

## General Interest

# Guidelines To Validate Control of Cross-Contamination during Washing of Fresh-Cut Leafy Vegetables

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MS 16-258: Received 24 June 2016/Accepted 23 September 2016/Published Online 24 January 2017

## ABSTRACT

The U.S. Food and Drug Administration requires food processors to implement and validate processes that will result in significantly minimizing or preventing the occurrence of hazards that are reasonably foreseeable in food production. During production of fresh-cut leafy vegetables, microbial contamination that may be present on the product can spread throughout the production batch when the product is washed, thus increasing the risk of illnesses. The use of antimicrobials in the wash water is a critical step in preventing such water-mediated cross-contamination; however, many factors can affect antimicrobial efficacy in the production of fresh-cut leafy vegetables, and the procedures for validating this key preventive control have not been articulated. Producers may consider three options for validating antimicrobial washing as a preventive control for cross-contamination. Option 1 involves the use of a surrogate for the microbial hazard and the demonstration that cross-contamination is prevented by the antimicrobial wash. Option 2 involves the use of antimicrobial sensors and the demonstration that a critical antimicrobial level is maintained during worst-case operating conditions. Option 3 validates the placement of the sensors in the processing equipment with the demonstration that a critical antimicrobial level is maintained at all locations, regardless of operating conditions. These validation options developed for fresh-cut leafy vegetables may serve as examples for validating processes that prevent cross-contamination during washing of other fresh produce commodities.

Key words: Antimicrobial wash; Cross-contamination; Fresh-cut produce; Leafy vegetables; Preventive controls; Validation

Water is extensively used in postharvest processing of fresh produce to cool, hydrate, clean, and transport product. However, if the water becomes contaminated with microbial pathogens, water can contaminate the produce. Antimicrobial chemicals added to the wash water can help to control microbial hazards, but the chemicals must be maintained in sufficient amounts. If not, the water can be a means of spreading microbial contamination in the production batch (18, 26, 30, 38, 58).

The purpose of this article is to examine the various factors and considerations for ensuring the effectiveness of antimicrobial washes, with an emphasis on developing practical guidelines for validation. The focus of the guidelines will be on fresh-cut leafy vegetables, as they are defined in the Arizona and California Leafy Greens Marketing Agreements (1, 8), including iceberg lettuce,

romaine lettuce, green leaf lettuce, red leaf lettuce, butter lettuce, baby leaf lettuce (i.e., immature lettuce or leafy greens), escarole, endive, spring mix, spinach, cabbage (green, red, and savoy), kale, arugula, and chard. It is anticipated that the guidelines can serve as an example for validating prevention of cross-contamination during washing of other fresh produce commodities.

Fresh-cut leafy vegetables have been linked to disease outbreaks resulting from infections with bacterial, viral, and protozoan pathogens (9, 56). The 2011 Food Safety Modernization Act (FSMA) authorized the U.S. Food and Drug Administration (FDA) to issue regulations for food producers that would require establishment of preventive controls for potential food safety hazards in their products (21 U.S. Code §350g).

As directed under FSMA, the FDA has issued regulations, two sections of which are “Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption” (50) and “Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food” (49). The first section, referred to as the “Produce Rule,” applies primarily to raw agricultural

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commodities that are not further processed and that may be eaten raw. The second section, referred to as the “Preventive Controls Rule,” applies to produce that is cut, peeled, or otherwise processed. Preventive controls apply to facilities but not to farms and some kinds of on-site packing operations. The preventive controls regulations must be implemented so that identified hazards are prevented or significantly minimized (21 CFR §117.135), and the preventive controls must be validated, based on scientific and technical information (21 CFR §117.160), to demonstrate that they do, in fact, prevent or significantly minimize the identified hazard. During production of fresh-cut leafy vegetables, microbial contamination that may be present on the produce can spread throughout the production batch when the product is washed, thus increasing the risk of illnesses. The use of antimicrobials in the wash water is a critical step in preventing such water-mediated cross-contamination; however, many factors can affect antimicrobial efficacy in the production of fresh-cut leafy vegetables, and the procedures for validating this key preventive control have not been articulated.

The risks from these microbial hazards need to be minimized to ensure delivery of safe products to consumers. It is essential that good agricultural practices are followed during growing and harvesting (48, 50), good manufacturing practices are followed in food production operations (49), and good hygiene practices are followed by handlers of fresh produce (17), and that facilities establish robust cleaning and sanitizing programs to prevent the introduction of microorganisms of human health concern onto raw and processed products.

A great deal of work has been devoted to controlling hazards in fresh-cut produce, and although data gaps remain, much has been learned and implemented toward improving processes for ensuring fresh-cut product safety, particularly with regard to the use of antimicrobials in wash water.

Although some antimicrobials can significantly reduce pathogen populations on the surface of fresh produce (36), if the product is contaminated, available antimicrobials are not always effective for eliminating microbial pathogens on the product (18, 20, 34, 35). Furthermore, antimicrobial treatments are less effective in reducing pathogens on leafy greens than on nonleafy vegetables (36). Antimicrobials are most effectively used to prevent transfer of microbial pathogens via wash water to noncontaminated produce, i.e., for preventing cross-contamination through water; however, the antimicrobials must be present in sufficient amounts to be effective. For chlorine, the most widely used antimicrobial in fresh-cut produce processing (20, 34, 35), maintaining an effective concentration can be challenging because the presence of organic matter reduces the availability of the active form of the chemical. Other factors, discussed below, may also have an impact on an antimicrobial’s efficacy. Demonstrating that the antimicrobial level in use is effective in preventing cross-contamination is the critical task to be accomplished through validation of this key preventive control.

#### ANTIMICROBIAL CHEMICALS IN FRESH-CUT PRODUCE WASHING PROCESSES

Antimicrobial chemicals often used in fresh-cut produce washing processes include chlorine (as sodium or calcium

hypochlorite), peracetic acid, chlorine dioxide, and ozone. Chlorine is the antimicrobial chemical most commonly used in fresh produce washing processes. Several reviews have summarized work on the effectiveness of antimicrobial chemicals and other treatments for controlling various pathogens in wash water and on various types of produce (18, 20, 34, 35, 56).

**Factors to consider in selecting antimicrobial chemicals.** The selection of an antimicrobial chemical for use in fresh-cut washing processes is based on several considerations. The antimicrobial chemical should have a broad spectrum of effectiveness against the microbial hazards that are reasonably foreseeable in the product. It must have the required regulatory approval and, if appropriate, must be in compliance with the U.S. Department of Agriculture’s National Organic Program standards (44). In addition to effectiveness and regulatory status, other considerations for antimicrobial selection may include (a) antimicrobial stability, (b) quality and sensory effects on the product, (c) worker safety and OSHA compliance, (d) corrosion effects on equipment, (e) waste water treatment, and (f) environmental impact. Table 1 compares several characteristics of commonly used antimicrobials for post-harvest washing of produce.

**Regulatory overview for antimicrobial chemicals used in the produce industry.** Antimicrobial substances can be regulated by the Environmental Protection Agency (EPA), FDA, or both agencies. Oversight is dependent upon the intended purpose of the antimicrobial agent:

- a. Control of microorganisms *in wash water*. When used to control microorganisms in the wash water, products are regulated by the EPA. However, if the facility further processes food, FDA clearance is also required.
- b. Control of microorganisms *on the food surface*. In general, the control of microorganisms on food surfaces is regulated by EPA for raw agricultural commodities (RACs) and by FDA for processed foods.

If the antimicrobial agent is used to control microorganisms in wash water in which only RACs are handled, EPA has jurisdiction. However, if the facility further processes food, FDA clearance is also required independent of whether the food treated is a RAC or whether it will be further processed. Figure 1 can be used as a guide to better understand regulatory jurisdiction for wash water antimicrobials. Full guidance documentation is available at the FDA Web site (46).

A limited number of antimicrobial agents have been approved for contact with fruits and vegetables (51, 52). When an agent is approved by the EPA, then a use-tolerance, or exemption from the requirement of tolerance, is listed in 40 CFR 180 (45). Others may be added at a later time. Antimicrobial agents may also be generally recognized as safe or may be cleared via food-contact notification. It is a violation of federal law to use these products in a manner that is inconsistent with their labels.

TABLE 1. Comparison of commonly used antimicrobial agents<sup>a</sup>

Key attributes	Hypochlorite	Peracetic acid	Ozone	Chlorine dioxide
Final rinse with potable water required	Yes	No <sup>b</sup>	No	Yes
pH must be controlled	Yes	No	No	No
Organic load tolerance	Very sensitive	Less sensitive	Very sensitive	Less sensitive
Off-gassing hazard potential	Yes at low pH	No	Yes	Yes
Approved for use in wash water for organic produce	See NOP <sup>c</sup>	Yes	Yes	See NOP
Mechanism of action	Oxidizer, metabolic poison	Oxidizer	Oxidizer	Oxidizer

<sup>a</sup> Always follow label instructions. Similar chemistries may have different claims or use requirements, depending on the product.

<sup>b</sup> A final rinse is not required when usage does not exceed 80 ppm in wash water.

<sup>c</sup> National Organic Program (44).

## PERFORMANCE OF WASHING PROCESSES

The produce washing process is affected by various physical and chemical factors. Many, if not all, can influence the risk of cross-contamination.

**Understanding cross-contamination.** Theoretically, cross-contamination can be mediated by water, by particles in the water, or by product-to-product contact. In water-mediated cross-contamination, it is thought that pathogenic microorganisms are washed off the surfaces of contaminated leaves, are transferred through the water, and then become attached to other leaves. A sufficient level and activity of antimicrobials in the water at all times and at all points of the wash system will reduce the risk of this form of cross-contamination. In particle-mediated cross-contamination, it is thought that small particles that harbor the contamination can transfer through the water and then attach to and contaminate leaves. The effectiveness of antimicrobials on these small particles may depend on the amount, size, and type of particle, as well as on time of contact with the antimicrobial. It is thought that wash water antimicrobials may be less effective in preventing particle-mediated cross-contamination. Product-to-product contact may also be a

potential mode of cross-contamination, perhaps promoted by high product loads in the wash system.

Using leafy greens as an example, cross-contamination can result from pathogen transfer from a contaminated leaf to an uncontaminated leaf through the wash water. To prevent it, antimicrobials must rapidly inactivate pathogens present in the wash water, before the pathogens can contaminate other leaves. The longer the pathogen survives in the wash water, the higher the probability that cross-contamination will occur. Pathogen survival, inactivation, and the potential for cross-contamination through water are affected by a number of factors, including pathogen type, population size, and physiological status; antimicrobial type, concentration, and activity; environmental conditions (e.g., pH); and other operating conditions, such as water temperature and turbulence. The hypothetical speed at which a pathogen can move from one leaf to another is affected by the agitation of the wash water (determined by equipment design), by the distance between the leaves, and perhaps by other factors that are not currently understood. It is important to avoid overloading the washing system with too much product, because overloading can decrease the distance between leaves, reduce the contact time of the antimicrobial, increase the frequency of direct contact

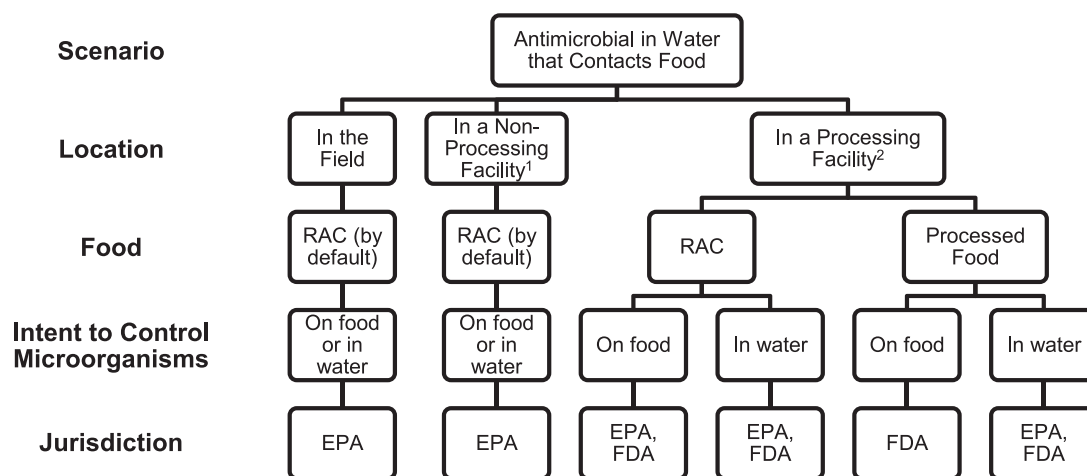


FIGURE 1. U.S. regulatory oversight of antimicrobials for control of microorganisms (46). <sup>1</sup> A place where RACs (raw agricultural commodities) are the ONLY food treated and the antimicrobial treatment activity does not change the status of the food as a RAC (e.g., washing). <sup>2</sup> A place where any of the following are happening: canning, freezing, cooking, pasteurizing, homogenizing, irradiation, milling, grinding, chopping, slicing, cutting, or peeling. Figure created by Ecolab, Inc. Please consult a regulatory representative to ensure product use compliance.



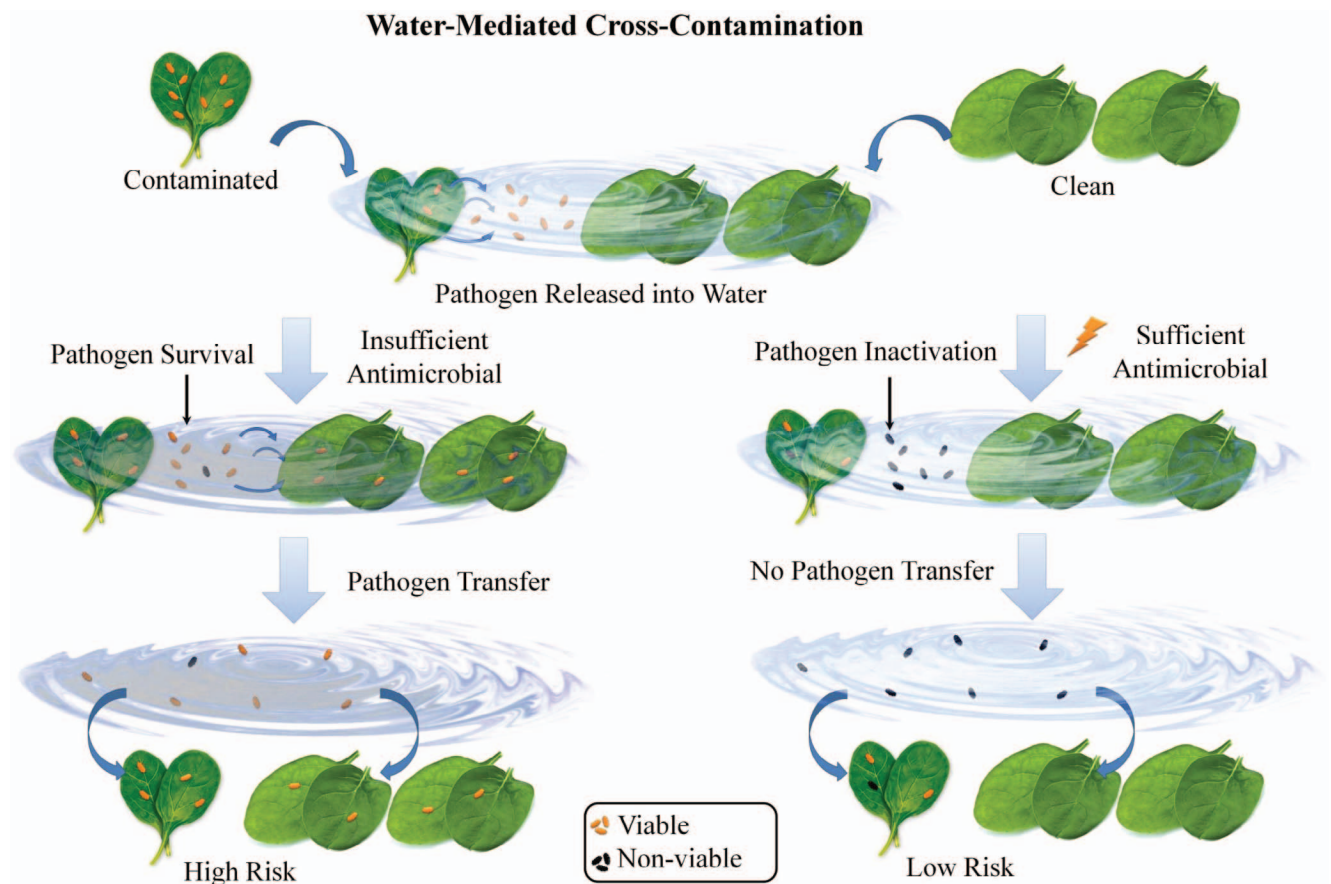


FIGURE 2. Illustration of microbial cross-contamination risk during washing of leafy vegetables. Contaminated leaves release pathogens into the wash water. With insufficient level of antimicrobial agent (left side of illustration), pathogens remain viable, survive, and are transferred to clean leaves. With sufficient level of antimicrobial agent (right side of illustration), pathogens are inactivated in the wash water, thus preventing cross-contamination to the clean leaves. Figure by Y. Luo and B. Zhang.

between leaves, and create localized areas of reduced antimicrobial concentration. All these can lead to an increased risk of cross-contamination.

In Figure 2, the risk of cross-contamination is compared in two scenarios, involving insufficient and sufficient levels of an antimicrobial chemical during washing of leafy vegetables. Contaminated leaves that may be present in the production batch can release microbial cells into the wash water. In the presence of an insufficient level of an antimicrobial agent, microbial pathogens in the wash water can remain viable and can be transferred to uncontaminated (clean) leaves. Although the production batch initially contained only a small proportion of contaminated leaves, the use of an insufficient level of antimicrobial agent in the wash water led to cross-contamination, a larger proportion of leaves carrying the pathogen, and a higher risk of illnesses. In the presence of a sufficient level of antimicrobial agent, microbial cells that are released from the contaminated leaves are inactivated in the wash water, thus preventing cross-contamination of the clean leaves. The presence of a sufficient level of antimicrobial agent also can reduce the number of viable microbial cells on the contaminated leaves, even though not all of them may be inactivated by the action of the antimicrobial agent. The combination of pathogen inactivation on the leaves, along with the prevention of

cross-contamination, results in a lower risk of illnesses. Of these two, the prevention of cross-contamination is the more important role of the antimicrobial agent in the wash water.

**Factors affecting antimicrobial efficacy.** The physicochemical characteristics of the wash water must be understood and maintained appropriately with respect to the specific antimicrobial chemical selected. Table 1 shows the relative sensitivity of several antimicrobials to pH and organic load conditions in the water. Although these physicochemical conditions may impact the efficacy of the different antimicrobials to varying extents, there are two factors independent of the wash water conditions that influence pathogen inactivation: the concentration of antimicrobial and its contact time with the pathogen. Both need to be considered simultaneously in the context of the processing operation to achieve optimal antimicrobial efficacy. Preventing pathogen cross-contamination during produce washing requires an antimicrobial concentration that inactivates pathogens as quickly as possible.

A common way to express antimicrobial effectiveness is the "CT value," where *C* is the antimicrobial concentration (in ppm) and *T* is the contact time (in minutes).

Thus, a CT value of 10 could be derived by exposure of the pathogen to an antimicrobial concentration of 10 ppm for

1 min, 1 ppm for 10 min, 2 ppm for 5 min, and so on. Pathogens can be compared for their sensitivity to a given antimicrobial treatment by their *CT* values. The *CT* values for inactivating waterborne pathogens have been used for many years to guide recommendations for waters for drinking and recreation (10).

Nevertheless, for fresh-cut produce, there are other characteristics of the process wash water that will impact antimicrobial effectiveness. Therefore, data relevant to disinfection of drinking or highly filtered recreational waters (swimming pools, water-theme parks, etc.) may not be applicable to produce wash water. Whereas antimicrobials are added to recreational waters to maintain them as pathogen-free—and so may have minutes or longer to react with contaminants—the primary purpose of antimicrobials in produce wash water is to prevent cross-contamination, which can occur in a much faster, near instantaneous time frame. Because the time required to prevent cross-contamination is measured in seconds or less, the effectiveness of antimicrobials added to produce wash water is almost entirely dependent on concentration.

It is difficult to recommend minimum antimicrobial concentrations that can be universally effective for all leafy greens operations and facilities, owing to the diversity of operating conditions and equipment configurations. Validation, tailored to specific wash systems and conditions, needs to be done to determine these concentrations.

The concentration of an antimicrobial agent (especially free chlorine), if not sufficiently replenished, usually declines rapidly in a fresh-cut vegetable washing operation as a result of its reaction with soluble organic materials present in the wash water. Major factors that contribute to the organic load in the wash water include the organic matter present in the dirt and soils, on the vegetable surface, as well as organic materials released from the cut edges or damaged areas. The decline in efficacy of a given antimicrobial agent at a particular concentration due to reactions with organic material is not the same for different types of antimicrobials, as listed in Table 1. Peracids will remain stable under conditions of high organic load, whereas ozone and sodium or calcium hypochlorite are more susceptible to degradation under similar conditions (58). See Appendices A and B for more specific considerations relevant to the use of chlorine and peracetic acid, respectively, as wash system antimicrobials.

Some of the operating conditions in a leafy greens facility that may influence the efficacy of the produce washing system to prevent cross-contamination include (a) antimicrobial type, (b) antimicrobial concentration, (c) pH, (d) water mineral hardness, (e) insoluble solids (particles), (f) soluble solids (such as leaf exudates and minerals from soil), (g) product type, (h) product quality, (i) product to water ratio, (j) cut size, (k) blade conditions, configuration, speed, (l) filtration, (m) temperature, (n) agitation speed, (o) submersion of product, (p) rate of water replenishment, (q) rate of antimicrobial addition, (r) variability of these conditions during the washing process, and (s) antimicrobial monitoring system.

## PROCESS VALIDATION FOR PREVENTIVE CONTROLS

Preventive controls should be properly implemented using approaches involving *process validation, monitoring, and verification*. Abundant information is available on these approaches (11, 23, 31), and a validation and verification framework relevant to the FSMA regulations has been developed (6).

**Process validation.** As described in the FDA regulation (49), validation is defined as obtaining and evaluating scientific and technical evidence that a control measure, combination of control measures, or the food safety plan as a whole, when properly implemented, is capable of effectively controlling the identified hazards. In an approach that has been thoroughly developed over many years for thermally processed foods (25, 32, 37), validation steps generally include the following:

1. identification of the target pathogen (typically the pathogen(s) considered reasonably likely to be in the raw material and with the greatest level of resistance to the treatment);
2. definition of the process to be validated, including identification of all critical factors affecting the efficacy of the process and all “worst-case” conditions (operational extremes within which the process is still acceptable) and limits for each of the critical factors within which the facility intends to operate the process;
3. identification of performance standards that the process must achieve, e.g., level of inactivation;
4. identification of an appropriate surrogate organism (i.e., one that has known resistance properties to the process that are commensurate with the resistance of the target pathogen) to be used in microbial inoculation studies;
5. design and performance of microbial inoculation studies under the worst-case conditions to determine the performance of the process to control the organism of concern; and
6. demonstration through the results of the microbial inoculation studies that the process, when operated at the worst-case conditions or limits, will meet the performance standards.

This approach, which has worked well for validating thermal processes for foods, can be challenging for fresh-cut leafy greens operations that use wash water. Some of the obstacles to validation include

- a. lack of a kill step in fresh-cut leafy greens washing processes, unlike in thermal processing;
- b. inability to introduce the target pathogen into the processing environment to perform the microbial inoculation validation studies;
- c. lack of surrogates known to demonstrate behavior in washing systems similar to target pathogens;
- d. the cost of performing microbial inoculation studies;
- e. uniqueness of wash water systems to each facility, with wide variability in process conditions, performance, and operational worst-case conditions, which limits the ability

to perform microbial inoculation studies outside of the operation; and

- f. the difficulty in replicating variability that the wash system can experience in a production day or over time.

Nevertheless, when designing a validation study for fresh-cut leafy greens, the focus should be not only on the level of the antimicrobial agent but also on any other factors that may affect its efficacy. Performance standards for validation may be based on different goals, for example, the absence of cross-contamination during washing, a log-reduction level in the wash water, or a log-reduction level on the product. The ultimate goal for an effective wash water system to achieve is the prevention of cross-contamination consistently at all points throughout the wash system. The anticipated changes in the physical and chemical composition of wash water and their potential impacts on antimicrobial efficacy must be understood. It is also necessary to define the target operating limits around the variables that will likely change during the process and identify the worst-case conditions.

Alternatives to the thermal process validation approach described above can provide useful options for wash water validation as well. For example, if a minimum acceptable temperature for a thermally processed canned product is known, then, instead of using surrogate microorganisms to demonstrate adequate lethality, temperature probes can be placed throughout the can to validate the process conditions that achieve the minimum acceptable temperature at the “cold spot” position in the can, i.e., the location that heats at the slowest rate. A similar alternative is to validate the position of the cold spot in the container and, in every production run, monitor the temperature at the cold spot during the thermal process until the minimum acceptable temperature is reached. Both options avoid the use of surrogates, but they require knowledge of the minimum acceptable temperature that the product must reach. The former is more useful in products and processes that are well established with little variability. The latter is particularly useful for products and thermal processes that have wide variability, batch to batch, in composition and operating conditions. For wash water validation, such alternative options would involve validating the point(s) in the washing process where antimicrobial levels are lowest and most vulnerable to cross-contamination.

**Fixed and variable conditions for validation of antimicrobial washes for leafy greens.** In any process, there are conditions that are fixed and those that are variable. For leafy greens washing systems, both fixed and variable conditions can affect the ability of the antimicrobial agent to prevent cross-contamination.

In a wash water flume system, conditions that are fixed generally include the washing equipment, the antimicrobial agent in use, the source of water, and the product type, e.g., variety of leafy vegetable, such as iceberg lettuce, cabbage, and curly spinach. Other conditions that may be fixed include additional water chemicals, water temperature, water flow rate, water filtration (i.e., equipment to remove

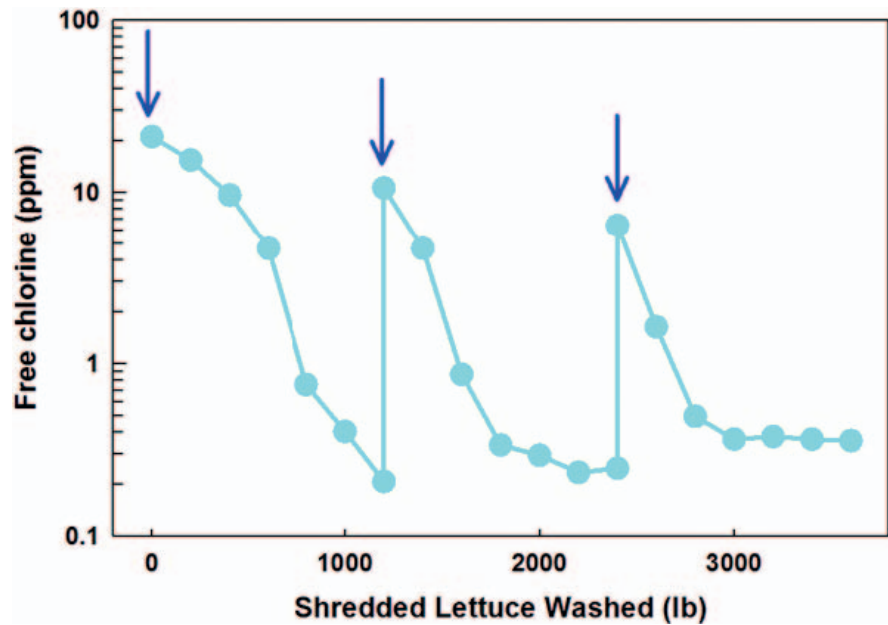
particulates from the water during operation), agitation rates, antimicrobial injection points, and the antimicrobial monitoring system. As long as these remain fixed and unchanging, they need not be considered further in the validation but do need to be documented. If these factors may change in the operation being validated, then they need to be included as variables that require testing in worst-case conditions. Any changes to fixed conditions during processing will need to be considered as to whether the system is still running within the parameters originally validated.

Conditions that are variable and controllable generally include, but are not limited to, product feed rate, water pH, water replacement rate, the product to water ratio, organic and mineral load, solids level, antimicrobial feed rate, and type of process applied to the product (e.g., chop, shred, and cut). There may be others. It is important to understand how these conditions affect the ability of the process to prevent cross-contamination and to understand the “worst” that these conditions can be in an acceptable production run. For example, published studies have reported the dynamic nature of chlorine concentrations that can exist in wash water (30, 59, 60). Furthermore, in a chlorine-based process, greater product feed rates are worse than lesser feed rates; higher pH is worse than lower; higher organic load is worse than lower; and so on, in considering each of the variable conditions for the system. Before performing a validation, therefore, it is important to know the worst that each of these conditions can be when the process is running, i.e., the conditions that create the greatest “challenge” for the ability of the antimicrobial to kill pathogens. This situation is complex and dynamic and can be different in different wash systems. The worst operating conditions may include all variables or a few of the critical variables at the same time. It may include the maximum capacity recommended by the equipment manufacturer, for each type of product. It is when the wash system is pushed to its acceptable limits during production, i.e., when the wash system is operating at the upper limit of all or some of its critical variables that affect antimicrobial effectiveness. If any of these conditions can occur at levels that defeat the ability of the process to prevent cross-contamination, then “critical limits” for the conditions must be set. These critical limits are the conditions beyond which the process is not allowed to run. Monitoring for these conditions must be readily and reliably performed. If the critical limits are exceeded, then corrective action must be taken.

Antimicrobial feed rate is an important variable and can be the source of the greatest process variability. Recent advances in wash water control systems have improved antimicrobial monitoring and dosing to reduce variability and minimize “spikes” (high levels) and “dips” (low levels) in antimicrobial levels. However, manual dosing and older automated systems are still in use, and wash water can experience wide swings in antimicrobial levels (see Fig. 3, showing variation in free chlorine level during processing). This can lead to a false “success” in a validation trial, particularly in validation option 1 (see below), if samples are collected during an antimicrobial spike and not during a dip. Validations of processes that are expected to have wide



FIGURE 3. Changes in free chlorine level that can occur during a leafy greens washing process. Arrows show points of dosing with additional chlorine during a processing run (Y.L., unpublished data).



variability in antimicrobial levels during normal operation should collect a sufficient number of samples to ensure that product exposed to such dips is targeted or at least included.

During normal operation, wash systems are usually dynamic. That is, at any given moment and at any position in the wash system, several variables will be at different concentrations or ratios than at other times or positions. Just as there are spikes and dips in antimicrobial concentration, there are likely localized highs and lows in, for example, product-to-water ratio, product flow rate and agitation, and turbidity and nonproduce solids. These must be considered during design of the validation study. Avoidable variability should be minimized during a validation trial, to provide better data on conditions under which cross-contamination can and cannot be prevented. However, some variability is expected to be unavoidable, and it can be accounted for by performing several validation trials (typically three), close monitoring of these dynamic variables, and collecting a sufficient number of samples or sensor readings during the trial (depending on the validation option selected, see below).

**Validation options.** Although information is available on the various considerations in wash water processes for preventing cross-contamination, there are knowledge gaps. For fresh-cut leafy greens, the target pathogen is likely *Salmonella*, pathogenic *Escherichia coli*, or *Listeria monocytogenes*, but others could be targets. The appropriate target organism and the level to which it must be controlled can be commodity-specific or can be driven by information specific to a region. Differences in antimicrobial resistance may be exhibited by different strains of pathogens. The efficacy of antimicrobials commonly used in industry wash water systems to inactivate the common target pathogens, and the conditions under which the antimicrobials have regulatory approval to be used, are well established under optimal (i.e., laboratory or pilot plant) conditions, but their efficacy is not always well established in realistic, scaled-up conditions in leafy greens

operations. Finally, whereas the critical factors affecting antimicrobial efficacy are generally known, the level to which combinations of critical factors can improve or decrease antimicrobial efficacy may not be known in detail (for example, the combined effects of organic load, water hardness, and temperature).

Despite all of the knowledge gaps and caveats cited above, we propose that wash water process validations can be performed through any of three options of customization for a specific operation's process. The options are described using chlorine as an example, but the principles apply to other types of antimicrobials as well. These three options involve demonstrating the following:

- Option 1. Cross-contamination is prevented under worst-case operational conditions, as shown by using product inoculated with a suitable surrogate.
- Option 2. Minimum antimicrobial level is achieved under worst-case operational conditions, as shown by using antimicrobial sensors.
- Option 3. Minimum antimicrobial levels are maintained in each processing run, without considering worst-case conditions.

Each option requires specific knowledge about the product, the process, and the equipment being used and is further described below. Option 1 involves microbiological experiments to demonstrate that a surrogate microorganism is effectively prevented from cross-contaminating product. Options 2 and 3 involve a demonstration that the antimicrobial agent can be maintained at effective levels during the wash process. All of the validation options involve use of the actual process equipment in the specific facility. Practical guidelines and strategies that may be used to validate antimicrobial washing processes according to options 1, 2, and 3 are provided in Appendix C.

**Option 1: microbiological validation using a surrogate.** An option 1 validation is performed using a

nonpathogenic surrogate for the pathogen. The validation is done in-plant, with the specific antimicrobial agent and the actual washing equipment under worst-case operational conditions. It can only be done safely by an individual who is knowledgeable in designing such specific microbiological studies involving the product, who is knowledgeable about the specific equipment and wash process, and who is qualified to conduct the study. This option allows an operation to set its own critical limits, allowing greater flexibility in process conditions before corrective action must be taken.

In an option 1 validation, the product is inoculated with a suitable surrogate to demonstrate the ability of the antimicrobial to prevent cross-contamination in the wash system. Surrogates are described as “nonpathogenic proxies for the pathogen of concern that have similar or more robust survival capabilities under the conditions being studied” (33). Surrogates can be biological (for example, *Clostridium sporogenes* in canned food thermal process validation) or chemical (for example, phosphatase destruction in validation of milk pasteurization). A surrogate is considered suitable if its behavior, when exposed to the antimicrobial agent (e.g., chlorine) at levels and in conditions that will occur in the wash water, is predictably proportional to that of the target pathogen.

At this writing, a suitable surrogate for validating antimicrobial washes has not been identified. Transfer from contaminated leaves to wash water (58) and attachment to cleaned leaves (24) have been studied as relevant behaviors in the survival of *E. coli* O157:H7 in fresh produce processes. Characteristics for surrogate behavior that might be relevant in a cross-contamination event include inactivation by the antimicrobial agent, as well as ability to detach from and attach to leaves in a washing process (14). When such a surrogate has been identified, an option 1 validation could be performed by product inoculation, representing the worst-case pathogen contamination load reasonably likely to be introduced to the process. The inoculated product must be identifiable and distinguishable from uninoculated product (for an example, see (7)). This type of study could be conducted as follows.

- a. The inoculated product is fed into the beginning of the process, which is operating under worst-case conditions.
- b. Samples of uninoculated product in the vicinity of inoculated product are captured at the end of the wash process and are analyzed for presence and levels of the surrogate. Where the inoculated product is fed into the process and where the uninoculated product samples are collected establish the beginning and end, respectively, of the process to be validated.
- c. The process is run at the following levels of antimicrobial feed rate:
  - i. none, or some low level of antimicrobial agent that will allow cross-contamination (i.e., a positive microbiological control);
  - ii. a very high level of antimicrobial agent that will not allow cross-contamination (i.e., a negative microbiological control);

- iii. the target level of antimicrobial agent that is being validated;
- iv. and, potentially, some level of antimicrobial agent that is higher than the target, in case the target level is actually unable to prevent cross-contamination.

A successful validation trial would demonstrate that the surrogate is detectable on uninoculated product in the “positive control” and is not detectable on the uninoculated product in the “negative control” and at the target level. The facility should perform at least three successful replicates of the validation trials. See Appendix C for an example of an option 1 validation strategy.

**Option 2: antimicrobial sensor validation.** The option 2 validation is also performed in-plant with the actual washing system operated under the worst-case conditions. In this validation approach, antimicrobial sensors are used to demonstrate that the system is continually in control under the worst operational conditions. This approach requires knowledge of the chemical or condition that must be monitored, the minimum level of this chemical or condition that reliably prevents cross-contamination, and the location(s) in the washing equipment where that chemical or condition may be at its lowest when operating under worst-case conditions. The scientific literature, laboratory and/or pilot plant studies, or other technical information may be used as the basis for this minimum level (see summaries of current literature in Appendices A and B for chlorine and peracetic acid levels, respectively). In reference to a specific antimicrobial level to prevent cross-contamination in produce wash water, the facility would be required “to demonstrate that it can consistently maintain that concentration under operating conditions” (49).

In a chlorine-based system, the chemical that must be monitored is “free available chlorine” (see Appendix A). Sensors that can monitor free chlorine levels in real time, independent of pH, turbidity or chemical oxygen demand (COD), temperature, or other conditions are commercially available. As of the time of this writing, the minimum level of free available chlorine that reliably prevents cross-contamination for all processes cannot be stated. However, levels to consider may be obtained from the research literature (see Appendix A).

An option 2 validation could be performed as follows.

- a. Preliminary trials are performed under worst-case conditions (as described above), positioning the sensors at many positions in the wash system in order to map those locations where the free available chlorine levels are lowest during operation and where wash water is most vulnerable to allowing cross-contamination.
- b. The sensors are positioned at those locations during the validation that simulates actual production conditions with product.
- c. Under worst-case operating conditions including product in the wash system, the antimicrobial feed rate is slowly raised, and the free available chlorine levels at those



locations are monitored until they all reach the target minimum level.

Ideally, as in option 1, the validation trial should be repeated at least three times to account for run-to-run variability. A successful validation trial would demonstrate an antimicrobial feed rate that achieves the minimum acceptable antimicrobial level at all sensor positions in the wash system at all times and under the worst-case operating conditions. After successful validation, the facility would monitor the variable operational conditions that require control, including antimicrobial feed rate, ensuring that they do not go beyond their critical limits. See Appendix C for an example of an option 2 validation strategy.

In both options 1 and 2, the critical operational limits are based on all other conditions being at the worst acceptable operational conditions, perhaps including variable or controllable conditions that must be operated within certain limits. In normal operation, it is unlikely that all operational conditions will be at the extreme of their acceptable levels at the same time. Hence, the validated antimicrobial feed rates will likely be higher than absolutely necessary at any given moment. Operations need to consider whether a constant antimicrobial feed rate may lead to excessive levels (e.g., hyperchlorination), potentially leading to employee comfort and safety issues. However, for wash systems that have low variability during operation (e.g., produce feed rate and produce-water ratio), this validation approach, when all operational conditions are at their acceptable extremes, minimizes the complexity of performing multiple validation trials under a multitude of possible operational conditions.

**Option 3: validation of sensor placement for minimum antimicrobial level.** Like option 2, the option 3 approach requires knowledge of the chemical or condition that must be monitored, the minimum level of this chemical or condition that effectively and reliably prevents cross-contamination, and mapping the washing equipment to identify the location(s) where that chemical or condition may be at its lowest when operating. However, unlike option 2, it does not require knowledge or monitoring of the worst-case operational conditions. It may be the best option for systems with wide variability in antimicrobial demand, such as frequent high and low product feed rates or intermittent antimicrobial addition, where the potential for excess antimicrobial agent could lead to product quality issues or exceeding the limits of use in accordance with the CFR and/or EPA label instructions. As in option 2, the antimicrobial sensors are positioned at many locations in the wash system to find those locations where the antimicrobial levels are lowest during operation. This should be performed multiple times, under a variety of operational conditions, until the facility has confidence in knowing those locations. A successful validation trial would identify the location(s) where antimicrobial levels are lowest and must be monitored continuously during operation to ensure that the level does not drop below the critical limit when product is present. This provides the greatest flexibility in controlling the

antimicrobial feed rate, because the feed rate is monitored under actual conditions and does not assume that all conditions may be at their worst. However, this also requires an antimicrobial control system that can respond quickly to changing conditions, and this may pose the greatest risk of exceeding a critical limit if conditions change quickly. See Appendix C for an example of an option 3 validation strategy.

If it is not practical to position sensors at the locations of lowest antimicrobial concentration during normal operation, then the validation must be conducted with a sensor that will be used for monitoring and that will demonstrate a reading consistently proportional to the minimum acceptable antimicrobial level (critical limit) at the other locations. For example, if the lowest concentration of antimicrobial chemical is in a generally inaccessible or impractical location in the washing system, a “monitoring” sensor could be placed in a more accessible or practical location (e.g., at the exit of the system). During the validation, both sensors would be monitored and their readings compared. If the monitoring sensor provides a consistent, reliable indication of the readings at the inaccessible sensor, then the reading at the monitoring sensor when the inaccessible sensor is at the critical limit becomes the new critical limit during operation. For considerations on chlorine monitoring specifically, see Appendix A “Monitoring and Control of Effective Chlorine Levels.”

**Monitoring and verification of process controls.** Monitoring and verification activities should be tied to the validation parameters. For example, if a system was validated using option 1 or 2, where a minimum antimicrobial feed rate was validated under worst-case operational conditions, then only the antimicrobial feed rate needs to be monitored during normal production, to ensure that it does not drop below the validated rate, i.e., critical limit. If other variable conditions needed to be set at maximum or minimum critical limits during the validation trial (e.g., pH, water replacement rate, or product to water ratio), then those also must be monitored during normal operation, at a frequency sufficient to demonstrate that those critical limits are also not exceeded during washing. If a system was validated using option 3, where positioning of a sensor is validated to monitor the minimum level of antimicrobial in the system, then only the antimicrobial level at the sensor needs to be monitored.

If a system exceeds a critical limit during normal operation, corrective action must be taken. Because rewashing the produce is not a reliable corrective action, the system must be stopped and brought back under control, and the affected produce must be collected and destroyed or reconditioned by a reliable treatment, such as cooking. Although some fresh produce commodities may be diverted to a thermally processed product, the corrective action for leafy greens will usually be destruction. An operational limit, which is set at a level above the minimum level, is useful to ensure that the process does not go below the critical limit and avoids the need for corrective action.

Periodically, verification must be performed to ensure that the system is functioning as validated. Typical verification activities will include a review of monitoring records and calibration or calibration checks of instruments used to control or monitor the system, especially antimicrobial feed rate controls and antimicrobial sensors. In addition, it may be advisable that an independent measure be performed; for example, collecting water samples at or near the antimicrobial low spot (if the system was validated using option 2 or 3) and verifying that the antimicrobial concentration is as expected. Chemical or physical tests can be performed to verify that other variables, critical to proper functioning of the wash system, are performing as expected. All of these verification activities should be recorded and reviewed.

Microbiological testing is rarely useful as verification that cross-contamination is being prevented, but a simple test can be used to verify that the system has not outright failed. Because the antimicrobial level must be high enough to prevent cross-contamination from occurring, it must be able to kill exposed pathogens almost instantaneously. Consequently, there should be no pathogens in any water samples collected during normal operation. Rather than test for pathogens, which are not expected to occur on fresh produce except rarely, an operation may test a water sample for viable gram-negative organisms, e.g., coliforms or *Enterobacteriaceae*. These organisms are common on fresh produce, but as in the validation trials, wash water samples should not have detectable levels of these organisms. Total or aerobic plate counts may provide erroneous results, because they will detect bacterial spores, which are not expected to be completely killed by the antimicrobial agent during washing. More studies are needed to investigate the acceptability of various microbial groups as indicators of wash water quality in fresh-cut produce facilities. Although detection of viable gram-negative bacteria in the wash water suggests that the antimicrobial level is too low to prevent cross-contamination (if bacteria can survive in the water long enough to be detected, they can also cross-contaminate produce), the absence of these bacteria is not verification that cross-contamination is being prevented, which is why the validation protocols described in this article are still necessary.

#### DATA GAPS AND RESEARCH NEEDS

Although some information is available on the use of antimicrobial washing to prevent cross-contamination, a great deal more is needed. Some research questions in need of additional investigations include

- a. identification of surrogate microorganisms for validating antimicrobial washing systems;
- b. minimum antimicrobial concentrations that will reliably inactivate target pathogens in produce wash water quickly enough to prevent leaf-to-water-to-leaf cross-contamination;
- c. minimum antimicrobial concentration required to inactivate pathogens in produce wash water as impacted by organic loading;

- d. correlation between chlorine (or other antimicrobial chemical) demand and commonly used organic load measurements (e.g., COD, turbidity, conductivity) for typical wash processes;
- e. cost-effective strategies and approaches to reduce organic load and particulate matter during produce washing;
- f. considerations for validation of processes that use different types of antimicrobials;
- g. studies to evaluate physical agents (e.g., UV-C light, ultrasound) for treatment of wash water and approaches for their validation; and
- h. studies to address how validation of commercial produce washing processes and systems can be performed in a laboratory or a pilot plant.

#### CONCLUDING REMARKS

Producers of fresh-cut leafy greens have the responsibility under FSMA to have controls in place that will prevent or significantly minimize hazards that are reasonably foreseeable in their products. It is necessary to ensure the effectiveness of these washes to prevent cross-contamination and to limit the spread of contaminants that may be present in the production system.

Microbiological cross-contamination in wash systems has been documented, yet there is still much to learn about limiting its occurrence. With more research, the conditions that promote cross-contamination, as well as the procedures that limit it, will be better understood. This document has presented the current knowledge and knowledge gaps regarding the safe production of fresh-cut leafy vegetables, focusing on prevention of cross-contamination during the wash process and on practical guidelines toward validating antimicrobial washing as a preventive control. Although these guidelines represent current thinking from industry, academia, and government technical subject matter experts, they are not intended to be legally binding on either the government or the regulated industry. Each company must establish its own specific validation protocols to evaluate wash system performance and is responsible for the efficacy of those systems.

#### ACKNOWLEDGMENTS

This article has been written as a result of discussions of the Wash Water Validation Group, composed of contributors from industry, academia, and government. The authors thank the other members of the Wash Water Validation Group for their expertise and discussions, which were essential to developing the technical concepts in the paper: Joe Holt (Earthbound Farms), James Gorny (Produce Marketing Association), Steven Lange (Ecolab), Tony Banegas (Ready Pac), John Gurrissi and Courtney Parker (Chiquita Brands), Loys Larpin (Aqua Pulse Systems), Felice Arboisiere (Yum Brands), Robert Brackett (Illinois Institute of Technology), Trevor Suslow (University of California–Davis), Keith Wariner (University of Guelph), Keith Schneider (University of Florida), Vincent Hill (Centers for Disease Control and Prevention), Tong-Jen Fu, Crystal McKenna, David Ingram, John Larkin, Mickey Parish, and Mary Lou Tortorello (U.S. Food and Drug Administration). The Wash Water Validation Group thanks the following persons for reviewing the manuscript prior to submitting it for publication: Devon Zagory (Zagory and Associates), Glenn Black (U.S. Food and Drug Administration), Ginger Povenmire and Micah Fuson (Apio), Ronald Wesley and James Zeigler (Ready Pac). The authors thank Sam Van Haute and Imca Sampers (University of Gent) and Ana Allende and Mabel Gil (CEBAS-CSIC) for

expert technical opinions. Support from the Center for Produce Safety, Illinois Institute of Technology Institute for Food Safety and Health, and the United Fresh Produce Association is gratefully acknowledged. These guidelines represent current thinking from industry, academia, and government technical subject matter experts and are not intended to be legally binding on either the government or the regulated industry. The opinions expressed in this article are solely those of the authors and not of their respective agencies or institutions.

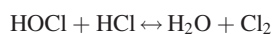
## APPENDIX A. USE OF CHLORINE IN WASHING PROCESSES

### UNDERSTANDING CHLORINE CHEMISTRY

Chlorine is a strong oxidizing agent and has powerful antimicrobial properties. It has been used for more than 100 years for disinfection of municipal water supplies (16). The commercially available forms of chlorine that are typically used in the produce industry are sodium hypochlorite and calcium hypochlorite (41). When added to pure water, they dissociate into two main chemical species: hypochlorous acid (HOCl) and hypochlorite ion ( $\text{OCl}^-$ ), as shown below in the reaction for sodium hypochlorite:



Although both HOCl and  $\text{OCl}^-$  have antimicrobial action, the effectiveness of HOCl is substantially greater than that of  $\text{OCl}^-$  (15). The proportion of the HOCl and  $\text{OCl}^-$  species existing in the water depends primarily on pH (see Fig. A1); therefore, it is critical to maintain the proper pH in order to achieve the greatest antimicrobial effectiveness. As illustrated in the figure, alkaline conditions ( $\text{pH} > 7$ ) cause  $\text{OCl}^-$  to predominate, whereas a pH below 7 shifts the balance toward HOCl. At very low pH, the chemistry favors formation of toxic  $\text{Cl}_2$  gas:



The pH of the produce wash solution needs to be maintained at appropriate levels to maximize the level of HOCl and achieve the highest antimicrobial efficacy, while maintaining a safe working environment. Chlorine chemistry in water is very complex, and proper understanding and handling is essential for ensuring not only effectiveness but also worker safety (13, 41, 42).

Chlorine can react with *inorganic* nitrogen-containing compounds such as ammonia to form chloramine compounds. Although the inorganic chloramines formed as a result have weaker antimicrobial effectiveness than HOCl, they have greater stability than HOCl; thus, the reaction has been used for the disinfection of drinking and recreational water supplies, where stability is an important consideration. Ammonia has been added purposely in some municipal water systems ("chloramination") to control the duration of effectiveness of chlorine (16). However, because chlorine levels are much higher in produce wash systems than in municipal water systems, and due to concerns about the human toxicity of chloramines, chloramination is not applicable to fresh produce wash water.

Chlorine can also react with *organic* nitrogen-containing compounds, for example, amino acids and proteins. Organic nitrogen compounds derived from soil and vegetable matter are likely to be present to various extents in leafy greens wash systems. Unlike the inorganic chloramines, these combined organic forms of chlorine (organic chloramines, or organochloramines) exhibit little

to no antimicrobial action. Organic nitrogen in wash water significantly reduces the antimicrobial action of the chlorine as a result of its conversion to organochloramine.

Different terms are used to describe the various forms of chlorine that can exist in wash water, and these terms can be confusing. Terms that describe chlorine in its *chemical* state are

- free chlorine—the sum of the concentrations of hypochlorous acid (HOCl), hypochlorite ion ( $\text{OCl}^-$ ), and dissolved chlorine gas present in water;
- combined chlorine—exists in combination with other molecules, e.g., inorganic and organic chloramines;
- total chlorine—the sum of free and combined chlorine.

Terms that describe chlorine in its *functional* state are

- available chlorine—any form of chlorine that has activity as an oxidizing agent, including free and combined forms;
- free available chlorine; also active free chlorine—the free chlorine that has activity as an oxidizing agent. In practical wash water applications, free chlorine, free available chlorine, and active free chlorine are synonymous.

Other terms identifying factors that significantly affect maintaining effective chlorine levels in wash systems:

- Chlorine demand—depletion rate of free available chlorine; rate of depletion will vary depending on several key factors, primarily affected by, but not limited to, the type and physical state of the commodity being processed, and the organic load level in the water.
- Organic load—amount of organic material, such as latex released from the leafy vegetable commodity, that is present in the water and that combines with and depletes the level of free available chlorine.
- Inorganic load—inorganic material present, such as minerals or ammonia, that react and deplete the level of free available chlorine.

Free available chlorine concentration declines rapidly upon introduction of organic materials into the wash solution. More than 60% of the total chlorine demand is often fulfilled within 5 min (55). This rapid reaction between chlorine and organic materials likely results in a discrepancy between the initial or targeted free available chlorine concentration and the residual chlorine concentration in leafy greens process water (19, 39, 59, 60).

### MONITORING AND CONTROL OF EFFECTIVE CHLORINE LEVELS

Several important factors need to be considered in wash system management to control the effective levels of chlorine: monitoring of organic load, precision and accuracy of the chlorine measurement, and frequency of chlorine monitoring and dosing.

**Monitoring of organic load.** As the wash run progresses, so does the accumulation of organic materials, and thus the chlorine demand, in the wash water (19, 28, 29, 59). The type and amount of organic load plays an integral role in the depletion of many antimicrobial chemicals. There are two key contributors to the organic load in wash water: dissolved solids and suspended solids. Dissolved solids react quickly with antimicrobial chemicals, whereas suspended solids contribute to a more prolonged depletion of antimicrobial chemicals over time. Both can contribute to cross-contamination.



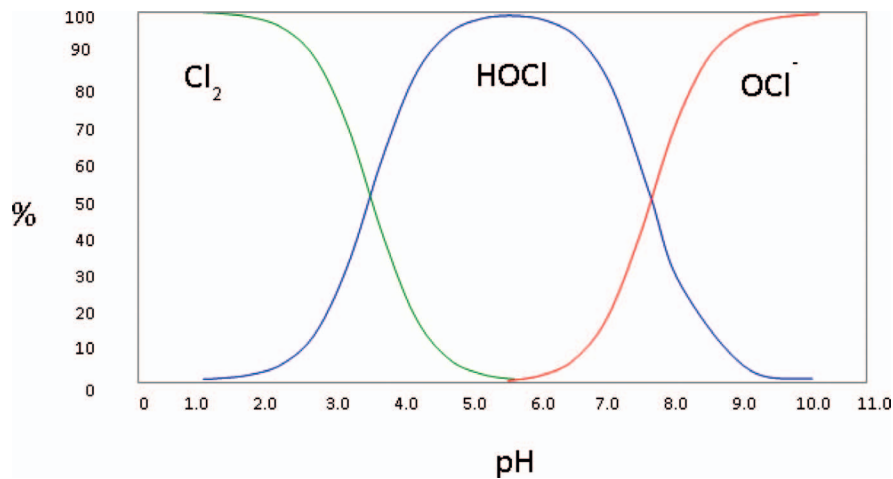


FIGURE A1. Effect of pH on proportion of chlorine in water. Green, chlorine ( $\text{Cl}_2$ ); blue, hypochlorous acid ( $\text{HOCl}$ ); red, hypochlorite ( $\text{OCl}^-$ ).

Organic load is a key parameter that should be monitored, especially in chlorinated wash systems. Increasing the rate of sodium hypochlorite addition may be required to reach the target free chlorine level and compensate for the increase in organic load in the wash system. Simply adding more antimicrobial chemical becomes inefficient, however, if the wash water's organic load gets too high. At this point, wash water should be changed or replenished with fresh water, sometimes referred to as make-up water. Understanding the dynamic changes in wash water quality is essential.

Listed below are commonly used approaches for monitoring organic load. With the exception of COD, they are not direct measurements of organic load. They can be considered to be indicators of organic load and may be impacted by a variety of influences. Studies should be performed to determine the degree of correlation of the indicator measurements with the chlorine demand due to the organic load.

- a. COD is a measurement of organic load and the chlorine demand in a system. The assay uses heat and a strong oxidizing agent under acidic conditions to oxidize organic material to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . It is performed by measuring the amount of the oxidizing agent consumed in the reaction through titration or photometry.
- b. Total suspended solids measures the amount of solids per volume of water. It is performed by filtering a volume of water and performing a dry weight measurement of the material captured on the filter.
- c. Turbidity is a measure of the clarity of water, i.e., the amount of light that is scattered by particles in the water. The term "turbidity" has been used loosely in the produce industry to refer to the organic load; however, the correlation between turbidity and organic load can be impacted by many factors.
- d. Conductivity is a way to quantify the capacity of water to transmit an electrical current, and it is affected by the presence of dissolved solids. It does not necessarily correlate with COD level, and a correlation determination should be performed for each wash process and product.
- e. Brix is an indicator of the sugar content present in the wash water, and it can be monitored via a refractometer. Although it is possible to use Brix as an index of the organic load for certain applications, e.g., cut carrot or tomato wash systems, it is generally a poor indicator for cut leafy greens wash water quality due to the low solids content of these types of produce.

**Precision and accuracy of chlorine measurement.** Methods of chlorine measurement can be compared for their accuracy and precision. Accuracy is defined as how close a measured value is to the *actual (true) value*. Precision is defined as how close the measured values are to *each other*. The precision and accuracy of the monitoring method(s) must be understood and documented as a prerequisite requirement of wash system validation. This should include periodic statistical evaluation of variances, appropriate calibration and the frequency of calibration of instrumentation, competency testing of the technicians responsible for the measurements, and documentation that any reagents used are within the method specification and shelf life.

There are many different ways to measure free chlorine (22), including

- a. Color changing test strips
- b. Colorimetric titration methods
- c. *N,N*-diethyl-*p*-phenylenediamine (DPD) methods
- d. Manual color wheels
- e. Photometric instruments
- f. Indirect electronic probes
- g. Ion-specific electronic probes

Each of these methods represents trade-offs between cost, simplicity of use, and accuracy and precision. For example, the test strip might be viewed as the simplest method to use: dip the strip in the water, and match the color to a preprinted chart. However, there is more that needs to be known:

- a. The increments of measurement on the preprinted chart must be considered. For example, they might be 5, 10, 15, 20, and 25 ppm; these increments imply an accuracy of no greater than  $\pm 2.5$  ppm (half the distance between the increments). So a reading of 10 is most likely between 7.5 and 12.5 ppm. If 10 ppm is the critical limit that has been set, then the operational limit must be set at least to the next increment higher, i.e., 15 ppm.
- b. The precision of the method must be considered. A test strip responds to the amount of chlorine presented to it, the time that the strip is left in the water, how the drop at the end of the strip is allowed to dry or is shaken off, and how long the technician waits before reading the color. All can contribute to the final color reading. This precision, or lack thereof, can contribute from 5 to 10 ppm of additional error in the reading of chlorine. If

an additional  $\pm 5$  ppm is assumed, coupled with the accuracy error, it is then necessary to have an operational limit of at least 20 ppm to assure that the system does not fall below a real value of 10 ppm.

- c. Another important consideration is pH. Test strips respond to any form of free chlorine, including hypochlorite ion, which has poor antimicrobial effectiveness. The pH of the water must be determined to ensure that the predominant chlorine species are the active ones.

#### Frequency of chlorine monitoring and chlorine dosing.

Given that chemicals in the water, in particular the organic materials that are brought in with the leafy greens, react quickly with free chlorine, the time frame between chlorine measurements must be aligned with the depletion rate of the free chlorine. For example, if one does not want the chlorine level to fall below 10 ppm, but chlorine is added only periodically, that periodic addition must compensate for the chlorine reaction with other chemicals. If one doses the system to 20 ppm and the chlorine concentration declines to 10 ppm in 10 min, both the frequency of dosing and the rate of monitoring free chlorine must be less than 10 min. The system dynamics for every individual part of a wash line must be understood and documented as a prerequisite for the wash system validation.

Because of the rapid loss of antimicrobial effectiveness during washing, replenishment of the antimicrobial chemical is critical. This can be accomplished either via manual input or automatic injection of concentrated antimicrobials. Current industry practices vary, and they include dosing strategies based on:

- addition of the antimicrobial chemical at regular intervals;
- addition after periodic concentration measurements;
- monitoring of the wash water by direct or indirect sensors.

Continuous monitoring in an automated system that controls dosing is preferred to ensure that the desired free chlorine level is maintained despite the chlorine demand of the system (3).

### PUBLISHED STUDIES REPORTING A LEVEL OF CHLORINE THAT PREVENTS CROSS-CONTAMINATION

Research on antimicrobial efficacy in wash water has a long history, with much more in the works as of this writing. Consequently, we are just beginning to understand the conditions and critical limits that can prevent cross-contamination in a washing system for even the most commonly used antimicrobial, chlorine. Determining the minimum chlorine concentration that is high enough to prevent pathogen cross-contamination and low enough to be of practical use is the essential task to be accomplished.

The available scientific literature is not definitive regarding the level of free available chlorine that must be maintained in the wash system at all times to prevent water-mediated cross-contamination. Because the measurement of chlorine in the various published reports is often not described precisely, it is sometimes difficult to draw conclusions. Residual free chlorine levels in wash water after organic loading can vary dynamically during an experiment (29, 59, 60), making interpretation of experimental results difficult. The literature suggests that approximately 10 ppm of hypochlorous acid at the optimum pH (6.5 to 7.0) is generally sufficient to minimize cross-contamination. Nevertheless, there are factors that might allow

lower levels to be sufficient, as well as factors that might require higher levels.

A major challenge is maintaining the target chlorine concentration during commercial operations with high organic loading (28–30, 54). In a laboratory setting, Luo et al. (30) evaluated a series of free chlorine concentrations, including 0, 1, 2, 5, 10, 15, 25, and 100 ppm, on pathogen survival and cross-contamination at pH 6.5 by washing cut lettuce that was inoculated with  $10^4$  CFU/g *E. coli* O157:H7 along with uninoculated lettuce at a ratio of 1:4. Although no pathogen survival was found in the wash water when the free chlorine concentration was at or above 5 ppm, a minimal free chlorine level of 10 ppm was required to prevent pathogen cross-contamination, at a pathogen detection limit of 0.36 MPN/g.

In a subsequent pilot plant study, Luo et al. (29) investigated the dynamic changes in free chlorine concentration as impacted by wash operations and the consequent effect on pathogen survival and cross-contamination in the presence or absence of a wash process aid. Spinach leaves inoculated with a nonpathogenic strain of *E. coli* O157:H7 ( $10^5$  CFU/g) were manually spread onto the conveyor belt adjacent to, but separated from, uninoculated lettuce at a 0.2:100 spinach-to-lettuce ratio. The spinach leaves and lettuce shreds were submerged simultaneously and, thereby, were mixed upon entry into the wash solution in the primary tank. Each test run (36 min), starting with a free chlorine level of 20 ppm, used approximately 1,620 kg of lettuce, with additional chlorine added after every 540 kg of lettuce washed. Water and lettuce samples were collected every 2 min during washing for testing water quality, free chlorine concentration, and pathogen cross-contamination. Data revealed that pathogen cross-contamination occurred when the residual free chlorine level was below 10 ppm, but not above 10 ppm.

Tomas-Callejas et al. (43) evaluated the effect of 25 ppm of free chlorine on cross-contamination of fresh-cut red chard leaves. Samples were inoculated with  $\sim 10^3$  CFU/g *E. coli* O157:H7 and  $\sim 10^4$  CFU/g *Salmonella* and were washed with uninoculated red chard leaves at a ratio of 4:100. No cross-contamination was noted using the traditional culture method with a detection limit of 1.5 log CFU/g, but a trace amount of cross-contamination was reported using a highly sensitive PCR method. Only one chlorine concentration (25 ppm) was tested. Although a residual free chlorine concentration was not reported, less than 25 ppm would be expected based on the reported experimental settings.

Shen (38) repeatedly washed inoculated spinach with uninoculated lettuce in a chlorine solution and monitored the changes in residual free chlorine and pathogen cross-contamination. No pathogen cross-contamination occurred during a drop from 40.8 to 9.4 ppm of residual free chlorine, but it was detected at 4.6 ppm or below. Lopez-Galvez et al. (26, 27) evaluated the potential for cross-contamination as a result of washing uninoculated lettuce in wash solutions that had previously been used to wash inoculated lettuce, in the presence and absence of various antimicrobials. These studies demonstrated the importance of antimicrobial presence in keeping wash water free of pathogens and in the avoidance of cross-contamination in subsequent washes. However, only one antimicrobial concentration was used in these studies, and the inoculated and uninoculated lettuces were washed sequentially.

Preventing pathogen cross-contamination requires the inactivation of pathogen cells as soon as they are dislodged from the contaminated produce surface. Zhang et al. (57) developed a novel microfluidic device that enabled the testing of pathogen inactivation kinetics within fractions of a second. Their results showed that 10 and 1 ppm of free available chlorine inactivated *E. coli*

O157:H7 in water free of organic material in 0.2 and 1.2 s, respectively.

Zhou et al. (59) evaluated the survival of *E. coli* O157:H7, *Salmonella enterica*, and *L. monocytogenes* in a simulated produce wash involving chlorine depletion by organic loading, followed by chlorine replenishment. Pathogens were added to the process and retrieved after a 30-s exposure. Results showed no pathogen survival in the presence of  $\geq 3.7$  ppm of residual free chlorine, regardless of organic load level. Given that the prevention of pathogen cross-contamination requires pathogen inactivation in the time frame of a second or less, a chlorine concentration that is higher than 3.7 ppm is likely necessary to prevent cross-contamination. This study also showed the critical importance of residual free chlorine concentration on pathogen survival, irrespective of the initial free chlorine concentration and organic load. As highlighted by Banach et al. (3), the reactivity between chlorine and organic matter will determine the residual chlorine concentration, and the latter is of paramount importance for microbial inactivation and should be monitored in situ during the process.

Taking a different approach, Gomez-Lopez et al. (19) also evaluated pathogen survival during the dynamic changes of free chlorine concentration as affected by the changes in organic load and chlorine replenishment. Results showed that the maintenance of a free available chlorine concentration of 5 ppm during washing of fresh-cut spinach kept the wash water pathogen-free during the entire testing period of 1 h. The authors recommended a minimum residual free available chlorine level of 7 ppm to be an effective treatment to inactivate *E. coli* O157:H7 under industrial conditions. The contact time between adding pathogens to the solution and sample collection was not reported, but longer than 5 s would have been expected based on the reported testing conditions.

A pathogen inactivation model developed by Van Haute et al. (53) was used to predict major factors affecting pathogen cross-contamination during lettuce washing in chlorinated wash water. The authors concluded that the rate of pathogen transfer to the wash water depends on the initial bacterial population of the contaminated produce, whereas pathogen inactivation in the wash water depends on the residual free chlorine concentration and is independent of the initial bacterial population. In other words, a higher residual free chlorine level is needed to avoid pathogen cross-contamination, if the initial contamination level is high.

In virtually all studies available, a pathogen contamination of 3 to 5 log CFU/g was applied, which may not reflect real-life situations. In addition, the ratio of contaminated to uncontaminated produce could also play an important role in pathogen cross-contamination. Furthermore, most studies used a batch wash system, which may not simulate commercial operating conditions, e.g., the dynamic changes in chlorine addition and depletion, pathogen transfer from the contaminated produce to wash water, pathogen survival in the water, or transfer to other uncontaminated produce. All of these factors could lead to either under- or overestimation of the chlorine concentration required to prevent pathogen cross-contamination. Furthermore, other factors, such as pH, can impact the form, stability, and efficacy of free chlorine. Thus, additional studies are needed to evaluate pathogen cross-contamination under conditions that fully simulate the dynamic changes of these important factors during commercial washing operations, such as in a pilot plant or commercial processing plant using suitable pathogen surrogates.

In summary, the published literature points to a concentration of 10 ppm of free available chlorine at the optimum pH range (6.5 to 7) as an approximate target for minimizing cross-contamination

in wash water. An increase or decrease in concentration may be targeted depending on process-specific operational factors.

## APPENDIX B. USE OF PAA IN WASHING PROCESSES

### UNDERSTANDING PERACID CHEMISTRY

Peracetic acid (PAA) is a water-soluble oxidative antimicrobial agent that is registered with the EPA for use in treating produce wash water. PAA is manufactured and supplied as an equilibrium mixture, with varying levels of PAA, acetic acid, and hydrogen peroxide, depending on the manufacturer. Regardless of the levels of the other components, only PAA is considered to be the active antimicrobial when used to treat produce wash water. PAA is certified for inactivation of pathogenic *E. coli*, *Salmonella*, and *L. monocytogenes* when applied at levels of 30 to 80 ppm. PAA does not require a postuse rinse with potable water if used at levels that do not exceed 80 ppm.

Because PAA is composed of an organic acid and hydrogen peroxide, it is most stable when kept at acidic pH and cold temperatures. PAA has an acid dissociation constant of 8.2, which allows it to be effective through the entire acidic range and slightly into the basic range, i.e., pH 0 to 7.5. As a result, pH is typically not a concern for adjustment or control when using PAA.

The exact mechanism of how PAA kills bacteria is not known; however, investigations to date allow for an understanding of the generalities of the mechanism and the likely sequence of events (2, 5). PAA has a chemical structure with a short-chain fatty acid, which allows it to target microbial cell membranes. Though not entirely composed of lipids, cell membranes are plentiful in fatty, lipid-type chemistry, for which PAA has an affinity. Once attracted to the cell membrane by the fatty acid nature of the acetate portion of the molecule, the oxidizing peroxyacid portion of the molecule causes disruption to the cell wall and ultimately opens up a penetration point. Once inside, PAA causes further disruption of cell function by oxidizing proteins, enzymes, and metabolites in the bacteria, ultimately disrupting all life functions and causing rapid death of the bacteria. The end products of this series of events are harmless acetic acid and peroxide, the latter of which further breaks down into water and oxygen.

### MONITORING AND CONTROL OF PAA

Options for PAA measurement exist as the following:

- a. Color changing test strips
- b. Colorimetric titration methods
- c. Photometric instruments
- d. Amperometric sensors

Test strips are rapid and simple to use; however, they generally do not afford great precision, because the lowest commercial offering has an operating increment of 5 ppm. This lack of precision requires a higher targeted operating level. Although systems are available to measure PAA level test strips based on color intensity, these systems need routine calibration and are costly on a per-test basis in comparison to other methods.

The most common, accurate, rapid, and economical method for routine PAA measurement is one of the colorimetric titration methods. Three primary titrimetric methods exist: permanganate (12), ceric sulfate (21), and iodometric (40). Although there are similarities among all three, the iodometric method affords the most rapid and direct method of PAA measurement owing to the



principle of suppressed hydrogen peroxide while measuring PAA first (accomplished using moderate acidic conditions and cold sample temperatures). Although dependent on the selection of titrant concentration for conducting the measurement, single-ppm resolution is achievable with minimal effort and adherence to best practice for titrant addition. There are no stable “combined” states of PAA, so that a single-step measurement is sufficient to determine the active, effective antimicrobial level.

Amperometric measurement makes use of an electrode, encased in a viscous salt solution (KCl) and held in place in a permeable membrane cap for interaction with PAA-containing solution. The membrane affords interaction with the PAA in solution, and the salt reacts with the PAA to generate electrons. The sensor electrode then completes the circuit, with the current generated used to correlate and measure the level of PAA. The sensors are verified and calibrated by comparison to one of the aforementioned titrimetric methods for PAA level determination.

PAA dosage control is most commonly handled by time-based peristaltic or metered pump dosing, with periodic PAA level checks by titration. With investment in equipment and adoption of a pump control system based on feedback from amperometric sensors, a system can be configured to operate at a target level. Verification of flume PAA levels by titration must still be performed; however, the system stability and adherence to the target level is greatly improved by comparison to a time-based, manually adjusted and controlled system.

With stock product concentrations of PAA typically in the 12 to 16% range, the point of addition of PAA to a wash flume system is critical to avoid overexposing product to high levels of PAA. This is best accomplished by input into the system at a point as far as possible from product contact, to afford sufficient mixing and dilution. This is typically accomplished by installing a supply line upstream of the recirculation pump.

It is also critical to consider flume system construction materials prior to implementing a PAA intervention program, because nonstainless (or soft) metals are not compatible with PAA, due to reactivity. Because modern food processing equipment generally uses stainless steel construction, this effectively minimizes the risk of incompatibilities.

Although all recirculating water-based produce wash systems are susceptible to organic load buildup in the wash water, PAA is only marginally sensitive to most organic loads present in the water. The level of sensitivity is dependent on the chemical nature of the organic load, with the greatest sensitivity to antioxidants (e.g., vitamins A, E, etc.) or sulfur-rich produce (e.g., onions). High levels of dissolved metals in the water will also deplete PAA levels, with greatest sensitivity to iron and copper.

## PUBLISHED STUDIES REPORTING EFFICACY OF PAA

To date, scientific investigations into the efficacy of PAA in produce wash water under various conditions have been conducted through laboratory or pilot-scale studies by various research groups. PAA has been registered with the EPA to provide a 3-log reduction of pathogenic *E. coli*, *Salmonella*, and *L. monocytogenes* in wash water when used at 30 to 80 ppm with a dwell time of 90 s. This primarily addresses water-mediated cross-contamination events, and future work is expected to continue to help refine the systems for produce washing and the specific conditions for a particular system for which this operating range is valid. As is always the case with EPA-registered products, label instructions must be followed as written to be in accordance with federal law.

Zhang et al. (58) published results wherein PAA, mixed peracids, and chlorine were tested with no organic load and with 10% organic load added to the test process water, with the intention of determining the risk reduction of cross-contamination from iceberg lettuce inoculated with *E. coli* O157:H7 to uninoculated lettuce. Results indicated that, in the absence of organic load, all interventions performed about equally but that, with the inclusion of 10% organic load, the efficacy of chlorine declined relative to the peracids. The authors concluded that all factors, such as organic load, fluid/produce ratio, antimicrobial type and concentration, and other variables, must be taken into account when conducting a validation, regardless of the intervention used.

## APPENDIX C. PRACTICAL GUIDELINES: STRATEGIES TOWARD VALIDATION OF ANTIMICROBIAL WASHES

Practical guidelines and steps that may be followed for validating antimicrobial washes for fresh-cut leafy vegetable processes are provided below. The preliminary steps involve statements of scope, objectives, and the identification of hazard, performance standard, and the fixed and variable process parameters. Following the preliminary steps are strategies toward performing validations according to options 1, 2, or 3.

### 1. Define Scope and Objectives

**1.1. Product.** Identify the product to be used in the validation. If the process is to be validated for multiple commodities, choose the commodity that will provide the greatest challenge to the antimicrobial’s ability to prevent cross-contamination. If the differences between commodities are great, the validated process may be excessive for the commodity presenting less of a challenge, and if so, separate validations may be needed.

**1.2. Wash system.** Identify the scope of the process to be validated. In multistage systems with more than one flume or wash tank, each flume or wash tank must be treated with antimicrobial agent. In a flume or wash tank, the beginning is typically where the product first enters the flume or wash tank, and the end is where the product exits the flume or wash tank. When multistage systems have independent antimicrobial dosing systems and water recirculation systems, separate validations for each flume or tank must be done. Static dump tanks can likewise be validated, with the scope being from when (instead of where) the produce enters the dump tank until when it exits.

### 2. Define Hazard and Performance Standard

**2.1. Pathogen hazard(s).** Identify the pathogen of concern. For fresh-cut produce, it is typically pathogenic *E. coli*, *Salmonella*, or *L. monocytogenes*, but it can be others. Choose the pathogen most likely to be a cross-contamination concern, i.e., most likely to be at the highest level or with the greatest resistance to the antimicrobial chemical being used.

**2.2. Performance standard.** Identify the level to which the target pathogen must be controlled. For purposes of this validation, a performance standard will be based on the absence of detectable cross-contamination during washing. Ideally, performance standards would be based on a maximum level of the target pathogen that is reasonably foreseeable; however, maximum levels typically are not known. There is precedent for use of a 100-fold safety factor in establishing the performance standard (see (47)). Nevertheless, since the realistic contamination level is not known, the validation should be performed at an inoculum level to ensure

that the positive and negative controls perform correctly, demonstrating that cross-contamination occurs without an antimicrobial agent and is controlled by the sufficient level of an antimicrobial agent (see section 4.1).

### 3. Define Process Parameters

**3.1. Identify the fixed process parameters.** The fixed process parameters are those that remain constant in every process run; for example, the dimensions of the flume and source of water.

**3.2. Identify the variable process parameters.** The variable process parameters are those that can vary between process runs and during a process and that will be monitored and potentially controlled during the validation, for example, product feed rate, water flow rate, water replenishment rate. For each variable process parameter, identify the extreme level or condition that would provide the greatest challenge to the antimicrobial but that would still be acceptable in a process run, for example, the highest level of turbidity that can occur or that would be acceptable before making a correction. These worst-case conditions would be needed for option 1 or 2 validations.

### 4. Select Validation Option

#### 4.1. Validation option 1: use of a surrogate.

- a. Prepare surrogate-inoculated produce. Inoculation methods should be based on published protocols and recommendations (4, 33). The inoculated product should be easily distinguishable from the uninoculated product. For example, if validating a process for washing chopped romaine or iceberg lettuce, inoculated chopped red leaf lettuce will be easy to distinguish. The produce should be inoculated to achieve the performance standard level. Just before use, determine microbial counts of the surrogate suspension and the inoculated produce quantitatively for the level of surrogate detectable. Report both values in the validation report.
- b. Set up the system to be validated. Ensure that all fixed process parameters are as expected and that all variable process parameters can be measured accurately, monitored, and recorded throughout the validation run. Begin running the system, but do this without antimicrobial agent or product. Adjust the process to those worst-case conditions that can be achieved without product.
- c. Begin product feed and adjust all variable process parameters to predetermined worst-case conditions. Aim for constant conditions where feasible. Where conditions will vary during the run, aim for minimizing and measuring variability. Record all conditions of variable parameters throughout the validation study.
- d. Negative control tests:
  - i. Without antimicrobial agent: Collect several product samples ("product control") and place into neutralizing solution. For example, if chlorine will be used as the antimicrobial agent in the validation, sodium thiosulfate in buffer could be a neutralizing solution. Test these for the surrogate to ensure that none is detectable in the test product.
  - ii. With a high level of the antimicrobial agent: Adjust the antimicrobial feed rate to a level that is high enough to ensure prevention of cross-contamination. Add a predetermined amount of the surrogate-inoculated product along with uninoculated product to the beginning of the process being validated. At the end of the process (e.g., exit of the flume), collect a predetermined amount of uninoculated and inoculated product, each in triplicate, and place separately into neutralizing solution. Test these for the presence of the surrogate. These are the negative controls 1 (uninoculated) and 2 (inoculated). Collect a predetermined amount of water samples in triplicate to verify that no surrogate is detectable in the wash water.
- e. Validation of antimicrobial level:
  - i. Next, adjust the antimicrobial feed rate to the level that is to be validated ("test level"). Ensure that the level is stable. Add separately a predetermined amount of the surrogate-inoculated product along with uninoculated product to the beginning of the process. Ensure that the inoculated and uninoculated products are not in contact with each other prior to entering the washing system. At the end of the process, collect a predetermined amount of uninoculated and inoculated product, each in triplicate, and place them separately into neutralizing solution. These are the "test samples" 1 (uninoculated) and 2 (inoculated). Do not allow the uninoculated and inoculated samples to touch during or after collecting. Test them quantitatively for the level of surrogate that is detectable. Collect a predetermined amount of water samples in triplicate to determine levels of the surrogate if present in the wash water.
  - ii. If additional antimicrobial feed rates (test levels) are to be evaluated, repeat the testing procedures for each.
- f. Positive control tests:
  - i. Finally, add a predetermined amount of the surrogate-inoculated product along with uninoculated product to the beginning of the process, without an antimicrobial agent. At the end of the process, collect a predetermined amount of uninoculated and inoculated product, each in triplicate, and place separately into neutralizing solution. These are the positive controls 1 (uninoculated) and 2 (inoculated). Do not allow the uninoculated and inoculated positive control samples to touch during or after collecting. Test them quantitatively for the level of surrogate that is detectable.
- g. The validation trial should be repeated to obtain three successive replicate validation trials.
- h. Ensure that the system is cleaned and sanitized between the validation trials.
  - i. Acceptable results to meet performance standard:
    - i. The enumeration results for the surrogate inoculum should be at the level expected. If much lower than expected for the performance standard, the validation trial has failed. If higher than expected, the trial may or may not have to be repeated depending on whether the test levels of the antimicrobial agent have been successful (trial is successful) or not (trial must be repeated). The level recoverable from the inoculated product is for information only, because the goal of the validation is to demonstrate the prevention of cross-contamination, not the reduction of bacterial counts on contaminated products.
    - ii. The levels and conditions of all variable conditions throughout each run should be at or worse than predetermined levels and conditions. If not, then the validation will be limited to the levels and conditions of the parameters during the trial. If it is possible for any of the variable levels or conditions to occur at worse levels during a normal run, then the worst condition experienced during the validation runs becomes a critical limit for that variable.
    - iii. The surrogate should not be detectable in water samples collected with the negative controls or at the test level. The

water samples should also be negative for gram-negative bacteria. If viable surrogate or gram-negative bacteria are detectable, the test level has failed.

- iv. The surrogate should not be detectable in the product control. If it is, the validation trial has failed.
- v. The surrogate should not be detectable in negative control 1 (uninoculated product). If it is, it is unlikely that lower levels of antimicrobial chemical would have been successful, and the trial has failed. The test results of negative control 2 (inoculated product) are for information only.
- vi. The surrogate should be detectable on both positive controls 1 and 2. If not detectable, then the validation trial has failed. If the surrogate is not detectable on positive control 1 (uninoculated product), then the surrogate inoculation level must be increased or sample collection procedure must be adjusted to detect cross-contamination. If the surrogate is not detectable on positive control 2 (inoculated product), then additional investigation is needed to understand why before repeating the trial.
- vii. If all negative and positive control results are as expected, and the conditions and levels of the process parameters during the trial were as expected, then the lowest test level where the surrogate is not detectable on any of the uninoculated samples, in all three trials, is the validated critical limit for antimicrobial feed rate. If the surrogate is detectable at a higher test level, then an investigation is needed to determine why, before considering the trial a success.

#### **4.2. Validation option 2: use of antimicrobial sensors and worst-case conditions.**

- a. Map the wash system using antimicrobial sensors: Obtain and position calibrated antimicrobial sensors in all positions in the water system where the antimicrobial agent is likely to be at its lowest level during normal operation. Use as many sensors as practical.
- b. Begin running the system without product or an antimicrobial agent, and bring all variable conditions to worst-case conditions or levels. Begin product feed at worst-case level, and readjust all variable process parameters to predetermined worst-case conditions. Aim for constant conditions where feasible. Where conditions will vary during the run, aim for minimizing and measuring variability. Record all conditions of variable parameters throughout the validation study.
- c. Begin the antimicrobial feed rate at its lowest level, wait for equilibrium to be established, and record sensor readings. Incrementally raise the antimicrobial feed rate, wait for equilibrium to be established, and record sensor readings. Stop when all sensors are at or above the established minimum antimicrobial level. Record the value of the antimicrobial feed rate.
- d. Second and third trials can be run immediately afterwards, if desired. Stop antimicrobial feed, ensure that all variable parameters are at predetermined conditions and that variability is as low as practical, and then repeat the previous procedure, beginning with the antimicrobial feed rate at the lowest level.
- e. Acceptable results to meet the performance standard:
  - i. The levels and conditions of all variable conditions throughout each run should be at or worse than predetermined levels and conditions. If not, then the validation will be limited to the levels and conditions measured during the trial. If it is possible for any of the variable levels or conditions to occur at worse levels during a normal run, then the worst level or condition experienced

during the validation runs becomes a critical limit for that variable.

- ii. The lowest antimicrobial feed rate that achieves equilibrium at or above the established minimum antimicrobial level at all sensors in all three runs becomes the critical limit.

#### **4.3. Validation option 3: use of antimicrobial sensors, without worst-case conditions.**

- a. Map the wash system using antimicrobial sensors. Obtain and position calibrated antimicrobial sensors in all positions where the antimicrobial agent is likely to be at its lowest level during normal operation.
- b. Begin running the system with product and an antimicrobial agent and record all sensor readings. Record all conditions of variable parameters throughout the validation study.
- c. Run the system under multiple conditions of the variable parameters, continuously monitoring the sensor readings. It is not necessary to know and run the system under worst-case conditions; rather, it is advisable to run the system under as many acceptable variable conditions as possible.
- d. The trial is completed when it is confirmed where the lowest level of antimicrobial agent exists in the wash system.
- e. If there are different locations where the lowest antimicrobial levels exist under different conditions, then either multiple lowest-level locations must be monitored, or the spot that provides the most conservative reading.
- f. If the sensor can be positioned at the lowest-level location during normal operation, then it becomes the monitoring point, and the established minimum antimicrobial level is the critical limit.
- g. If the sensor cannot be positioned at that spot during normal operation, a "monitoring" location will need to be selected, i.e., somewhere where a sensor can be positioned during normal operation and where the sensor readings are proportional and predictable to the lowest level sensor.
- h. In that case, the highest sensor readings at the monitoring location, when the lowest-level sensor was at the established minimum antimicrobial level during the validation trials, becomes the critical limit.

### **5. Additional Considerations**

Validators should keep in mind a few key points to help ensure generation of repeatable, reliable data.

#### **5.1. System precleaning for the validation study.**

- a. Existing sanitation standard operating procedures (SSOPs) should be consulted for wash system cleaning and sanitation procedures prior to the validation. If SSOPs do not exist, validators should consult with their chemical supplier or other qualified individual to develop SSOPs relevant to the specific needs of the system. An effective wash system maintenance program requires system clean-outs at regularly scheduled intervals.

#### **5.2. Representative wash system operation.**

- a. Efforts should be made to ensure that the wash system is operated during validation in a manner that replicates as closely as possible the actual operating conditions. For example, typical industry practice is to not offer for public consumption any product used in validation studies. This practice can cause delays in staging working product at the front end of the process and may not reflect actual production practices.

#### **5.3. Relationship of antimicrobial addition and monitoring points.**

- a. Antimicrobial application points should be installed in such a



way as to ensure that the product is not fed directly in front of a control sampling point. Adequate mixing time should be achieved, and properly located testing points should be used.

#### 5.4. Proper use of probes.

- a. If an amperometric probe is used to determine and control the antimicrobial agent, its operational requirements must be met, as specified by the probe manufacturer. For example, the required flow rate across the probe and stable response time (typically 30 s to 3 min, for currently available probes) should be understood. Calibration of probes in dynamic flow conditions, or within the first 2 h of operation, is not recommended.
- b. In any wash system using electronic determination of antimicrobial residual level, an alternate manual determination methodology should be concurrently validated with the probe or analyzer.

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