APPENDIX C:
Product Testing Protocol

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Disclaimer

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Introduction

This appendix provides guidance for product testing as specified in the *Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain*.

The purpose of this document is to provide guidelines to assist users in developing and executing a product testing protocol when irrigation water that exceeds generic *E. coli* water quality standards or when Type B →A water treatment fails to achieve performance criteria is used on lettuce or leafy greens and product from the affected block(s) must be tested for the presence of STEC (including specific tests for *E. coli* O157:H7) and *Salmonella*.

Product Testing Protocol

Target Organisms

- STEC (including specific tests *E. coli* O157:H7) and *Salmonella*.

Timeline

- Conduct a root cause analysis (as described in Appendix F) to determine what may have caused the failure to achieve performance criteria.
- Collect samples as close as possible to the actual harvest day - consider testing less than 8 days before harvest; 4 days or less is strongly encouraged. The closer to the actual harvest day, the more likely to detect any issues with irrigation water quality.

Sampling block size:

- How the measurement of a designated sampling block is determined should be based on plant density and site-specific conditions as opposed to overall acreage only.
- The smaller the block, the more likely to identify issues before any product is harvested. Block sizes may range from 1 to 5 acres per sampling block.

Sampling Protocol

- Consider 60 subsamples (n=60) per sampling block. If a sampling block is determined to be 1 acre in size, increase the number of subsamples per additional acre added to that specific sampling block. Sixty subsamples should generate a total sample volume of at least 1,500 grams for tender leaf items; a more intensive sampling approach for head items should be considered. For instance, a minimum of 30 head-punch cores sampled in a small block configuration or biased sampling where there is an indication of potential issues within a specific area of a sampling block.
- The larger the volume of individual samples, the greater the total volume of the sample, which increases the potential to detect any issues. Statistical probability guidance, based on research, suggests that all n=60 subsample material be used for lab analysis to provide a 95% confidence in the results.
- Consider increasing the volume of each subsample, in particular in areas susceptible to food safety hazards (i.e., borders of flood irrigated fields) for specific risk-based sampling where hazards exist or have occurred.
- Take tissue samples at random locations as follows:
    - o Walk the designated block in whichever pattern best suits existing conditions and ensures the sample is representative of the designated block. Recent research suggests that a "Z" pattern may be more protective and improve sensitivity. Sampling pattern within a designated block may vary based on the properties and condition of the block. For example, if the block is long and narrow, a "Z" pattern may be the best approach, and if the

block is square, a "W" or "S" pattern may be more appropriate.

OR

- o Alternative method per the Interstate Technology and Regulatory Council (IRTC) recommended soil sampling practices: Divide field into grid and conduct systematic random sampling within each grid starting at a random location and continuing to each grid in a serpentine direction. Randomly select your sampling locations along your pre-determined sampling pattern (e.g., "Z", "W", "X", etc.) within the sampling block.

- Using aseptic sample collection techniques, cut leaves near the ground from the edible portion of plants. Focus on leaves that would come into contact with harvesting tools, harvesting equipment, or harvest workers' gloves. Do not trim and discard leaves that would not be included with harvested product but focus on the areas of the plant/field that would be at greatest risk for crop contamination including but not limited to the following: inner leaves, outer leaves, and wrapper leaves. Additionally, when assessing the possibility of contamination via furrow irrigation water or animal intrusion, specific care should be taken to collect leaf samples from beds at the irrigation discharge point of the field.
- Place each sample in a sterile container or sealable sample bag and include the specific sampling location in documentation.
- Place samples in cooler with adequate ice packs, but do not freeze. A double layer sheet of craft or butcher paper as a barrier between samples and gel-ice is helpful to prevent tissue freeze injury. If using water-based ice (not recommended), ensure product is protected from potential cross-contamination from melting ice.
- Fill out the chain of custody form with the sample collection information.
- You may choose to utilize a third-party laboratory for collection of the sample or just to have the actual test performed. Regardless of your choice, make sure your laboratory is utilizing a validated method to conduct pathogen testing (BAM, AOAC, etc.); it is recommended that laboratories utilize validated methods based on sample mass to enrichment buffer ratios.
- Samples must be transported in a timely manner and at the right temperature as required under your specific sampling method protocol. For instance, less than 48 hours if the arrival temperature is assured to be between 33˚F and 41˚F.
- Review testing results before harvest of the field if the product is destined for fresh market or when you are ready to determine what product enters a processing or distribution facility.

Measurement Criteria

- No confirmed positives for STEC (including *E. coli* O157:H7) or *Salmonella*. Confirmation of a presumptive positive can be achieved by either negative molecular marker or by negative cultural confirmation.

Remedial Actions

Remedial Actions may vary depending on how sampling blocks are defined. Some corrective actions may include the following:

- Destroy and do not harvest all products from blocks which do not pass the above criteria.
- Clean and sanitize all equipment utilized to destroy the crop upon exiting the field.
- Do not replant the field with edible food crop production for the remainder of the season in which pathogens are detected.
- Document all remedial actions. All documentation must be available for verification from the grower responsible party.

Resources and Rationale

This protocol provides minimum guidelines to consider for product testing, they are based on current scientific knowledge, feedback from food safety experts as well as current sampling protocols produce companies have shared with Western Growers. At this time, there is not a statistically validated and practical sampling design and sample size approach at the field level. Ongoing and future research efforts should assist in refining this document in the future.