**Microbial Food analysis**

: **Reasons for microbial food analysis.1**

 To meet certain set standards-

· To estimate the shelf-life of the product-

· To determine quality of the food-

· -For public health purposes

**: 2.The organisms to look for**

***i) Indicator organism)***

Definition: an indicator organism or group of organisms is one whose numbers in a product reflect the success or failure of "good manufacturing practices". Coliform group of microorganisms and *Escherichia coli* are commonly used as indicator organisms

***ii) Index organism***

Definition: an index organism is one whose presence implies the possible occurrence of a similar but pathogenic organism. *E. coli* is used an index organism and its presence indicates possible presence of pathogenic enterobacteriaceae e.g. *Salmonella sp*.

***iii) Food poisoning organisms***

The are two types of food poisoning organisms

· Those which produce toxin in food-

Those which cause the decease by infection

**a)** Those which cause intoxication must grow in food in large numbers and produce enough toxin and when consumed together with food cause intoxication. The most common microorganisms in this category includes, *Clostridium botulinum*, *Staphylococcu* *aureus and toxigenic fungi e.g. Aspergillus flavus*

***b)*** *Infectious microorganisms*

Definition: Organisms whose presence in small numbers in food or water and when consumed can cause infection. In this case the food acts as a vector but not necessarily as a growth medium.Infectious organisms can be transmitted by various ways including man to man and are said to be contagious. Organisms in this group includes; *Vibrio cholerae* O1, *Salmonella typhi*, *Shigella sonnei*, *Bacillus anthracis*…

***iv) Spoilage organisms***

Definition: Spoilage organisms are the organisms whose growth in the food creates undesirable characteristics in that food. Any microorganism which is not intentionally added into food or intentionally allowed to grow in food so as to impart certain qualities in that food is considered a contaminant. Growth of the contaminant in that food will spoil the food making it unfit for human consumption. Some useful microorganisms e.g. lactic acid bacteria are considered as spoilage organisms when in beer, wine and fruit juices but not in milk.

**How to analyze**

***i) Quantitative analysis***

Serial decimal dilution-

Aerobic plate count-

Pour plate count-

Total viable count-

Most Probable Number (MPN) method-

Yeast and Molds count-

Anaerobic bacteria (anaerobic jar)-

***ii) Qualitative analysis***

Presence or absence of a specified microorganism e.g

*Salmonella sp.*-

*E. coli-*

-*V. cholerae* O1

**Food Sampling**

The adequacy and condition of the sample or specimen received for examination are of primary importance. If samples are improperly collected and mishandled or are not representative of the sampled lot, the laboratory results will be meaningless.

The composition and nature of each lot affects the homogeneity and uniformity of the total sample mass. The collector must determine the proper statistical sampling procedure, according to whether the food is solid, semisolid, viscous, or liquid, at the time of sampling whenever possible, submit samples to the laboratory in the original unopened containers.

 -If products are in bulk or in containers too large for submission to the laboratory, transfer representative portions to sterile containers under aseptic conditions.

**-**Sterilize one-piece stainless steel spoons, forceps, spatulas, and scissors in an autoclave or dry-heat oven.

Use of a propane torch or dipping the instrument in alcohol and igniting is dangerous and may be inadequate for sterilizing equipment.

Use containers that are clean, dry, leak-proof, widemouthed-

sterile, and of a size suitable for samples of the product. Containers such as plastic jars that are leak-proof may be hermetically sealed.

- Deliver samples to the laboratory promptly with the original storage conditions maintained as nearly as possible. When collecting liquid samples, take an additional sample as a temperature control.

Check the temperature of the control sample at the time of collection and on receipt at the laboratory.

-Dry or canned foods that are not perishable and are collected at ambient temperatures need not be refrigerated.

-Transport frozen or refrigerated products in approved insulated containers of rigid construction so that they will arrive at the laboratory unchanged.

Collect frozen samples in prechilled containers. Place containers in a freezer long enough to chill them thoroughly. Keep frozen samples solidly frozen at all times.

-Cool refrigerated samples, in ice at 0-4°C and transport them in a sample chest with suitable refrigerant capable of maintaining the sample at 0-4°C until arrival at the laboratory. Do not freeze refrigerated products. Unless otherwise specified, refrigerated samples should not be analyzed more than 36 h after collection.

- Make a record for all samples of the times and dates of collection and of arrival at the laboratory.

**Collection of samples**

1-A sample, consisting of a specified number of sample units (usually five) drawn at random from each lot, shall be taken.

2-Each sample unit shall consist of at least 100 ml or g, unless stipulated in the method.

 . 3-Collect original unopened container wherever possible

4-If the product is in bulk, several sample units can be collected from one container, while ensuring that the total number of sample units are not collected from one container.

. 5-Employ aseptic techniques in collecting the sample units

6-Keep the sample unit refrigerated (0-4̊ C) or frozen, depending on the nature of the product, during transport.

frozen, to thaw during shipment. 7-Do not allow sample units, that are usually

**Handling of Sample**

During storage and transport, the following shall apply:

1- With the exception of shelf stable products, keep the sample units refrigerated (0-4 ̊C). Sample units of frozen products shall be kept frozen.

2-Thaw frozen samples in a refrigerator or under time and temperature conditions which prevent microbial growth or death

.3- Analyze sample units as soon as possible after receipt in the laboratory

**Receipt of samples**

***Sample collection****:* As soon as the sample arrives at the laboratory, the analyst should note its general physical condition. If the sample cannot be analyzed immediately, it should be stored.

***Condition of sampling container:*** Check sampling containers for gross physical defects Carefully inspect plastic bags and bottles for tears, pinholes, and puncture marks. If sample units were collected in plastic bottles, check bottles for fractures and loose lids. If plastic bags were used for sampling, be certain that twist wires did not puncture surrounding bags. Any cross contamination resulting from one or more of above defects would invalidate the sample.

***Labeling and records:***Be certain that each sample is accompanied by a completed copy of the Collection Report and officially sealed with tape bearing the sample number, collecting official's name, and date.

***Storage****:* If possible, examine samples immediately upon receipt. If analysis however, store frozen samples at -20°C until examination. Refrigerate unfrozen perishable samples at 0-4°C not longer than 36 h. Store nonperishable, canned, or low-moisture foods at room temperature until analysis

***Thawing :***Use aseptic technique when handling product: Before handling or analysis of sample, clean immediate and surrounding work areas. In addition, swab immediate work area with commercialgermicidal agent. Preferably, do not thaw frozen samples before analysis. If necessary to tempera frozen sample to obtain an analytical portion, thaw it in the original container or in the container

in which it was received in the laboratory. Whenever possible, avoid transferring the sample to a second container for thawing. Normally, a sample can be thawed at 2-5°C within 18 h. If rapid thawing is desired, thaw the sample at less than 45°C for not more than 15 min. When thawing a sample at elevated temperatures, agitate the sample continuously in thermostatically controlled

water bath

***Mixing:*** Various degrees of non-uniform distribution of microorganisms are to be expected in any food sample. To ensure more even distribution, shake liquid samples thoroughly and, if practical, mix dried samples with sterile spoons or other utensils before withdrawing the analytical unit from a sample of 100 g or greater. Use a 50 g analytical unit of liquid or dry food to determine aerobic

plate count value and most probable number of coliforms. Other analytical unit sizes (e.g., 25 g for Salmonella) may be recommended, depending on specific analysis to be performed.

***Weighing:*** aseptically and accurately (± 0.1 g) .