Overview of Statistical Sampling: Limitations and Use to Determine Process Control and Lot Disposition

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Why Am I Here?

- § 117.165 Verification of implementation and effectiveness.
  - (a) *Verification activities.* You must verify that the preventive controls are consistently implemented and are effectively and significantly minimizing or preventing the hazards. To do so you must conduct activities that include the following, as appropriate to the facility, the food, and the nature of the preventive control and its role in the facility's food safety system:

Why Am I Here?

- § 117.165 (a)(2). Product testing, for a pathogen (or appropriate indicator organism) or other hazard;
- § 117.165 (a)(3). Environmental monitoring, for an environmental pathogen or for an appropriate indicator organism, if contamination of a ready-to-eat food with an environmental pathogen is a hazard requiring a preventive control, by collecting and testing environmental samples

What Does the Regulation Tell Us?

- FDA achieved regulatory flexibility by requiring the food manufacturer to decide the appropriate microbiological testing regime that will be implemented in their facility
- With increased flexibility comes increased responsibility

What Does It Imply?

- Food manufacturers need expertise in the principles, interpretations, and limitations of microbiological testing for verification
  - If not, they will have to rely on "safe harbor" approaches and not likely to reap the benefits of the data they are collecting
  - My goal is to briefly introduce some of the key concepts

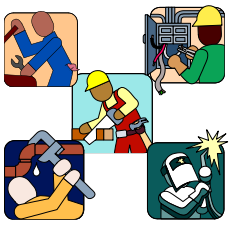
Microbial Testing

- Paradox
  - Detection methods one of the most investigated and used aspects of food microbiology

- Sampling methods one of the most poorly understood aspects of food microbiology

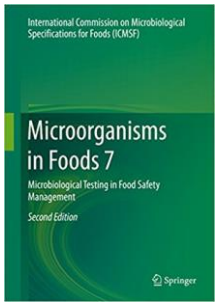
Microbial Testing

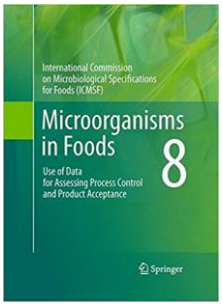
- **Set of technologically-based, statistically-based tools**
  - **"Hold and Release" of batches/lots**
  - **Process control**
  - **Environmental**
  - **Investigational sampling**
  - **Surveillance**
- **Need right tool for the right job**



Microbial Testing

- **Lot Testing:**
  - **Goal is to determine if a lot is "safe"**
  - **Is effectively a CCP/monitoring activity**
  - **Based on "within lot" testing**
- **Process Control Verification Testing:**
  - **Goal is to assess whether a food safety system is functioning as intended**
  - **A verification activity**
  - **Based on "between lot" testing**





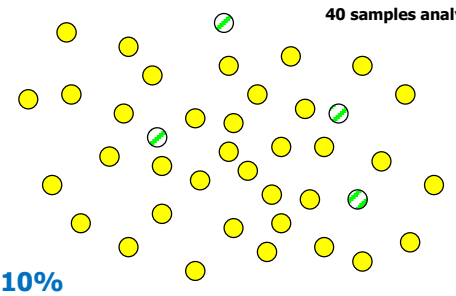
Types of Microbiological Testing

- **Evaluating the microbiological safety or quality of food involves two general classes of sampling plans**
  - **Attribute:**
    - **Two class: "presence/absence"**
    - **Two class: "stratified numerical" (e.g. <100 vs. ≥100)**
    - **Three class: Numerical with inclusion of marginally acceptable (e.g., <10 vs. ≥10 - <100 vs. ≥100)**
  - **Variables:**
    - **Direct use of quantitative data**
- **These basic forms include both culture-based and "omics"-based testing**

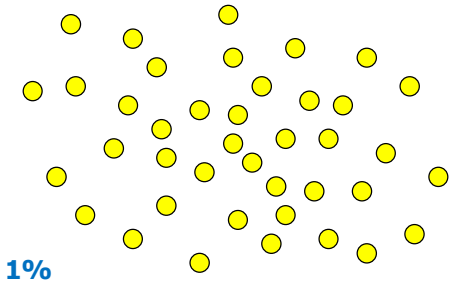
Microbial Testing

- **Utility, effectiveness and cost of testing is dependent on:**
  - **Defect rate***
  - **Method's lower limit of detection* (i.e., when is zero zero?)**
  - **Inherent cost of analysis**
  - **Sensitivity required**
  - **Distribution (variance) of contamination**
  - **Sample unit size**
  - **Analytical unit size**
  - **The degree of confidence required (i.e., 90%, 95%, 99%?)**

Concept of Defect Rate



Concept of Defect Rate



Concept of Defect Rate

- How many tomatoes would you need to test if you wanted to be 95% confident that you would detect contaminated tomatoes if defect rate was 30%? If you needed to be 99% confident? If the defect rate was 0.5%

Shortcut Formula for Number of Samples Needed

- Example 1: 30% of tomatoes are defective
  - 95% Confident of detecting at least one positive sample:
    - 2/0.3 = 7 tomatoes
  - 99% Confident
    - 3/0.3 = 10 tomatoes
- Example 2: 0.5% of tomatoes are defective
  - 95% Confident of detecting at least one positive sample :
    - 2/0.005 = 400 tomatoes
  - 99% Confident
    - 3/0.005 = 600 tomatoes

Defect Rates

- The concept of defect rates has major implications in both food safety and food defense
- The lower the defect rate:
  - The lower the cost effectiveness of testing programs
  - The greater the probability of accepting a contaminated lot of food
- Foods with a high defect rate are easy to detect but once it falls below 2% likely that contamination will go undetected

Microbial Testing

- Microbiological testing is inherently "probabilistic" in nature
  - Cannot prove a negative, only disprove it
  - However, negative findings are useful to determine
    - The likelihood that contamination is below some preset value
    - What level of confidence one can have in the results
  - The only way to absolutely ensure total absence of a microorganism is to test the entire lot of a food

Concept of Lower Limit of Detection

- We tend to think about sampling as something that is done on the farm, in a food facility, or at a port of entry
- However, it is equally important to understand that laboratory protocols also involve sampling
  - Typically one or more analytical units are taken from a sample
  - Preparing the sample for analysis alters the distribution of the contaminant
  - Compositing of analytical units alters method's sensitivity

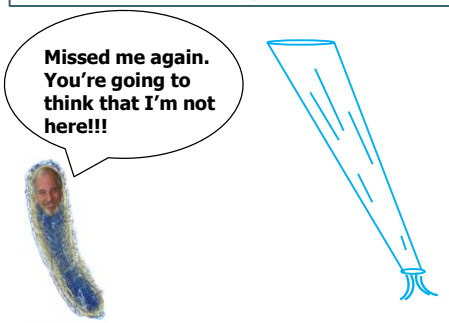
Requirements for Detection

- Two primary factors determine the lower limit of detection (LLD) for a microbiological method
  - Number of target microorganisms that must be present to distinguish "signal" from "noise"
  - Due to the particulate nature of microorganisms, the likelihood that the microorganism is actually present in an analytical unit
- Same principles are true for traditional and rapid methods

Requirements for Detection

- The size of the analytical unit is a key determinant
- In order to be 99% confident that at least one cell will be present in the analytical unit
  - 1 µl: MLC = 4.3 Log(CFU/ml)
  - 10 µl: MLC = 3.3 Log(CFU/ml)
  - 100 µl: MLC = 2.3 Log(CFU/ml)

Sampling Dilemma



Lower Limits of Detection

- 1 µl: MLC = 4.3 Log(CFU/ml)
- 10 µl: MLC = 3.3 Log(CFU/ml)
- 100 µl: MLC = 2.3 Log(CFU/ml)

- To get around this physical limitation, need to:
  - Increase the size of the analytical unit being examined
  - Increase the number of analytical units being examine
  - Enrich the sample to reach these levels
  - Concentrate the sample to reach these levels
- Not about the sensitivity of the method

Enrichment

- Anything that impacts the ability of the enrichment to achieve the needed levels of the target microorganism will impact effectiveness of the method
  - Inherent growth rate
  - Incubation time
  - Incubation temperature
  - Injury
  - pH
  - Antimicrobials
  - Microbial competition
  - Others

Example: Impact of pH and Enrichment Time/Temperature on Detection

- Increasing trend in microbiological testing to try to reduce the size or duration of the enrichment step
- Traditional approach is a 1:9 dilution followed by 24 - 48 h incubation
- Reports of shortening to 6 hours using a 1:2 dilution

Example: Impact of pH and Enrichment Time on Detection

- **Example: Detection of *L. monocytogenes* after enrichment**
- **Assumptions:**
  - **Composite sample mixed with non-selective enrichment broth and incubated at 25º and 37ºC**
  - **Initial concentration of *L. monocytogenes* in enrichment broth is 1 CFU/1500 ml (Log(CFU/ml) = -3.2)**
  - **Growth of *L. monocytogenes* determined using the USDA Pathogen Modeling Program**
  - **Probability of acceptance (P_a) calculated using ICMSF model for presence/absence testing using a 2-class attribute plan**
  - **Detection made using 50 µl analytical unit in conjunction with plating**

Example: 37C

Incubation Time (h)	Probability (%) that a lot would be accepted based of the results of the enrichment at 37ºC											
	Pred Log(CFU/ml)	pH 5.0			Pred Log(CFU/ml)	pH 6.0			Pred Log(CFU/ml)	pH 7.0		
		n = 1	n = 3	n = 5		n = 1	n = 3	n = 5		n = 1	n = 3	n = 5
3	-3.00	99.99	99.98	99.97	-2.17	99.96	99.89	99.81	-1.69	99.89	99.66	99.43
6	-2.84	99.99	99.98	99.97	0.09	93.44	81.58	71.23	1.32	35.49	4.47	0.56
12	-0.59	98.58	95.81	93.12	4.61	0.00	0.00	0.00	>7.0	0.00	0.00	0.00
18	1.67	12.37	0.19	0.00	>7.0	0.00	0.00	0.00	>7.0	0.00	0.00	0.00
24	3.93	0.00	0.00	0.00	>7.0	0.00	0.00	0.00	>7.0	0.00	0.00	0.00

Example assumes that one, three, or five 50-µl portions of the enrichment broth assayed by plating assay at the designated incubation times

Example 2 – 25C

Incubation Time (h)	Probability (%) that a lot would be accepted based of the results of the enrichment at 25ºC											
	Pred Log(CFU/ml)	pH 5.0			Pred Log(CFU/ml)	pH 6.0			Pred Log(CFU/ml)	pH 7.0		
		n = 1	n = 3	n = 5		n = 1	n = 3	n = 5		n = 1	n = 3	n = 5
3	-3.00	99.99	99.98	99.97	-3.00	99.99	99.98	99.97	-3.00	99.99	99.98	99.97
6	-3.00	99.99	99.98	99.97	-2.35	99.98	99.93	99.88	-1.90	99.93	99.73	99.65
12	-3.00	99.99	99.98	99.97	0.23	91.08	75.57	62.69	1.12	50.72	13.05	3.36
18	-2.31	99.97	99.92	99.86	2.81	0.00	0.00	0.00	4.13	0.00	0.00	0.00
24	-1.25	99.69	99.07	98.45	5.39	0.00	0.00	0.00	>7.00	0.00	0.00	0.00

Example assumes that one, three, or five 50-µl portions of the enrichment broth assayed by plating assay at the designated incubation times.

Distribution Within a Lot

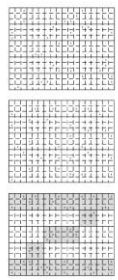
- **The effectiveness of microbiological sampling is traditionally based on the assumption that the contamination is randomly distributed through out a batch or lot**
- **In reality, this is often not the case**
- **When not randomly distributed the assumption that one can sample anywhere within the batch may not be true**

Example: Pre-Harvest Testing

- **One of areas of increased microbiological testing is the pre-harvest testing of produce (e.g., leafy greens)**
- **Little information on impact of the sampling methods that would be most effective**
- **Stimulated our interest being able to compare approaches**

Pre-harvest Testing

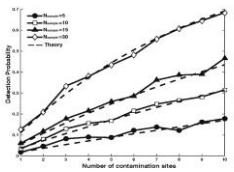
- **Evaluated three of the more commonly used sampling methods**
  - **Random**
  - **Stratified Random**
  - **Z-pattern**
- **Evaluated performance by**
  - **Theoretical probability**
  - **Simulation modeling (100 simulations with 100 iterations per simulation)**



Pre-harvest Testing

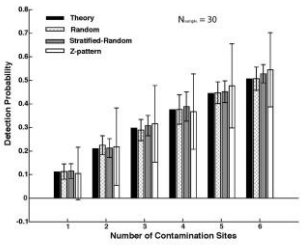
- **The mean probability of detecting at least one contamination site as a function of:**
  - **Number of samples taken**
  - **Number of contamination sites in the field**

Mean probability of detecting at least one contamination site

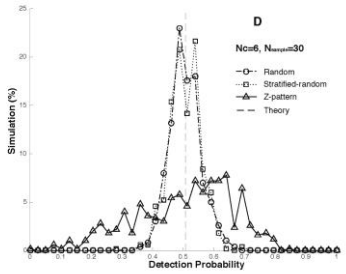


Pre-harvest Testing

- **When consider performance variability using simulation modeling there are differences among the methods based on standard deviations of simulations detecting at least one positive sample**



Pre-harvest Testing



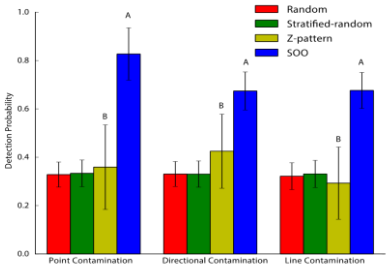
Pre-harvest Testing: SOO

- **Potentially, could use expert knowledge of contamination sources to increase detection probabilities**
- **Studied hybrid sampling method termed "samples of opportunity" which combines random sampling with targeted sampling of part of the field based on likely geometry of contamination from an identified source**

Pre-harvest Testing: SOO

6 contamination sites

30 samples



Sampling Methods

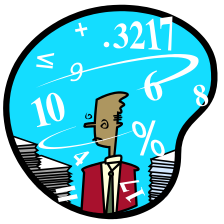
- **The concept discussed for field sampling hold true for sampling of food batches/lots**
- **The use of stochastic simulation modeling is proving to be a powerful tool for better understanding the relative effectiveness of different sampling methods**
- **With modern software, more rigorous sampling protocols can be considered and evaluated in the field with laptop computer**

Assessing Zero by Testing

- Verifying adherence to a "zero tolerance" must start by "operationalizing" zero by specifying a sampling plan and testing protocol to verify compliance, i.e., an official method
- Establishes a non-zero value based on sensitivity of the sampling plan

Zero Tolerance

- Once an official method has been established, it easy to calculate what the effective non-zero risk an agency or an industry is actually willing to tolerate



Methodological/Policy Challenge

- Thirty years ago, the vast majority of microbiological foodborne disease cases were considered sporadic
- In today's world of "whole genome sequencing," we are identifying smaller and smaller outbreaks that are geographically diffuse and often involve a span of multiple years
- At the same time the scale of food production and food manufacturing continues to increase (e.g, millions of servings per day)

Methodological/Policy Challenge

- In many instances the defect rates associated with many foods are well below the limits of practical sampling methods and sampling plans
- Right now the most sensitive means for detecting foodborne pathogens is by feeding consumers
- So why are we doing all this testing?
- It is time for serious scientific and food safety policy discussions on how we can better optimize our food safety systems based on risk

Take Home Messages

- Statistically based microbiological testing is an important tool for verifying effectiveness of food safety system but do not prove that a product is pathogen-free
- Primary investment needs to be in preventive controls and sanitation
- Expertise in effective sampling methods lags behind detection methods
- Rational used of microbiological testing requires establishment of risk-based, technologically-feasible performance standards that were supposed to be part of the Food Safety Modernization Act of 2011.