PRACTICAL 12 SAMPLING AND ANALYSIS OF MICROBIAL LOAD ON FOOD CONTACT SURFACES

Structure

12.1 INTRODUCTION

Unit 10 in the theory course (MFN-003) focussed on sanitation and hygiene in food service establishments. Going through this practical you would have realized, that sanitation is not just cleanliness, it is much more than that. A food or equipment can be free of visible dirt and still be carrying microorganisms or chemicals that can cause food borne disease or spoilage of food. So in this practical, we will focus on sampling and analysis of microbial load on food contact surfaces. The methods of swabbing for determining total microbial counts in selected food processing environments and equipments will be highlighted.

Objectives

After studying this practical and undertaking the exercises given herewith, you will be able to:

- discuss the importance of sampling the food processing environment,
- describe the different techniques employed for counting the microbial load on food contact surfaces and air of processing environment, and
- determine the total microbial count in selected food processing environments and equipments using the method of swabbing.

12.2 NEED FOR MICROBIOLOGICAL ANALYSIS OF ENVIRONMENT SAMPLES

Once the food is harvested, it passes through various operating steps before reaching the consumer. Do you recall reading about these steps in the Principles of Food Science Course, Unit 13? These steps include –

- harvesting and transportation to the food processing facilities,
- separation of desirable part of the food from raw material like removing of skin from the food or potatoes, etc.
- cleaning of food items themselves and also of equipment and surfaces, and
- processing and packaging of food items.

Contamination of food items can take place during these steps depending upon the hygienic and sanitary practices applied. Processing environment has an important contributory role in the addition of food microbiota. Heavily contaminated processing environment generally results in the poor quality food products and also pose a threat with food borne pathogens. To avoid these problems, therefore, it is essential to sample the processing environment, i.e., *floors, drains, equipments and utensils, contact surfaces, air, storage sites, personnel hands and clothing* etc. for microbial quality. Analysis of environment samples allows investigators to evaluate secondary contamination sources in the processing environment. Results of analysis also helps the processor to know the efficacy of cleaning and sanitization procedure, to trace the source of microbes in the processing facility and also to define the critical control points in the food processing operation. This may also helps in establishing the efficient HACCP (Hazard Analysis Critical Control Point) Plan. Remember, we learnt about HACCP in the theory Booklet in Unit 13.

12.3 ENVIRONMENTAL SAMPLING: METHODS AND TECHNIQUES

Environmental sampling can be done for total microbial load or for some specific pathogens or spoilage organisms. The medium used is chosen accordingly. Different media like Brain Heart Infusion (BHI) broth, Plate-count agar with or without antibiotics, *Pseudomonas* isolation agar etc. can be employed, depending upon the purpose. Criteria for acceptable microbiological results from food contact surfaces depend on the food being processed in the facility. On the basis of nature of the site, degree of contamination and microbiological information sought, different sampling techniques can be employed for processing surfaces and air sampling. These sampling techniques are discussed next.

First let us learn about the methods we can employ for processing surface samples.

12.3.1 Surface Sampling

Food contact surfaces (e.g. storage tank, packaging material, ripening room, utensils, equipments, refrigerators etc.) which directly or indirectly (walls, floors, enclosures, workers' garments) contact the food may result in its contamination. Therefore, maintenance of hygienic state of these surfaces is must. Microbiological analysis of these surfaces can be carried out by following methods:

a) *Swab Method*

Swab method is the oldest and widely used method in food and dairy industry and was developed by *W.A. Manheimer* and *T. Ybanez* in 1917. A sterile cotton swab is used which is made up of wound cotton head on a 12-15 cm long wooden stick. It is moistened with a sterile rinse solution and used for rubbing the surface to be examined. Figure 12.1 illustrates the swab method.

Figure 12.1: Swab method for analysis of contact surfaces

Swabbing is the most commonly used method to sample food contact surfaces. It is generally used for surfaces having high contaminant counts. Swab samples can be taken from any surface of the food processing facility like chillers, coolers, freezers, utensils, holding tanks, packaging machines, meat slicers, floor, walls, drains, working table, interior of a pipe or equipment piece etc. and analyzed by plating technique for total plate count. The exposed swab is kept back in the test tube containing a suitable diluent and kept in a refrigerator till used for plating. The organisms in the diluent are counted by SPC or any other method used for enumeration, as discussed earlier. Calcium alginate swabs can also be used in place of cotton swabs.

Sterile 0.85% saline can be used to rinse the swab. It is used to hold microbial cell temporarily in stasis so that no change in number occur between the sampling and plating events.

The advantages and disadvantages of swab method are tabulated herewith:

Advantages	Disadvantage
suitable for flexible, uneven and heavily contaminated surfaces.	removal of organisms depend on the texture of the surface and nature and types of the flora.
rapid, simple and inexpensive method to assess microbiological flora of food surfaces and utensils.	

(b) *Contact Plate (RODAC – Replicate Organism Direct Agar Contact) Method*

Contact plates are special petri plates containing suitable agar media with a raised agar surface above the rim of the plate. For sampling, the plate is inverted and agar is pressed against the sampled surface. The plate is rolled while applying the pressure and is then incubated after replacing the cover at appropriate temperature and for appropriate time according to the purpose (Refer to Figure 12.2). Margin figure shows the growth on the contact plate. The colonies on each plate are counted. When surfaces to be examined are cleaned with detergents, neutralizer (lecithin, Twin 80 etc.) have to be incorporated in the medium. Figure 12.2 illustrates the contact plate method.

Figure 12.2 : Contact plate method

However, there is a disadvantage of using this method, which is highlighted next.

Disadvantage:

- These plates are suitable for sampling the surfaces having low contamination, like pre-cleaned and sanitized surfaces. This is because no dilution is made and if contamination is heavy, it would result in the overcrowding of the plate and make the interpretation difficult. Swabs are better when the contamination level is more.

(c) *Sponge Method*

In the sponge method, sterilized sponge with 45 x 5 cm contact surface and free from antimicrobial agent is used. Aseptically, it is moistened with 10 ml rinse solution and rubbed against the surface to be sampled. It is kept in a sterile plastic bag and analyzed by transferring to a suitable enrichment media for detecting the pathogen or by plating after making the dilution for quantifying the microbiota.

The advantage of this method is highlighted herewith:

Advantage:

- The method is suitable when the surface to be swabbed is large or low incidence of microbes in the environment.

(d) *Agar Syringe/Agar Sausage Method*

Agar syringe method involves 100 ml syringe, which is filled with agar. A layer of agar is pushed beyond the end of the barrel by means of a plunger and pressed against the surface to be examined. The exposed agar layer is cut, incubated and examined for colony count. In agar sausage method, plastic tubing is used instead of a modified syringe.

The limitations of this method are highlighted herewith:

Limitations:

(i) It is applicable to surfaces with low contaminants.

(ii) Problem of spreading colonies may occur.

(iii) Colony counts may be low because clumps or chains of cells will yield single colonies.

(e) *Direct Surface Agar Plating Method*

In the direct surface agar plating method, molten agar is placed on to the surface to be assessed. Upon hardening, it is incubated after keeping in the petri plate and analyzed. It is not used frequently for routine analysis of food plant surfaces.

(f) *Sticky Films*

Sticky films or tape are pressed against the surface to be assessed. Exposed film/tape is then pressed on the agar plate and analyzed after incubation.

(g) *Swab or Agar Slant Method*

The method involves sampling with cotton swabs that are transferred directly to slants.

Other methods include use of ultrasonic devices (used to release microorganisms from surface into diluent) and spray gun (spraying of washing solution on to surface followed by its plating).

The discussion presented above focused on the surface sampling methods. Next, we shall review the air sampling methods.

12.3.2 Air Sampling

Microorganisms may be present in air of processing facility and have an impact on the quality and safety of foods processed and handled there. To avoid food contamination during processing and post processing, it is important to measure the microbial load in air. Different methods can be used for air sampling. Most common ones are sedimentation, impingement and filtration. Let us get to know about these methods.

(i) *Sedimentation* — It is the simplest method, which involves exposing agar plates in air for specified time at a location to be sampled. Microorganisms will sediment on the plate by gravitational force and forms colonies on incubation. Results are, however, influenced by the size of particles and by the speed and direction of air flow. Figure 12.3 illustrates the air exposure method. The margin figure shown growth on agar medium after air exposure.

Figure 12.3: Air exposure method for analysis

(ii) *Air Samplers*, e.g., all glass impinger and the Andersen sieve samplers may be used. Volume of the air sampled is known. In all glass impinger, air is pulled through a capillary orifice by vacuum and impinges on the diluent placed in the impinger. Microorganisms are trapped in the liquid and plated for SPC. The method does not work well when numbers of organisms are too small.

In the Andersen sampler, air is drawn through one or more sieves by vacuum and the microorganisms are trapped on the surface of agar plate. After incubation of plates, enumeration of microbial load is done.

(iii) *Filtration* — Air stream may also be filtered through a micro-filter. Microorganisms are released from the filter using a suitable diluent and the microbial load is counted.

With the knowledge about sampling methods, let us get started with the exercises given in this practical. There are 2 exercises in this practical.

EXERCISE 1

PREPARATION OF BACTERIAL SMEAR

Date : .....

Aim : To assess the sanitary quality of different contact surfaces in food environment by swabbing method.

Requirements

Samples of Surfaces : Refrigerator, cutting board, fruit bin, table top, utensils, glassware, equipments etc.

Reagents : 5 ml sterile 0.85% saline in screw capped test tubes, PCA plates, PCA antibiotic plates.

Equipments & Glassware : Sterile cotton, antibiotic plates, swabs, pipettes, bunsen burner.

Composition of PCA plates (Plates count agar)

Glucose	1 gm
Tryptone	5 gm
Yeast Extract	2.5 gm
Agar	15 gm
Distilled Water	1000.0 ml

pH 7.0

Composition of PCA plate (Plate count agar)*

Theory/Principle:

Look up section 12.2 for theory on swabbing method. Based on your understanding about the method, about the swab and the contact surface from which sampling is done. Write the principle of swabbing method in the space provided herewith.

Procedure:

Now carry out the exercise using the steps enumerated herewith:

1. Collect swab samples from the suitable sampling sites e.g. refrigerator shelves, fruit bin or door gasket, cutting board, kitchen counter top, utensils etc.
2. For swabbing, moisten a swab head in the broth and press out the excess solution against the interior wall of the test tube.
3. Rub the swab head over a portion of the sampling site gently and rinse it in broth. Press out excess solution.
4. Rub the same swab on another portion of same sampling site and rinse in the broth. Repeat the process for remaining portions of the sampling site.

5. Break off the swab and put only swab head in the screw capped tube.
6. Store samples in the refrigerator prior to use.
7. Mix the sample by rolling in between hands and make dilutions from 10^{-1} through 10^{-5}.
8. For total aerobic plate count, plate 0.1 ml each of the undiluted sample and dilution tubes on to PCA plates and incubate at 37°C for 24 hours.
9. For yeasts and moulds, spread 0.1 ml of undiluted sample and dilution tubes (10^{-1} and 10^{-2}) on PCA – antibiotics plates. Incubate the plates at 28 ± 2°C for 5 days.
10. Count the colonies and calculate CFU/100 cm^2 surface area or CFU per site for total aerobic plate count and yeast and mould counts.

Precautions:

1. Plates used should be prepared 24 hours before use to avoid spreading of colonies due to moisture in plates.
2. Swab samples should be taken aseptically.

Observations and Results:

Record your observations in the format given herewith:

Sample	**Observations**		**Result**
Dilution	**No. of Colonies** **Factor PCA**	**PCA-Antibiotics**	**CFU/cm^2**
			Total Aerobic count **Yeast & Mould count**

Inference/Conclusion:

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Submit the exercise for evaluation.

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Counsellor Signature

EXERCISE 2

ANALYSIS OF AIR OF PROCESSING FACILITY FOR MICROBIAL LOAD

Date : .....

Aim : To analyze the air of processing facility for microbial load.

Requirements

Medium : Nutrient agar and Potato dextrose agar plates.

Equipments & Glassware : Incubator, bunsen burner.

Theory/Principle:

Microbiological analysis of air can be performed by sedimentation, impingement and filtration techniques. Microbes in the processing and storage area becomes airborne due to different activities in the processing facilities, like, dry ingredient handling, water spraying and vigorous air movements. Improper filtration of air entering the processing area or recycling air from the area of raw product to finished product area can result in food contamination. Poor air quality in packaging area can lead to post process contamination. This all makes it necessary to determine the microbial load in air and take necessary actions to reduce it.

Procedure:

Now conduct the experiment following the steps enumerated herewith:

1. Label the Petri plates with nutrient agar and potato dextrose agar.
2. Expose the plates uncovered in air at sampling site for 5 minutes.
3. Replace the cover and incubate nutrient agar plates at 37^{o}C for 24 hours and potato dextrose agar plates for 4-5 days at 28$\pm$ 2^{o}C.
4. Count the colonies and identify them.

Precautions:

1. Plates poured about 24-48 hours before use should be used.
2. Time of exposure in air should be same for all the samples.

Observations and Results:

Record your observations in the format given herewith:

Observations	Result
Number of colonies on nutrient agar	
No. of colonies on potato dextrose agar	

Inference/Conclusion:

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Review Questions

1. Explain the importance of sampling the environment of processing facility.

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2. Discuss the methods used for sampling the contact surfaces.

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3. Name the ways by which air of the processing unit can be sampled. What are the limitations of sedimentation method?

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Submit the exercise for evaluation.

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Counsellor Signature