

Microbiological Criteria and Sampling Guidelines

Microbiological Criteria

A criterion is a measure on which a judgment/decision can be made. A microbiological criterion will stipulate that a type of microorganisms, group of microorganisms or toxin produced by a microorganism must either be not present at all, be present in only a limited number of samples, or be present as less than specified number or amount in given quantity of a food or food ingredient.



Components of a Microbiological Criterion

A statement describing identity of food

A statement of contaminant of concern (microorganisms/group of microorganisms and/or toxin)

Analytical method to be used for detection, enumeration, or quantification of contaminant of concern

The sampling plan

The microbiological limits considered appropriate to the food and commensurate with sampling plan

Types of Microbiological Criteria

Mandatory and advisory criteria

A mandatory limit is one that the food cannot exceed. Food that does not meet criterion must be subjected to some action e.g., it may be rejected by purchaser, destroyed, reprocessed, sold as an inferior grade, or diverted to a use where contaminant is not of concern. Certain mandatory criteria also may result in loss of license to process food when limits are consistently exceeded. Advisory criteria serve as an alert to deficiencies in processing, distribution, storage, or marketing.

Standards

- A microbiological standard is a microbiological criterion that is a part of a law, ordinance, or administrative regulation.
- Failure to comply with it constitutes violation of law, ordinance, or regulation and will be subject to enforcement policy of regulatory agency having jurisdiction.

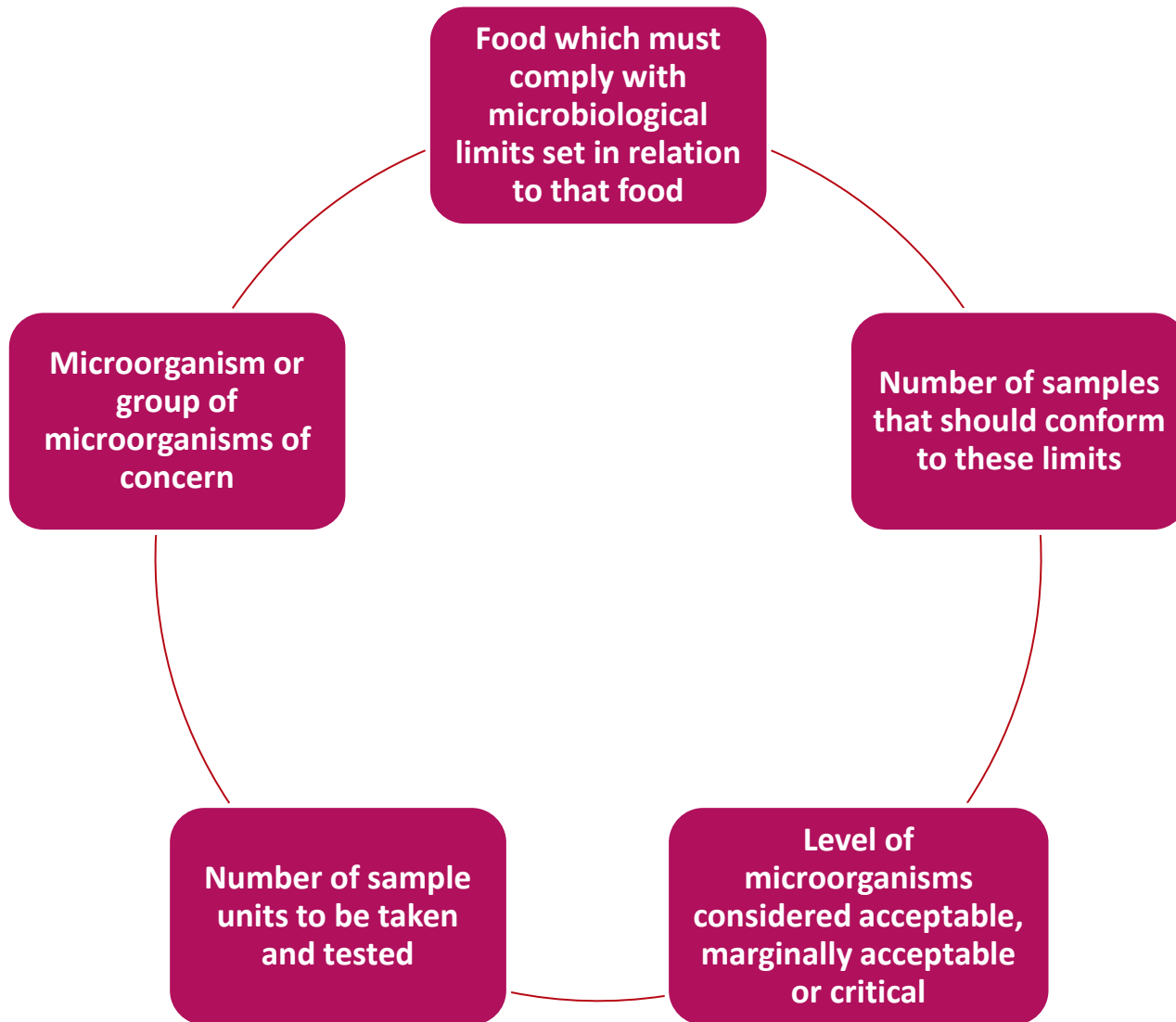
Guidelines

- A microbiological guideline is a criterion that is used by food industry/regulatory agency to monitor a manufacturing process.
- Guidelines function as alert mechanisms to signal whether microbiological conditions prevailing at critical control points or in finished product are within normal range.

Specifications

- A microbiological specification is a microbiological criterion that is used as a purchase requirement whereby conformance with it becomes a condition of purchase between buyer and vendor of a food/ingredient.

Microbiological Criteria for Acceptance or Rejection of Sample Lots



Sampling and Sampling plan

 **Why do we have to set the sampling plan and collect samples?**





 **Control of microbiological hazard in foods**

 **Education and training**

 **Inspection of facilities and operations**

Sampling Plan

Sampling plan: a statement of criteria of acceptance applied to a lot (batch) based on appropriate examinations of a required number of sample units by specific methods

-  Number of units**
-  Sampling procedure**
-  Decision criteria (Microbiological criteria)**
-  two or three class plan**

Sampling Plans and FSSAI Microbiological Standards for Milk and Milk Products

Sampling plan and interpretation

Following terms are used by International Commission on Microbiological Specifications for Foods (ICMSF) are defined and used in development of FSSAI standards for different milk and milk products.

n = number of sample units which must be examined from the batch/lot of food to satisfy the requirements of particular sampling plan.

c = maximum allowable number of defective sample units. This is the number of sample units, which may exceed microbiological limit specified by m .

m = Represents an acceptable level and values above it are marginally acceptable in terms of sampling plan.

M = Microbiological criterion which separates marginally acceptable quality from unsatisfactory/potentially hazardous quality. Values above M are unacceptable in terms of sampling plan and detection of one or more samples exceeding this level would be cause for rejection of lot.

Valid samples

Representative

 **representative of the batch/lot of products**

 **batch?** 

 **lot?**

 **Representative samples depend on ?** 

 **Aseptically collected**

 **Sterile equipment**

 **Stored properly**

How large a sample is required?

Physical limitation

 How many samples can be tested per day

Population

 types of samples









 Heterogeneous

 Homogeneous

 Estimation  lot size

 Number of unit + 1

Steps for choosing sampling plan

-  **Select the measurement**
-  **Define the sampling unit**
-  **Determine: consumer or producer risk to ensure the lot quality**
-  **Obtain an estimate process**
-  **Select a plan that meet the risk and lot quality**
-  **Calculate operating characteristic curve (probability of acceptance)**
-  **Apply the plan on the lot**
-  **Maintain record on process or make change the plan if necessary**

Sampling plan

Variable sampling plan

 The probability distribution is required
(normal distribution)

 Fewer samples are required

Attribute sampling plan

Two class plan

Three class plan

 Do not know the distribution

 Larger samples are required


Two class plan

 **Simple plan used to designate acceptable or unacceptable batch/lot of food**

 **Specification: n, c, m**

 **n = the number of sampling units from the lot**

 **c = the maximum allowance number of sample units that exceed the microbiological criterion**

 **m = the maximum number or level of relevant bacteria (CFU/gm or ml) (std. acceptable criteria)**

Sampling plan in beef

Salmonella	$n=20$	$c=0$	$m=0$
Coliform	$n=5$	$c=2$	$m=10^2$

More stringent plan

 increasing number of unit (n)

 reducing the maximum allowance number (c)

 **Salmonella** **$n=20$** **$c=0$** **$m=0$**

 **All samples must be negative**

 **If one sample is positive, entire lot should be rejected**

 **To make more effective sampling plan**

 **increasing number of unit (n)**


Three class plan


 Used to designate acceptable/marginally acceptable/unacceptable food

 Specification: n, c, m, M

 n = the number of sampling units from the lot

 c = the maximum allowance number of sample units that exceed the microbiological criterion

 m = the maximum number or level of relevant bacteria (CFU/gm or ml) (Threshold value)

 M = the maximum number that use to separate marginally acceptable/unacceptable quality food (Maximum permitted microbial level)

Sampling plan in raw chicken

APC	$n=5$	$c=5$	$m=5 \times 10^5$	$M=10^7$
<i>E. coli</i>	$n=5$	$c=2$	$m=11$	$M=5 \times 10^2$

 **Acceptable:** C samples have M/O level between m and M , other samples have level of M/O less than m

 **Satisfactory:** All samples have M/O level less than M

 **Unacceptable:** If one or more samples have level of M/O more than M

Aerobic plate count (APC)

$$n=5 \quad c=5 \quad m=5 \times 10^5 \quad M=10^7$$

 None of the unit n more than M

 If any sample more than, entire lot is rejected

 unacceptable

 c units can more than m

 acceptable

 marginally acceptable (Satisfactory)





 To make more effective sampling plan

 increase n




 reduce m

Sampling plans and microbiological limit in foods

Poultry product

	<i>n</i>	<i>c</i>	<i>m</i>	<i>M</i>
 APC	5	3	10 ⁴	10 ⁵
 <i>S. aureus</i>	5	1	10 ²	10 ⁴
 <i>E. coli</i>	5	2	10	10 ²
 Salmonella	10	0	0	-

Dried milk

 Mesophilic count	5	2	5x10 ⁴	2x10 ⁵
 Coliform	5	1	10	10 ²
 Salmonella	15	0	0	-

Microbiological criteria and their interpretation

Three categories of microbiological quality have been assigned based on Total plate count, levels of indicator organisms (Coliform count and yeast & mold count) and number /presence of pathogenic bacteria.

- **Satisfactory**= If a maximum of c/n value are between m and M , rest of values observed are $< m$ (means results are within limits of acceptable microbiological quality and no action is required).
- **Unsatisfactory**= If one or more of values observed are $> M$ or more than c/n values are between m and M means results are outside acceptable microbiological limits linked with hygiene indicators (Total plate count, Coliform count and Yeast and mold count) and indicative of poor hygiene/handling practices.
- **Potentially hazardous**= If one or more of values observed are $> M$ or more than c/n values are between m and M (results are outside acceptable microbiological limits linked with *E. coli*, *Salmonella*, *B. cereus*, *C. perfringens*, *L. monocytogenes* and are indicative of serious food safety concern and immediate remedial action should be initiated.

Sample Size, Storage and Transport Standards & Reference Methods of Testing

- 1. Each sample unit taken for submission to laboratory should be of sufficient size to allow appropriate analysis .**
- 2. A minimum sample size of 100 g or 100 ml is usual.**
- 3. Food samples obtained by an authorized officer as a result of a consumer complaint, or as part of an investigation of suspected food poisoning incident, may only be single samples.**
- 4. Food samples provided to testing laboratory should have undergone only minimum change in their microbiological status since point of sampling.**
- 5. Knowledge of aseptic sampling techniques and use of appropriate sampling tools are necessary.**

- 6. It is also important that temperature during transport and storage of food samples is appropriate to minimize microbial growth.**
- 7. Chilled foods should be transported in an insulated container and frozen foods kept frozen.**
- 8. Every effort should be made to minimize period between sampling and analysis.**
- 9. The sampling for microbiological testing as per the sampling plan in standards is to be ensured at manufacturing units as per guidelines given in IS: 11546:1999 (ISO 707:1997).**

S. No	Product	Temperature before and during transport (°C)	Minimum sample size #
1	Pasteurized milk, Cream & Flavored milk	0 to 4	100 ml or g
2	Sterilized & UHT Milk, Cream, Flavored Milk, Evaporated Milk	Ambient, max. 30	100 ml or g
3	Sweetened Condensed Milk (SCM)	0 to 4	100 g
4	Pasteurized Butter	-18 or lower	50 g
5	Dried Products: Milk Powder, Whey, Edible Casein/Ice Cream Mix	Ambient, max. 30	100 g
6	Ice Cream, Frozen Desserts, Milk Lolly, Ice Candy	-18 or lower	100 g
7	Processed Cheese & Cheese Spreads	4 to 8	100 g
8	Other Types Of Cheeses	4 to 8	100 g
9	Yoghurt, Dahi, Chakka & Srikhand	0 to 4	100 g
10	Paneer	0 to 4	100 g
11	Khoa	0 to 4	100 g

Guidelines for sample size, storage & transport temperature for milk & milk products

Microbiological requirements		Reference test procedure	Reference
Total Plate Counts (TPC)		General guidance for the enumeration of microorganisms – Colony Count Technique at 30°C	IS 5402, 2002/ ISO:4833:1991
Coliform Count		General guidance for the enumeration of Coliforms	IS: 5401: PART-1; 2002/ ISO: 4831:1991
<i>E. coli</i>		Detection of bacteria responsible for food poisoning part – isolation, identification and enumeration of <i>E. coli</i>	IS:5887 (PART I) -1976 (Reaffirmed as 1995)
<i>Salmonella</i>		Methods for detection of bacteria responsible for food poisoning	IS:5887:PART-3; 1999/ ISO: 6579; 1993
<i>S. aureus</i>		Methods for detection of bacteria responsible for food poisoning – part - 8 horizontal method for enumeration of coagulase - positive staphylococci (<i>S. aureus</i> and other species) - section 1 technique using Baird – Parker agar medium)	IS:5887 :PART 8; SEC 1 :2002 /ISO 6888- 1:1999
Yeast & Mould Count		Method for Yeast and Mould Count of foodstuff and animal feeds.	IS:5403-1999
Spore Count	Aerobic	Specification for sterilized milk	IS:4238-1967 (Reaffirmed 1999)
	Anaerobic	Methods for detection of bacteria responsible for food poisoning part-IV Isolation and identification of <i>C. welchii</i> , <i>C. botulinum</i> and <i>B. cereus</i> and enumeration of <i>C. welchii</i> , <i>C. botulinum</i> and <i>B. cereus</i>	IS:5887 PART IV; 1976
<i>Listeria monocytogenes</i>		Microbiology of Food and Animal Feeding Stuffs –Horizontal Method for the Detection and Enumeration of <i>Listeria monocytogenes part-1 detection method</i>	IS:14988:PART1; 2001 ISO:11290-1;1996

Lot of food does not comply with standards if number of defective samples units is greater than c, or level of microorganism in a food any one of the sample units exceeds M.