2022 CPS Symposium
Executive Summary: Key learnings every company should know

The 2022 CPS Symposium featured produce safety research presentations and industry discussions on a wide range of critically important produce safety challenges. This executive summary can be used to look inward at your own produce safety programs to examine current strategies and ensure they reflect the current science. Executives can also look outward and engage customers, trade groups and regulators to set the framework for trust-building discussions and data-sharing opportunities to advance mutual produce safety objectives and enhance consumer confidence in fresh produce. This summary is organized into three areas to guide executives in the action levels they need to take: (1) results that merit immediate action from your produce safety team, (2) results that reinforce current best practices and represent opportunities for renewed training or finetuning and lastly, (3) findings you should be aware of and monitor as further research enables practicable operational value.

1. **Results that merit immediate actions from your team.** New learnings from the 2022 Symposium critical to your company's journey to more effective produce safety are briefly described below:
   - ***When it comes to Cyclospora testing, always confirm presumptive positives***. Before testing irrigation water samples for *Cyclospora*, it is important to understand the current scientific literature on environmental *Cyclospora* and the decisions that can be made given the limitations of this testing. Currently, no single DNA-based testing method is capable of discerning *C. cayetanensis* from other genetically related parasites commonly found in growing environments (e.g., *Eimeria*) [*Mattioli 2019*, *Ortega 2019*, *and Kniel 2019*]. It is imperative to confirm presumptive PCR-positive tests using multiple *C. cayetanensis*-specific mitochondrial or nuclear genetic sequences.
   - ***Hollow fiber filters improve capture of Cyclospora oocysts in irrigation water samples***. Detecting *Cyclospora* offers several technical challenges; among them collecting enough oocysts to permit DNA-extraction needed for PCR testing. Hollow fiber filters permit large volumes of water to be passed through the system to filter out oocysts in sufficient numbers to support DNA extraction [*Ortega 2019*]. Increased availability of oocysts and DNA permits more extensive *Cyclospora* research leading to more sensitive and selective detection tools.
   - ***Think your equipment is clean and sanitized? Biofilm formation on equipment represents an important challenge in controlling Listeria risks.*** Rough, porous surfaces on harvesting, packing or processing equipment offer *Listeria monocytogenes* (Lm) and other microorganisms niches to reside in and deposit biofilms that permit them to survive inadequate cleaning and sanitation and even grow in production environments. A study of Lm and biofilm control conducted in seven peach packinghouses revealed that in some of the facilities, lines washed and sanitized right after production ended and left till start-up the next morning surprisingly had *higher* aerobic plate counts than

what was found during the production shift [*Dawson 2019*]. Evidently, bacteria grew overnight on the "sanitized" equipment, meaning that the equipment really had not been cleaned sufficiently to remove either the bacteria or the organic materials that can fuel growth. In other words, you cannot sanitize equipment that has not been thoroughly and properly cleaned. The data also show that on "rough" surfaces (e.g., brushes, sponges, plastics) biofilms can form overnight and protect to the extent that viable bacteria "hidden" within the biofilm can be recovered even after 25 minutes of exposure to 200 ppm sodium hypochlorite. These results should stimulate reconsideration of the timing of cleaning and sanitation relative to restarting lines. Currently, the best management practice is to rigorously, frequently and consistently clean equipment and production environments with detergents and agitation to eliminate organic residues and thoroughly sanitize surfaces, permitting optimal time for exposure to the labelled, lethal concentrations of sanitizer to ensure Lm and other bacteria (and viruses) are killed. It is important to validate the efficacy of your cleaning and sanitation strategies.

- ***A new tool to permit more effective temperature control.*** Think you are cooling your products properly? You might want to think again as surface temperatures are not always indicative of core produce temperatures. Low cost ($450-700) infrared cameras integrated into a cell phone permit reliable, non-contact, non-invasive, real-time measurement of core and surface product temperatures [*Mis Solval 2020*] to help control spoilage and pathogen growth. Infrared cameras can also enable identification of "hot spots" and permit better temperature control in distribution facilities.

2. **Results that reinforce current best practices.** Building knowledge is a continuous process. The Symposium featured projects that built upon our knowledge base to provide opportunities to fine-tune best practices:
   - ***Hazard analysis and risk assessment are currently your best tools for managing Cyclospora.*** Surveys of open water sources in California and Florida yielded about 10-percent presumptive *Cyclospora* positives. Follow on confirmation testing indicated these samples were largely genetically related species of *Eimeria* and *Isospora [Ortega 2019]*. Similar water sampling in Georgia yielded forty-seven presumptive *Cyclospora* positives yet confirmation testing showed these to be false positives [*Mattioli 2019*]. Earlier CPS projects surveyed open irrigation water sources in the U.S. Southwest and found low levels of *Cyclospora* [*Lopez 2018*]. Though there are a number of hypotheses, our knowledge base is limited as to how *Cyclospora* moves into our production environments. Currently, our best tool for managing *Cyclospora* is a thorough understanding of potential human fecal contamination hazards and risks of transfer to water sources used to grow, harvest, pack and cool fresh produce. In other words, "know your water sources" and manage human-to-product transfer via adherence to good management practices (GMPs).

- ***Pay attention to your use of quat-based sanitizers.*** FDA has suggested operators rotate sanitizers to prevent pathogens from becoming tolerant to common quat-based sanitizers. An earlier CPS project suggested the development of genetically heritable tolerance is unlikely [*Wiedmann 2019*]. A new research project [*Deng 2019*] examined 25,000 strains of Lm isolated in U.S. food processing operations for the bcrABC gene which confers increased tolerance to quat-based sanitizers (benzalkonium chloride). 94.6-percent of the isolates were found to harbor the bcrABC gene. However, Lm strains isolated from produce environments harbor the bcrABC gene less frequently. Importantly, genetic tolerance to quat-based sanitizers can only be developed if the bacteria have "fuel" (carbon and nitrogen sources) to support growth and are subject to sublethal concentrations of sanitizer for a period of time (as little as 24 hours). Therefore, consistent, and thorough cleaning to eliminate residual organic materials that support Lm growth, the use of properly titrated quat-based sanitizers (as dictated by the label) at manufacturer-recommended label concentrations, and trained sanitation workers that ensure proper, consistent use make quat resistance unlikely.
- ***Effective use of wash water disinfectants can help you control the risks represented by illness-causing human enteric viruses***. The MS2 bacteriophage shares characteristics of the human enteric hepatitis A virus and can be used as a surrogate to study the efficacy of preventive controls to mitigate cross contamination by enteric viruses in produce wash water systems [*Sánchez 2019*]. Research demonstrates that commonly employed wash water practices, a one-minute contact time with 5-20 ppm sodium hypochlorite or 2-3 ppm chlorine dioxide, is sufficient to control enteric viruses.

3. **Concepts that bear watching**. It is always important to be on the lookout for emerging concepts that could improve efficiencies, reduce costs, and protect your product safety. The 2022 Symposium featured new concepts addressing an array of current industry challenges:
   - ***Help may be on the way***. Packinghouses and processing operations often rely on electronic equipment (e.g., optical sorters, scales, metal detectors and electric motors) that are sensitive to cleaning and sanitation chemicals and water. LED antimicrobial blue light (aBL), wavelength 405 nm, can be used to achieve a 100 to 10,000-fold reduction of Lm on otherwise difficult to clean and sanitize surfaces [*Diez-Gonzalez 2020*]. Antimicrobial efficacy can be optimized by adjustments to distances between the light source and the target and to exposure times. Knowledge gaps remain regarding the optimization of light intensities versus heat generation and product quality impacts, but this new approach bears monitoring.
   - ***Using viruses to kill bacteria: encouraging results for a natural approach to managing Lm***. Bacteriophages are like viruses that infect and kill bacterial cells. A commercially available cocktail of bacteriophages, Listex^{TM}, consistently achieved a 100-fold reduction of Lm in produce under laboratory conditions. It was also demonstrated that applications of sufficient doses of phages (10^6 to 10^7) through water sprays were achievable in commercial operations [*Allende 2019*]. Listex is approved for use in the

U.S. as a processing aid, and an organic version of the product is also available. The use of bacteriophage cocktails may be a valuable tool going forward to reduce Lm on products susceptible to supporting postharvest Lm growth [*Schaffner 2019* *and* *Strawn 2018*]. Further work on performance consistency, product delivery and measurement of quality effects remains.

- ***Lm growth on produce - you just knew there would be an app someday***. ListRisk is an app being developed for leafy greens that will help operators identify factors contributing to postharvest growth of Lm [*Allende 2019*]. The model is being constructed with over 600 data points and uses temperature, pH, water activity and other factors to predict growth potential for Lm on leafy greens.
- ***Ultrafine bubble technology may hold the key for more effective wash water sanitation***. Ultra-fine bubble technology may be a mechanism for delivering wash water sanitizers more effectively in wash systems. Research using ozone in combination with ultrafine bubble technology demonstrated promise for industry use [*Upadhyay 2020*]. Ultrafine ozone-containing bubbles reduced Lm levels by 10 to 250-fold on the surface of lettuce, apples, and celery with exposure times of one to two minutes at 4°C without damaging product quality. This proof-of-concept result merits monitoring as knowledge is gained, e.g., temperature versus stability, capacity to produce ultrafine bubbles, costs, etc. in various wash systems.
- **Cold plasma to disinfect wash water?** Remember the four states of matter – solid, liquid, gas and plasma? Bet you missed the plasma state, didn't you? Think of solid ice. When heated, the solid turns to a liquid (water). Applying more heat, water turns into steam, a gas. If even more heat is applied to the gas, a plasma forms which is a highly reactive state consisting of high temperature electrons, ultraviolet photons, cooler charged and neutral atoms and other reactive chemicals that have antimicrobial properties. Research was presented that broadly outlined the potential for cold plasma wash water control [*Fridman 2019*]. Treatments produced up to 10,000-fold reduction of inoculated bacteria in simulated wash water mixtures and 10 to 100-fold reductions of inoculated bacteria on the surface of selected commodities. Early in development, much needs to be learned regarding water treatment capacity, modes of action (e.g., low pH versus reactive chemistries), performance in actual industry wash water chemistries and costs of the technology on a per pound basis.

Acknowledgements*: The Center for Produce Safety thanks all the dedicated researchers that presented their project results, the session moderators, breakout discussion leaders and the students who helped in various capacities to make event logistics efficient. More detail on the research projects can be found at www.centerforproducesafety.org. This Executive Summary document is meant to inform and provoke thought, with an eye towards inspiring industry senior executives to examine their own company's produce safety programs and to use this research to generate discussions with their own produce safety team to meet the objective of driving science and risk-based improvements. It is not meant as a directive on what must be done to produce safe food. If you have additional questions, please feel free to contact Bonnie Fernandez-Fenaroli [info@centerforproducesafety.org]. Thank you.*