

Microbiological Criteria

Food industry Standards:

- Good Manufacturing Practices (GMPs)
- Written Standard Operating Procedures (SOPs)
- Hazard Analysis Critical Control Points (HACCP)
- Used to assess product quality
- Prediction of shelf life of food.
- Utility of the food for a particular purpose
- Used to assess product safety

Who Establishes Microbial Criteria?

U.S. FDA: Enforcement of Food, Drug and Cosmetics Act
– Adulterated food, food additives, sanitizers, seafood

USDA: Based on several Inspection Acts
– Food Safety And Inspection Service (FSIS)
– Meat, poultry, eggs, processing plants, grain, vegetables

CDC: reporting

EPA: enforcement of water, air, insecticide regulation

State health departments

Natl. Academy of Science

Recommendation of Zero tolerance



History

- 1795: “ An act against Selling Unwholesome Provisions”
- 1798: US Public Health Service (started with milk laws)
- 1900: USDA established
- 1906: Pure Food and drug Act
- 1931: US FDA established
- 1938: Federal Food, Drug and Cosmetic Act
- 1953: Dept of Health, Education, and Welfare
- 1968: FDA, CDC, EHSA HSMHA, NIH all under HEW

Today there are 50 agencies, with FDA as main enforcer and USDA provides Food Safety Inspection Service, along with NMF, OHSA, EPA, and FTC

Types of Criteria

- Standards: Mandatory
 - Law containing limits for pathogens of public health significance
 - Some limits on non-pathogens
- Guidelines: Advisory
 - Standard not available
 - Assess processing efficiency at critical points
 - Monitoring of manufacturing process.
 - Production sector not retail.
- Specification: purchase requirement
 - written description that sets acceptable levels



Establishment of Criteria

- Risk Assessment to determine health hazard
 - Population at risk?
 - Costs/benefits of detection, processing, etc.
 - Potential abuse at consumer level
- Evaluate:
 - Growth potential of pathogen in food
 - Related microflora
 - Effects of processing/storage/distribution on contamination
- Reliability of detection methods
- Establish where criteria should be applied



Criteria Requirements:

- Describes specific food or food ingredient
- Describes detection method
- Defines Sampling Plan
- Sets Microbiological limits
- Organisms or toxins allowable at specified concentrations in specified amount of food

H.A.C.C.P.

= Hazard Analysis of Critical Control Points

- NASA program for safe food
 - Pillsbury Co. (1959)
 - End product testing not possible
 - Preventive quality control
 - Control raw materials, process, distribution, and storage.
- Made public (1971)
- Food industry (1985)
 - "HACCP Principles for Food Production"
 - HACCP widely accepted (1995)
 - Food industry now required to implement HACCP program



Farm to Table concept to include consumers

HACCP: lists points in process where hazards can occur and designs prevention

1. Hazard analysis: Food placed in risk categories (based on product users, food cooked/uncooked, processing)
2. Define CCPs (heating, cooling, pH)
3. Set critical limits: specify time and temperature
4. Monitor CCPs: continuous if possible
5. Define corrective action: recall, product hold
6. Record keeping
7. Verification: sample analysis, on site review
8. Consumer education

Sampling Plans

1. **Defines sampling procedure**
2. **Decision criteria**
 - Statement of acceptance/rejection criteria
 - **Statistically** based on examination of required number of random samples
3. **Types of Plans:**
 - Variables Plan: requires normal distribution in food produced under uniform conditions
 - Attributes Plan: Two class vs. three class plans
(Preferred when no knowledge of processing or past performance record)

Two-Class Sampling Plan

Determines acceptable vs. not acceptable lot

Three components:

1. n= total # of sample units,
2. c=max # of positive samples allowed,
3. m=max allowable concentration for positive

Example:

If n=5, c=2, m=100: For one lot, not accepted if 3 or more samples out of a total of 5 sampled have > 100 fecal coliforms.

(If c=0, this is a zero tolerance standard)

Three Class Sampling Plans

Four components: n, c, m, and M

M=concentration at or above which in any sample is unacceptable and lot will be rejected

- Establishes max concentration in any sample units to determine levels of quality and/or safety.
- Specific quantity separates allowable from not allowable

For example: M=1000, n=5, c=2, m=100 fecal coliforms: not to exceed 1000 in any sample or not to exceed 100 for 2 samples out of 5 total

Microbiological Criteria

Product category	Test parameters	Case	Plan class	per g			
				n	c	m	M
"Roast" beef	<i>Salmonella</i>	12	2	20	0	0	
Pâté	<i>Salmonella</i>	12	2	20	0	0	
Raw chicken	APC	1	3	5	3	5×10^5	10^7
Cooked poultry, frozen, ready to eat	<i>S. aureus</i>	8	3	5	1	10^3	10^4
Cooked poultry, frozen, to be reheated	<i>S. aureus</i>	8	3	5	1	10^3	10^4
	<i>Salmonella</i>	10	2	5	0	0	
Chocolate/confectionery	<i>Salmonella</i>	11	2	10^6	0	0	
Dried milk	APC	2	3	5	2	3×10^4	3×10^5
	Coliforms	5	3	5	1	10	10^2
	<i>Salmonella</i> ^b (normal routine)	10	2	5	0	0	

Stringency

Food borne pathogens are grouped into one of three categories based on the severity of hazard:

1. severe hazards
2. moderate hazards with potentially extensive spread
3. moderate hazards with limited spread

Severity based on the hazard to consumer :

- pathogenic microorganisms
- toxins or toxic metabolites
- quality deterioration to an unacceptable state
- types and numbers of microorganisms

Increased stringency required for severe hazard

- increasing n (total # sample) for set number of c (# positives)

Zero Tolerance

- Mandated for *Salmonella* spp. in ready-to-eat foods by USDA
- *E. coli* O157:H7 in fresh ground beef
- Guideline for Zero Tolerance of *Listeria*
- Monitored by Food Safety Inspection Service (USDA/FSIS)

The Indicator Concept (Fecal Coliform)

- Difficult to test for specific pathogens
- Coliforms as surrogate for fecal contamination
- von Fritsch used *Klebsiellae*
- Escherich (1892) described *Bacillus coli*
- Schardinger suggested *E. coli* as indicator of fecal pollution.
- Eijkman (1904) differentiated "fecal coliforms" from environmental coliforms by growth at 46°C



Indicator Concept:

1. Easily and rapidly detectable
 - Distinguishable from other flora
 - Detectable in all types of foods
2. Reliable association with pathogen/spoilage
 - Growth and death rate parallel with pathogens or spoilage
 - Direct negative correlation with product quality/safety
 - Growth not be affected adversely by other flora
 - Absent in pathogen-free, unspoiled food

Indicators of Food Quality

- DMC (milk)
- Total, Standard or Aerobic Plate Counts (APC)
 - More spoilage organisms = shorter shelf life
 - Used to monitor GMP, compliance with guidelines
 - Surface contamination (aerobes)
- Fecal coliforms
 - Estimate fecal-associated bacteria
 - Indicates fecal contaminatin
- Metabolic products of microorganisms
 - Organoleptic properties indicate degree of decomposition
 - Histamine, lactic acid, putresine etc.
 - Aerobes H₂S in sealed products

Metabolic Products

Metabolites indicate a **potential hazard** rather than on direct test for pathogenic or indicator microorganisms.

- Thermostable DNase in foods indicate *S. aureus* contamination
- UV light on grains detect alfatoxin produced by *Aspergillus*
- Alkaline phosphate: a natural constituent of milk that is inactivated during pasteurization
- Organoleptic evaluation detects metabolites
- Cadaverine and putrecine, ethanol, histamine

Fecal Indicator Organisms

Indicate fecal contamination and human pathogens

- Should occur only in intestine
- Should occur in very high numbers in feces
- Resistance to the external environment stresses
- Easy and reliable detection even at low numbers in sample

History

- von Fritsch: *Klebsiellae* as surrogate for human fecal contamination of water supplies.
- Escherich (1892) described *Bacillus coli*
- Schardinger: *E. coli* as indicator of fecal pollution.
- Eijkman (1904) differentiated "fecal" vs. environmental coliforms by incubating samples at 46°C

Fecal Coliforms and E.coli

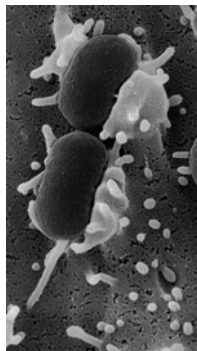
1. **Total Coliform:** gram-negative asporogeneous rods, ferment lactose (acid/gas) in 48 hours at 35°C
2. **Fecal Coliform:** gram-negative asporogeneous rods ferment lactose (acid/gas) in 48 hours at 44-45°C
3. ***E. coli*:** gram-negative asporogeneous rods that ferment lactose (acid/gas) in 48 hours at 44-45°C with dark, metallic colonies on Endo-type agar

Fecal coliforms include four genera of family Enterobacteriaceae: ***Citrobacter*, *Enterobacter*, *Klebsiella*, and *Escherichia***

E. coli most indicative.

Escherichia coli

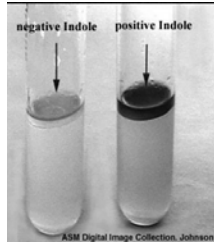
- Warm-blooded animals
- Resist bile salts
- Survives in water and acid
- **Confirmatory tests:**
 - MaConkey's Agar
 - IMViC
 - Rapid test: MUG



Dr. Brett Finlay

IMViC assay:

- Indole +: **Tryptophanase**
Tryptophan cleaved to indole, pyruvic acid, ammonia.
Kovács reagent. (red color)
- Methyl Red +: acid from glucose
- Vi negative: Voges-Proskauer detects acetyl-methyl-carbinol
- Citrate negative: cannot use citrate as sole carbon source



LST-MUG

Glucuronidase assay

- 94% of *E.coli* produce (not O157)
- Enzyme cleaves methylumbelliferone glucose (MUG) to release fluorescent compound 4-methylumbelliferone
- Incorporate in lactose medium (LST) to enumerate coliforms and test for EHEC

Most Probable Number

- Statistical analysis
- Dilutions in lactose enrichment broth
- Replicate tubes (usually 3 or 5) at each dilution
- Positives: Turbidity biochemical analysis (Lactose with gas)

MPN calculated from # positive results at each dilution as determined by statistical table or MPN Calculator

Example: 5 tube, 3 dilutions MPN

MPN Most Probable Number Enumeration:

Water sample

- Total coliform: MPN Lactose Enrichment 35°C



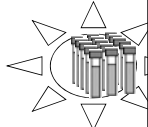
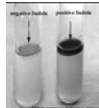
- Fecal Coliform: MPN Lactose Enrichment

44.5°C

- *E. coli*: Chromogenic assay (MacConkey 1ViC)



MUG assay



Most Probable Number

Pos. Tubes			MPN/g
0.1g	0.01g	0.001g	
5	3	0	79

Or 79/g

Pos. Tubes			MPN/g
10g	1.0g	0.1g	
5	3	0	0.79

Or 79/ 100g

Alternative Indicators

Enterococci:

- Both warm- and cold -blooded animals also plants.
- More salt/cold tolerant than coliforms
- *E. faecalis*. and *E. faecium* are also heat resistant.
- Specific applications: Used to identify poor GMP

Coliphages:

- Viruses specific for intestinal indicators
- *E. coli* stain C as host (Need mixed indicators?)
- Useful to detect enteric viruses in water
- Chlorine resistant

Limitations

Indicators

- Pathogens (viruses) may live longer than indicator
- Destroyed by treatment (heat, irradiation and freezing) pathogens resist
- Some pathogens (vibrios) lack fecal association
- Animal coliforms not always indicative of human disease

MPN

- Not a direct count: provides estimate
- Faster-growing, non-coliforms may overgrow
- Injured cells grow more slowly and produce false negatives
- Inhibitors in sample limit growth
- Time consuming and labor intensive assay

Pathogen or Toxin Detection

- Applied when repeatedly implicated in outbreaks
- **Low infective dose:** “zero tolerance”
- Do not correlate with fecal (National Shellfish Sanitation Program)
- More practical with better biotechnology
