EHEC/STEC Colloquium on Produce Safety and Testing Systems

Prepared for:

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Acknowledgements

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Western Growers

Produce Marketing Association

California Leafy Greens Research Board

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Forward

In the early to mid- 1990s large salad processors began to periodically test agricultural inputs and/or product for *Escherichia coli* (*E. coli*) O157:H7. At that time, and realistically up to late 2006, rapid commercial kit methods validated or performance tested on lettuce and leafy greens for *E. coli* O157:H7 were largely non-existent. Despite examples of sporadic human illness and regional U.S. and global outbreaks associated with fresh produce involving non-O157 Shiga toxin-producing *E. coli* (STEC), testing was only for the enterohemorrhagic *E. coli* (EHEC) O157:H7 subtype.

After the *E. coli* O157:H7 outbreak on spinach in 2006, the programmatic pre-harvest testing of leafy greens greatly increased. Although it was widely known that other non-O157:H7 STEC were also isolated from consumers' bags of spinach associated with the 2006 outbreak lot code, the focus of commercial lot acceptance testing was limited to the O157 subtype. Several limiting methodological factors in the detection and confirmation of STEC as frequent human pathogens associated with farm-gate produce as well as the predominance of the O157:H7 subtype in U.S. outbreaks at the time, dictated the general industry attitude towards focused testing. This focus was either self-determined or required by customer qualified-supplier specifications.

Since that time there has been a rapid expansion of platforms, kits, pathogen targets, and diversity of approaches to lot acceptance criteria. In parallel with the experience and policy development at the Centers for Disease Control and Prevention (CDC), the U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS), and within the meat industry, there has been a gradual but accelerating shift in product testing criteria and policies for the group of pathogens that includes EHEC and STEC. Some recently used commercial kit test systems screen for the top-seven EHEC (O157, O26, O45, O103, O111, O121, and O145) based on the premise that these subtypes are responsible for over 85% of clinical cases. However, due to the increasing recognition of diverse STEC in clinical cases, many commercial service labs have more recently been using detection and lot acceptance systems that employ the minimal cardinal diagnostic genetic markers for this group – namely, presence of *eae* (intimin; attaching and effacing) and *stx* (either of two key forms of Shiga toxin) in an enrichment culture. Testing for these genetic markers was often coupled with other confirmatory tests including immuno-diagnostic rapid tests (dip-strips or lateral flow devices) and /or immuno-detection tests for the actual Shiga toxin.

Presence of these two markers alone has resulted in frequent crop destruction involving many acres and substantial economic loss at the individual grower level. Some of these crop destruction decisions have arguably been made with only marginal risk-based data; reaction to a presumptive positive rarely allows for more than a fleeting deliberation or pursuit of unequivocal cultural confirmation. Crop perishability and the reality that current methods for cultural confirmation among the non-O157 STEC may take as long as 8 − 18 days or may never be successful in a presumptive positive enrichment, preclude using culture techniques in lot acceptance decisions.

In the absence of a clear, understandable, and consistent policy, there has been building resentment among the grower-suppliers regarding the necessity for uniform crop destruction or recalls in every case of *eae* and *stx* detection alone or other associated molecular evidence. In a mixed enrichment culture, these markers may not be present in the same cell, and since they are not unique to EHEC/STEC, may be carried in a different genus of related bacteria. This fact and the associated fact that not all environmental STEC are equally infectious or recognized as pathogenic to humans has

provoked renewed and passionate discussion surrounding the need to develop and standardize criteria for rapid virulence profiling that would be necessary before a lot acceptance or destruction decision is finalized. It is for these reasons that Western Growers, Produce Marketing Association, California Leafy Greens Research Board and the Center for Produce Safety collaborated to sponsor an EHEC/STEC Colloquium on Produce Safety and Testing Systems organized and moderated by Dr. Trevor Suslow, extension research specialist from the University of California, Davis. Our goal and purpose was to bring together produce industry associations and suppliers with public health, regulatory, and academic experts in the study of genetic diversity, pathogenicity, detection methodology, and epidemiology of this specific group of pathogens associated with serious foodborne illness to discuss the broad ramifications of product testing design and implementation. The anticipated outcome was the development of recommendations relative to rapid virulence profiling and its application to routine compliance and lot acceptance testing for fresh produce. It is our contention that the regulatory, scientific and academic communities have an obligation to consolidate and clarify the available information on risk associated with the diverse STEC group and present this information in a guidance format that can help form industry-based standards of practice.

The Colloquium was intended as a first step towards fulfilling this obligation for the produce industry and those committed to stewardship of the agricultural environment and associated regional landscape.

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Colloquium Notes:

Background

As marketplace and consumer expectations for pathogen testing on fresh produce have increased over the past five years, in particular, growers and handlers have been challenged to incorporate a broader spectrum of potential food-borne pathogens as a prerequisite to contracts and sales. Arguably, the greatest challenge and source of frustration has surrounded testing product for the presence of potential human pathogens among the numerous types of *E. coli* that may be found in production environments and occasionally on product. Severe limitations in time and affordable technologies for accurate detection of those types most capable of causing severe human illness and death has led to repeated incidents of product destruction. Making the decision to destroy potentially contaminated crops within a limited framework may result in substantial individual economic losses. In the absence of a clear or uniformly accepted solution, keeping consumer protection as the highest priority, the best available starting point is a better understanding of the nature of this group of bacteria and the challenges faced by the imperfect test methods currently accessible to growers.

Introduction

Since the 2006 spinach *E. coli* O157:H7 outbreak, microbial testing of fresh produce products has increased both in the production and handling sectors. This testing has focused primarily on *E. coli* O157:H7 and *Salmonella*, but other highly toxigenic and pathogenic *E. coli* have more recently been recognized as important contaminants associated with fresh produce foodborne illness outbreaks. The focus on *E. coli* O157:H7 during the early development of preharvest and postharvest testing programs was well justified. In the United States, *E. coli* O157:H7 was believed to be the major cause of Hemolytic Uremic Syndrome (HUS), a serious consequence of infection which can lead to permanent kidney failure. However, in early 2000's, the CDC reported that the number of human illnesses caused by members of the diverse group of STEC, collectively exceeded those cases of illness, HUS, and sometimes death caused by *E. coli* O157:H7. As surveillance testing, improved testing methods, and public awareness of this broader group of pathogenic *E. coli* increases, the fresh produce industry has struggled to implement practical and cost-effective microbial testing programs. Often with limited specificity and accuracy, these test results are evaluated by companies in an effort to confidently make decisions intended to protect both consumers and their business integrity. As product testing for pathogenic STEC continues to evolve, Western Growers seeks to provide its members with an understanding of contamination risk factors, surveillance testing and research efforts, improved rapid detection methods and the U.S. Food and Drug Administration's (FDA) regulatory approach to testing for these pathogens.

What is STEC?

E. coli are a diverse group of bacteria consisting of different subgroups categorized by various taxonomic and illness-causing characteristics. *E. coli* may be grouped as serotypes (biochemical traits) or pathotypes (pathogenic/infectious and toxin traits). Within each subgroup there are numerous strains differentiated by an extensive list of genetic variations. *E. coli* strains are similar to dog breeds in that, although they belong to a larger category of dogs, each breed has unique traits that distinguish it from other breeds (Ingerson-Mahar, 2011). Similarly '*Escherichia coli*' designates a category of

bacteria, but each *E. coli* strain exhibits a variety of traits, and, similar to cross-breeding in dogs, can mix traits among themselves during reproduction (Ingerson-Mahar, 2011). They can also acquire new traits from other related but non-*E. coli* bacteria or from bacterial viruses (bacteriophage *or "*phage") some of which may present serious new medical concerns following infection (i.e., the transfer of multiple antimicrobial resistance genes).

Serotyping is one way to differentiate among *E. coli* isolated from water, soil, compost, domestic animals, wildlife, produce, humans, or any other source of interest. Rather than being recognized by color, size, or other visible characteristics, *E. coli* strains are commonly matched or differentiated by biochemical tests, one of which is their serological reactions to highly specific known reactive markers present on the bacterium's surface. These various markers are designated by letters (e.g. the O and H in **O**157:**H**7) that signify the main group followed by numbers indicating the particular type of marker compound (the O**157**:H**7**). The key relevance of these classification details will be explained later in this document but are among the critical keys to linking foodborne illness cases to each other and in validating the epidemiology and common-source/root cause trace-back in an investigation.

While most *E. coli* are harmless, one subgroup − enterohemorrhagic *E. coli* are widely recognized for causing serious illness, potentially lifelong medical consequences, and frequently death. While several types of *E. coli* can cause illness (i.e., via toxin production or other mechanisms), those producing Shiga toxin (Stx) have a high risk of causing life-threatening human illness. There are approximately 300 *E. coli* strains capable of producing various types of Stx. These *E. coli* are designated as Shiga toxin-producing *E. coli*; however, it is important to note that not all *E. coli* carrying the genetic code for Stx (the gene is designated *stx* and there are multiple known genetic variants) will produce the toxin or have the other key genetic traits essential to be human pathogens. Although much has been learned about the genetic determinants and mechanisms that lead to STEC infection, the complexity of STEC pathogenicity is not fully understood. It is generally accepted that in order to be considered a potential pathogen, STEC must have certain 'cardinal traits'. These are the ability to produce at least two gene products (referred to as virulence factors): one type of Stx and one of several attachment proteins that allows STEC to bind to specific human cells (Mathusa, 2010).

Pathogenic STEC include EHEC as well as other Shiga toxin-producing *E. coli*. The most common and best characterized EHEC and STEC capable of producing serious illness and death in humans is *E. coli* O157:H7. The Center for Disease Control and Prevention has collected 19,406 STEC that have been obtained from ill humans (referred to as human isolates). Sixty-nine percent of this collection are *E. coli* O157. Other STEC representing a minority (1.4 − 7.0%) of those isolated from humans include the serotypes O26, O103, O111, O45, O121, and O145. Although *E. coli* O157:H7 is the most common STEC in the U.S., disease from non-O157 STEC infection increased from an incidence of 0.12 per 100,000 population in 2000 to 0.95 per 100,000 in 2010 while incidence of O157 STEC infections decreased from 2.17 to 0.95 per 100,000 (Gould, 2013). Some of the increase in incidence of non-O157 STEC-associated disease is clearly explained by improved techniques for detection and recovery from complex samples. In addition, to ensure better representation of the non-O157 subtypes associated with human illness, the public health system began requiring submission of all human stool samples showing evidence of actual Shiga toxin presence to a state laboratory. However, it appears that a significant contribution to the increased disease incidence rates, and therefore recognition of the broad group of STEC in human illness, is an actual increase in STEC prevalence in food and the environment.

The six major non-O157 STEC (O26, O111, O103, O121, O145, and O45), often referred to by the U.S. Department of Agriculture FSIS as the "big six", are responsible for 82% of all non-O157 STEC-associated cases (Gould, 2013). Recently, these six and *E. coli* O157:H7 have been termed the "big seven". In addition to these six non-O157 STEC, the FDA also screens various food products including fresh produce for several other strains (O113, O104, O91, and O128). Food implicated in non-O157 STEC outbreaks include milk, salad bar, punch, apple cider, sprouts, ice cream, berries, beef, smoked wild game, sausage, venison, and iceberg and Romaine lettuce (Buvens, 2011; CDC, 2013; Ethelberg, 2009; Farrokh, 2013; FIOD, 2006; Frank, 2011; Mathusa, 2010; Rounds, 2012; Taylor, 2013).[1]

Where does it come from?

STEC are facultative anaerobes meaning they grow best in environments without oxygen (i.e. in warm-blooded animals' lower intestines), but can also thrive in environments with oxygen (i.e., in water, on surfaces, in food, etc.) For reasons that are not entirely clear, STEC prefers the lower intestine of some warm-blooded animals over others; however, for most animals (and for some humans), colonization and residence does not lead to illness. These infected animals are referred to as reservoirs, carriers or hosts for the pathogen. Dairy and livestock cattle, deer, elk, and pigs serve as the primary reservoirs/hosts for STEC, but STEC are also found in horses, sheep, goats, wild boar, cats, dogs, rabbits, rats, gulls and other birds (Benjamin, 2014; Dunn, 2004; Feder, 2003; Franklin, 2013; Gilbreath, 2009; Kilonzo, 2013; Oporto, 2008; Sánchez, 2009; Wallace, 1997). Primary hosts for different STEC subgroups or presence in a common potential reservoir may differ by location (i.e., may be found in sheep in one country/region, but not in another); however, cattle seem to be a preferred host independent of location (Callaway, 2009; Hussein, 2007). Other animals such as slugs, frogs, and nematodes may also transfer STEC to plants through fecal matter, saliva, etc. (Dipineto, 2010; Kenney, 2005; Sproston, 2006). People become infected with STEC when consuming contaminated food and water or when STEC are transferred by the fecal-hand-oral route during contact with infected animals and people.

What are the risk factors for STEC contamination of fresh produce?

Although site-specific risk factors leading to STEC contamination in the produce production environment are difficult to fully predict, the primary potential sources of STEC are point sources in the environment around fresh produce fields. Knowledge of potential sources of water contamination and the activities occurring on land adjacent to the production field is critical for assessing and minimizing the risk of STEC contamination. Because STEC's primary reservoirs are warm-blooded animals, fields that are adjacent to agricultural animal operations or use a water source close to an animal operation have an increased risk of contamination (Amézquita-López, 2012: Cooley, 2007; Cooley , 2013; Millner, 2008). In addition to close proximity to potential contamination sources, other factors that may affect the risk for contamination spreading to crops from adjacent land use include physical barriers separating fields from potential contamination and whether or not the potential contamination is uphill or downhill, upwind or downwind from the crop, or the potential for chronic or seasonal movement of wildlife between areas of animal and crop production. Wildlife in addition to domestic animals may also serve as potential contamination sources. Aside from direct contamination from wild and domestic animal feces, additional contamination sources are those that are routinely named in

[1] http://www.outbreakdatabase.com/search/?organism=Non-O157+STEC

good agricultural practices and food safety guidance documents – agricultural water, equipment, soil amendments, and workers – all of which, if contaminated, can transmit STEC to fresh produce.

Adjacent land use is not the only potential risk factor of STEC contamination. Other environmental factors such as rainfall may affect the risk of STEC dispersal from point or non-point sources and environmental dissemination leading to contamination. Harvesting while it's raining or following heavy rainfall may increase the amount of soil on equipment, workers' tools and clothing, and on the harvested product, which subsequently increases the contamination risk (unpublished industry observations). Other suspected weather-related risk factors include warm weather followed by rain close to the time of harvest. Risk factors may also be dependent on the surface traits and nutritional characteristics of a fresh produce commodity (i.e., smooth and waxy vs. rough and wettable; sugar and nitrate content). This may explain why some commodities grown in the same fields using the same practices (e.g., irrigation, soil amendments) experience different rates of positive STEC detection in product surveillance testing.

STEC occurrence in fresh produce

STEC have been detected on various crops including cantaloupe, chives, cilantro, hot peppers, lettuce, parsley, spinach, sprouts, and tomatoes (Feng, 2013). USDA sampling as part of the agency's Microbiological Data Program (MDP) and FDA sampling as part of their routine microbiological surveillance between 2002 and 2012, have resulted in isolation of 133 STEC strains from produce of which 52.6% and 21.1% came from spinach and lettuce, respectively (Feng, 2013). Based on the MDP and FDA's surveillance results from 2008 − 2012, the prevalence rate of STEC in spinach ranged from 0.22 to 0.95%. Although a few of these strains were taxonomically aligned with pathogenic subgroups, most serotypes isolated had no history of being associated with clinical samples. This prevalence rate is much higher than reported prevalence rates from industry testing. However, unlike USDA and FDA surveillance testing, most industry testing conducted during this same period specifically targeted *E. coli* O157:H7, and not, until more recently, the broader category of pathogenic STEC. In addition, even if decisions not to harvest or withdraw from the market were made based on non-specified STEC detection, culture confirmation was often not attempted or attempted but unsuccessfully. Another possible cause for the data disparity is that industry surveillance testing is conducted during production − most often just prior to harvest while the FDA tests product taken from the marketplace generally at the point of consumer sales and near the end of labeled "Use by Date". By experience, it is commonly found that recovery of trace contamination, especially in packaged greens, is improved with product storage, especially at warmer temperatures. Companies that test finished product prior to market release also have much lower prevalence rates than either the MDP or the FDA's surveillance testing results. In a study conducted by the USDA, 16 of 2,462 produce items tested positive for STEC (Cooley, 2013). Forty STEC strains were recovered from those 16 samples, but only 3 of those strains also contained a protein critical to the ability for STEC to bind to and infect human cells. Based on these findings, it is questionable whether a general positive STEC test, in the absence of other essential genetic factors simultaneously present in the same cell, necessarily means there is a public health threat.

What actions are being taken by the fresh produce industry to address positive STEC test results?

In a rapidly evolving product testing services marketplace, more laboratories offer various versions of STEC testing and some growers are routinely testing their products voluntarily or in response to customer requirements. However, depending on the specific test platform, product shelf life and

associated time constraints force companies to make decisions on whether or not to harvest or accept incoming products based on test results indicating pathogen presence without confirmation that the genetic markers detected were collectively present in a single cell, and therefore meeting the diagnostic criteria as a likely human pathogen. The most common grower response to a positive preharvest STEC test is to destroy the crop, which often must occur without confirmation that the detected STEC is from a recognized pathogenic subtype. Similarly, handlers and processors will destroy product or, when possible, divert raw incoming product to processing that includes a lethal "kill step". Most packaged products that test positive for STEC, responsibly presumed to be accurate, are commonly sent to the landfill.

What other actions have been triggered by STEC concerns?

Although no longer a common practice, additional action may include destruction of non-crop vegetation and riparian areas because these areas are viewed as potential habitat for animals that have been identified as potential STEC sources. Unnecessary crop and vegetation destruction works against company and industry agricultural sustainability and environmental stewardship initiatives that are widely supported by the grower community. Trees and other vegetation serve as windbreaks that reduce the transfer of dust aerosols, protect soil and water, and reduce physical injury to crops. Vegetation, such as hedgerows, also creates an environment that encourages the establishment of beneficial microorganisms contributing to healthy crops as well as microorganism that may be detrimental to STEC persistence and growth.

What is FDA doing about STEC?

The U.S. federal government has no microbiological quality criteria for fresh produce. Florida is the only state with a standard that designates product with >100 CFU/g generic *E. coli* as adulterated, but does not specify what action to take if levels exceed the standard. In the United Kingdom, Ireland, and Germany, generic *E. coli* levels in ready-to-eat foods cannot exceed 100 CFU/g and in Switzerland, the limit is 10 CFU/g. France and Brazil set limits using fecal coliforms (1,000 and 100 CFU/g, respectively) – a broader category of bacteria that includes *E. coli* as well as other bacteria potentially associated with animals' gastrointestinal tract and fecal matter.[2]

Although the U.S. does not have a standard for fresh produce microbiological quality, the FDA has engaged in or sponsored various surveillance programs and commodity-specific assignments where fresh produce is sampled at various points along the marketing chain and tested for STEC among other pathogens. Currently, the FDA considers STEC to be pathogenic if a single STEC cell contains particular combination of genes: one or more genes that code for Stx and one or more genes that code for proteins that allow STEC to bind to and enter human cells. If testing confirms the presence of genes that fit these criteria in the same bacterium, then that produce item is considered adulterated and is recalled. In addition to this definitive criterion for determining if STEC is pathogenic, the FDA may recall product if combinations of other defining genetic markers or virulence genes are present in a sample. Although the agency has an internal strategy for determining when to recall product, it is a complex protocol that still contains some subjective judgments when unusual strains are found. This current strategy has the significant challenge of taking too long for it to be used in industry for perishable horticultural foods that would necessitate a "hold and release" policy and the cold storage capacity to

[2] These different bacterial indicator standards may be confusing, but for practical purposes fecal coliform and *E. coli* are treated as equivalent.

execute such a policy. In contrast, current industry testing methods are not standardized in applying analysis for definitive genetic material and/or protein(s) in the sample. Without the knowledge provided by culture confirmation and application of the more complex methods and genetic arrays used by the FDA, industry is left with uncertain risk management that has undoubtedly led to unnecessary and substantial crop losses.

What is needed to make STEC testing meaningful?

STEC testing using a non-specific or inadequately validated presence/absence diagnostic method is not sufficiently or sustainably informative since not all STEC are pathogenic. Rapid test methods that identify STEC presence are effective screening tools; however, without confirmation of pathogenicity, positive samples will continue to be taken as an actionable benchmark for contaminated product. It is recognized that the status quo situation requires very conservative decision-making to first protect the consumer as well as the business' integrity and sustainability. As previously stated, most STEC require at least two specific genes to be considered pathogenic, so finding these genetic markers in a sample, as most rapid presence/absence testing methods do, does not unequivocally determine pathogenicity. In order to be a human pathogen and, therefore, a potential public health threat, an individual STEC cell must contain several essential combinations of genes that code for regulatory mechanisms and proteins associated with its ability to cause human illness. Determining STEC contamination requires the use of a combination of test methods to screen and then confirm the presence and location of disease-associated genetic markers in a single cell. However, current test methods confirming broad association with STEC serotypes and/or pathogenicity are not available in all service laboratories and are somewhat time-consuming with limited applicability for testing highly perishable products. Though new technologies are rapidly becoming available, the time required for the FDA's genetic marker screening and culture confirmation testing currently exceeds most fresh produce products' expected quality shelf-life. By the time screening and confirmation testing is completed, the tested products are often no longer in the marketplace and have been consumed or disposed of.

To be effective for industry, test methods must provide rapid results with high sensitivity and specificity so that false positives and false negatives are minimal. Several new detection tools have recently come to market improving on previous methods (e.g., conventional culture-based protocols, first generation polymerase chain reaction) for confirming STEC contamination (Son, 2014). One such tool is Neogen Corporation's NeoSeek™ that uses a combination of genetic material- and mass-based technologies to develop a genetic profile for bacteria directly from a food sample enrichment broth. The sample's bacterial genetic profile is then compared with the known genetic profile of seven reference STEC (O26, O45, O103, O111, O121, O145, and O157) to identify and differentiate any STEC in the sample within 24 hours.[3] Another tool is Roka BioScience's Atlas® System that within a 12-hour timeframe detects a highly correlated surrogate marker for key determinative genetic markers that define the regulatory view of virulence in STEC. Most importantly this surrogate marker is highly diagnostic for these actionable and determinative genes being located in a single bacterial cell.[4] While additional in-use experience is needed for produce applications, promising technologies such as these and other emerging validated commercial test methods provide industry with the ability to make risk-based, cost-effective decisions about product contamination instead of needlessly destroying crops based on presumptive positive test results.

[3] NeoSeek™ STEC confirmation http://www.neogen.com/FoodSafety/NS_STEC.asp
[4] Roka Science Atlas® System http://rokabio.com/products/atlas-system/

Summary

In USDA and FDA testing from 2002 – 2012, STEC was detected on 133 fresh produce items. In recent years, industry testing of pre-harvest and incoming or finished product for STEC has also increased. STEC detection is the most frequent pathogen group encountered in routine testing of leafy greens and several culinary herbs. However, most industry testing involves tests targeting a narrow or overly simplistic definition of STEC without necessarily the ability to determine whether or not pathogenic STEC are present. Faced with this uncertainty growers and handlers have no responsible choice other than to destroy the crops or packed product without further testing to confirm STEC pathogenicity. Although the FDA tests fresh produce items for particular STEC, they recognize that the process they must employ is excessively time consuming for routine industry surveillance and impractical for product market decisions. New rapid methods have recently been developed that may allow detection and identification of pathogenic STEC in product samples. Utilization of these rapid methods will provide industry with better decision-making tools to reduce unnecessary crop destruction.

Recommendations and Practical Implications

There is broad recognition among the food safety research community, especially those most involved in STEC biology, genetics, and epidemiology, and many leading public health officials and administrators that not all STEC are "created equal" with regard to their potential to cause human illness. Despite this acknowledgement found throughout the scientific literature, where fresh produce consumption is concerned federal policy makers will be unlikely to narrow the definition of adulteration or the scope of specific STEC serotypes or subgroups. The complexity and plasticity (the abilities to acquire new traits and cause infection) of this diverse bacterial group is truly mind-boggling. The risk potential for under-reported and unrecognized variants to cause illness to our most susceptible populations is too high for the FDA to relax its position. Industry has worked too hard to build credible and increasingly effective food safety systems to jeopardize consumer confidence in, as yet, unproven technologies. A break-through 'titanium-clad' solution and practical relief for growers, handlers, and processors is not on the immediate horizon. Despite this gloomy forecast, we are very optimistic that rapid and definitive test methods will be forthcoming as STEC research enters a new phase of genome sequencing expanding to individual strains in large STEC collections isolated specifically from human cases. It is highly likely that unique genetic signatures of STEC associated with human infections will be discovered and used to develop improved test methods reducing the time-to-decision and greatly refining what is included and excluded from risk considerations. In the meantime, there are a few key steps producers can take to responsibly limit their liability and minimize unnecessary crop losses:

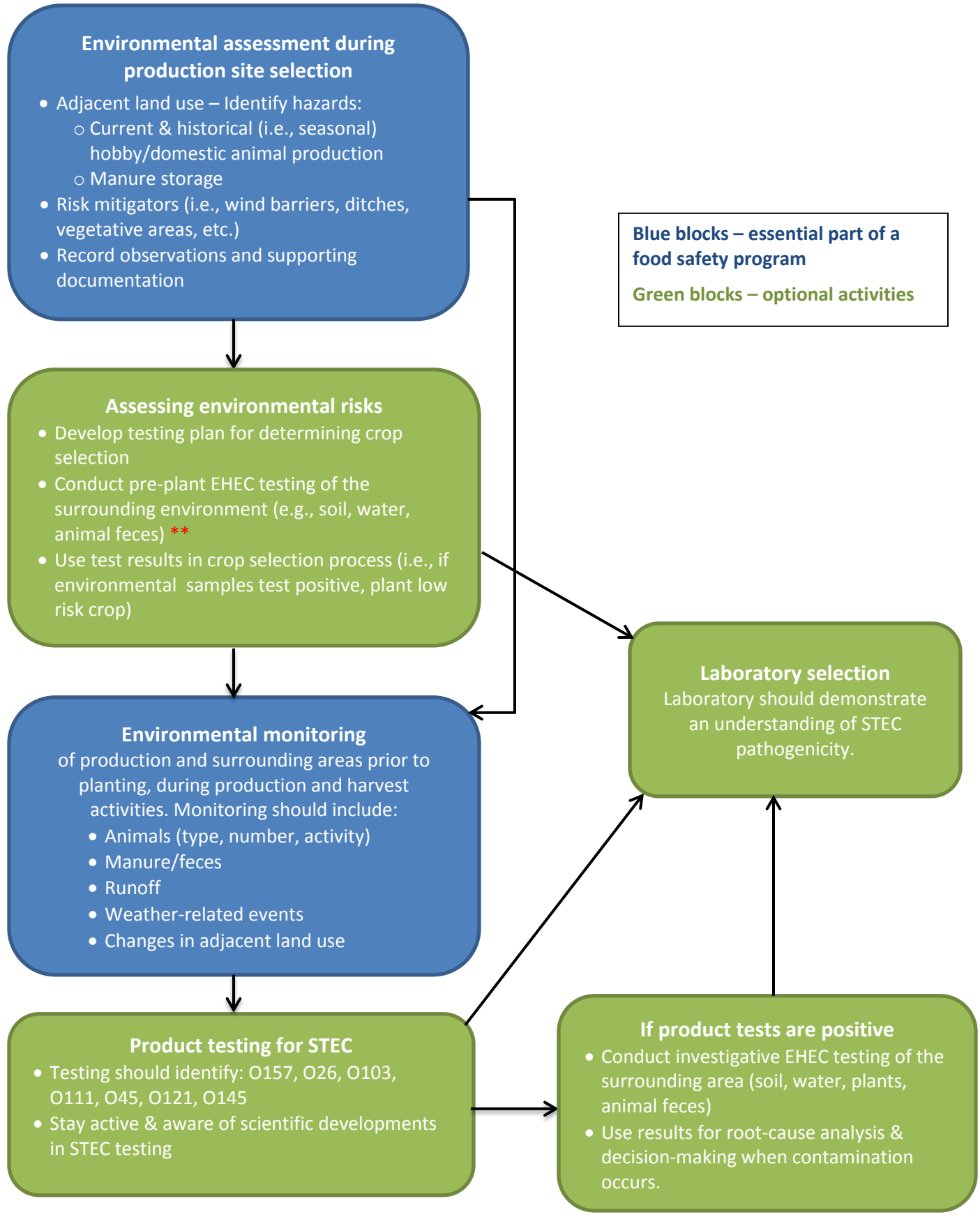
- Because STEC is principally associated with livestock, producers and landowners may devote extra effort to production site selection in cases where hobby or domestic animal production or manure storage is in close proximity to production sites. It is prudent to closely evaluate these settings even when within current guidance recommendations.
    - When/If a preharvest testing program reveals a STEC positive then investigative testing to detect EHEC vs. STEC may help with root-cause assessments and decision-making.

- Be aware that STEC contamination from adjacent seasonal grazing land or feeding operations may be transferred (e.g. run-off and bioaerosols) even well after animals have been moved to other locations.

- Animal head-count on adjacent land during production or at harvest may not be the relevant timeframe as even their recent historical presence may influence transfer of STEC from adjacent lands.

- If STEC testing is part of your program, select a lab offering services that are at least definitive for the top seven EHEC or broader EHEC groupings. This will provide a better resource for making decisions than testing based solely on the two FDA-designated 'cardinal traits' (one Stx type and an attachment protein) without the benefit of culture confirmation.

- Stay active and aware of scientific developments in STEC diagnostic testing and consider conducting investigative sampling of potential or suspected STEC sources using modern technologies when results within 48 hours are acceptable.



****Serious considerations should be given to potential liabilities associated with use of pre-planting environmental testing for crop selection.**

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