Product Testing: Considering the Issues

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While product testing for pathogens has a long history as part of food safety protocols in the food industry, technical challenges as well as the complex nature of the fresh produce industry make it a thorny issue to address. In a perfect world, our products' safety could be verified with product testing, but that is not our reality. While product testing *can be* a part of any fresh produce food safety program, it should never be considered the "be all, end all" substitute for risk-based food safety programs. The produce industry has long pursued prevention of contamination as a preferred strategy over product testing to identify contaminated products. This has been the basis for our industry's focus on the development of risk and commodity based Good Agricultural Practices or GAP programs at the farm level and Hazard Analysis Critical Control Point (HACCP) programs during processing to manage potential contamination risks.

The produce industry has historically used microbial testing to measure irrigation water quality, verify wash water sanitation and measure the effectiveness of equipment and facility sanitation practices. Identifying a specific risk and selecting practices to manage that risk logically leads to the next question of how to measure the effectiveness of that management practice. In some cases, that measurement involves microbial testing.

However, when it comes to actual pathogen testing in raw or finished produce products, the issue is not so cut and dried. There are several technical, operational and product quality challenges surrounding product testing, including:

- the challenges of testing fresh produce items because of their highly perishable nature and complex composition;
- the selectivity and sensitivity of the pathogen tests commercially available;
- the question of whether to test raw or finished products; and,
- the challenges surrounding effective and significant product sampling.

As a food category, produce is unique. When considering how – or even if – pathogen testing in raw or finished products has value as a food safety tool in your operation for the products you produce, it is important to account for these unique characteristics and understand the opportunities and challenges involved in executing a product testing program. Just as cruicial is knowing how you will use the resulting data to make food safety and business decisions. This paper strives to expose the reader to some of the key technical and operational factors to consider. The paper focuses on the most common questions asked by producers when faced with the question of whether to test or not test products.

1. **How does the perishable nature of produce affect product testing schemes?**

Fruits and vegetables are perishable, and testing can impact our ability to meet shipping requirements. The perishable nature of many fruits and vegetables often dictates that these products must be harvested and shipped within 12 to 72 hours so that they can be received in distribution centers around the country with approximately 10 days of shelf life remaining. This generally permits adequate time to distribute produce to retail or foodservice operations and then on to consumers with acceptable product quality.

Our entire logistics chain is built around the perishability of our products and the objective of delivering the freshest fruits and vegetables possible. We pride ourselves on getting product to market quickly. Likewise, our buyers and end consumers value this speed. However, as buyers increasingly request finished-product testing, the produce industry has struggled to find workable programs; principally because of the time required to process test results before product can be released to the marketplace. Our industry is rife with stories of operators who have suffered significant market losses because product testing took up valuable time.

To reduce testing time, most growers and processors that have testing programs have begun using rapid, DNA-based tests that can offer results within 24 to 48 hours. Unfortunately, although these tests can be very useful, they have proven to be less than 100 percent conclusive. It turns out that "positives" are not always positive and, in some cases, samples that are positive can be missed. Many producers automatically re-test product that has already undergone a rapid test just to ensure results are accurate. This secondary testing is called confirmation testing and while necessary, adds time and cost to the equation.

To offer perspective on time required for product testing, consider the following activities:

- Raw or finished products are sampled and these samples are shipped to a microbiology testing laboratory which can take up to one day (depending on where the field or production facility is located relative to the testing lab and if express delivery systems can be used).

- Once received, the testing lab needs to process the samples. This generally requires an enrichment step that permits the microorganisms present in the sample to grow to ultimately improve our ability to detect them. This step can take an additional 12 to 24 hours, depending on the procedures employed by the laboratory, the length of the enrichment cycle used, the target organisms and the volume of samples the lab is processing at one time.

- Most laboratories currently employ DNA-based testing. This testing is based on detecting DNA fragments in the sample using DNA primers developed from human pathogens like *E. coli* O157:H7 or *Salmonella*. In a very general sense, these DNA primers serve to find pathogen DNA fragments and enable their multiplication so the presence of the pathogen can be detected. There are a number of unique variations in how various laboratories execute this rapid, DNA-based testing, but once the samples have been processed, performing the actual tests should only take only 2 to 4 hours to complete and verify the results.

- The overwhelming majority of product tests result in "negative" findings, i.e. the test fails to detect the presence of signature DNA fragments associated with known pathogens. When this occurs, for all practical purposes, testing is complete, the laboratory notifies the producer the sample is negative and the producer can release the product for its intended purpose. However, if initial DNA-based testing results in "can't rule outs", "molecular positives", "initial positives" or "presumptive positive" results (depending on the terminology you and your lab use), further confirmatory testing is generally ordered. While rapid DNA-based screens have the advantage of being fast, they are not always selective enough, i.e. a positive initial test may not actually be positive for that pathogen and the test may have only detected a closely related strain. There are a number of different options for performing confirmation testing (see Question 5) and, of course, they each have benefits and limitations. However, confirmation testing generally adds an additional 24 to 72 hours to complete.

In best case scenarios where samples can be taken and delivered to the lab within a few hours and DNA-based rapid screening reveals "negative" results, a minimum of 24 hours is required to deliver results back to the grower or processor. In reality, this timeframe is more like 24 to 36 hours or perhaps a bit longer to permit a quality assurance step by the testing laboratory to be sure the data are correct. If the initial testing results in a "positive" sample, confirmation testing can take anywhere from an additional 24 to 72 hours for full complete results. With many produce commodities, 2to 3 days, let alone another 3 to 4 days for confirmation testing, can mean the difference between product that can be sold into the market at market value and product that must be disposed of owing to advanced age and/or post-harvest quality defects that develop over time.

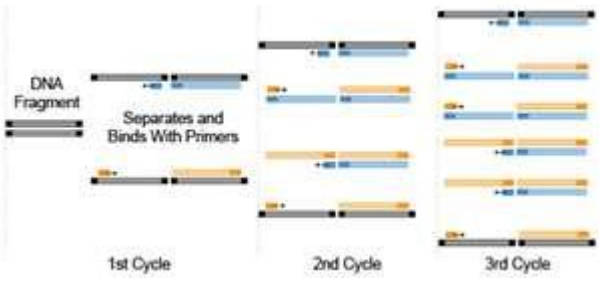
The time element is an especially challenging issue for fresh-cut produce. Processing sets off a complex chain of biological reactions that result in discoloration, textural changes, flavor degradation or nutritional content loss. Because of this, many processors and customers operate on a "one day from fresh" policy, or at the most 48 hours from packing to shipment. So the 1 to 2 day timeframe required just to perform rudimentary testing raises serious logistical issues. If further confirmatory testing is needed, those additional 3 to 4 days can make many fresh-cut products unmarketable.

2. **How accurate are pathogen tests?**

It is important to understand the sensitivity and selectivity of the product tests you may choose to employ. When thinking about the appropriate role of product testing, one must consider that not all pathogen tests are created equal and that they each have benefits and challenges. The chemical and biological complexity of fruits and vegetables in addition to the fact that they are grown in the environment and subject to an array of potential contaminates makes pathogen testing a difficult challenge. There are a variety of tests commercially available today for specific pathogen targets or pathogen groups, e.g. Enterohaemorrhagic *E. coli* (EHEC), Shigatoxin-producing *E. coli* (STEC), *E. coli* O157:H7, *Salmonella* or other potential pathogens or indicators of pathogens. When evaluating the appropriateness of a specific test type or protocol, it is important to consider whether a test meets one's particular needs in its specificity (the ability to distinguish between closely related bacteria) and/or its sensitivity (the ability to detect various bacterial species at a required level or concentration).

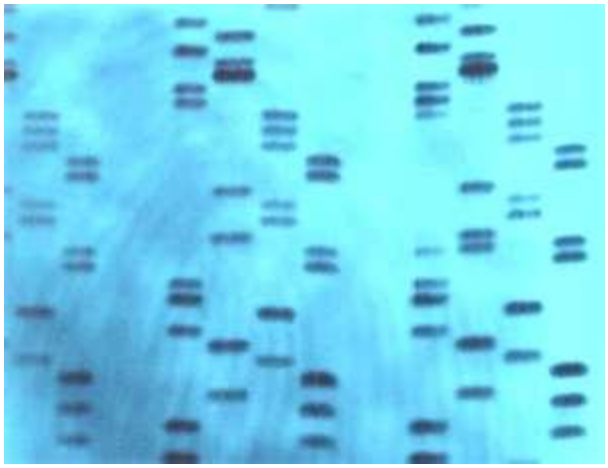
As discussed earlier, when product testing has been adopted by specific produce industry segments or individual companies, the tendency has been to use molecular or DNA-based testing owing to the speed with which results can be obtained. However, it is important to understand what these tests can and cannot tell you. The concept of PCR was developed in 1983 by Kary Mullis who was later awarded the Nobel Prize 10 years later for his work. PCR simply permits the rapid amplification of DNA fragments so that you can go from only a few copies of a single piece of DNA to millions in a matter of minutes. So why is PCR useful for detecting pathogens in fruits or vegetables? Basically, the technology permits the laboratory analyst to use specific pieces of DNA to determine if a pathogen is present in a product sample without having to isolate the pathogen and grow it out; i.e. it's fast, relatively cheap and meets our supply chain requirements. The science behind PCR is complex and technical reviews are readily available elsewhere. The description of PCR that follows here is a generalization and describes some key factors that can impact the sensitivity and selectivity of rapid, PCR-based tests and ultimately determine how results are interpreted or used in making critical and costly operational decisions.

PCR uses heat to cause DNA fragments isolated and prepared from microorganisms in the product sample to "melt" or open up. If you think of DNA as basically a double stranded helix; picture the strand "un-zipping" down the middle and you get a pretty good picture of what the melting process looks like. The key to identifying whether pathogen DNA is present is the primer DNA. Primer DNA is simply a small piece of DNA that helps start or "prime" DNA replication. In this case the primer DNA is prepared so that it matches the sequence of a known pathogen gene. Most often, scientists have used genes thought to be critical to the ability of a pathogen to cause illness in humans, e.g. one of the shigatoxin genes from *E. coli* O157:H7, to develop a primer DNA. They determine the gene sequence and look for a section of the DNA that is unique to *E. coli* O157:H7 strains but hopefully not present in any other bacteria. The more unique the piece of DNA is to the pathogen, the more specificity the PCR test will have for identifying the presence of pathogen DNA in a produce sample. Often, multiple primer DNAs, or multiplexes are used on a single sample so that greater specificity can be achieved, i.e. the probability of a positive test result being correct increases if more than one unique pathogen DNA fragment can be identified in the sample. DNA primers made to specific pathogen genes are developed commercially to be used in testing and testing laboratories typically buy them much as they would any other chemical in a typical lab test.



Once the DNA melts, primers for the target pathogen are added to the mixture of melted sample DNA. Because the primer DNA is homologous or has the same structure as the target pathogen

DNA, if the sample contains pathogen DNA, the primer can attach to the single stranded sample DNA. The combination of the melted sample DNA and the bound pathogen primer DNA permits DNA synthesis to occur. This synthesis or replication is catalyzed by an enzyme called polymerase. This enzyme was originally discovered and isolated from a bacterium that grows near volcanic hot spots under the ocean, in other words it works at the high temperatures used to melt the sample DNA. The polymerase enzyme uses the melted sample DNA with attached probe as a template to replicate the entire piece of melted DNA very quickly as the temperature is cooled in the reaction. The end result is that the mixture now has two copies of the pathogen DNA. When the reaction temperature is increased by the thermo cycler, the DNA again melts, primer again attaches to the melted DNA sites corresponding to the pathogen primer DNA and the replication begins again. This cycle of melt, bind primer, replicate DNA can go on very quickly so that in the short space of 30 to 60 minutes, many millions of copies of the target pathogen DNA can be made. In some PCR systems, a piece of DNA that permits light emission is included in the primer DNA so that during the replication process light is released which can be measured. This light or "beacon" is the measure which tells the analyst that pathogen DNA is present and ultimately that the sample is "positive". Alternatively, following PCR, the replicated DNAs can be visualized by using a process called Pulse Field Gel Electrophoresis or PFGE. The copies of DNA pieces generated by the PCR reactions can then be loaded onto a gel and separated using electrical current, hence the name, pulse field gel electrophoresis. The DNA fragments migrate within the gel based on their size and their electrical charge when subject to an electrical field. The result is a DNA fingerprint; DNA fragments that form bands arranged in patterns that can be dyed and visualized, i.e. a fingerprint. Using PFGE, sample DNA fingerprints can be compared to fingerprints of known pathogens and samples that contain pathogen DNA can be readily identified.

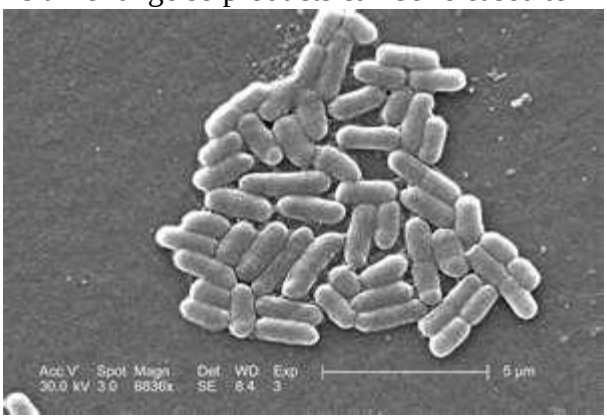


PCR is an elegant and powerful tool, but it also may have some limitations in detecting pathogens in produce samples. It was mentioned earlier that while these PCR tests have the benefit of being fast, they may not conclusive by themselves, i.e. the rapid test may be "positive" for a target pathogen, but confirmation testing fails to validate the initial test. If the DNA primer used to find pathogen DNA in the sample is not absolutely unique to the target pathogen, then it is possible that the test may detect other related, but not necessarily pathogenic species. So when making the decision to use PCR testing for pathogens, it is critical that growers and processors work with the testing labs to understand the specificity of the primers being used, how many different primers are being used and the confirmation rates the labs have experienced, i.e. what percent of the tests run with PCR that indicate "positive" results are actually confirmed via BAM or other secondary tests. For some tests, the conversion rate of initial PCR positives confirming positive when subjected to microbial plating methods can be surprisingly low. It is important to

recognize these issues and weigh them carefully if you intend to use initial positive test results to make harvest decisions or ship or do not ship decisions based on a single test.

3. **What is the purpose of the enrichment step and why is that important?**

Whether employing PCR testing it is necessary to use an enrichment step in preparing produce samples for analysis. As already described here, enrichment is used to permit the microorganisms that may be present on the fruit or vegetable tissues to "recover" from that less than ideal growing environment thereby making it easier to detect pathogen presence. If the enrichment programs and purification steps that address the sensitivity of the test have not been properly developed and calibrated, you run the risk of missing the presence of pathogens and obtaining false negative results. Studies of various enrichment periods indicate that optimum enrichment times can vary based on the physiological condition of the pathogen, and can run anywhere from 8 to 20 hours. However, many commercial test protocols specify the lower end of this time range so products can be released to meet supply chain demands. Clearly, further research is needed to permit better definition of enrichment practices and their impact on testing selectivity and sensitivity. It also begs the question that if a pathogen needs a prolonged enrichment period in ideal growth conditions to "recover" and grow, would that pathogen really be capable of eliciting illness if consumed (see below)? Again, it is important to understand how your testing laboratory has selected the enrichment periods they are using for your samples and the validation data to support their conclusions.



4. **Can the type of product affect the test methods?**

Produce items are chemically and biologically diverse. This diversity further complicates the sensitivity and selectivity of various product testing methodologies. Most obvious is the fact that different commodities vary substantially in terms of chemical composition; for example, a tomato is chemically very different from iceberg lettuce, which in turn is quite different from a green onion. These compositional differences can mean that testing methods need to be fine tuned based on the commodity being tested. It has been shown that specific plant metabolites (most often pigments associated with product color) can interfere with PCR detection reactions possibly resulting in "false negatives". It is important to note that very few commercial pathogen tests have been validated on a commodity-specific basis.

Another important aspect of the complexity of testing for pathogens in raw produce is the fact that the exterior surfaces of fruits and vegetables have a vibrant microbial ecology; a number of microbial species are natural inhabitants of fruits and vegetables. Many of these are beneficial bacterial species that can actually protect its host from infection by plant pathogens, and perhaps even human pathogens. However, they represent a challenge when designing pathogen tests. The presence of multiple microbial species in an enriched sample can make detection of pathogen DNA more difficult. Therefore, most testing labs now use magnetic beads coated with an antibody that binds target pathogens so they can be selected away from complex mixtures of different bacteria. These antibody-coated beads offer an elegant technical solution to limit non-pathogen organisms from contaminating samples.

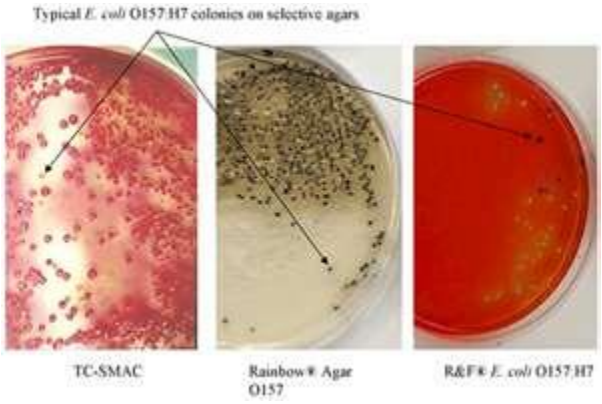
Basically, an antibody that recognizes a specific surface protein or structure on the external surface of a pathogen is bound to a simple magnetic bead. When added to an enriched mixture of various bacteria, the antibody binds the targeted pathogen. After a predetermined time period that permits the antibody-coated beads to thoroughly interact with the mixture of bacteria, a magnet can be used to attract the beads and remove them from the mixture along with any bound pathogens. This method has been considered by many to be critical to permitting pathogen detection in complex mixtures of bacteria. However, the selectivity of the antibodies used on the magnetic beads is critically important along with the use of the proper concentrations of beads. It is important for those using these methods in their testing programs to understand the selectivity of this immuno-capture method.

5. **What is "confirmation" testing and why is it important to do it**?

The reliability of rapid, DNA-based testing has improved over the last few years and with continued research will continue to evolve. Where product testing has become more common in commodities like leafy greens, the industry has seen numerous instances where PCR screening methods have yielded "molecular positive" results that were subsequently not verified using alternative test methods Indeed, while rapid PCR-based screening tests have the advantage of being fast, they are not always selective enough, i.e. a positive initial test may not be indicative of a pathogen but could be a closely related strain. For example, it is not uncommon to find initial PCR positives of *Salmonella* turn out to be related species like *Klebsiella* or *Acetobacter*. Some labs simply use another round of PCR-based screening using an entirely different set primer DNAs from the initial test. The strategy is based on the premise that the probability of the "positive" sample actually being positive increases as you increase the number of pathogen-specific genes you target and find. Once again, the validity of this approach is dependent upon the specificity of the DNA primers employed. This approach does have the advantage of speed as confirmation testing using a second PCR-based screening can be accomplished within hours.

Other laboratories employ traditional Food and Drug Administration (FDA) Bacteriological Analytical Manual or BAM methodologies. BAM methods are based on microbial plating techniques similar to those we all learned in high school or college. Bacteria are genetically programmed to grow or utilize specific carbon and nitrogen sources. Specific media formulations have been developed to identify various pathogens by how they grow on these media. These methods have the advantage that they are very well understood from a microbiology perspective and they result in a visual bacterial culture that can be used for even

further verification steps. However, BAM methods have a disadvantage in that they can take up to 48 to 72 hours to complete. Additionally, some interpretation of microbial colony morphologies and color changes is not necessarily cut and dried and requires considerable experience.



6. **Does finding a "positive" necessarily mean consumption of that product could cause illness?**

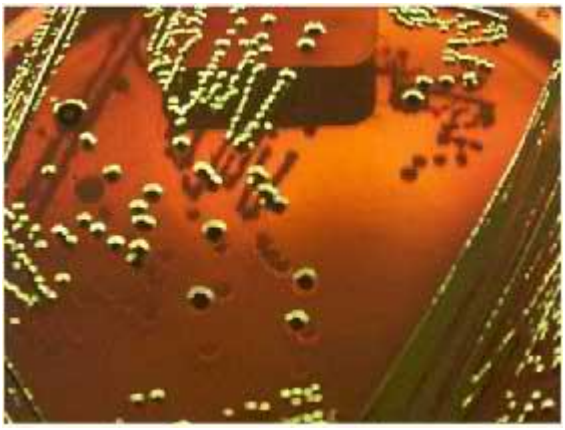
Without question, in today's environment and with our current scientific knowledge, products with confirmed pathogen positives should be removed from commerce. Public health concerns trump every other consideration and producers must report the contaminated product via the Reportable Food Registry. However, perhaps not all "positives" uncovered by enrichment and PCR testing on fruits or vegetables may actually be capable of subsequently causing illness. Many of those pathogens most often associated with foodborne illnesses ideally require the warm, high moisture and nutrient-rich environment of the human digestive tract. The temperature and humidity of the growing or storage environment the fruit or vegetable resides in can fluctuate dramatically, creating an inhospitable environment for the pathogen. Meanwhile, nutrients that might support the bacteria are, in contrast, much less accessible on produce than what might be encountered in the human gut. Therefore, while a human pathogen might survive for some specified period of time on the surface of a fruit or vegetable, they are not in ideal conditions, compromising their viability and perhaps their ability to subsequently grow or cause illness.

Indeed, in studies where pathogens have been purposely placed on the surface of a produce item and permitted to remain there for a period of time, researchers often lose the ability to detect the pathogen after a matter of days. Often, the only way the pathogen can be detected is via enrichment and sometimes only by using molecular technologies as living cultures cannot be recovered. Rather than thriving on the plant surface, the pathogen either goes into a dormant or stationary growth state or becomes associated with other microbial strains in what is commonly referred to as a biofilm. The question is whether a human pathogen in this state could ultimately cause illness if ingested by a human.

As described already, in order to detect the presence of the pathogens in produce the samples are placed in a nutrient-rich culture medium, allowing pathogen cells to "recover" and grow in ideal

conditions so that enough cells can be obtained and sufficient DNA extracted to perform the PCR-based tests described in previous sections. Pathogens sampled from the surface of a fruit or vegetable that are in a slowed metabolic condition or are dying may in fact recover in such enrichment conditions and be induced to grow if sufficient time is provided. We must consider, if a pathogen has been physiologically injured by the inhospitable environment on a plant or food surface, but can essentially be "rescued" by using laboratory culture methods, would that pathogen have actually been able to cause illness if the product had been consumed?

This question can also be examined genetically. We have seen in recent years that confirmed positive produce samples can not only contain different pathogenic strains, e.g. *E. coli* O157:H7 plus *E. coli* O145 but also different variants within *E. coli* O157:H7 that may only differ by the presence of a single gene, e.g. the presence or absence of one of the shigatoxin genes. In other words, we don't always find a genetically homogeneous pathogen type. Why is this relevant? It is possible that current PCR testing methods focused on a select group of genes involved in pathogenicity might not be sufficient to describe the actual virulence of the detected pathogen. For example, if a PCR test designed to detect *E. coli* O157:H7 is based on the two shigatoxin genes and perhaps some of the genes involved in attaching the organism to the human intestine.



This is clearly an important question that requires concerted research effort in the future. The question is particularly intriguing when one considers the sampling deficiencies that will be discussed below and the comparatively few illnesses associated with produce items versus the number of serving consumed by the public each day.

7. **Is a zero tolerance policy still scientifically valid?**

This is certainly a valid question to ask. The Federal Food, Drug and Cosmetic Act of 1938 established a "zero tolerance" standard, i.e. if any food is found to contain a pathogen it is considered to be "adulterated". Today, we know much more about how some human pathogens cause illness, their natural distribution in the environment and the particular dose rates required resulting in symptoms in humans. Additionally, as we have discussed here, we can now detect bacteria at very low levels that scientists and regulators in the 1930's wouldn't have even dreamed of.

Clearly and unequivocally, the goal of food producers, government and the public health community should always be to reduce the risk of any pathogen contamination and related human illness that could conceivable occur. Consumers expect our products to be safe each and every time they purchase food for their families. However, it seems reasonable to consider that the one size fits all approach of declaring a zero tolerance standard for all pathogens might no longer be valid. For example, we know that new strains of *E. coli* have emerged in the last three decades, most notably, *E. coli* O157:H7. It is thought that this strain can cause significant human health issues at levels as low as 10 cells, especially in the young, old or in immunocompromised populations.

By way of contrast, we now recognize more than 2,000 strains of *Salmonella,* and current thinking points to the likelihood that the dose rates to cause illness are much higher with most *Salmonella* strains than with *E. coli* O157:H7. Further, *Salmonella* infection is generally not lethal (although immune-compromised individuals face increased risk) and we're learning that *Salmonella* may be more common in the environment than originally thought. So, while a zero-tolerance approach for a pathogen such as *E. coli* O157:H7 may be very appropriate, today's science may or may not justify such a strict standard for other, perhaps less devastating pathogens.

8. **Which pathogens should the produce industry test for?**

It seems intuitively obvious that if you are going to test fruit or vegetable products then you would want to test for any and all pathogens that could conceivably be present. However, if you examine that question in light of the issues already presented in previous sections, that assertion is less apparent. Adding tests, adds time and cost. It also presents significant testing challenges in that enrichment steps that might be appropriate for say, *Salmonella*, may not be appropriate for a different pathogen strain. It seems a more prudent approach might be a risk-based testing strategy, i.e. we need to determine which pathogens need to be tested on a commodity-specific, and perhaps even a location, basis.

There are a number of human pathogens that have never been associated with produce items, so these are easy to eliminate from consideration. A more limited collection of bacterial, protozoan and viral pathogens have been associated with foodborne illness outbreaks linked to produce over the last 20 years. In some instances, patterns seem to emerge. For example, *Salmonella* is more consistently associated with tomatoes and melons, *E. coli* O157:H7 with leafy greens, Hepatitis A with green onions or berries, and *Shigella* in leafy herbs. However, there are also a number of examples where these relationships do not hold up, e.g. it is not uncommon to also find *Salmonella* on leafy greens. To manage the time element of produce logistics as described earlier in this document and to best utilize resources, it is again important to avoid a "one size fits all approach." Technology may very well present a solution to this question as we look to the future as test methods are being designed to detect multiple serovars and strains of pathogens simultaneously.

9. **What are some of the challenges in setting up meaningful sampling programs?**

The specificity and selectivity of the actual pathogen tests employed are only half of the equation in a product testing scheme. The other, and perhaps most difficult half is the sampling program. While there are many potential issues with actual pathogen tests, in many ways developing a sampling methodology that can achieve statistically significant confidence levels is more troublesome.

It is impractical to test every tomato in a field or every leaf in a head of lettuce as all the marketable product would be destroyed in the process. Instead, the number of samples collected, their distribution, the frequency of collection, the amount collected and other factors need to be carefully calculated if a "sample" is to be created that represents the entire production lot. This is important because the object of product testing is to create confidence that a specific production lot is not contaminated with a potentially harmful human pathogen.

Based on the millions of pounds of produce that are harvested, packed or processed, shipped and consumed each day by millions of people throughout the country without illness, we can assume that the frequency of pathogen contamination is quite low. To add weight to this assumption, data from buyer-mandated product testing of some commodities and FDA/U.S. Department of Agriculture (USDA) surveillance product testing also reveal that contamination is indeed a low-frequency event. Therefore it is imperative that sampling methods be constructed so that we can detect even these low-frequency events. Further, from some of these recent product testing programs we know that contamination, when it does occur, is not uniform. If contamination is found in a field, it tends to be random and isolated. For example, there have been several documented occasions over the last few years where field-level raw product testing of leafy greens has resulted in a "positive" test result, indicating a pathogen may be present in a specific production block or lot. Although sampling programs can vary from grower to grower, many have adopted the sampling methods employed for pesticide residue testing. In general, a 10 to 20 acre production lot of a leafy green is sampled by taking 60 total samples (25 to 150-grams each) from 15 specific locations (4 samples per location arranged like the points of a compass; north, south, east and west) across the field in a "Z pattern". The idea is that the samples thus taken represent some of the block's edges, and traverse the interior of the acreage as well. These samples are generally combined into a composite sample, sent to a testing laboratory and then a subsample of this composite sample is tested for pathogens.

When a "positive" for a specific pathogen is found in a composite sample, often the grower, processor or a food safety specialist will go back to that production lot, perform an observational risk assessment and establish a formal sampling grid in an attempt to determine how widespread the contamination is and perhaps even identify how the contamination might have occurred. In the overwhelming majority of these instances, despite intensive individual plant sampling and testing, the initial positive test results are not repeated; thus the

"needle in a haystack" analogy often associated with product testing.

Sometimes it is hard to visualize the obstacles in setting up an effective product sampling program. To assist the reader, envision a spinach field. A typical commercial spinach field being grown for bagged spinach has more than 4 million plants per acre, with anywhere from 4 to 6 harvestable leaves per plant. That's 20 million individual leaves per acre. A typical planting lot might be 10 to 20 acres in size; meaning at least 200 million leaves are contained in a 10 acre block. The current 15-point, 60-site composite sample practice employed by many producers might comprise 2,000-3,000 leaves, meaning that only a fraction of the material in any production block is actually tested. Let's say that the field was contaminated by a flock of birds migrating from a feeding lot and landing in our spinach field for a few minutes. If you don't happen to sample the specific area where the birds landed or indeed the specifically affected or contaminated plant or even further the specific contaminated leaves, i.e. find that needle in the haystack, you could conclude that the field was not contaminated even though your sampling program was not really sufficient to draw that conclusion.

The question then becomes, why not just test more material? The problem there is determining how much more material to test, and in what location in the field. Remember, these contamination events are random, of low frequency and isolated. One could take a thousand samples from that same production block and only minimally increase the relative amount of product tested relative to the total amount of product in the production lot and could just as easily fail to sample the exact location(s) in the field where the potential contamination resides. Also, since product testing is destructive, any product used for testing is gone and not available for harvest.

Finished-product testing is analogous to the example given here on field-level testing. Today's automated packing machines run at speeds up to 100 bags per minute, and sampling 10, 20 or even 100 bags per line per hour only represents a fraction of the total material being processed. From these examples one can understand the inherent problem with developing statistically significant sampling programs that permit the producer to assign confidence levels that support a conclusion that the product in question is free of contamination.

Once again, as it was with test method benefits and challenges, it is important for producers and buyers to understand the limitations of current sampling programs. It will be interesting to see how sampling technologies and strategies change as research and development impact this area. We are just now beginning to see sampling protocols using "swabs" that can be dragged through large portions of fields to effect sampling a greater proportion of the product in a field non-destructively. Similarly, environmental vacuums similar to what is used to detect weaponized to microorganisms might be used to essential "vacuum" fields to accomplish a more statistically meaningful sample

10. **Where should products be sampled during production?**

In any discussion on the proper role of product testing, it is important to understand the implications of testing based on where the product is sampled. In recent years, when companies have implemented product testing, we have seen product testing programs developed around three different points in the supply chain: in-field or preharvest, post-harvest or at receipt at the cooling facility and finally finished product testing prior to shipment. The choice as to where to sample has been largely made based on each companies estimation on how best to manage sampling programs and manage "hold and release" decisions within their business operations. In general, in-field testing has the advantage of permitting the sampling to take place 2 to 7 days prior to harvest so that the samples can be processed at a lab, tested for pathogens, results returned to the grower and the results acted upon. As already indicated in a previous section, that process can take up to 2 days for a "negative" result so that the practical implication is that the field can be "cleared" and harvest can commence without interruption to business operations. In the event that a "positive" rapid test result is determined, there is still time to perform confirmation testing and once those results are obtained harvest can either be canceled (if the positive is confirmed) or started on time (if the preliminary "positive" test is not confirmed).

In-field or preharvest testing has the timing advantages just described but also permits the sampling and testing to occur prior to the next financial investment into the crop, i.e. harvest and cooling costs. Additionally, with the crop still in the field, the "shelf-life clock" is not started until after the test results are obtained. Additionally, if confirmation testing does reveal a "confirmed positive" for a pathogen, the affected product remains in the field, permitting follow-up studies on the cause for contamination and minimizing disposal costs and eliminating the possibility that harvested or processed products could inadvertently be shipped to the consuming public.

However, while there are some advantages to in-field raw product testing, field-level testing can still be highly disruptive to the supply chain. Harvest windows for various products can often be very narrow due to rapidly changing market opportunities. Delaying harvest to permit product testing can have significant impacts on profitability. This strategy also leaves a potential window of vulnerability, i.e. if the raw product is tested in the field 2 to 7 days prior to harvest, any contamination that might occur after sampling but before harvest could go undetected.

There are also communications challenges to implementing an in-field testing program. In the leafy greens industry, the industry has witnessed instances where one company might be doing in-field sampling and another may not even though they are harvesting or obtaining products from the same field or even production lot. If the company that is doing testing finds a "positive" test and confirms that positive but the second company is unaware of these results, we have seen instances where the first company does not harvest the product but the second company does and subsequently has to recall products it inadvertently put into the market. In-field, preharvest testing requires active and complete communication between growers, shippers and processors to

ensure that test results are shared in a timely fashion prior to harvest. Therefore, while in-field or raw product testing has some advantages over sampling at other subsequent stages in production, it's not without potential problems that can be difficult to manage if broadly implemented without proper consideration and planning.

When dealing with processed products, sampling the raw product at receipt at the cooling facility is similar to in-field testing in that the product has not yet been processed or packed in its final form. Thus, all of these costs are not yet fully invested although the harvest, cooling and transportation costs from the field are expended prior to obtaining test results. Testing raw products at receipt may also require additional cold storage space to "hold" products while awaiting testing results. Sampling product at receipt is significantly different from field-level testing in that the product has already been harvested so that the "shelf-life clock" is ticking and because the product has been removed from the field, positive test results may be more difficult to evaluate going back to production (although some producers use GPS-based tracking systems on containers that do permit location of harvested materials to field locations). A large advantage of testing products at receipt is that the test results represent the product as it sits in your cold storage area, i.e. assuming you control your storage area to limit cross contamination, you remove the uncertainty of a contamination event happening after field sampling has occurred. Similar to field level sampling, if "positive" samples are detected and confirmed, the affected product can be immediately isolated and disposed of thus eliminating any public health concerns.

Finished product testing is more closely associated with typical "test and hold" testing programs that you might find in non-perishable foods and other commodities when shelf life is not a driving issue. Test and hold finished product testing in produce has been difficult for the produce industry to execute because of the perishable nature of the products. Holding finished products for 24 to 36 hours in order to obtain initial test results from PCR methods can be challenging although it has been shown by several companies to be manageable. However, any initial PCR "positives" that require additional confirmation testing extends the "hold" period by one to four days depending on the confirmation testing methods employed thus limiting the sales options for the product if shown to be ultimately "negative" due to aging and likely rendering the product unmarketable. Finished product testing has a distinct advantage over in-field and at-receipt testing owing to the sampling occurring just prior to shipment, i.e. the samples should be reflective of any contamination that may have occurred up to being placed in the final, protective package. In practice, finished product testing has thus far been largely limited to processed or packaged products since they are protectively packaged in their final format. In contrast, commodity products while contained in cartons or perhaps even wrapped in some form of packaging are not viewed as necessarily being "protected" from potential contamination that might occur during distribution. Of course, finished product testing is also the most expensive option for producers as virtually all of the costs involved with that product have been expended so that if a "positive" test were uncovered, having to dispose of that product in its finished form is considerably more expensive that at earlier stages of production.

Final Thoughts

It should now be apparent to the reader that product testing can be a useful tool to verify risk-based food safety programs. However, it is not without its challenges both scientifically and operationally. It is critically important to understand what you wish to accomplish, the value product testing might bring to your program, how you will use the results and how you will communicate those results ***before*** implementing a product testing program. It is also important to indentify a testing laboratory that meets your program needs. The technology can be complex and it is changing quickly. It is in your best interests to identify a testing partner that can clearly describe the tests being performed and understands what they mean. Ultimately, you will have to make significant business decisions based on product test results and you want to have confidence that you are making the best decisions for your company and for your customers.