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
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- *Sampling protocol*
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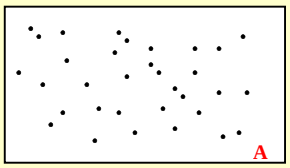
Sampling problems

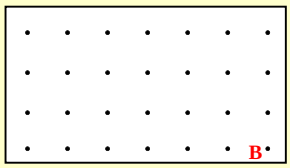
- uneven distribution of through food of microorganisms
- not even truly random
- responsible for the inherent errors in methods (sampling and test)
- how are they distributed?

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

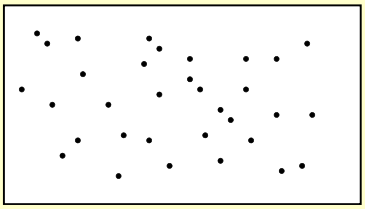
Organisms in foods?





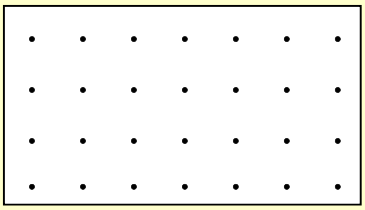
A, B or C ?

Organisms in foods



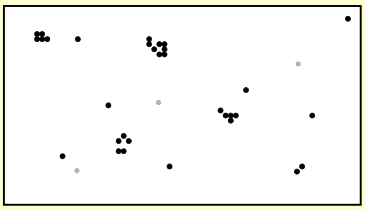
RANDOM

Organisms in foods



REGULAR

Organisms in foods



CONTAGIOUS

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, ICMSF7

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)
  - determined from surveys and product monitoring
- *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

Degree of concern relative to utility and health hazard	Conditions in which food is expected to be handled and consumed after sampling, in the usual course of events		
	Reduce degree of hazard; increase shelf-life	No change in hazard; no change in shelf-life	May increase hazard; reduce shelf-life
No direct health hazard (utility)	Case 1, 3-class $n = 5, c = 3$	Case 2, 3-class $n = 5, c = 2$	Case 3, 3-class $n = 5, c = 1$
Health hazard Low, indirect (indicator)	Case 4, 3-class $n = 5, c = 3$	Case 5, 3-class $n = 5, c = 2$	Case 6, 3-class $n = 5, c = 1$
Moderate, direct, limited spread (*S. aureus*)	Case 7, 3-class $n = 5, c = 2$	Case 8, 3-class $n = 5, c = 1$	Case 9, 3-class $n = 10, c = 1$
Moderate, direct, potential extensive spread (*Salmonella*)	Case 10, 2-class $n = 5, c = 0$	Case 11, 2-class $n = 10, c = 0$	Case 12, 2-class $n = 20, c = 0$
Severe, direct (*C. botulinum*)	Case 13, 2-class $n = 15, c = 0$	Case 14, 2-class $n = 30, c = 0$	Case 15, 2-class $n = 60, c = 0$

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

Environmental sampling

- 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

Environmental sampling

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

Environmental sampling

- 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)

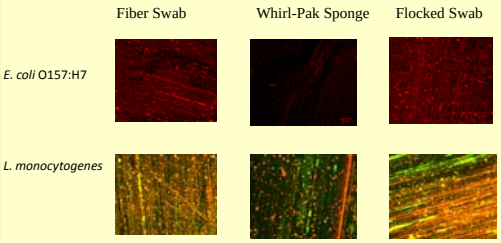
Environmental sampling

- 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

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



-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

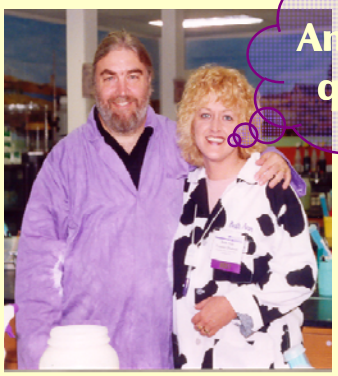
- Agar plate technique (for air sampling):
  - time of sampling
  - agar type
  - agar surface area
  - air flow
  - other factors

Environmental sampling

- Automated air samplers:
  - time of sampling and agar type

SAS Air Sampler

Material for Hand Washing Demonstration



How are *sampling* and *microbiological analysis* important to management of food safety?

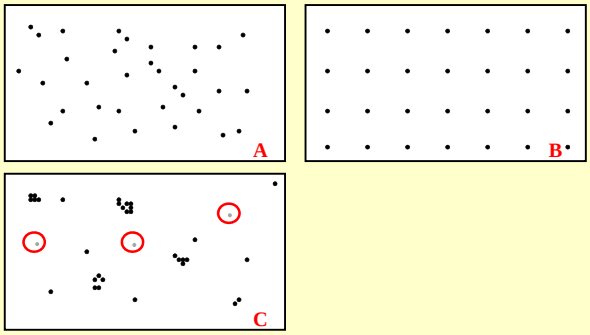
Issues and risk

- 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

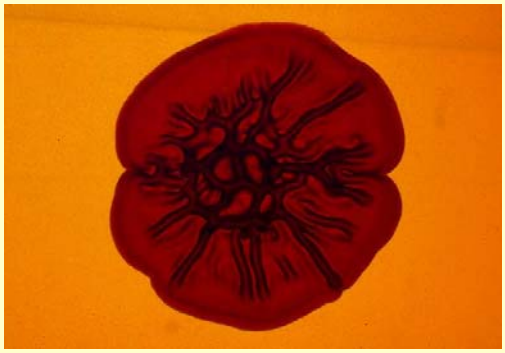
Issues and risk

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

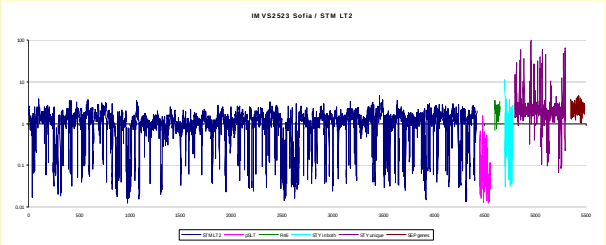
SALMONELLA and *Salmonella*

- *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



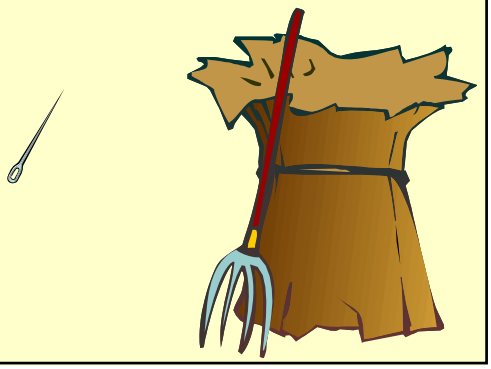
Salmonella Sofia



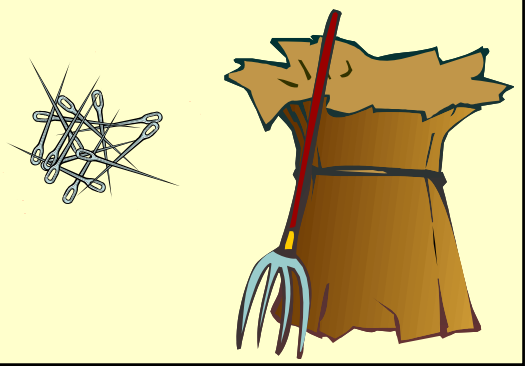
What is required to assist food safety management?

- 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)

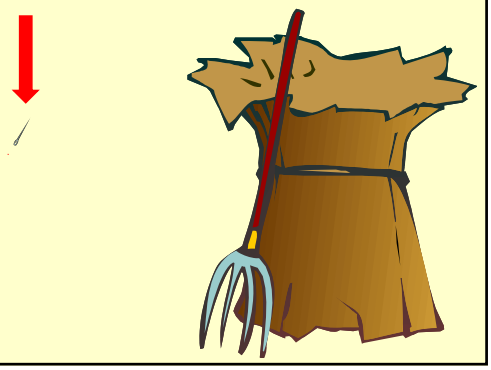
Analyte



Bacteria



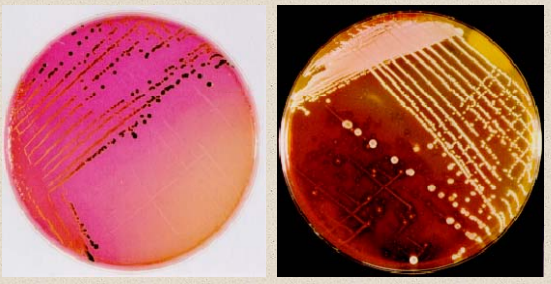
Viruses



What is required to assist food safety management?

- Issues, continued
  - Darwinian selection: H_2S, lactose

Lactose-positive *Salmonella*



What is required to assist food safety management?

- Issues, continued
  - Darwinian selection: H_2S, lactose
  - cultural selection (test methods, harmonisation)
    - competition, bias
    - Y&M diluent (salt), *Salm.* and dyes
  - enumeration
    - risk assessment needs numbers
    - numbers – of what?

What test do I use?

- What works for you!
- Rapid/alternative vs traditional
- Plate media (chromogenics)
- PCR

BAX vs Culture

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