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Field Sampling – Truth and Consequences

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What does your pre-harvest sampling plan tell you? What should you keep in mind as you develop your plan and interpret the results? A group of experts shared some thoughts to get you started.

There are several approaches to sampling that may be appropriate, depending on your specific objective:

Single Sample of Multiple Composites

- Uses: Risk assessment trending, on-going verification of GAPs
- Cons: If an individual sample already has a low contamination rate, further diluting that sample by including it as part of a composite can decrease the likelihood of the contamination being detected. If 10 individual samples are composited into a single composite sample, take note that the 'n' in this situation would be considered as n=1 *not* n=10.

Multiple random samples (with or without compositing) per lot

- Uses: Gross defect, Risk assessment trend, on-going verification of GAPs
- Cons: High cost of samples

Two stage sampling

- Uses: Risk assessment trend, on-going verification of GAP, Root cause investigation
- Cons:

Some common misconceptions around sampling & testing include:

- *You can test safety into food.*
    - You can't. There are two reasons for this:
        1. Food sampling is inherently destructive. If you needed to test 100 cell phones, you could plug each one into a testing device and run diagnostics. At the end of that process you'd still have 100 cell phones left to sell. If you test 100 leaves of lettuce, you have zero leaves of lettuce left to sell.
        2. When foodborne pathogens are present in a batch of product, they are very likely present at a low prevalence. Therefore, even with a high number of samples (n) the probability of finding the pathogen is quite low (though that likelihood *can* be calculated if you know the true prevalence).

    In other words, pathogen testing is not a control measure, and the statistics associated with testing don't support its use as a means of "lot acceptance". Finding a 'positive' tells you a great deal. Finding a 'negative' doesn't tell you much.

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- *Testing is useless because you'll never find any contamination.*
    - This isn't true, either. A well designed sampling plan, implemented consistently over time may help reduce risk, depending upon the plan and upon certain assumptions about pathogen prevalence and concentration.

- *If I get a negative test result, that means there are no pathogens in the raw product.*
    - No: "Not detected" findings may not indicate 'no pathogens' or low risk; instead it may indicate a sampling plan that is collecting too few samples, or methods that are not optimized to find the target. As per the first point above, it may just indicate that you were not "lucky" enough to pick the sample that had the pathogens.

- *n60 is the best sampling plan.*
    - n60 is not magic - the % defect (e.g., the contamination rate) & lot size matter, as does the size of each "n" in finding what you are looking for. The origins of n60 come from the original ICMSF volume 2, where the most rigorous plan proposed in the book was a sampling plan which collected 60 samples. In general terms, it found that while 60 is good, 30 is not as good, and 120 would be better. See also "these go to 11" from *This Is Spinal Tap* (https://youtu.be/KOO5S4vxi0o)

- *Sample size doesn't matter.*
    - It actually matters a lot. Testing a sample size of 375 g requires much higher sensitivity, but enrichment methods need to be validated at this level (USDA 2019). A sample size of 25 g is much less sensitive, but for practical reasons, 25 g samples are often selected.
    - Note that taking more samples from more locations can better detect point sources of contamination, while taking larger samples is better at detecting low-level systematic contamination, dependent on the adequacy of enrichment

- *The Z sampling plan is the best plan for walking a field.*
    - There is no universal "best sampling plan", and there is at least one peer reviewed publication (Xu & Buchanan, 2019) that shows that the Z sampling plan will miss contaminants in fields that occur at the points where the Z will never reach.

- *If I go from a larger to a smaller field lot size (with the same n), there is a greater likelihood that I will find a positive (if present). For example, I went from sampling a 5-acre lot to a 1-acre lot, therefore I'm more likely to find a positive.*
    - It depends. If the source of contamination is limited to a single point (e.g. a single fecal contamination event), chances of finding it are higher if that contamination occurred in the 1-acre lot vs. the 5-acre lot using the same n. If the contamination is widespread and evenly distributed across multiple fields, chances of finding the contamination are no greater in the smaller field using the same n.

- *A "negative test" is definitely a "negative test" and I can be confident in that result.*

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  - Not all test methods are the same. Incubation times, enrichment media ratios, limit of detection, and type of technology (PCR, cultural, lateral flow, ELISA, etc.) all influence the possibility of getting a positive. Bacteria grow differently under different conditions, and a few extra hours of incubation may make the difference between a "not detected" and a presumptive positive. For these reasons it's imperative that the test method is validated for your sample size and for the produce item you are sampling (the matrix).
  - Not all laboratories are the same – they should be accredited for the testing method you wish to use and ideally, the lab and staff performing the test should have experience handling your product and the analyte.
- Modeling and statistics are so complicated no one can understand it.
  - They can be complicated. But anyone can understand the bottom line. See the graph and figure on the last page.

What does a "good" sampling plan do?

- A good plan will provide information about risks, such as those posed by an individual field or a series of fields and/or practices.
- A good sampling plan will enable you to make better decisions over time.
  - Test results should not be used in isolation but will rather allow you to visualize risk over time, areas, and products.
- It will give you confidence that contamination will be detected (if present), or at least help you understand the limitations of the plan.
  - This can increase customer confidence in your food safety program
- A good sampling plan with a historical data set (and a root cause analysis mindset) can inform meaningful decisions around what to do when subsections of a ranch (contiguous growing area) test positive and negative.
- A useful plan is adaptive. When we identify riskier situations through testing (events, seasons, harvest practices, products, etc.) we can adapt preventive programs to manage risk, and adapt the sampling plan in light of new information.

Take-Home Point: Don't let "the perfect be the enemy of the good". Improving the plan that you have is a good idea. Testing just to test doesn't make sense and is a waste of money. Just because sampling does not reduce risk to zero, a better plan will still reduce risk more than what is being done today.

So, how do you choose a plan that will be "good" for you? What questions should you ask yourself when constructing and implementing a sampling and testing plan?
For more details and context, review:

- ICMSF. 1986. Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications
- Jarvis. 2007. On the compositing of samples for qualitative microbiological testing. Letters in Applied Microbiology 45: 592-598
- Lopez-Velasco et al. 2015. Factors affecting cell population density during enrichment and subsequent molecular detection of *Salmonella enterica* and *Escherichia coli* O157:H7 on lettuce contaminated during field production. Food Control 54: 165-175
- United Fresh Produce Association. 2020. Key Questions Around Sampling and Testing Fresh Produce

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- United Fresh Produce Association, 2010 Microbiological Testing of Fresh Produce
- USDA FSIS. 2019. Understanding and evaluation of microbiological sampling and testing.
- Xu & Buchanan. 2019. Evaluation of sampling methods for the detection of pathogenic bacteria on pre-harvest leafy greens. Food Microbiology 77: 137-145

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Some visuals to help understand the statistics (from Jarvis. 2007. Letters in Applied Microbiology 45: 592-598)

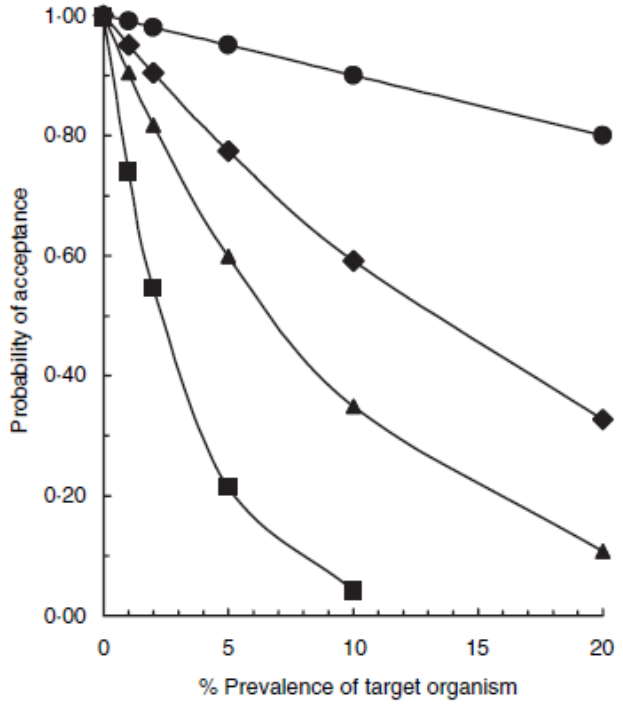

Figure 1 Operating characteristics curves for probability for acceptance of samples having a prevalence of contamination from 0% to 20% and various numbers (*n*) of samples examined from $n = 1$ (●), $n = 5$ (◆), $n = 10$ (▲) and $n = 30$ (■).

In this graph, you can see that as you decrease the prevalence of a pathogen, the likelihood of finding it decreases. For example, even if 5% of a crop is contaminated, there is still a 20% chance (1 in 5) that you'll "clear" the lot with an N30 sampling plan.

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Table 3 Number of sample units to be tested with negative results in order to be able to give a 90%, 95% or 99% assurance that the sample units comply with a criterion for freedom from a defined target organism in a given number of sample units [based on eqn (2)]

True incidence of defective sample units (%)	Number (n) of sample units required to be tested		
	$P_{(x=0)} = 0{\cdot}90$	$P_{(x=0)} = 0{\cdot}95$	$P_{(x=0)} = 0{\cdot}99$
10	22	28	44
5	45	58	90
2	114	148	228
1	229	298	458
0·5	459	598	919
0·1	2301	2994	4603
0·05	4604	5990	9208
0·01	23 025	29 956	46 049

Do you want to be 99% sure that you find the contamination in your lot (which in truth has a 1% contamination rate)? You'll need to take 458 samples (e.g. n = 458). These are the individual sample units to be tested, and they should not be composited.

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