WORKSHOP A

Microbiological Sampling and Testing in Food Safety Management

Microbiological Criteria for Foods

Criterion?
A “principle taken as a standard in judging”

Pocket Oxford Dictionary
Definitions

**A standard**
- A criterion specified in law or regulation.
- It is a criterion which the food must meet by law, enforceable by a regulatory agency.
- Most useful when epidemiological evidence identifies a food as a common vehicle of transmission of disease.
- Less useful or questionable on the basis of 'high count, low quality', as safety and quality not always related.

Definitions

**A guideline**
- A criterion used by the food industry or a regulatory agency to monitor the acceptability of a product or process
- Normally advisory, but may be mandatory, in terms of corrective action by the manufacturer
- Liquid egg products...

Definitions

**A specification**
- Commercial criterion.
- Requirement of purchaser to be met by vendor as a condition of acceptance.
- May be mandatory or advisory.
Elements of a criterion
- Food to which criterion applies
- Contaminants of concern
- Analytical methods to be used
- Sampling protocol
- Limits to be applied
- Last two most difficult to decide upon

Use of criteria
- Only establish and implement when there is a need and when criterion shown to be effective and practical.
- Criteria should:
  - accomplish what they are meant to do
  - be technically feasible
  - be administratively feasible

Use of criteria
- Used to assess or achieve one or more of the following:
  - safety of food
  - adherence to manufacturing processes and controls
  - suitability of a food/ingredient for a particular purpose
  - shelf-life of a food
Decisions

- In establishing criteria, must consider:
  - evidence of hazard to health, spoilage or shelf-life
  - microbiology of the raw material
  - effect of processing
  - likelihood and consequence/s of microbial contamination and/or growth during subsequent handling and storage

Decisions - 2

- In establishing criteria, must consider:
  - category of consumer at risk
  - reliability of available methods
  - cost/benefit associated with application of the criterion

Selection for safety

- epidemiological evidence
- susceptibility of food to contamination
- survival of organism in food
- growth of organism in food
- treatment prior to service
- susceptibility of probable consumers
Common criteria

- **Pathogens**
- **Marker organisms**
  - indicator organisms - indicate that faulty practices may have occurred, which adversely affect safety or shelf-life
  - index organisms - suggest the presence of a pathogen or toxin

Markers

- ‘Standard’ plate counts
  - indication of process efficiency
  - gauge likelihood of shelf-life
- **Coliforms**
  - indicate post-process contamination
  - poor sanitation/hygiene
  - *not* indicators of faecal contamination

Markers - 2

- **Enterobacteriaceae**
  - similar role to coliforms
  - considered more sensitive
  - popular in Europe
- **Escherichia coli**
  - indicator of potential human or potential faecal contamination
  - possible pathogen presence
- Others...?
Test Methods

- Use ‘standard’ or reference methods
- Available or developed by:
  - AS (Standards Association of Australia)
  - ISO (Codex Alimentarius)
  - AOAC (FDA-BAM, USA)
  - IDF (dairy applications)
  - ICMSF (reference)
  - APHA (reference)

And over to Dr. Beth!
Elements of a criterion

- Food to which criterion applies
- Contaminants of concern
- Analytical methods to be used
- *Sampling protocol*
- Limits to be applied
- Last two most difficult to decide upon

Sampling problems

- uneven distribution of through food of microorganisms
- not even truly random
- responsible for the inherent errors in methods (sampling and test)
- how are they distributed?

Testing Problems

- Surveillance
  - 201,000 vs. 5,720,000
- Reporting
  - Food company *Listeria* testing
  - Cleaning for clients
- Ready to Eat (RTE) food
  - Refrigeration
  - No cooking
  - *Listeria monocytogenes*
- Brand recognition
- Standardization of Testing
  - Global Harmonization Initiative (GHI)
  - Global Food Safety Initiative (GFSI)
- Marbles in the yard
Organisms in foods?

A, B or C?

Organisms in foods

RANDOM

Organisms in foods

REGULAR
Organisms in foods

Sampling plans

- Attributes sampling
  - Assumes little or no knowledge about specific product
  - Rejection based on number of positive results among the samples tested

Attribute sampling

- Developed by ICMSF
- Each sample classified according to either two or three grades of quality
- Presence/absence = 2-class
- Acceptable
  - Marginally defective = 3-class
- Defective
- Latter for quantitative data
2-class plan

- Defined by three values \((n, m, c)\)
  - \(n\) = number of samples to be tested
  - \(m\) = count above which sample is defective
  - \(c\) = number of samples which may exceed \(m\) before batch is rejected
  - No analytical tolerance

Stringency

- How can we make the plan ‘safer’, or more stringent?
  - Change \(m\) (or \(M\))?
  - Change \(n\)?
  - Change \(c\)?
  - Consider discussion in 8.7 and 8.10, ICMSF

3-class plan

- Defined by four values \((n, m, M, c)\)
  - \(n\) and \(c\) defined as before
  - \(m\) = count at which sample is marginal in quality, and which most test samples should not exceed
  - \(M\) = count above which sample is defective
**m and M**
- **m** defined as a level which is both *acceptable* and *attainable* in the food
  - under GMP (& HACCP)
- **M** defined as a hazardous level of contamination, indicative of, or resulting in:
  - untimely spoilage
  - obvious mishandling, poor hygiene
  - infectious dose of pathogen

**3-class plan**
- acknowledges the uneven distribution of organisms in foods
- most of a given batch acceptable, while part may be only marginally acceptable

**‘Case’ sampling plans**
- Developed by ICMSF
- Depend on type of hazard and likely change, if any, in the degree of hazard
- five risk categories
- three potential changes
- fifteen cases
- more stringent case plans are usually selected for sensitive foods which are (or may be) destined for high-risk populations
**Risks and changes**

- **risks (= hazards)**
  - no health hazard
  - low, indirect
  - moderate, direct, limited spread
  - moderate, direct, potential spread
  - severe, direct

- **changes**
  - none, increased, decreased

---

<table>
<thead>
<tr>
<th>Degree of concern relative to utility and health hazard</th>
<th>Reduce degree of hazard; increase shelf-life</th>
<th>No change in hazard; no change in shelf-life</th>
<th>May increase hazard; reduce shelf-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>No direct health hazard (utility)</td>
<td>Case 1, 3-class</td>
<td>Case 2, 3-class</td>
<td>Case 3, 3-class</td>
</tr>
<tr>
<td>Health hazard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low, indirect (indicator)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate, direct, limited spread (S. aureus)</td>
<td>Case 7, 3-class</td>
<td>Case 8, 3-class</td>
<td>Case 9, 3-class</td>
</tr>
<tr>
<td>Severe, direct</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate, direct, extensive spread (Salmonella)</td>
<td>Case 10, 2-class</td>
<td>Case 11, 2-class</td>
<td>Case 12, 2-class</td>
</tr>
<tr>
<td>Severe, direct</td>
<td>Case 13, 2-class</td>
<td>Case 14, 2-class</td>
<td>Case 15, 2-class</td>
</tr>
</tbody>
</table>

---

**Limits**

- **Establishing limits**
  - survey to determine distribution of desired microbiological parameter
  - what is practically achievable under conditions of good manufacturing practices
Zero tolerance

- ‘Knee-jerk’ reaction to certain risks
- No sampling plan can guarantee compliance
- Plan with $c = 0$ not necessarily most stringent, e.g. $n = 95, c = 1$ is more stringent than $n = 60, c = 0$
- *Salmonella*?
- *Listeria*?

Probabilities
Acceptance/Rejection

- In practice, two errors arise from any sampling plan, referred to as the producer’s and consumer’s risks
- Can vary acceptance/rejection parameters and risks by changing plan stringency
- Refer to 6.6 to 6.8, 7.2, ICMSF7

ICMSF7

- Microorganisms In Foods 7:
- Microbiological Testing In Food Safety Management
Environmental Sampling

Environmental sampling

- **Who's in charge?**
  - EPA
    - Public drinking water
  - ATF
    - Alcoholic beverages
  - USDA
    - Meat, poultry or poultry products
  - FDA
    - Everything else

- **Programs**
  - Sanitation Standard Operating Procedures
  - How will a plant clean?
  - Good Manufacturing Practices
  - What practices must be followed?
  - Hazard Analysis and Critical Control Points
  - Where are the risks?
Environmental sampling

- Why sample the environment?
  - Product safety assurances
  - Shelf-life issues
  - Regulatory mandates
  - Other issues
- Environmental monitoring for food processing plants
  - Establish baseline for each area
  - Continuous Statistical Process Control for environment

Factors to consider:
- Most environmental organisms are stressed
  - Nutrient depletion (starvation)
  - Drying (osmotic stress)
  - Temperature variations
  - pH variations

Target organisms
- Product type
- Plant layout and location
- Air flow
- Employee traffic

What to test
- Product non-contact surfaces
- Product contact surfaces
- Air
- Employees
Environmental sampling

How
- Swabs
- Sponges
- Rinse
- Agar contact method (RODAC or HYcheck)

Swabs and Sponges:
- Surface area
- Swabbing technique
- Diluent
- Swab/Sponge type

Agar contact method (RODAC or HYcheck):
- Agar types
- Agar surface
- Other factors
- DE Neutralizing Agar
Recovery Comparison Research

Whirl-Pak® Sponge
- 25 ml of diluent is added to sponge, extra squeezed out, 100 cm² sampled, sponge returned to bag.

Survival of *L. monocytogenes* vs. *Escherichia coli*

- *E. coli O157:H7*
- *L. monocytogenes*

- Sponge sampling method recovers the greatest (*P* < 0.05) number of bacteria from all surface types.
- Flocked swab was more effective than the swab (*P* > 0.05) in the number of organisms recovered.

**While we are able to recover the organisms by sampling, we are leaving many organisms behind...**
Environmental sampling

- Agar plate technique (for air sampling):
  - time of sampling
  - agar type
  - agar surface area
  - air flow
  - other factors

Automated air samplers:
- time of sampling and agar type

Material for Hand Washing Demonstration
How are sampling and microbiological analysis important to management of food safety?

Issues and risk

- Attribution
  - Where does it come from?
- Source?
- Food to humans (emphasis)
- Origin to food
  - Food animal?
  - Environmental source?
    - Primary production
    - Processing environment
Issues and risk

Sampling
- number of samples and confidence
- nature of foods

Organisms in foods?

Issues and risk

History through processing
- *Bacillus cereus* and rice
- What does a low count mean?

Criteria
- are they applicable? Foodservice?
- food manufacturing versus foodservice
- product testing versus process management
Issues and risk

- Prevalence
  - Rate of contamination
  - Exposure assessment
- Population
  - Level of contamination
  - Refine exposure assessment
- *Salmonella* and *SALMONELLA*
  - Enteritidis versus Sofia

*Salmonella* and *Salmonella*

- *Salmonella Enteritidis*
  - Colonises chickens very well
  - Highly virulent in chickens
  - Illness in humans
  - Major public health concern
- *Salmonella Sofia*
  - Colonises chickens very well
  - No disease in chickens
  - No illness in humans
  - No public health concern

*Salmonella Enteritidis*
What is required to assist food safety management?

- Traditionally, detection
  - presence or absence (or is it?)
- Issues
  - acceleration
    - how fast can we/do we need to go?
    - low numbers, physiological state (injury)
    - background flora, matrix
    - need recovery, amplification (growth)
What is required to assist food safety management?

- Issues, continued
  - Darwinian selection: H₂S, lactose
  - Cultural selection (test methods, harmonisation)
    - Competition, bias
    - Y&M diluent (salt), *Salmonella* and dyes
  - Enumeration
    - Risk assessment needs numbers
    - Numbers – of what?

Lactose-positive *Salmonella*

What is required to assist food safety management?
What test do I use?
- What works for you!
- Rapid/alternative vs traditional
- Plate media (chromogenics)
- PCR

BAX vs Culture
- USDA protocol
  - BAX screening, plate positives
- Previous studies, spiked samples
- Analysis:
  - natural carcass rinses
  - plate ALL samples
Results

- 360 rinses tested
- 213 BAX vs 193 culture-positive
  - 181 TP, 135 TN, 32 ‘FP’, 12 FN
  - 5.6% FN, 15% FP
  - FNs definitive, but FPs??
  - if all false are truly false, equivalent?

Summary

- Search for the ‘truth’ in testing
  - Sampling: how many, subsampling
  - Testing
    - many impacts on testing (target, background)
    - information from testing (quant vs qual)
    - what is there and how much (of each)?
  - Truth informs risk assessment and meaningful food safety plan design

Anyone have questions?