery products other than filling is concerned mainly with possibility of spoilage due to growth molds since the bakery temperature is sufficient to kill fungal spores subsequent spoilage usually caused by mold contamination from the atmosphere or from wrapping material.

#### **Requirements**

Bread Sample, sterile petri plates, lacto phenol cotton blue solution, glass slides and cover slips, incubator, compound microscope

#### **Procedure**

Moistened bread sample was kept in sterile petri plates

It was incubated at room temperature for 5 days

Microscopic observation of different molds was carried out

#### **Results**

#### **Conclusion**

The microflora carried by bread consist of penicillium, aspergillus, mucor, hizopus and some bluish green spots.

#### **References**

1. [How to make Microbiological analysis of food - Method of testing - YouTube](#)
2. [Microbiological Examination of Food - YouTube](#)

## Practical No. 6

### Microbiological examination of different food samples (Part 2)

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#### Objective

Microbiological examination of fruits and vegetables

#### Introduction

Fresh fruit and vegetables normally carry a surface flora of micro-organisms some of them play role in spoilage. Microbiological examination of these foods is not easily carried out. But in case of fruits any vegetable handling is involved eg. Salad preparation an examination of contamination is advisable.

#### Examination of potato and tomato

The interior part of the intact healthy fresh fruit and vegetables are usually sterile therefore such tissue removed aseptically can be used as culture media without heat treatment in incubation shows tissues to be sterile. Since skin of most vegetables and fruits *e.g.*, Tomato contains all microorganisms present count of organisms on such vegetables should be expressed in term of area of skin examination.

#### Requirements

Tomato and potato, sterile knife, sterile buffered distilled water, sterile petri plates and pipettes, 95% ethanol, plate count broth, brilliant green lactose bile broth

**Table: Composition of plate count broth**

Chemicals	Quantity
Peptone	10 g
Lactose	10 g
Sodium taurocholate	20 g
Sodium Chloride	5 g
Distilled water	1 L
pH	7.5

All ingredients were dissolved and 1.3% brilliant green [aqueous solution – 1ml] was added. Durham's tube was put in test tube and medium was sterilized at 110 °C for 40 min.

**Table: Composition of brilliant green lactose bile broth**

Chemicals	Quantity
Glucose	40 g



Peptone	10 g
Agar	18 g
Distilled water	1 L
pH	6.8 to 7

Sterilize by autoclaving at 110 °C for 10 min.

## **Procedure**

### **I. Plating of surface of tomato**

#### **Procedure**

1. One-centimeter square area of skin and adjoining flesh was cut with a sharp knife.
2. It was grounded with and tissues were transferred to 99 ml of buffered distilled water and shaken vigorously.
3. Dilutions were prepared (1:10, 1:100, 1:1000).
4. Duplicate plates were incubated at room temperature for 5 days or at 30 °C for 2 days.
5. In the same way plates were poured with agar for yeast and molds and incubated at room temperature for 2 days or 5 days as per requirement.

## **Results**

### **II. Test for sterility of inner tissue**

#### **Procedure**

1. One side of well sound tomato was wetted with 95% ethanol, burned off later and thoroughly flamed.
2. The flamed skin was pierced with sterile end of pipette and 3 ml juice and pulp was drawn out.
3. 1 ml of juice and pulp was blown in each of 2 tubes of plate count broth.
4. Tubes were incubated at room temperature for 5 days or 30 to 32 °C for 2 days and then it was observed for growth.
5. Similarly double and single strength tubes of BGLBB were inoculated with dilutions prepared from tomato juice and pulp in order to calculate MPN.

## **Results**

### **Conclusion**

This shows that the inner tissues of tomato were not sterile

### **Reference**

1. [Microbiological examination of foods - YouTube](#)

## **Practical No. 7**

### **Assessment of surface sterilization by swab/rinse method (Part 1)**

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#### **Objective**

To assess surface sanitation by swab method

#### **Introduction**

There are various agents of contamination such as air, water, soil and they leave their imprints on the surfaces exposed to them thereby causing the entry of micro-organisms through surfaces such as working shelf, laboratory shelves, equipment surface as well as uncovered glassware and other laboratory required ware in the experimental assays. Therefore, there is a need to devise methods to assess the surface sterility so that necessary germicidal action can be taken by using various physical and chemical agents, such as steam, detergents (alkali, QAC, acids), antiseptics etc.

#### **Principle**

Two commonly used methods to determine surface contamination are Swab and Rinse method. In the first method, i.e., Swab Method, there is a need of a swab made up of non-absorbent cotton and this method is used for flat exposed surfaces such as working desktops or laboratory shelves. These are also used in industry for checking the efficacy of Clean In Place (CIP) cleaning. This method is based on a simple principle i.e. lifting of microorganisms from a surface then diluting the samples into various serial dilutions followed by pour plating and incubating at 37 °C.

#### **Requirements**

Reagents:

Sterile Nutrient agar, Ringer's solution in sterile dilution blanks (9 ml) and in swabs.

Equipment:

Bunsen burner, wide mouth test tubes, sterile petri plates, nutrient agar, non-absorbent cotton, thread, glass rod, pipettes.

#### **Preparation of swabs**

Take clean glass rods slightly longer than the wide mouth tubes and wipe with the help of spirit. A small amount of non-absorbent cotton is draped in the form of a bud at one end of the glass rod and tied with the help of a thread. The tube is then plugged with cotton or a cap is tightened. Number of swabs prepared is directly proportional to the number of surfaces to be assessed (Readymade sterile swabs can be used too).



Fig. Wide mouth tube containing Swab

### Preparation of rectangular predetermined glass rod bound surface area

A glass rod was taken and bent with the help of flame into a dimension of  $20 \times 10 \text{ cm}^2$ . This frame was used to limit the area selected for swabbing.

### Preparation of Ringer's solution

Prepare as mentioned and disperse in volumes of 20 ml and 10 ml each in wide mouth tubes containing swab and test tubes for dilution blanks respectively. Then they are plugged tightly and autoclaved at  $121 \text{ }^\circ\text{C}$  for 15-20 min.

**Table: Composition of Ringer's solution**

Component	Quantity (g/L)
NaCl	9
KCl	0.42
Anhydrous $\text{CaCl}_2$	0.24 (0.48 g in case of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ )
Sodium bicarbonate	0.20
Distilled water	1 L

### Procedure

1. In this method, select any two areas (for e.g., working shelf and laboratory shelf) to assess the surface sterility.
2. Take the glass rod rectangle and swab the area within it, twice or thrice with the help of same swab at one location.
3. Then, press the swab gently pressed along the sides of wide mouth tube and take it out.
4. This is the stock solution to be used further for serial dilutions and pour plating. Repeat for different locations.

### Serial Dilution and Pour Plating

1. All the stock solutions, i.e., two from swab are suitably diluted with a sterile ringer's

diluent and pour plated using nutrient agar.

2. After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 37 °C for 24 h.
3. At the end of the incubation period, select all of the petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated **too many to count (TMTC)**. Plates with fewer than 30 colonies are designated **too few to count (TFTC)**. Count the colonies on each plate. A Quebec colony counter should be used.
4. Calculate the number of bacteria (CFU) per millilitre or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

$$\text{Number of bacteria per ml} = \frac{\text{Number of colonies}}{\text{dilution} \times \text{amount plated}}$$

5. Record your observations.

### Observation

Location	Dilution used	No. of Colonies	Total CFU/200 cm <sup>2</sup>

### Results

Record your results and find the average number of cells/ml or cm<sup>2</sup> by adding the results from all of your plates and dividing by the number of plates. Then relate cleanliness to microbial load and comment on the prevailing conditions or process efficacy.

### Precaution

1. All the glassware should be clean and sterile.
2. The area to be tested should be swabbed twice or thrice with the same swab.
3. The dilutions should be made properly and under sterile conditions.
4. The counting should be done accurately.

### References

1. [Surface Sampling Swab - YouTube](#)
2. [Swab culture test procedure Microbiology - YouTube](#)

## **Practical No. 8**

### **Assessment of surface sterilization using swab/rinse method (Part 2)**

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#### **Objective**

To assess surface sanitation by rinse method

#### **Introduction**

There are various agents of contamination such as air, water, soil and they leave their imprints on the surfaces exposed to them thereby causing the entry of micro-organisms through surfaces such as working shelf, laboratory shelves, equipment surface as well as uncovered glassware and other laboratory required ware in the experimental assays. Therefore, there is a need to devise methods to assess the surface sterility so that necessary germicidal action can be taken by using various physical and chemical agents, such as steam, detergents (alkali, QAC, acids), antiseptics etc.

#### **Principle**

Two commonly used methods to determine surface contamination are Swab and Rinse method. In the first method, i.e., Swab Method, there is a need of a swab made up of non-absorbent cotton and this method is used for flat exposed surfaces such as working desktops or laboratory shelves. These are also used in industry for checking the efficacy of Clean In Place (CIP) cleaning. This method is based on a simple principle i.e. lifting of microorganisms from a surface then diluting the samples into various serial dilutions followed by pour plating and incubating at 37 °C.

#### **Requirements**

Reagents:

Sterile Nutrient agar, Ringer's solution in sterile dilution blanks (9 ml) and in swabs.

Equipment:

Bunsen burner, wide mouth test tubes, sterile petri plates, nutrient agar, non-absorbent cotton, thread, glass rod, pipettes.

#### **Preparation of rectangular predetermined glass rod bound surface area**

A glass rod was taken and bent with the help of flame into a dimension of 20×10 cm<sup>2</sup>. This frame was used to limit the area selected for swabbing.

#### **Preparation of Ringer's solution**

Prepare as mentioned and disperse in volumes of 20 ml and 10 ml each in wide mouth tubes containing swab and test tubes for dilution blanks respectively. Then they are plugged tightly

and autoclaved at 121 °C for 15-20 min.

**Table: Composition of Ringer's solution**

Component	Quantity (g/L)
NaCl	9
KCl	0.42
Anhydrous CaCl <sub>2</sub>	0.24 (0.48 g in case of CaCl <sub>2</sub> .6H <sub>2</sub> O)
Sodium bicarbonate	0.20
Distilled water	1 L

### Procedure

1. Select clean milk can, conical flask or beaker for assessing efficacy of cleaning agent used.
2. Add 10 ml of sterile diluent into the conical flask / beaker (100 ml in case of milk can) and swirl gently.
3. Transfer back to the sterile test tube and plug.
4. This is the stock solution to be used for serial dilution and pour plating further.

### Serial dilution and pour plating

1. All the stock solutions, i.e., two from swab are suitably diluted with a sterile ringer's diluent and pour plated using nutrient agar.
2. After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 37 °C for 24 h.
3. At the end of the incubation period, select all of the petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated **too many to count (TMTC)**. Plates with fewer than 30 colonies are designated **too few to count (TFTC)**. Count the colonies on each plate. A Quebec colony counter should be used.
4. Calculate the number of bacteria (CFU) per millilitre or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar

$$\text{Number of bacteria per ml} = \frac{\text{Number of colonies}}{\text{dilution} \times \text{amount plated}}$$

5. Record your observations.

### Observation table

Sample	Dilution used	No. of colonies	Total CFU/ML

### Results

Record your results and find the average number of cells/ml or cm<sup>2</sup> by adding the results from all of your plates and dividing by the number of plates. Then relate cleanliness to microbial load and comment on the prevailing conditions or process efficacy.

### Precaution

1. All the glassware should be clean and sterile.
2. The area to be tested should be swabbed twice or thrice with the same swab.
3. The dilutions should be made properly and under sterile conditions.
4. The counting should be done accurately.

### Reference

[EnviroTrans™ Swab Rinse Kit - YouTube](#)

## **Practical No. 9**

### **Assessment of personal hygiene**

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#### **Definition**

The word Hygiene has evolved from the Greek term “Hygeia” which means “Goddess of Health”. Hygiene can be defined as, “The science and art which is associated with the preservation and promotion of health”.

#### **Concept of personal hygiene**

Personal hygiene includes different habits i.e., washing hands and brushing teeth which keep bacteria, viruses and fungal far away from our bodies. Moreover, these habits will help us to protect our mental health and activity. Also, good personal hygiene will help us to keep feeling good about ourselves. Since those who do not take care of their personal hygiene i.e., dirty clothes, body odor and bad breath will suffer from discrimination and this will mainly lead to mental problems. But the most important point in this subject is that all people have their own hygiene but some people do it better than others, this mainly depends on each person’s culture, society and family norm.

#### **Scientific knowledge base**

1. Physical hygiene is necessary for comfort, safety, and well-being.
2. Ill patients require assistance with personal hygiene.
3. Several factors influence a patient’s hygiene practices, such as culture and age.
4. Good hygiene techniques promote normal structure and function of tissues.
5. Apply knowledge of pathophysiology to provide preventive hygiene care.

#### **Factors influencing hygiene**

1. Social pattern
2. Personal preferences
3. Body image
4. Socio-economic status
5. Health beliefs and motivation
6. Cultural variables
7. Developmental stage
8. Physical condition

#### **Table: Assessment of personal hygiene**



Self-Care Ability	Skin	Feet and Nails
Oral cavity	Hair and hair care	Eyes, ears, and nose
Use of sensory aids	Hygiene care practices	Cultural influences

### **Diagnosis**

Common diagnoses associated with hygiene:

1. Activity intolerance
2. Bathing self-care deficit
3. Dressing self-care deficit
4. Impaired physical mobility
5. Impaired oral mucous membrane
6. Ineffective health maintenance
7. Risk for infection

### **Planning**

1. Goals and outcomes
  - a. Partner with the patient and family
  - b. Measurable, achievable, individualized
2. Set priorities based on assistance required, extent of problems, nature of diagnoses
3. Teamwork and collaboration
  - a. Health care team members
  - b. Family
  - c. Community agencies

### **Implementation**

1. Use caring to reduce anxiety, promote comfort.
2. Administer meds for symptoms before hygiene.
3. Be alert for patient's anxiety or fear
4. Assist and prepare patients to perform hygiene as independently as possible.
5. Teach techniques and signs of problems.
6. Inform patients about community resources.

### **References**

[Personal hygiene ppt \(slideshare.net\)](#)

## Practical No. 10

### Biochemical test for the identification of bacteria (Part 1)

---

#### Objective

To identify the bacteria using various biochemical tests

#### Introduction

There are many biochemical tests available for bacterial identification. Few of them are required to be carried out depending upon the bacteria. The commonly used biochemical tests are as mentioned below

- (a) Catalase test
- (b) Coagulase test
- (c) Oxidase test

#### (a) Catalase test

#### Purpose

The catalase test facilitates the detection of the enzyme catalase in bacteria. It is essential for differentiating catalase-positive *Micrococcaceae* from catalase negative *Streptococcaceae*. While it is primarily useful in differentiating between genera, it is also valuable in speciation of certain gram positives such as *Aerococcus urinae* (positive) from *Aerococcus viridians* (negative) and gram-negative organisms such as *Campylobacter fetus*, *Campylobacter jejuni*, and *Campylobacter coli* (all positive) from other *Campylobacter* species.

#### Requirements

Microscopic slide, petri dish, inoculating loop, dropper or Pasteur pipette, 3% H<sub>2</sub>O<sub>2</sub>, bacterial colony

#### Procedure

1. Place a microscope slide inside a petri dish. Keep the petri dish cover available.
2. Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18-24 h colony and place it onto the microscope slide. (Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction).
3. Using a dropper or Pasteur pipette, place 1 drop of 3% H<sub>2</sub>O<sub>2</sub> onto the organism on the microscope slide. Do not mix.
4. Immediately cover the petri dish with a lid to limit aerosols

5. Observe for immediate bubble formation ( $O_2 + \text{water} = \text{bubbles}$ ). Observing for the formation of bubbles against a dark background enhances readability.

### **Examples**

1. Catalase positive bacteria: *Staphylococcus spp*
2. Catalase negative bacteria: *Streptococcus spp*

### **b. Coagulase test**

#### **Purpose**

The coagulase test differentiates strains of *Staphylococcus aureus* from other coagulase-negative species. *S. aureus* strains are capable of coagulating plasma in the tube test and will produce clumps of cells in the slide test.

The coagulase test can be performed using two different procedures

1. Slide test
2. Tube test.

The slide test is simple, giving results within 10 s, but it can give false negatives. The tube test is the definitive test; however, it can take up to 24 h to complete. For both tests, clumping or clots of any size indicate a positive response. While *S. aureus* is the most commonly isolated coagulase positive organism, there are several other species of *Staphylococcus* which are positive for coagulase activity. *S. schleiferi* and *S. lugdunensis* may give positive results in the slide test for bound coagulase, and *S. schleiferi* and *S. intermedius* may give positive results in the tube coagulase test.

#### **Requirements**

Microscopic slide, bacterial colony, blood plasma

#### **Procedure**

1. Prepare a suspension of bacterial cells.
2. Allow to mix into a drop of rabbit plasma on a microscope slide
3. Observe for clot formation

#### **Interpretation**

Formation of a clot will be noted within 24 h for a positive response. As the bacteria multiply in the plasma, they secrete staphylocoagulase. Staphylocoagulase initiates blood coagulation by activating prothrombin. Staphylocoagulase adheres to fibrinogen, forming a complex that cleaves fibrinogen into fibrin, bypassing the blood clotting cascade and directly causing a clot of fibrin to form.

#### **Examples**

1. Coagulase positive bacteria: *Staphylococcus aureus*

2. Coagulase negative bacteria: *Staphylococcus epidermis*, *Staphylococcus saprophyticus*

### **(c) Oxidase test**

#### **Purpose**

The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized color product.

#### **Requirements**

Filter paper, Kovacs oxidase reagent, inoculating loop, bacterial colony

#### **Procedure**

There are many method variations to the oxidase test. These include, the filter paper test, filter paper spot test, direct plate method, and test tube method.

#### **Filter Paper Test Method**

1. Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry.
2. Use a loop and pick a well-isolated colony from a fresh (18 to 24 h culture) bacterial plate and rub onto treated filter paper.
3. Observe for any color changes.

#### **Interpretation**

1. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 s.
2. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 s.
3. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 min.

#### **Examples**

1. Oxidase positive bacteria: *Pseudomonas*, *Vibrio cholera*
2. Oxidase negative bacteria: *E. coli*, *Klebsiella*, *Salmonella*.

#### **Reference**

1. [Bacterial Identification Tests: Catalase Test - YouTube](#)
2. [Coagulase Test - Amrita University - YouTube](#)
3. [Oxidase Test - YouTube](#)

## **Practical No. 11**

### **Biochemical test for the identification of bacteria (Part 2)**

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#### **Objective**

To identify the bacteria using various biochemical tests

#### **Introduction**

There are many biochemical tests available for bacterial identification. Few of them are required to be carried out depending upon the bacteria. The commonly used biochemical tests are as mentioned below

- (a) Indole test
- (b) Citrate test
- (c) Urease test

#### **(a) Indole test**

##### **Purpose**

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC (indole, MR-Vp Citrate) procedures, a battery of tests designed to distinguish among members of the family Enterobacteriaceae.

##### **Requirements**

Tryptone broth, bacterial culture, incubator, Kovac's reagent, test tube

##### **Procedure**

1. Inoculate the tube of tryptone broth with a small amount of a pure culture.
2. Incubate at 37 °C for 24 to 48 h.
3. To test for indole production, add 5 drops of Kovac's reagent directly to the tube.

##### **Interpretation**

1. A positive indole test is indicated by the formation of a pink to red color (“cherry red ring”) in the reagent layer on top of the medium within seconds of adding the reagent.
2. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

##### **Examples**

1. Indole positive bacteria: *E. coli*, *Vibrio cholera*
2. Indole negative bacteria: *Klebsiella*, *Salmonella*, *Shigella spp.*

#### **(b) Citrate Test**

##### **Purpose**

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator. The citrate test is often part of a battery of tests used to identify gram-negative pathogens and environmental isolates.

### **Requirements**

Bacterial culture, needle, incubator

### **Procedure**

1. Use a fresh (16-18 h) pure culture as an inoculation source.
2. Pick a single isolated colony and lightly streak the surface of the slant. A needle is the preferred sampling tool in order to limit the amount of cell material transferred to the agar slant (*avoid using liquid cultures as the inoculum source*).
3. Citrate utilization requires oxygen and thus screw caps, if used, should be placed loosely on the tube.
4. Incubate at 35°C (+/- 2°C) for 18 to 48 h (*some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium*).

### **Interpretation**

Citrate positive

1. Growth will be visible on the slant surface and the medium will be an intense Prussian blue.
2. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green color to blue.

Citrate negative

1. Trace or no growth will be visible.
2. No color change will occur. The medium will remain the deep forest green color of the uninoculated agar.
3. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant.

### **Examples**

Citrate positive bacteria: *Klebsiella spp.*

Citrate negative bacteria: *E. coli.*

### (c) Urease test

#### Purpose

The test is used for identifications of bacteria *Proteus vulgaris* which is a fast producer of enzyme urease. Urease attacks on urea, a waste product in urine and produces ammonia.

#### Requirements

Urea broth or urea agar medium, bacterial colony, test tubes, incubator

#### Procedure

1. Preparation of urea agar medium
2. Use a heavy inoculum from an 18 to 24 h pure culture to streak the entire slant surface  
(Do not stab the butt as it will serve as a color control).
3. Incubate tubes with loosened caps at 35°C.
4. Observe the slant for a color change at 6 h, 24 h, and every day for up to 6 days.

#### Interpretation

1. Urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt (Note: Any degree of pink is considered a positive reaction).
2. Rapidly urease-positive *Protease* (*Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 h of incubation.
3. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 h, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days).
4. The culture medium will remain a yellowish color if the organism is urease negative.

#### Examples

Urease positive bacteria: *Proteus* spp., *Morganella morganii*

Urease negative bacteria: *E. coli*,

#### Reference

1. [Bacterial Identification Tests: Indole Test - YouTube](#)
2. [Citrate Test Microbiology - YouTube](#)
3. [Urease Test Microbiology - YouTube](#)

## Practical No. 12

### Schemes for detection of food borne pathogens (Part 1)

#### Objective

To identify the various schemes required for detection of food borne pathogens

#### Introduction

Foodborne pathogens can lead to serious outbreaks irrespective of the region. This leads to the spread of disease, more so in infants and aged individuals. Rapid detection becomes important to contain the spread of the pathogen before it leads to a serious outbreak. Various techniques have been evolved to detect the foodborne pathogens. The detection methods have been classified into different groups along with their principles, advantages and disadvantages. The following figure shows the schematic representation of the methods for the detection of pathogens. ELISA, enzyme linked immuno sorbent assay; DAS, double antibody sandwich; PCR, polymerase chain reaction; LAMP, loop mediated isothermal amplification; RSS, restriction site specific; RT, real time; ECL, electrochemiluminescence; FRET, fluorescence resonance energy transfer; IMS, immunomagnetic assay; ICA, immune chromatic assay; FTIR, Fourier transform infrared spectroscopy.

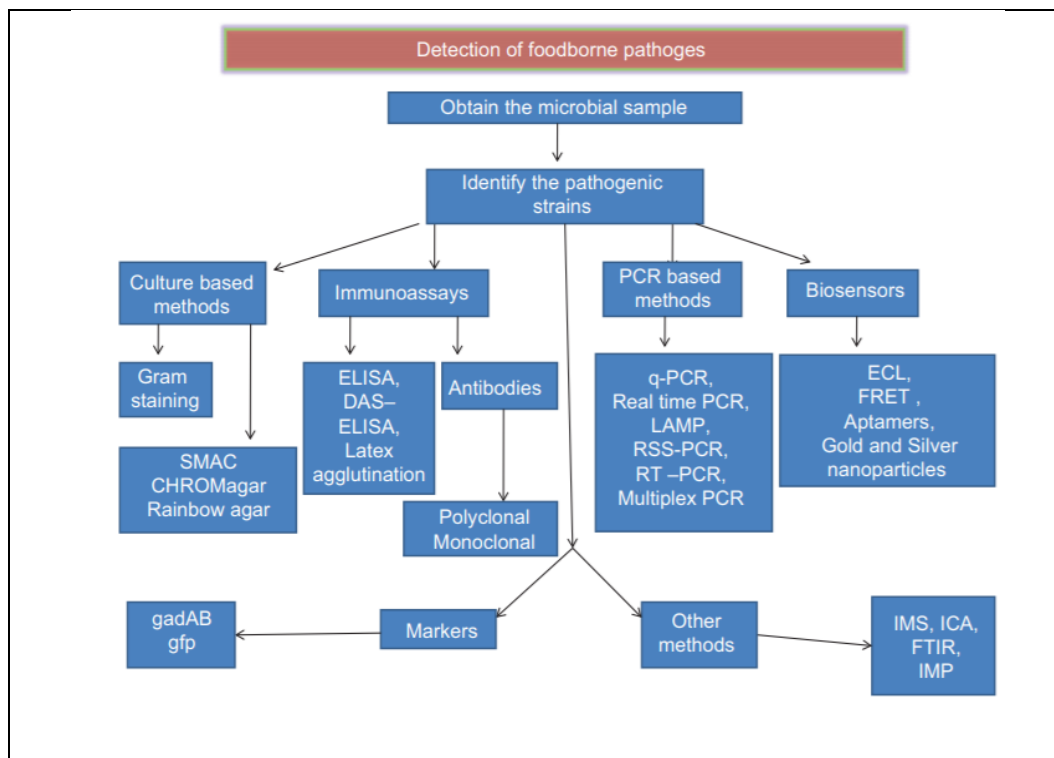


Fig. Schematic representation of the methods for the detection of pathogens



## Reference

1. [Detection of pathogens in food - conventional detection methods - part 1 - food microbiology - youtube](#)
2. [Detection of pathogens in food - rapid detection methods - part -2 - food microbiology - youtube](#)

## **Practical No. 13**

### **Schemes for detection of food borne pathogens (Part 2)**

---

#### **Objective**

Detection of food borne Gram-positive and Gram-negative pathogenic bacteria using Gram-Staining Technique

#### **Principle**

Gram stain was originally described by Christian Gram in 1884. When the bacteria are stained the Gram-positive bacteria stain with crystal violet and are not de-colored with acetone iodine, while Gram-negative bacteria are de-colored with acetone iodine and hence take up the color of the dye carbol fuchsin. The difference in staining is due to the difference in the cell wall structure. Gram positive bacteria have a thick layer of peptidoglycan in their wall while gram gram-negative bacteria have a thin layer. This is why the gram-positive bacteria cause the retention of crystal violet.

#### **Procedure**

Following steps were done in the staining

1. Make a smear and fix it by passing the slide rapidly over a flame.
2. Cover the slide with, crystal violet and allow it to act for 30 s.
3. Pour off and wash freshly with iodine solution. Cover with fresh iodine solution and allow to act for 30 s.
4. Pour off the iodine solution and wash freely with acetone iodine. Cover with acetone iodine and allow it to act for about 30 s (until the stain stops carrying out).
5. Wash thoroughly with water.
6. Counter- stain with dilute carbol fuchsin for 30 s.
7. Wash with water, blot and dry.
8. See under the microscope with oil immersion lens.

Following organisms were used for monitoring the quality control.

**Positive Control:** Staphylococcus aureus

**Negative Control:** Escherichia coli

#### **References**

[Gram Positive vs Gram Negative Bacteria - Gram Staining Technique - YouTube](#)

## **Practical No. 14**

### **Preparation of plans for implementation of FSMS - HACCP, ISO: 22000 (Part 1)**

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#### **Objective**

Preparation of plans for implementation of FSMS

#### **Introduction**

FSMS Plan or Food Safety Management System (FSMS) plan is a set of standards established to direct and control aspects of food safety. There are many international FSMS like HACCP, ISO 22000, FSSC (Food Safety System Certification) 22000 and many more. However, these are voluntary certifications and the FSMS as defined in the Food Safety and Standards Act, 2006 means the adoption Good Manufacturing Practices (GMPs), Good Hygienic Practices (GHPs), Hazard Analysis and Critical Control Point (HACCP) and such other practices as may be specified by regulation. FSMS plan is required during application for new FSSAI License or renewal of FSSAI License.

#### **Requirement**

The Food Safety and Standard Act, 2006 requires all food businesses to have a FSMS plan. Even if a food business has a Food Safety Certification from HACCP or ISO or FSSC, a FSMS plan as per the Food Safety and Standards Act is required. However, for those food businesses having HACCP or ISO or FSSC certification, preparing a FSMS plan would be easy as there are many areas that overlap.

Further, the requirement for FSMS plan is also not dependent on type or size of business. Hence, all food businesses require a FSMS plan. But, depending on the size and nature of the business, the size and complexity of the FSMS plan would vary.

#### **Preparing a FSMS plan**

All FSMS Program must have a FSMS plan, flow chart for the process and an inspection checklist. FSMS plan is an important document that defines each of the steps in the operation, the applicable/identified hazards, method for controlling the hazard/threat, critical limit, method for monitoring for critical threat, corrective action, responsibility and record keeping. A sample FSMS plan is attached below:

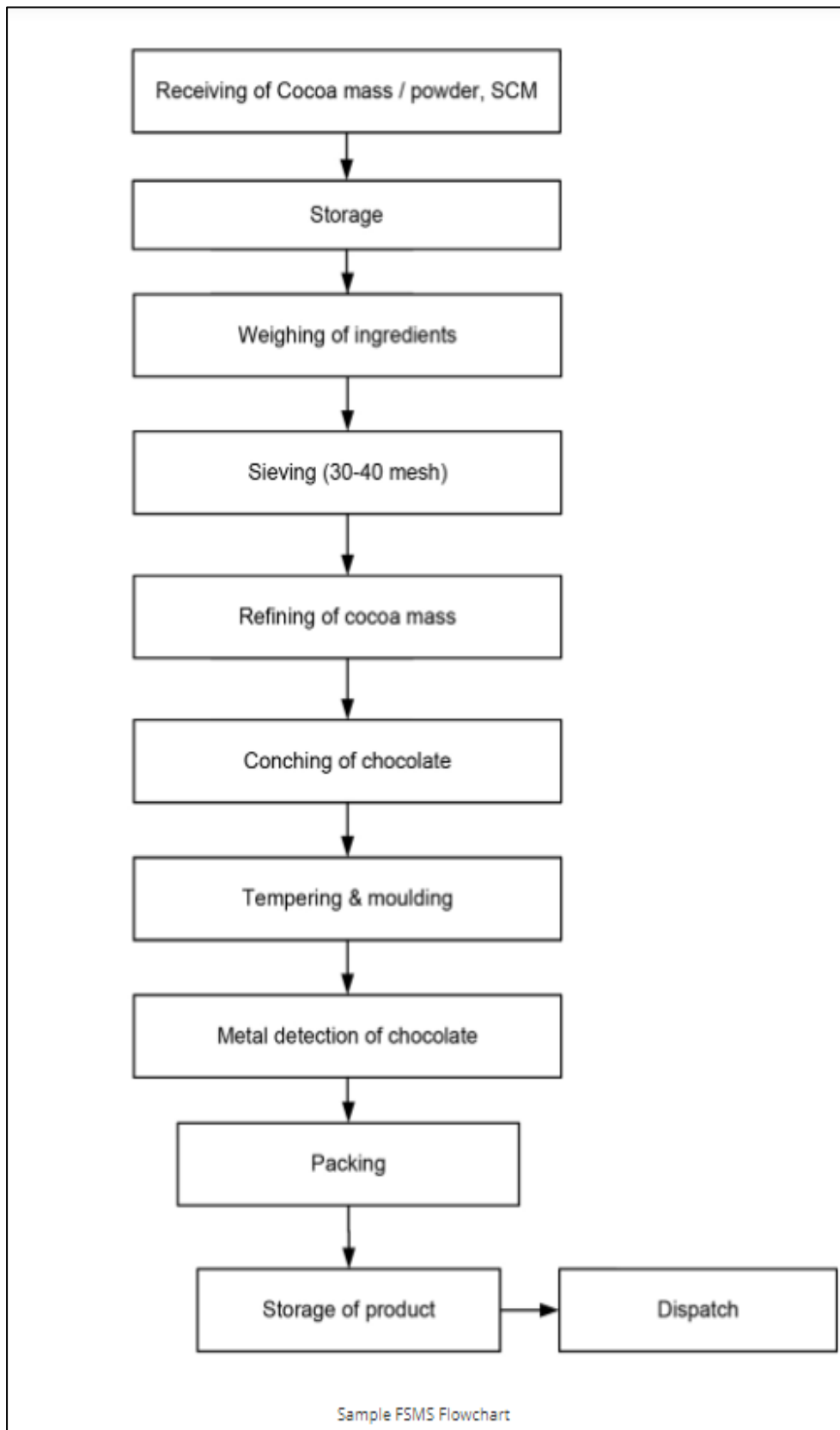
Operational Step	Hazard	Control Measure	Critical Limit	Monitoring Method	Corrective Action	Responsibility	Record
Receiving	Microbial Growth in RTS received	Receive below the danger zone	4°C +/- 2 degrees	Incoming Receipt Check	Reject Lot	Purchase Manager	Incoming Material Receipt Log
Storage	Microbial Growth in Food to be consumed raw	Store below the danger zone	Fish 4°C +/- 2 degrees for 7 days	Daily Monitoring of Freezer Temperature	Inform Chef, and re adjust freezer temperature	Sanitation Officer	Refrigerator, Freezer Temp Log
Preparation	Microbial Contamination through food contact surface, and handlers	<ul style="list-style-type: none"> <li>Restrict ill employees;</li> <li>Control bare hand contact</li> <li>Separate raw from ready to eat food</li> </ul>	Nil	Thrice a day checks	Inform Chef, reject lot if contamination suspected	Sanitation Officer	Food Area Checklist
			Nil				
			Always				
Preparation	Microbial Growth during Process 1 Food preparation	Work at 4°C or below	4°C +/- 2 or below and within 2 hours	Chef to control Time and temperature	reject lot	Chef	Raw Foods Preparation Log
Cooking	Bacterial, Parasitic, Viral Survival during Cooking	Cook to Product Internal Temperature, Time	Product core temperature 75°C +/- 2 degrees for 60 secs	Chef to control Time and temperature	reheat till requirements satisfied	Chef	Cooked Food Preparation Log
Cooling	Microbial Growth during Cooling of foods, Process 3	Quick chilling to below danger zone	Cool food from 75°C to 5°C within 2 hours	Chef to control Time and temperature	reject lot	Chef	Raw Foods Preparation Log
Reheating	Microbial Growth during Re heating of foods, Process 3	Bring and hold to Safe zone	Reheat to 75°C for 60 sec	Chef to control Time and temperature	reject lot	Chef	Cooked Food Preparation Log
Holding	Microbial Growth during Holding of foods, Process 1	Hold below danger zone	5°C or below and use within 4 hours	Chef to control Time and temperature	reject lot	Chef	Raw Foods Preparation Log
Holding	Microbial Growth during Holding of foods, Process 2, 3	Hold above danger zone	75°C or above and use within 4 hours	Chef to control Time and temperature	reject lot	Chef	Cooked Food Preparation Log

### FSMS flowchart

A FSMS flowchart is a flowchart representation of the process undertaken by the food business. It is important to have a FSMS flowchart to have a good understanding of the process and flow of the activities.

## Sample flowchart for chocolate manufacturing

Manufacturing of chocolate



## Inspection Checklist

The last and final part of a FSMS plan is the FSMS inspection checklist. Using the FSMS inspection checklist, an authorized person periodically checks the points of inspections and marks his/her observation. Based on the FSMS plan, correction actions are taken based on the observation present in the checklist.

Please mark as Status as appropriate		
<input checked="" type="checkbox"/>	In order	<input type="checkbox"/> X noting order
* If Status is marked not in order, please provide target completion date		
Sr. No.	Particular/Point of Inspection	Observation
1	Whether daily records of quantity of fruits, vegetables, fish, milk etc. is maintained or not.	
2	Whether periodic cleaning and disinfection of store is carried out and record thereof is maintained.	
3	Whether staff engaged in handling of food stuff is having basic knowledge of importance of health, hygiene and contamination of food etc.	
4	Facility for keeping perishable food products is available.	
5	Facility for frozen food products is maintained.	
6	Containers used for storage are made of non-toxic material.	
7	Exhaust system in food store room in good working condition, and kept clean.	
8	No person handling food is suffering from any infection or contagious disease.	
9	The working area is well ventilated and well lit	
10	Facility for keeping the food items covered.	
11	Proper pest-proof measures taken to prevent infestation of pests.	
12	Adequate facilities for toilets, hand wash and footbath, with provision for detergent/bactericidal soap etc.	

Sample FSMS Checklist

## FSMS affidavit

While obtaining a FSSAI License, food business operators were required to submit a FSMS Affidavit in Non-Judicial Stamp Paper. The FSMS affidavit stated that the food business has in place a food safety plan to ensure the safety and standards of food and undertakes to put in place a FSMS as per the food safety Act. However, in the interest of improving ease of

doing business, the FSMS affidavit has been replaced by an FSSAI Undertaking by way of self-declaration on plain paper. Hence, presently the FSSAI accepts only undertaking by way of self-declaration.

### **References**

[FSSAI- food safety management systems - YouTube](#)

## **Practical No. 15**

### **Preparation of plans for implementation of FSMS – HACCP, ISO 22000 (Part 2)**

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#### **Objective**

Preparation of plans for implementation of FSMS – HACCP, ISO 22000

#### **A) FSMS HACCP plan procedure**

The FSMS HACCP Plan procedure delineates the steps needed to develop, implement, and maintain each of the company's HACCP plans. The procedure also helps the company develop HACCP plans that meet statutory, regulatory, and customer requirements. The procedure pertains to all processes that directly and indirectly affect the safety of the company's food products.

#### **FSMS HACCP plan procedure activities**

1. Developing a HACCP plan
2. Implementing a HACCP plan
3. HACCP plan review
4. HACCP plan revision

#### **FSMS HACCP plan worksheet template**

Top Management should appoint a HACCP Team, consisting of persons having knowledge and expertise to develop a FSMS HACCP plan worksheet template and including diverse disciplines (including but not limited to production, inspection, sanitation, microbiology, and engineering). The HACCP Team should develop a HACCP plan (using the HACCP Plan Worksheet as a guide) for each product process, based on the seven HACCP principles:

- *Principle 1* – List food safety hazards and possible control measures associated with each step in the flow diagram(s) in columns 2 and 3 of FS1080-1 HACCP plan worksheet.
- *Principle 2* – For each hazard identified in the hazard analysis, list critical control points – measures in place to prevent, eliminate, or reduce the hazard to an acceptable level (e.g., cooking, chilling, formulation control) – in column 1 of the plan worksheet.
- *Principle 3* – Establish critical limits for each CCP identified and record in column 4. A “critical limit” is what separates acceptable products from unacceptable ones.
- *Principle 4* – Establish monitoring procedures for each CCP, to determine if the operation is within critical limits at that point.
- *Principle 5* – Establish planned corrections and corrective actions (deviation handling) for each CCP and record these in column 10 of FS1080-1.



- *Principle 6* – Establish verification steps for each monitoring activity and record this information in column 11 of the HACCP Plan Worksheet.
- *Principle 7* – Establish required records and recordkeeping/documentation procedures and record them in columns 9 and 10 of the worksheet.

## HAZARD ANALYSIS AND CRITICAL CONTROL POINTS PLAN

FORMAT NO.:

PRODUCT ID	PRODUCT DESCRIPTION	MATERIAL STORAGE LOCATION / AREA / ROOM								
METHOD OF STORAGE MATERIAL										
HACCP NO	PROCESS	HAZARD	CONTROL MEASURE	CRITICAL LIMITS	OBJECT MONITORING	METHOD OF MONITORING	MONITORING FREQUENCY	MONITORING BY	ACTIONS	AUDITING
SAFETY OFFICER SIGNATURE & DATE : _____					M.R. SIGNATURE & DATE : _____					

### B) FSMS - ISO plan procedure

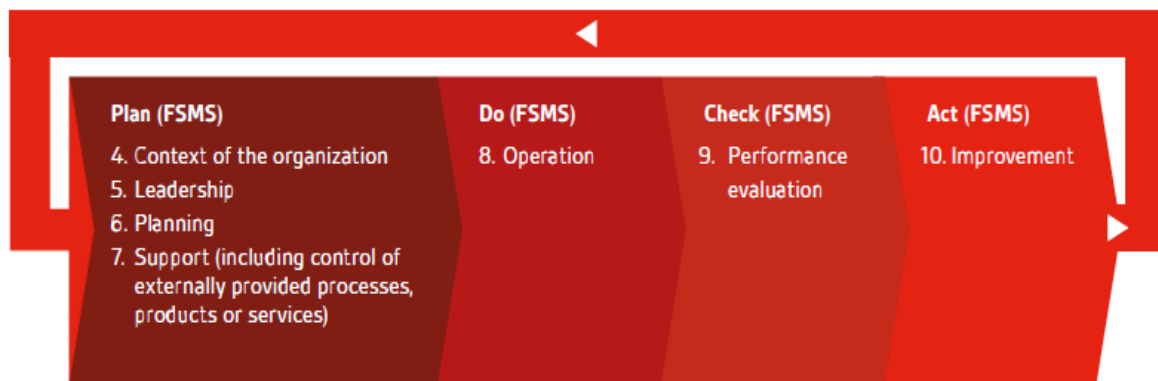
Food safety hazards can occur at any stage in the food chain making adequate control throughout the food chain essential. By combining PDCA (Plan-Do-Check-Act cycle) to manage business risk with HACCP to identify, prevent and control food safety hazards, ISO 22000 helps organizations to reduce exposure to risk and improve safety. Certification to the standard provides additional benefits to organizations throughout the food chain:

1. Improved control over food safety activities
2. Customer, statutory and regulatory compliance
3. Facilitated market growth
4. Increased customer, stakeholder and consumer confidence in products
5. Improved risk management
6. Integration with other ISO management systems

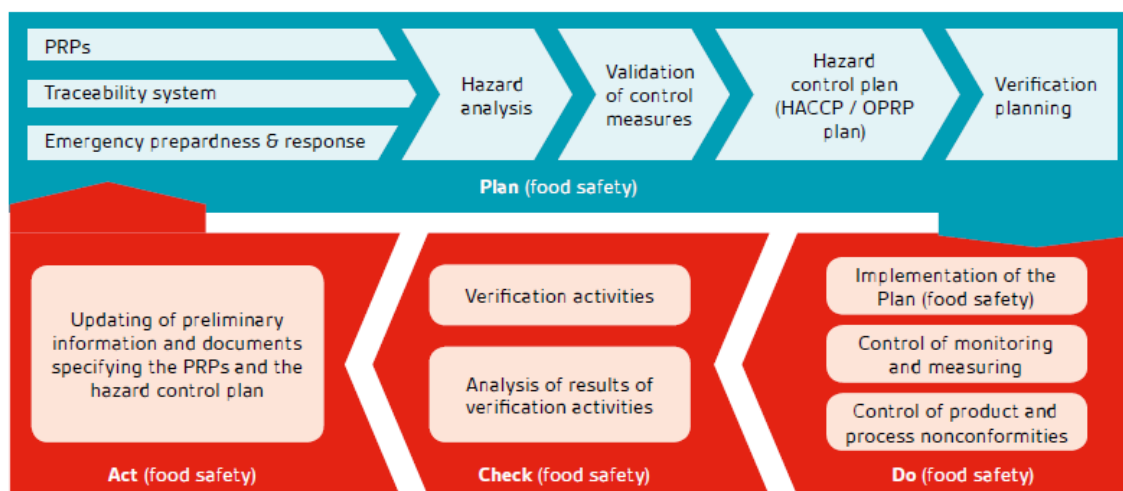
An ISO 22000 food safety management system (FSMS) can be implemented in small, medium and large-sized food organizations from all aspects of the food chain (food and ingredient manufacturers, retailers, wholesalers, agricultural producers, transport, logistics and storage providers, packers, equipment and packaging manufacturers, caterers etc.).

This combines both organizational and operational risk management into one management system. Organizationally, this approach provides the opportunity to consider all the different things that might impact your company, both good and bad. This allows you to prioritize the objectives of your FSMS so that it's implemented in a way that can accommodate the effects of these risks should they occur. On the operational side, risk-based thinking and implementation is based on the principles of HACCP traditionally associated with food safety management.

### Organization planning and Control of ISO 22000



### Operational planning and control of ISO 22000



### References

1. [Planning and Implementation of HACCP System in Sea food Processing by Dr. Abhishek Thakur, CoF - YouTube](#)
2. [HACCP & ISO 22000: Food Safety Management System - YouTube](#)