Principles of Statistical Design for Microbiological Sampling

Martin Cole

Data Collection and Utilization in Risk Assessment and Management Decisions

College Park Sept 14th, 2004
Overview

• Definitions and Uses
• Sampling plans
• ICMSF Cases
• Indicators
• To Test or Not
• Relationship to FSOs
• Summary
A microbiological criterion defines the acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area, or lot.
Microbiological Criteria
Components

- Microorganisms and reasons for concern
- Analytical methods to be used
- Sampling plan and size of analytical units
- Microbiological limits
- Numbers of units to be in conformity

Establishment and Application- CAC / GL 21 - 1997
Uses of Microbiological Criteria

- Assess the safety of food
- Verify/validate procedures in HACCP
- Demonstrate adherence to GMP/GHP
- Demonstrate the utility (suitability) of a food or ingredient for a particular purpose
- Establish the keeping quality (shelf-life) of certain perishable foods
- As a regulatory tool to drive industry improvement
- To achieve market access
- **As a Control measure to Achieve a Performance criteria or FSO**
Testing as a Regulatory Tool/Market Access

Eg US FDA FSIS Pathogen reduction/HACCP Reg

• Testing of carcasses by industry for Biotype I E.coli
• Salmonella testing by USDA

Eg ‘Moving Window’ for E.coli

• Variables testing based a limit (M) that cannot be exceeded
• Warning value (m) must not be exceeded more than 3 times (c)
• In moving window 13 tests (n=13)
• Values of m and M plus sampling rate commodity specific
<table>
<thead>
<tr>
<th>Type of testing</th>
<th>Purpose</th>
<th>User</th>
<th>Sample type</th>
<th>Sampling plan</th>
<th>Microbes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptance</td>
<td>Lot inspection</td>
<td>Government</td>
<td>End products</td>
<td>Attributes</td>
<td>Pathogens, indicators</td>
</tr>
<tr>
<td>Acceptance</td>
<td>Verification, lots (batches) of known history</td>
<td>Government industry</td>
<td>End products</td>
<td>Attributes</td>
<td>Pathogens, indicators</td>
</tr>
<tr>
<td>Monitoring, checking</td>
<td>CCPs, lines</td>
<td>Industry</td>
<td>Line samples</td>
<td>Variables, attributes</td>
<td>Indicators</td>
</tr>
<tr>
<td>Environmental sampling</td>
<td>Line, environments</td>
<td>Industry</td>
<td>Residues, dust, water</td>
<td>Targeted, to find source of contamination</td>
<td>Indicators</td>
</tr>
<tr>
<td>Verification</td>
<td>HACCP</td>
<td>Industry</td>
<td>End products</td>
<td>Attributes</td>
<td>Pathogens, indicators</td>
</tr>
<tr>
<td>Surveillance</td>
<td>Compliance</td>
<td>Governments, industry</td>
<td>Products in commerce</td>
<td>Attributes (usually n=1)</td>
<td>Pathogens</td>
</tr>
<tr>
<td>Investigation</td>
<td>Food chain</td>
<td>Governments, industry</td>
<td>All types of samples</td>
<td>Investigational, rarely statistically based</td>
<td>Pathogens</td>
</tr>
</tbody>
</table>
Types of Acceptance Criteria

• **Standard**—a mandatory criterion that is part of a law or ordinance.

• **Guideline**—an advisory criterion issued by a control authority, industry association, or food producer to indicate what might be expected when best practices are applied.

• **Specification**—Part of a purchasing agreement between a buyer and supplier of a food; such criteria may be mandatory or advisory according to use.
Sampling Plans

- Define the probability of detecting a microorganisms or other hazards in a lot
- None can ensure the absence of a particular hazard
- Should be administratively and economically feasible
Types of Microbiological Sampling Plans

Attributes plans:
Qualitative analytical results (presence/absence) or quantitative results that have been grouped (e.g. <10 cfu/g, 10 to 100 cfu/g, >100 cfu/g)

Variables plans:
Non-grouped quantitative analytical results
Require distributional assumptions be made
Two-Class Attributes Sampling Plans

Two-class sampling plans designed to decide on acceptance or rejection of a lot consist of

- **n** – number of sample units to be chosen independently and randomly from the lot
- **m** – a microbiological limit (i.e. in cfu/g); a sample is defined to be positive, if its microbial content exceeds this limit
- **c** – maximum allowable number of sample units yielding a positive result (presence/absence testing) or exceeding the microbiological limit m; for pathogens c is usually set to 0
Two-class sampling plan:
OC Curve for Two-Class Plans

Operation characteristics (OC) or performance for two-class sampling plans:

Probability of lot acceptance calculated for possible proportions defective in lot

Plot of OC curve to visualize
- sampling plan performance
- dependency on n and c
Probability of Acceptance by Proportion Defective

- $n=5$, $c=0$
- $n=10$, $c=0$
- $n=20$, $c=0$
## Two-Class Plans (c=0): Probabilities of Acceptance

<table>
<thead>
<tr>
<th>Composition of Lot</th>
<th>Number of Sample Units Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>% Acceptable</td>
<td>% Defective</td>
</tr>
<tr>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>
Three-Class Attributes Sampling Plans

Three-class sampling plans consist of

- **n** – number of sample units to be chosen independently and randomly from the lot
- **m** – a microbiological limit that separates good quality from marginally acceptable quality
- **M** – a microbiological limit above which sampling results are unacceptable or defective
- **c** – maximum allowable number of sample units yielding results between **m** and **M** (marginally acceptable); the number of sample units allowed to exceed **M** is usually set to 0
Three-class sampling plan:

Proportion marginally acceptable

Proportion defective
**OC Function for Three-Class Plans**

Operation characteristics (OC) or performance for three-class plans:

Probability of lot acceptance depending on two proportions

- marginally acceptable: between $m$ and $M$
- defective: above $M$

OC function plotted as a three-dimensional graph
ICMSF Cases

15 cases which reflect:

- Degree of risk
- Conditions of use
- Intended Population
### Risk categorization matrix

**Food handling conditions**

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Health hazard**

- A
- B
- C

- Increased risk
Categories of hazards

• **A) Moderate:**
  - *S. aureus* toxin
  - *V. parahaemolyticus*
  - *B. cereus*
  - EPEC

• **B) Serious:**
  - *Salmonella* (non typhi)
  - *Shigella*
  - *Listeria monocytogenes*

• **C) Severe:**
  - EHEC (STEC, VTEC)
  - *V. cholerae O1*
  - EPEC for infants
### Plan Stringency (Case) in Relation to Degree of Health Concern and Conditions of Use

<table>
<thead>
<tr>
<th>Type of Hazard</th>
<th>Reduce Degree of Hazard</th>
<th>Cause No Change in Hazard</th>
<th>May Increase Hazard</th>
</tr>
</thead>
<tbody>
<tr>
<td>No direct health hazard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Utility (general contamination)</td>
<td>Case 1</td>
<td>Case 2</td>
<td>Case 3</td>
</tr>
<tr>
<td>Health Hazard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low, indirect (indicator)</td>
<td>Case 4</td>
<td>Case 5</td>
<td>Case 6</td>
</tr>
<tr>
<td>Moderate, direct, limited spread</td>
<td>Case 7</td>
<td>Case 8</td>
<td>Case 9</td>
</tr>
<tr>
<td>Moderate, direct, potentially extensive spread</td>
<td>Case 10</td>
<td>Case 11</td>
<td>Case 12</td>
</tr>
<tr>
<td>Severe, direct</td>
<td>Case 13</td>
<td>Case 14</td>
<td>Case 15</td>
</tr>
</tbody>
</table>
Suggested Sampling Plans for Severe, Direct Health Hazard and Conditions of Use

<table>
<thead>
<tr>
<th>Conditions of Use</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduce Degree of Concern</td>
<td>Case 13</td>
</tr>
<tr>
<td></td>
<td>$n = 15, c = 0$</td>
</tr>
<tr>
<td>Cause No Change No Concern</td>
<td>Case 14</td>
</tr>
<tr>
<td></td>
<td>$n = 30, c = 0$</td>
</tr>
<tr>
<td>May Increase Concern</td>
<td>Case 15</td>
</tr>
<tr>
<td></td>
<td>$n = 60, c = 0$</td>
</tr>
</tbody>
</table>
Choosing a Sampling Plan for a Specific Application

Is the organism in question to be measured by presence or absence tests (+/-) or count or concentration tests?

If +/- tests, a 2-class plan is required

Is it possible to accept the presence of this organism in the food?

If no, then c=0
Choose n to give the desired probability

If yes, then c>0
Choose n and c to give the desired probability

If count or concentration tests a 3-class plan is preferred

Choose the n and c values to give the desired probability
Indicators

- Should indicate something:
  - Contamination
  - Survival
  - Recontamination
  - Growth

- Should be easy to determine
- Should behave as pathogen (growth, survival) when used instead of testing for pathogen
- Cannot be relied upon as "proof" that pathogen of concern is absent
Pathogen not measurable

- Example: < 1 *Salmonella* / 10 kg of dried egg-product

- Enterobacteriaceae are good indicators of
  - adequate pasteurisation and
  - control of recontamination
Indicators are measurable

- Example: Absence of Enterobacteriaceae in 1 g of egg-product

a) case 7 : n = 5, c = 2 * (use : biscuit)
b) case 8 : n = 5, c = 1 (dried egg)
c) case 9 : n = 10, c = 1 (use : tiramisu)

* if adequate heating is assured, no testing is necessary
Salmonella criterion for dried egg products

- case 11 : \( n = 10 \), \( c = 0 \), 25g samples

- lots containing 1 S. per 83 g
- will be rejected with 95% probability

lots containing < 1 S. per 7.7 kg
will be accepted with 95% probability

A producer would need to test 565 end-products to verify that he would meet this criterion
No indicators available

- Example: <1 C. botulinum in 1000 ton of low-acid canned meat product
  - Reliance on
  - Process Criteria (bot cook)
  - and GMP

No Microbiological Criteria
To Test or Not to Test?

Severity of the hazard(s)

New information linking the food to illness

Whether the food is
  Commonly involved in disease
  Primarily destined for a sensitive population
  From a country with endemic disease of importance to food safety

History of consistency and compliance

Distribution of contaminant(s)
  Homogenous, heterogeneous, stratified

Ability to sample
  Sufficient numbers
  Random sampling
## Tightened or Reduced Testing

<table>
<thead>
<tr>
<th>Warranting increased frequency of sampling and/or more stringent sampling plan</th>
<th>Warranting reduced frequency of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The food operation</strong></td>
<td><strong>The operation has an effective system of control based on GHP and HACCP</strong></td>
</tr>
<tr>
<td>An audit indicates the operation does not have an adequate system of controls based on GHP* and HACCP†</td>
<td></td>
</tr>
<tr>
<td>Records indicate a deviation at a CCP‡ in the HACCP plan has occurred</td>
<td></td>
</tr>
<tr>
<td>Information indicates the operation has used an ingredient from a source that has caused problems in other similar operations</td>
<td></td>
</tr>
<tr>
<td>Food from the operation has recently been implicated in illness</td>
<td></td>
</tr>
<tr>
<td><strong>The food</strong></td>
<td><strong>Records indicate the operation is under control</strong></td>
</tr>
<tr>
<td>The composition of the food differs from other foods of the same type and an increase in a hazard is likely to occur under expected conditions of storage and distribution</td>
<td></td>
</tr>
<tr>
<td>Previous tests frequently unsatisfactory</td>
<td></td>
</tr>
<tr>
<td>Routine tests for indicators have revealed a trend toward increased contamination</td>
<td></td>
</tr>
<tr>
<td>The food has a history of being a source of foodborne illness</td>
<td></td>
</tr>
<tr>
<td><strong>Food from the operation has a favorable history of safety</strong></td>
<td></td>
</tr>
<tr>
<td>The composition of the food differs from other foods of the same type and the potential hazards will decrease or be eliminated during expected conditions of storage and distribution</td>
<td></td>
</tr>
<tr>
<td>Previous tests satisfactory</td>
<td></td>
</tr>
<tr>
<td>Routine tests for indicators show continuing control</td>
<td></td>
</tr>
<tr>
<td>Rarely involved in foodborne illness</td>
<td></td>
</tr>
</tbody>
</table>
**Tightened or Reduced Testing**

| The food has been found to be a source of a newly emerging pathogen or new type of an existing pathogen | Not primarily intended for sensitive populations |
| Circumstances suggest involvement of this type of food in a recent outbreak | The parameters necessary for controlling food-borne illness are well known and widely applied |
| A food that traditionally has been for the general public is to be directed toward a sensitive population | |
| New type of food or new formulation with reason to be concerned about a microbiological hazard | |
| Examination results from different laboratories are in conflict and disposition of the food is in question | |

**Country or region of origin**

| Food control systems are in question | The food control systems are known to be equivalent for control of the food or ingredients in question |
| Endemic or epidemic situations exist that increase concern for consumers of the food | Endemic or epidemic situations do not exist that would increase concern for consumers of the food |
Problems

• Application of sampling statistics based on random distribution to situations which contamination is not random

• Use of too few samples to draw valid conclusions
  ➢ Only meaningful if data indicates non-compliance
  ➢ Negative results have little value

• Re-sampling of product that failed initial test

• Many regulatory standards ignore principles of establishment of criteria
  Example: Zero tolerance can be a deterrent to testing
‘Zero tolerance’

Science vs Risk Communication

- No feasible sampling can ensure complete absence of a pathogen

- Plans where c=0 not necessarily most stringent
  eg 5% Defects $ n=95 $ c=1 vs $ n=60 $ c=0

- Sampling assume random distribution through the lot

- Not yet commercially viable to market some foods completely without pathogens
Microbiological Criteria in Relation to FSOs

Alternative approach for quantitative data:

- Distributional assumption for sampling results e.g. log-normal with standard deviation known from previous experience
- Determine proportions acceptable, (marginally acceptable), and defective for possible mean log cfu/g
- Calculate acceptance probabilities and plot against mean log cfu/g
Probability Density

Log cfu/g

$P_a$  $P_d$  $m$
Probability Density

Log cfu/g
Proportion defective, $P_d$

Mean Log cfu/g
Proportion defective, $p_d$

Mean Log cfu/g
Proportion defective, $p_d$
OC curve
n = 10,
c = 2
$OC$ curve
$n = 10, c = 2$
OC curve
n = 10,
c = 2
OC curve

\( n = 10, \ c = 2 \)
$OC$ curve
$n = 10,$
$c = 2$
OC curve
\( n = 10, \ c = 2 \)
$OC$ curve
$n = 10, \ c = 2$
$OC$ curve
$n = 10, c = 2$
OC curve
\( n = 10, \quad c = 2 \)
$OC$ curve
$n = 10, c = 2$

Probability of acceptance

Mean log cfu/g
OC curve
$n = 10, 
 c = 2$
Performance of Sampling Plans

Sampling plan stringency, steepness of OC curve, location of critical lot qualities (95% probability of rejection, 95% probability of acceptance) depend on

- Plan specifications \( n \) and \( c \)
- Microbiological limits \( m \) and \( M \)
- Standard deviation \( s.d. \)
- Difference \( M-m \) in relation to \( s.d. \)
Probability of Acceptance by Mean Log cfu/g (s.d.=0.8)

- n=5, c=0, m=100 cfu/g
- n=10, c=0, m=100 cfu/g
- n=20, c=0, m=100 cfu/g
Probability of Acceptance by Mean Log cfu/g (s.d.=0.8)

n=5, c=0, m=100 cfu/g
n=10, c=0, m=100 cfu/g
n=20, c=0, m=1 cfu/g
Probability of Acceptance by Mean Log cfu/g (s.d.=0.8)

- n=5, c=0, m=1 cfu/25g
- n=10, c=0, m=100 cfu/g
- n=20, c=0, m=1 cfu/g

Probability of Acceptance

Mean Log cfu/g
Probability of Acceptance by Mean Log cfu/g
3-Class Plan: n=5, c=1, m=1000 cfu/g, M=10000 cfu/g

s.d.=0.8
s.d.=0.4
s.d.=0.2
ICMSF Three-Class Plans: Mean CFU/G Rejected With 95% Probability

<table>
<thead>
<tr>
<th>Case</th>
<th>n, c</th>
<th>Mean CFU/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 4</td>
<td>5, 3</td>
<td>5128 cfu/g</td>
</tr>
<tr>
<td>Case 5</td>
<td>5, 2</td>
<td>3311 cfu/g</td>
</tr>
<tr>
<td>Case 6</td>
<td>5, 1</td>
<td>1819 cfu/g</td>
</tr>
<tr>
<td>Case 7</td>
<td>5, 3</td>
<td>3311 cfu/g</td>
</tr>
<tr>
<td>Case 8</td>
<td>5, 1</td>
<td>1819 cfu/g</td>
</tr>
<tr>
<td>Case 9</td>
<td>10, 1</td>
<td>575 cfu/g</td>
</tr>
</tbody>
</table>

With:
m = 1000 cfu/g, M = 10 000 cfu/g,
and standard deviation s.d. = 0.8
ICMSF Two-Class Plans: Mean CFU/G Rejected With 95% Probability

<table>
<thead>
<tr>
<th>Case 10:</th>
<th>Case 11:</th>
<th>Case 12:</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n=5, c=0 )</td>
<td>( n=10, c=0 )</td>
<td>( n=20, c=0 )</td>
</tr>
<tr>
<td>1 cfu / 32g</td>
<td>1 cfu / 83g</td>
<td>1 cfu / 185g</td>
</tr>
<tr>
<td>Case 13:</td>
<td>Case 14:</td>
<td>Case 15:</td>
</tr>
<tr>
<td>( n=15, c=0 )</td>
<td>( n=30, c=0 )</td>
<td>( n=60, c=0 )</td>
</tr>
<tr>
<td>1 cfu / 135g</td>
<td>1 cfu / 278g</td>
<td>1 cfu / 526g</td>
</tr>
</tbody>
</table>

With:
\( m = 0 \) cfu / 25g,
and standard deviation s.d. = 0.8
Sampling Plans and FSOs: Example

Food Safety Objective:

≤100 *Listeria monocytogenes* per g in cold-smoked salmon at time of consumption

Cases and sampling plans:

No inactivation, growth assumed not to occur
case 11: n = 10 samples with c = 0 and m = 100 cfu/g

No inactivation, growth assumed to occur
case 12: n = 20 samples with c = 0 and m = 100 cfu/g

CODEX ALIMENTARIUS COMMISSION, August 2001, CX/FH 01/6 ANNEX 3.2
Performance of Sampling Plans for *Listeria Monocytogenes*

Assumption: standard deviation $s.d. = 0.8$

Case 11: $n = 10$ samples with $c = 0$ and $m = 100$ cfu/g
Mean cfu/g rejected with 95% probability: 30 cfu/g
Mean cfu/g accepted with 95% probability: 1 cfu/g

Case 12: $n = 20$ samples with $c = 0$ and $m = 100$ cfu/g
Mean cfu/g rejected with 95% probability: 13 cfu/g
Mean cfu/g accepted with 95% probability: 0.5 cfu/g
| A     | B          | C          | D          | E          | F          | G          | H          | I          | J          | K          | L          | M          | N          | O          | P          | Q          | R          | S          | T          |
|-------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 1     | OC Curve for proportion defective: | n = 19 c = 0 | | | | | | | | | | | | | | | | | | |
| 2     | | | | | | | | | | | | | | | | | | | | |
| 3     | Probability density function (PDF) for log counts. Distribution mean = 0.80 and sign 1.48 | | | | | | | | | | | | | | | | | | | |
| 4     | OC Curve scaled to relate mean log count to m | | | | | | | | | | | | | | | | | | | |
| 5     | | | | | | | | | | | | | | | | | | | | |
| 6     | | | | | | | | | | | | | | | | | | | | |
| 7     | | | | | | | | | | | | | | | | | | | | |
| 8     | | | | | | | | | | | | | | | | | | | | |
| 9     | | | | | | | | | | | | | | | | | | | | |
| 10    | | | | | | | | | | | | | | | | | | | | |
| 11    | | | | | | | | | | | | | | | | | | | | |
| 12    | | | | | | | | | | | | | | | | | | | | |
| 13    | | | | | | | | | | | | | | | | | | | | |
| 14    | | | | | | | | | | | | | | | | | | | | |
| 15    | | | | | | | | | | | | | | | | | | | | |
| 16    | | | | | | | | | | | | | | | | | | | | |
| 17    | | | | | | | | | | | | | | | | | | | | |
| 18    | Input data | P(accept) | | | | | | | | | | | | | | | | | | |
| 19    | Mean       | 1.48      | 0.05       | | | | | | | | | | | | | | | | | | |
| 20    | sigma      | 0.80      | | | | | | | | | | | | | | | | | | |
| 21    | m          | 2.00      | | | | | | | | | | | | | | | | | | |
| 22    | M          | n/a       | | | | | | | | | | | | | | | | | | |
| 23    | a          | 10        | | | | | | | | | | | | | | | | | | |
| 24    | c          | 0         | | | | | | | | | | | | | | | | | | |
| 25    | | | | | | | | | | | | | | | | | | | | |
| 26    | | | | | | | | | | | | | | | | | | | | |
| 27    | | | | | | | | | | | | | | | | | | | | |
| 28    | | | | | | | | | | | | | | | | | | | | |
| 29    | | | | | | | | | | | | | | | | | | | | |
| 30    | | | | | | | | | | | | | | | | | | | | |
| 31    | | | | | | | | | | | | | | | | | | | | |
FSOs specify a maximum frequency or concentration of a pathogen, toxin or metabolite in a food to provide a desired level of protection, but does not specify how this is obtained
Microbiological criteria could specify the same limit as an FSO or performance criterion (PC) but includes a sampling plan, test method, etc.
Microbiological criteria are only one of the several tools available to achieve FSOs, but because of the limitations of sampling and testing, are not the preferred method of control.
Summary and Conclusions

Limitations of Microbiological Testing

- Often not practical to test a sufficient number of samples
- Non-random sampling may cause incorrect conclusions to be drawn
- Identifies outcomes, not causes or controls
- No feasible sampling plan can ensure absence of a pathogen
Summary and Conclusions

Uses of Microbiological Testing

- Establish baseline data
- Control ingredients
- Verify control of HACCP/GHP system(s)
- Identify highly contaminated lots
- Assessing control of the environment
- Verify compliance of PC and FSO (within limits of sampling and testing)