WESTERN GROWERS SCIENCE

Primer: Preharvest Pathogen Testing of Leafy Green Products

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Version 0

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Pathogen testing of Leafy Greens

Microbial testing is a common component in many food safety programs for leafy green producers and has increasingly been requested as a condition of sale. In recent years, pre-harvest product testing has also been required for import marketing by regulatory bodies, such as the Canadian Food Inspection Agency (CFIA). In 2022, the Leafy Green Marketing Agreement (LGMA) Appendix C: Pre-harvest Product Sampling and Testing Protocol was updated to include more specific guidance on risk-based pre-harvest tissue testing for routine lot acceptance qualification as well as situational testing as a response to an observed field or adjacent land risk. The update was to provide a construct for pre-harvest testing consistency across the industry and to provide a sampling plan compliant with recently developed outbreak-predictive models, which informed the CFIA import requirement. This document provides general considerations when conducting and evaluating existing pre-harvest leafy green testing programs.

Designing a testing program

It seems simple to take a sample and get a test result, but evaluating what it means can be more nuanced once a result is in hand. All microbial testing plans are designed with a (i) purpose, (ii) limit of predictive statistical power and (iii) physical limits of detection for the analytical method. Understanding the informative scope as well as the limitations of a testing program is important when analyzing the results. Is the testing program designed to find gross (high-level, widespread) contamination, identify trace contamination, ensure the product is pathogen-free or some other outcome? Identifying how the testing program was designed and whether the underpinning question to be answered is achievable will help define what you learn from the testing data. For example, the often-expressed expectation that testing will ensure the product is pathogen-free is inherently invalid.

In leafy green production, most samples are taken as pre-harvest tissue samples that are generally obtained <7 days from harvest. This pre-harvest approach is intended to help identify contaminated field products before entering handling, processing and distribution into the supply chain. Over the past decade, this has become a common requirement in customer specifications. To collect a sample, trained samplers walk a planned pattern through fields while taking small grabs of the product (‘n’ subsamples) from various parts of the plant (top, middle, bottom) and positions on the planted bed (edge or central) within the lot to create one larger composite sample to be analyzed in a microbial testing laboratory. The final size of the sample to be analyzed in the lab is made of the ‘n’ grabs and is often at least 375 grams (g). The “lot” to be tested can vary in size between growers and customer requirements but is usually set based on the number of acres, plantings, water distribution systems, number of pounds of product and/or harvest plans. Statistical models provide guidance on the influence of lot size on the level of confidence one should have during an analysis of the results.

What are current pre-harvest programs designed to find?

Today’s pre-harvest testing programs have largely been designed to identify randomly distributed–rather than highly localized–contamination events with a sensitivity of detecting 1 colony forming

unit (CFU) per pound distributed across a production lot unit. While it is difficult, if not impossible, to know the level and type of contamination in a field in advance of implementing a sampling plan (i.e., uniform vs. point source), these assumptions are often used since this level of contamination was derived from leafy greens lot-data implicated in a multi-state outbreak. Model development necessitated an assumption for uniform contamination and calculated the outbreak-responsible level (1 CFU/lb randomly distributed across the whole field). Statistically, the size of the "lot" does not matter, up to the limit of the actual outbreak lot scale, and the size of the total sample to be tested (g) drives the power of the sampling efforts. Appendix C: Pre-harvest Product Sampling and Testing Protocol provides an analysis of two common pre-harvest plans to show the theoretical power of the testing program, dependent on assuming 100% accuracy of the testing method in its ability to detect the target contaminant (Table 1).

Total Sample size	Grabs (n)	Size of grab (g)	Probability for detecting widespread, randomly distributed 1 CFU/lb contamination across a field
375g	60	6.25g	57%
1500g	60	25g	97%

Table 1: recreated from Western Growers Preharvest Product Testing Sampling Scenario Analysis Version 2: 06/14/2022**.**

Table 1 helps communicate the theoretical power of common 'n=60' pre-harvest testing plans using a 375g sample and a 1500g sample described in Appendix C. By increasing the mass of the total sample to be tested, a 1500g plan would find a 97% probability of detecting a distributed uniform contamination event vs. the 57% probability with the smaller, 375g sampling plan. In either scenario, it is critical that the proper number of grabs ('n') is taken randomly within a field, and that the entire mass of grabs/sample (e.g., 375g, 1500g) is analyzed in the lab for the target.

How to evaluate your test results

Food safety management in fresh produce is complex due to open environments where it is impossible to predict or control all factors that may impact the presence of foodborne pathogens. Good agricultural practices and dedication to food safety are paramount for growers and processors of these items. Pathogen testing programs serve a role for the verification of food safety practices and identification of scenarios that may contribute to illness events. Understanding the power of a testing program is the first step since it equips food safety managers to be able to interpret what their pathogen data does and does not mean about the safety of their system and/or product.

Food safety data sharing and analysis invites a new paradigm in food safety management, increasing learning opportunities with the potential to identify mitigation strategies faster than ever before. With the advent of food safety data-sharing programs such as those housed on the food safety data platform GreenLink®, there is an increased need to fully understand the power of a sampling program, the test method performance and the confidence in the abilities of these two factors to detect the target. Effective analysis of results in a data-sharing platform requires a prerequisite equivalency in the platforms and sampling approaches being used in the testing programs. Technological advancements for detecting and visualizing microbial risks will allow us to address food safety issues faster than ever before. Understanding what data represents in these

efforts is critical to proper analysis, comparability between datasets and driving efforts toward improved efficiency and performance.

For questions please contact:

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