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## Assessing Effective Quality Controls of Chlorination for Produce Postharvest Wash Water

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### Research Article

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#### ABSTRACT

Postharvest produce sanitation is an important consideration in reducing food safety risks. Evaluation of current on-farm sanitation practices may reveal factors that affect the effectiveness of current sanitizers with fruits and vegetables. The goal of this research is to determine and validate commercially available quality controls for postharvest rinse water to support small farms. In this study we evaluated and compared two commercially available portable Oxidative Reduction Potential (ORP in millivolts) meters and three types of free chlorine test strips as an on farm quality control tool to monitor process wash water sanitation. The quality controls were evaluated using different types of synthetic process wash water (soil, vegetation, and vegetation / soil and challenge water) at varied turbidity concentrations that were inoculated with *E.coli* O157:H7. Results indicate that different types of wash water solutions can influence the free residual chlorine levels. Data suggests ORP range 650-800 RmV and free chlorine test strips used under low turbid wash conditions can be used as a qualitative tool to monitor the free residual chlorine levels for small farm postharvest sanitation management as a best practice for food safety.

### INTRODUCTION

The Centers for Disease Control and Prevention estimates that 48 million people annually in the United States become sick from foodborne related illnesses<sup>[1]</sup>. Furthermore, in 2013 the CDC reported fresh produce as the major cause of foodborne illness. Microorganism contamination can occur at any facet of the produce chain starting on the farm. Having Good Agriculture Practices (GAPs) on farm can help reduce contamination of spoilage and disease-causing microorganisms. Increased attention has focused on management of agricultural water sources used in produce wash water and mitigation strategies to reduce food safety risks on-farm<sup>[2]</sup>.

Proper management of postharvest sanitation on produce is an essential practice to reduce and minimize potential transmission of foodborne pathogens and spoilage organisms in fresh produce. There have been several produce food safety outbreaks (e.g. leafy greens, cantaloupe, and sprouts) where improper management of the produce wash water was a contributing factor. Effective use of sanitizers postharvest can assist with the reduction of harmful bacteria. Understanding the factors that can make sanitizers less effective on produce is crucial to maximizing preventive controls.

Chlorine is currently the most utilized produce rinse agent used by small and medium sized growers. There are many commercially available chemical paper test strips that detect and estimate the range of free chlorine in water solutions. Considerations include: budget, sensitivity and accuracy, correct ppm ranges for corresponding chlorine dosages in water, sampling directions, and the impact of pH, turbidity, and temperature of the process wash water. Current research recommends chemical paper test strips as a method for sanitation monitoring<sup>[3-5]</sup>. However, no publication specifies approved manufacturers nor appropriate chlorine test strip ranges. Another point of concern, commercially available chlorine test strips are designed for

pools and clear drinking water which are both filtered of debris compared to grossly turbid process wash water. Paper test strips are not designed for agricultural water applications and their potential for interfering variables.

One major problem with agricultural water is the turbidity due to organic matter and soil. At low and moderate turbidity levels, chlorine test strips give accurate results. High turbidity of suspended solids may interfere with accurate color matches and false measurements. Chlorine test strips are thus a good pre-check practices or for low turbid process water applications since vegetation and debris impact accuracy of readings. Other variables for farmers to consider when choosing a chemical paper test strip include: different manufacturers vary in specificity, increased accuracy and sensitivity increase with price. Good management of sanitation practices follows optimal frequency of changing process wash water based on turbidity level and/or has a second monitoring method of ORP sensor meters during processing following the test strip pre-check <sup>[6]</sup>.

Oxidation-reduction potential (ORP) is an alternative method to measuring direct chlorine concentrations. ORP measures the tendency a chemical species has to acquire electrons. A strong oxidizer draws electrons away from the cell membrane and the destabilized membrane structure collapses leading to cell death. Thus ORP probes measure the antimicrobial potential in millivolts (mV). Research has demonstrated that ORP, contact time, and antimicrobial efficacy are affected by temperature, turbidity, and pH. The optimal pH range for disinfection with chlorine is 6.5-7.5 since lowered pH is correlated with higher percentage of free hypochlorous acid (HOCL) <sup>[3,4]</sup>. ORP offers real-time sanitation monitoring of antimicrobial activity of chlorinated water. Established research has recommended an ORP set point of 650-800 mV as an effective antimicrobial range that reduces spoilage bacteria and pathogens such as *E.coli* and Salmonella in a short contact time of a few seconds <sup>[3,4,6]</sup>.

Small agricultural operations lack the analytical resources and tools necessary to conduct traditional quality control analysis. The objective of this research is to compare and evaluate free chlorine monitoring tools such as commercially available handheld ORP meters and free chlorine paper test strips for effective water sanitation in postharvest wash practices to assist farmers in choosing appropriate water monitoring tools.

## MATERIALS AND METHODS

### Inoculum preparation

A pathogenic strain of *Escherichia coli* O157:H7 ATCC 43895 was grown overnight in 10 mL of Tryptic Soy Broth (TSB) (Remel Thermo Fisher Scientific, Lenexa, KS, USA) at 37 °C. A 1% inoculum was made with 0.5 mL overnight *E. coli* O157:H7 inoculum transferred into 49.5 mL TSB and incubated at 37 °C for 18 hours. After incubation, the 18 culture was centrifuged at 1500 rpm x g for 15 minutes. The cell pellet was re-suspended, washed with Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA, USA), and centrifuged at 1500 rpm x g for 15 minutes. The final cell pellet was suspended in 5mL of Butterfield's phosphate buffer to obtain an initial cell concentration of 10<sup>9</sup>-10<sup>10</sup> CFU/mL determined with a 1.99 OD600 (Spectronic21D UV-Visible spectrophotometer, Milton Roy, Rochester, NY, USA). Final concentration was confirmed by serial dilution plating on Tryptic Soy Agar (TSA) (Remel Thermo Fisher Scientific, Lenexa, KS, USA) incubated for 24 hours at 37 °C.

### Postharvest process wash water preparation

Different postharvest process wash water was designed to reproduce and simulate small commercial farm conditions. Five process wash water solutions were created as follows: Clean Water; Soil in water; Cucumber suspended solids "SS" in water; Soil and Cucumber "CS" in water; and Organic Challenge water. Different organic matter was added to the treatment wash solutions using ultrapure water (Barnstead Nanopure Infinity™, Thermo Scientific, Dubuque, IA, USA) to the following turbidities Nephelometric Turbidity Units (NTU): 0 NTU, 25 NTU, 50 NTU. Turbidity was measured using a portable HACH turbidity meter (portable HACH 2100Q Turbidimeter, HACH Company, Loveland, CO, USA).

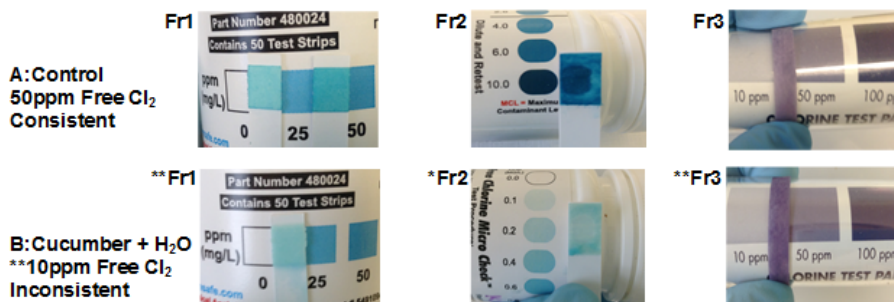
Topsoil from the University of Massachusetts Agriculture Extension Crops Research and Education Farm (South Deerfield, MA, USA) was collected during the harvest season 2012. The soil was sieved to separate small twigs, insect parts, and rocks from the soil. Resulting finely sieved soil was diluted with ultrapure water to predetermined turbidities and autoclaved. Cucumbers were obtained in bulk from a local commercial market (Amherst, MA, USA). Cucumbers were blended into a puree then sieved and filtered to obtain concentrated cucumber juice. The stock cucumber juice and soil material was then diluted with ultrapure water to 25 NTU or 50 NTU and then autoclaved.

Organic Challenge water with 2.5 g/L total organic carbon (TOC) and 2.5 g/L total dissolved solids (TDS) was used as an extreme case of background interference. The challenge water was prepared by dissolving 2.5 g of sodium chloride (Fisher Scientific, Fair Lawn, NJ, USA) for TDS and 2.5 g of tannic acid (Fisher Science Education, Nazareth, PA, USA) for TOC into 1.0 L of ultrapure water, pH adjusted to 7.0 ± 0.2 with 1 M NaOH (Fisher Scientific, Fair Lawn, NJ, USA) <sup>[7]</sup>. The challenge wash water solutions were autoclaved at 121 °C for 15 minutes and cooled to ambient temperature.

### Inoculum Procedure

Process wash water solutions were exposed to different concentrations of chlorine bleach (Clorox Company, Oakland, CA, USA) at 0 ppm and 50 ppm for 2 minutes before inoculation. Challenge water was exposed to an additional chlorine concentration

of 200 ppm. Chlorine levels were measured using the Water Works Free Chlorine Check Ultra High “Fr1” (Industrial Test Systems, Rock Hill, SC, USA) and LaMotte free chlorine test strip “Fr3” (LaMotte, Chesterton, MD, USA) before inoculation (**Figure 1A**). “Fr2” was not used since the limit of detection caps at 10 ppm (**Figure 1B**). The wash solutions were then inoculated to a final concentration of 107 CFU/mL *E. coli* and allowed to homogeneously distribute for 2 minutes. Residual free chlorine concentrations were measured with a free chlorine test strips Fr1, Fr2 and Fr3 after inoculation during the 2 minutes. Water samples were immediately neutralized with 100 µl sodium thiosulfate 1 N solution (1N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, Fisher Scientific, Fair Lawn, NJ, USA) [8]. HACH Free & Total Chlorine Test Kit (Model CN-80, HACH Company, Loveland, CO, USA) was used to validate the neutralization process in parallel with non-inoculated process water solutions due to biosafety hazards. Microbial reduction was confirmed with serial dilution plating on TSA incubated for 24 hours at 37 °C. The experiment was repeated in triplicate.



**Figure 1.** Cucumber Matter Affects Free Chlorine Strips Readings.

### Physicochemical analysis

Oxidation Reduction Potentials (mV), free chlorine concentration (ppm), turbidity (NTU), and temperature were monitored separately without pathogens for biosafety concerns. Samples were continuously agitated using a magnetic bar and stir plate. Readings before and after chlorination were recorded every 30 seconds up to 8 minutes or until measurements stabilized. Free residual chlorine was analyzed in each sample immediately after ORP readings keeping within a maximum 10 minute chlorine exposure window. HACH Free & Total Chlorine Test Kit (Model CN-80, HACH Company, Loveland, CO, USA) was used as the standard of measurement for free chlorine concentrations which follows the Diethyl-p-Phenylenediamine (DPD) method. The DPD method is an industry standard for colorimetric chlorine measurements [3,8-11]. All physicochemical analysis was conducted according to standard protocols and within manufacturers’ instructions. Specifications of instruments listed as follows: The two handheld ORP units were: “ORP#1” bench top (Thermo Scientific Orion Star A221 Portable ORP Meter) and “ORP#2” handheld (Eutech Instruments OAKTON Handheld ORPTestr 10) for ORP. The free chlorine test strips evaluated were: Free Chlorine test strips “Fr1” = Water Works Free Chlorine Check Ultra High: #480024 (Industrial Test Systems, Rock Hill, SC, USA); “Fr2” = HF Scientific Free Chlorine Micro Check: # 09940 (Watts Water Technologies Company, Fort Myers, FL, USA); “Fr3” = LaMotte Chlorine Test Papers: #4250-BJ (LaMotte, Chesterton, MD, USA). Analysis was repeated in triplicate. One-way analysis of variance (ANOVA) tests were performed on the ORP data set to determine statistical significance and standard deviations with P < 0.05 representing a significant difference using GraphPad® Software (La Jolla, CA, USA). Free residual chlorine test strips were statistically analyzed using the Fisher’s exact test.

## RESULTS

### *E. coli* survival in synthetic process water treated with chlorine bleach

Confirmed residual free chlorine concentrations coincided with a  $7.45 \pm 0.054$  log reduction of *Escherichia coli* O157:H7 ATCC 43895. All solutions with the exception of the Organic Challenge water maintained levels of free chlorine. Soil in water maintained free chlorine levels of 25-50 ppm. Cucumber in water maintained 1.2-11 ppm free chlorine. Mixture of cucumber and soil in water maintained 10-13 ppm free chlorine. All challenge water studies measured 0 ppm free chlorine resulting with no reduction in *Escherichia coli* O157:H7. A full summary of all data collected can be found in (**Table 1 and Table 2**).

### Water physicochemical conditions

The physicochemical parameters of ORP and free chlorine concentrations were evaluated in turbid process waters for indications of sanitizer antimicrobial efficacy. Results are summarized in **Table 1 & 2**.

**ORP meters:** Portable Orion ORP#1 meter and handheld pocket ORP #2 gave consistent results in process wash water controls and organic matter solutions and the values were not statistically different from each other. ORP measurements of antimicrobial activity was consistent with establish sanitizer activity range of 650-800 mV range [3,6]. The organic matter greatly reduced ORP below threshold while maintaining effective microbial reductions. This reinforces the prevailing research suggestion for a higher ORP operating limit as a safety barrier: if more organic matter had introduced, all the free chlorine would have been inactivated, leaving the process water vulnerable for contamination.

**Table 1.** Comparison of Averaged ORP Measurements with Reduction of E.coli O157:H7 by Free Residual Chlorine Bleach in Organic Matter Wash Solutions.

Wash Solutions	Sanitizer treatments: NaClO (ppm)		Residual Free Cl (Avg HACH)	Target: 650-800 mV		E. coli O157:H7*
				ORP# 1	ORP# 2	
Clean H2O	Water, 0 NTU	0 ppm	0 ppm	444.9 ± 45.6	455 ± 38	-
	Water, 0 NTU	50 ppm	50 ppm	679.5 ± 45.3	705 ± 47	+
Soil + H2O	Soil @ 50 NTU	0 ppm	0 ppm	520.9 ± 61.2	490 ± 43	-
	Soil @ 25 NTU	50 ppm	25 ppm	687.0 ± 26.4	699 ± 21	+
	Soil @ 50 NTU	50 ppm	50 ppm	708.2 ± 24.2	718 ± 20	+
Cucumber + H2O	Cucumber, 50 NTU	0 ppm	0 ppm	344.2 ± 72.7	315 ± 26	-
	Cucumber, 25 NTU	50 ppm	11 ppm	822.8 ± 30.5	824 ± 21	+
	Cucumber, 50 NTU	50 ppm	1.2 ppm	573.9 ± 42.5	556 ± 18	+
Cucumber + Soil	Cucumber/Soil, 50 NTU	0 ppm	0 ppm	375.0 ± 21.6	362 ± 48	-
	Cucumber/Soil, 25 NTU	50 ppm	13 ppm	764.9 ± 13.7	781 ± 18	+
	Cucumber/Soil, 50 NTU	50 ppm	10 ppm	833.1 ± 37.0	816 ± 15	+
Organic Challenge H2O	Challenge water, 0ppm Cl	0 ppm	0 ppm	220.1 ± 17.2	223 ± 24	-
	Challenge water, 50ppm Cl	50 ppm	0 ppm	209.9 ± 25.6	224 ± 31	-
	Challenge water, 200ppm Cl	200 ppm	0 ppm	211.8 ± 1.2	210 ± 1	-

\* Indicates survival of *E. coli* O157:H7 in a sample wash water solution. (+) Reported > 7 log reduction (CFU/ml), (-) Held a microbial load > 7 (log CFU/ml)

**Table 2.** Comparison of Free Chlorine Test Strips in Turbid Organic Matter Wash Solutions (50 NTU).

Wash Solutions	Initial chlorine load added to the process wash water	Residual Free Chlorine (ppm)			
		HACH	Fr1	Fr2	Fr3
Clean H2O	0	0 ppm	0	0	0
	25ppm	25 ppm	25	>10	Between 10-50
	50ppm	50 ppm	50	> 10	50
Soil + H2O	0	0 ppm	0	0	0
	25ppm	25 ppm	25	> 10	Between 10-50
	50ppm	50 ppm	50	> 10	50
Cucumber + H2O	0	0 ppm	0	0	0
	25ppm	4 ppm	50	2.0	10
	50ppm	10 ppm	< 25	0.2	50
Cucumber + Soil	0	0 ppm	0	0	0
	25ppm	5 ppm	< 25	2.0	Between 10-50
	50ppm	25 ppm	< 25	0.6	50
Organic Challenge H2O	0	0 ppm	0	0	0
	25ppm	0 ppm	0	0	0
	50ppm	0 ppm	0	0	0

Each sample was prepared in triplicate for reproducibility. Turbidity was prepared at 50NTU. Ranges for each commercial free residual chlorine test strip (ppm):FR1: 0, 25, 50, 100.; FR2: 0, 0.1, 0.4, 0.6, 0.8, 1.2, 1.5, 2.0, 2.6, 4.0, 6.0, 10.; FR3: 10, 50, 100, 200.

**Free chlorine Test strips:** Commercial test strip data is summarized in **Table 2**. Without the presence of turbidity, the strips Fr1, Fr2, and Fr3 correlated to the free residual chlorine levels reported by the HACH analysis (see **Table 2**). Fr1, Fr2, Fr3 (results given in sequential order) measured 0 ppm, 0 ppm, 0 ppm in unchlorinated water and 50 ppm, >10 ppm, 50 ppm in chlorinated 50 ppm control clean water. When soil was introduced to the water, all 3 commercial test strips performed to their manufactured ranges and were consistent with the HACH control measurements. Soil Water prepared with 25 ppm at 50 NTU correlated with the response capabilities of the commercial strips, FR1: 25 ppm, FR2: >10 ppm, and FR3: between 10-50 ppm. However, the presence of cucumber matter in the wash solutions make a significant difference in the ability of the test strips to accurately detect free residual chlorine levels. A residual level of 4 ppm free chlorine in Cucumber Water produced FR1: 50 ppm, FR2: 2.0 ppm, and FR3: 10 ppm readings; all 3 test strips failed to correlate to the HACH reading of 4 ppm. At residual level of 10 ppm free chlorine in Cucumber Water from the HACH results produced FR1: <25 ppm, Fr2: 0.2 ppm, and Fr3: 50 ppm; suggesting that Fr1 was a match, Fr2 and Fr3 non-match compared to the HACH reading. Cucumber and Soil process wash water gave inconsistent results when compared to the HACH residual control measurements. A residual level of 5 ppm free chlorine in Cucumber Soil Water produced <25 ppm, 2.0 ppm and between 10-50 ppm; Fr1 was match, Fr2 and Fr3 non-match. A residual level of 25 ppm free chlorine in Cucumber Soil water produced <25 ppm, 0.6 ppm, and 50 ppm; all 3 test strips did not match the HACH reading of 25 ppm of free residual chlorine. Organic Challenge water as previously stated inactivated all available free chlorine to 0 ppm. All 3 test strips produce corresponding measurements 0 ppm, 0 ppm, and 0 ppm, which was consistent with the HACH control measurements of 0 ppm.

## Water microbiological quality

The low turbidity water (ONTU) with free residual chlorine concentration of 50 ppm measured ORP 680.7-701 mV. Water with increased turbidity of soil 50 NTU at 50 ppm free residual chlorine measured 717-718.8 mV; cucumber 50 NTU at 1.2 ppm measured 584.3-560 mV; and cucumber and soil 50 NTU at 10 ppm measured 816-833.1 mV. In all cases, except Organic Challenge water, the presence of free chlorine of at least 1.2 ppm-50 ppm in turbid wash water demonstrated greater than a 7 log reduction of pathogenic *E.coli* O157:H7 ATCC 43895.

## DISCUSSION

The results shown in **Table 1** demonstrate that high turbidity in process wash water correlate with low free residual chlorine concentrations in the cucumber based wash solutions. This is consistent with other research findings that organic loads and suspended solids interfere with sanitizer antimicrobial activity [3,4,6,8,12-14]. A reduction of *E.coli* O157:H7 occurred when ORP readings and free residual chlorine readings indicated the presence of active free residual chlorine level was confirmed. This is consistent with other research where *E.coli* O157:H7, Non-O157 STEC, and Salmonella were reduced by 4.5 log CFU/mL in presence of free chlorine [14]. The presence of free chlorine in this research reduced *E.coli* O157:H7 ATCC 43895 suspended in various turbid wash solutions. Portable ORP meters gave consistent results in control and organic matter solutions. The reduction of *E.coli* corroborated to ORP value as a monitoring tool indicating effective sanitizer activity when within 650-800 mV range. Results in **Table 1** indicate that 580 mV approximates the true minimum critical limit with 1.2 ppm free residual chlorine having effective antimicrobial activity. However, any increase turbidity would inactivate the low 1.2 ppm free chlorine. As a good agricultural practice, it is recommended that the chlorine sanitizer be maintained within the industry reported range of 650-800 mV to ensure food safety. Chlorine loses disinfection strength rapidly with the introduction of organic matter leading to greater potential of pathogen survival.

It is recommended to maintain the ORP value between 650-700 mV to maintain antimicrobial activity [4]. As seen in Suslow [6], organic load and chlorine concentration affects the readings of ORP. A comparison of different organic matter was performed on its effects on ORP. Clean Water with no chlorine measured and average of 444.5- 455 mV; with added 50 ppm bleach the ORP increased to an average of 680.7-701 mV. In the presence of soil, water with no chlorine averaged 475.3-457 mV similar in range with Clean Water. Soil water with chlorine added increased ORP levels. Soil water samples compared to the Control Clean water demonstrates that soil as an organic matter model does not interfere with free chlorine and ORP at the predetermined turbidity levels.

Cucumber water with no chlorine measured a low 303-350.0 mV. 25 NTU Cucumber water measured 11 ppm free chlorine with 830-834.9 mV. Fifty NTU Cucumber water measured 1.2 ppm free chlorine equated to a low 560-584.3 mV. Cucumber solids had a greater effect on inactivating free chlorine ions than soil matter in ORP measurements.

Chemical water test strips is a practical quick method of checking free chlorine levels. Results from **Table 2** demonstrate commercial test strips can be used accurately in process water that is clear, low turbid, and contain soil suspended solids. However, wash water samples with higher organic load indicate that different organic material can interfere with free chlorine strip accuracy. Cucumber solids in the water interfered with accuracy of the test. The blue or purple color change to corresponding legend did not match the measurements of the HACH control reading and thus provided false measurements. Cucumber vegetation had a greater impact to the accuracy of ORP and chemical test strip readings.

The Organic Challenge water was a method of controlling a known type of suspended solids in the water [7]. The total dissolved solids completely inactivated extreme range of sanitizer 50-200 ppm chlorine. Further investigation is needed to establish a method for organic matter since type of suspended solids in synthetic process water produce varied results.

## CONCLUSION

Our results showed that an increase in produce particulate, such as cucumber matter, in the wash water has an impact on the free residual chlorine levels. When used in high organic loaded wash water, commercially available ORP and free chlorine commercial test strip can result in inconsistent readings. Both ORP meters and paper test strips indicated antimicrobial activity of chlorinated water when compared to the HACH colorimetric control, so if there is inconsistent reading with high turbid vegetative process water than farmers can have a false sense of safety security. Test strips are an inexpensive, easy-to-use and rapid method to monitor free chlorine, but should be used as a qualitative tool for monitoring quality and not as a quantitative method.

As demonstrated in this research the presence of free residual chlorine will reduce microbial contamination. As a good agricultural practice, it is recommended that a sanitizer, such as sodium hypochlorite, be added to the recycled water sources used to wash produce. In small scale farm operations, commercially available quality control tools such as an ORP hand held unit or free chlorine test strips can be used as a qualitative assess that there is free residual chlorine present to reduce the risk of pathogenic contamination.

The use of chlorine sanitizer in produce wash water can help to significantly reduce food safety risk. Handheld ORP units

and free chlorine test strips are quality control tools that can qualitatively monitor the free chlorine to help control wash water. In this research the presence of chlorine doses as low as 1 ppm demonstrated a reduction of *E.coli*. Further research is needed to evaluate vegetative organic matter affecting chlorinated wash solutions and interferences with monitoring tools to understand their limitations so that these tools can be used quantitatively. There is a need for a standard model wash water system designed to mimic the organic loads that result from produce wash water in order to improve food safety validation studies. Total Dissolved Solids (TDS) should be considered in addition to turbidity to establish these types of model wash water systems. Proper maintenance of pH, organic matter, and free chlorine concentration with monitoring tools is critical in a postharvest sanitation program to prevent contamination of foodborne pathogens.

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