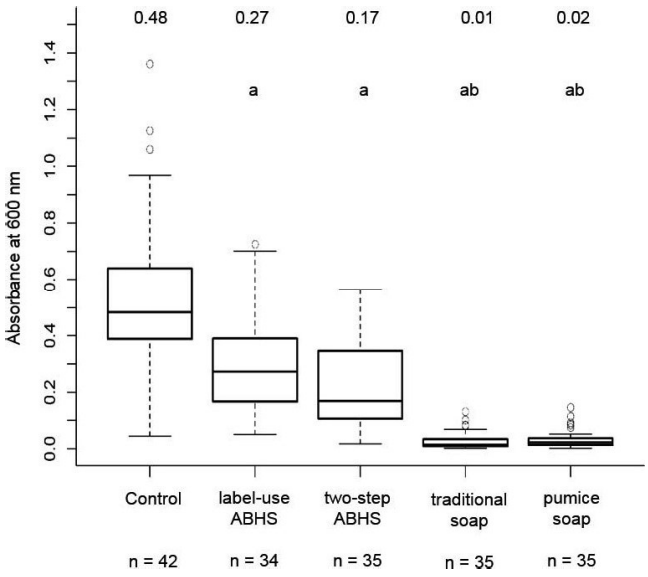


FIGURE 3. *Absorbance (at 600 nm) in hand rinsate samples from the control group and four intervention groups of workers harvesting tomatoes. For each study group, the boxes display the quartiles (25th, 50th, and 75th) and whiskers extend to 1.5 times the interquartile range. Any data points outside the whiskers are displayed individually as dots. The value above each study group box plot indicates the median absorbance ($A_{600}$). The control group samples were collected after farmworkers harvested tomatoes for 1 to 2 h. The four intervention groups had hand rinsates collected immediately after performing hand hygiene. a, significantly different from the control group ($\alpha = 0.05$); b, significantly different from the label-use ABHS and two-step ABHS groups ($\alpha = 0.05$)*

on hands than ABHS-based interventions (range, $A_{600}$ 0.02 to 0.73) ($P < 0.05$) (Fig. 3). These absorbance results confirm the trends seen in the "after intervention" photographs taken of hands (Fig. 4).

## DISCUSSION

The goal of this study was to assess the ability of two soap-based (traditional or pumice) and two ABHS-based (label-use or two-step) hygiene interventions, compared with a no-hand-hygiene control, to reduce microbes (coliforms, *E. coli,* and *Enterococcus*) and soil ($A_{600}$ of hand rinsate) on farmworker hands after harvesting produce. Without intervention, farmworkers' hands were contaminated with high concentrations of indicator bacteria and were heavily soiled after 1 to 2 h of harvesting tomatoes. All four hygiene intervention groups had lower concentrations of *Enterococcus* and *E. coli* on their hands than the control group. Furthermore, all four interventions yielded significantly less soil remaining on hands, soap-based interventions more so than ABHS-based interventions. Based on these results, ABHS can be viewed as a promising hand hygiene solution for produce handlers, even on soiled hands. To build on these findings, future studies could investigate the efficacy of ABHS for pathogen inactivation on soiled hands in a controlled setting (e.g., an experimental greenhouse).

Farmworkers' hands were heavily soiled and contaminated with high concentrations of indicator bacteria after 1 to 2 h of harvesting tomatoes. The control group results are supported by our previous field observational study of microbial contamination of produce, environmental samples, and farmworkers' hands *(23),* where we found that 16 to 41% of farmworkers' hands had detectable *E. coli,* 92 to 100% had detectable coliforms, and 70 to 99% had detectable *Enterococcus* bacteria, depending on the type of produce harvested. The lower percentage of samples positive for *E. coli* than of samples positive for coliforms and *Enterococcus* is expected, as *E. coli* is a gram-negative species of bacteria indicative of fecal contamination from a warm-blooded animal, whereas *Enterococcus* spp. (a genus of gram-positive bacteria) and coliforms (a general group of bacteria) are larger, more general categories of indicator bacteria. It is unlikely that the presence of these indicator bacteria is simply a result of poor sanitation and hygiene practices among the farmworkers given that they washed their hands with soap and water before beginning harvest and their sole activity was harvesting produce. It is more likely that farmworkers' hands are accumulating organic matter and indicator bacteria present in the agricultural environment (e.g., on plants, soil, or produce bins). Both coliforms and *Enterococcus* are naturally present in the guts of animals *(5, 36),* but they are also present in the environment *(36)* and could be introduced into the agricultural environment through various pathways (e.g., irrigation water, soil amendments, or contaminated tools or equipment). Similarly, the *E. coli* seen on some farmworker hands after harvest may indicate recent fecal contamination from a warm-blooded animal *(36)* or may indicate past environmental contamination, as *E. coli* is known to be persistent in the environment *(41).*

Farmworkers in all four intervention groups had lower concentrations of *Enterococcus* and *E. coli* on their hands than those in the control group. These results indicated that all four interventions were efficacious at reducing the concentrations of viable microbes on hands. The soap-based interventions likely reduced bacterial concentrations because soap is, by definition, an emulsifier, meaning it suspends hydrophobic compounds and, with them, any particles and microbes. These particles and microbes are then removed when hands are rinsed. These traditional soap and pumice soap intervention results are consistent with the results from a pilot study of a hand hygiene intervention using foam soap on soiled farmworker hands *(13).* The ABHS-based interventions likely reduced bacterial concentrations because ethanol, the active ingredient in the ABHS, is an effective antimicrobial agent *(3, 24).* These results suggest that ABHS can be an efficacious hand hygiene method, even on soiled hands. Although the soap-based and ABHS-based interventions work by different mechanisms, they were both efficacious at reducing microbes on soiled hands.

No intervention resulted in lower concentrations of coliforms than in the control group. Given the high variability of coliform concentrations in the control and all intervention groups and the generally small reductions (0 to 2 log) in coliforms previously reported with hand washing with foam soap and ABHS in the field *(13),* a larger sample size would likely have been needed for these interventions to demonstrate a statistically significant difference in coliform

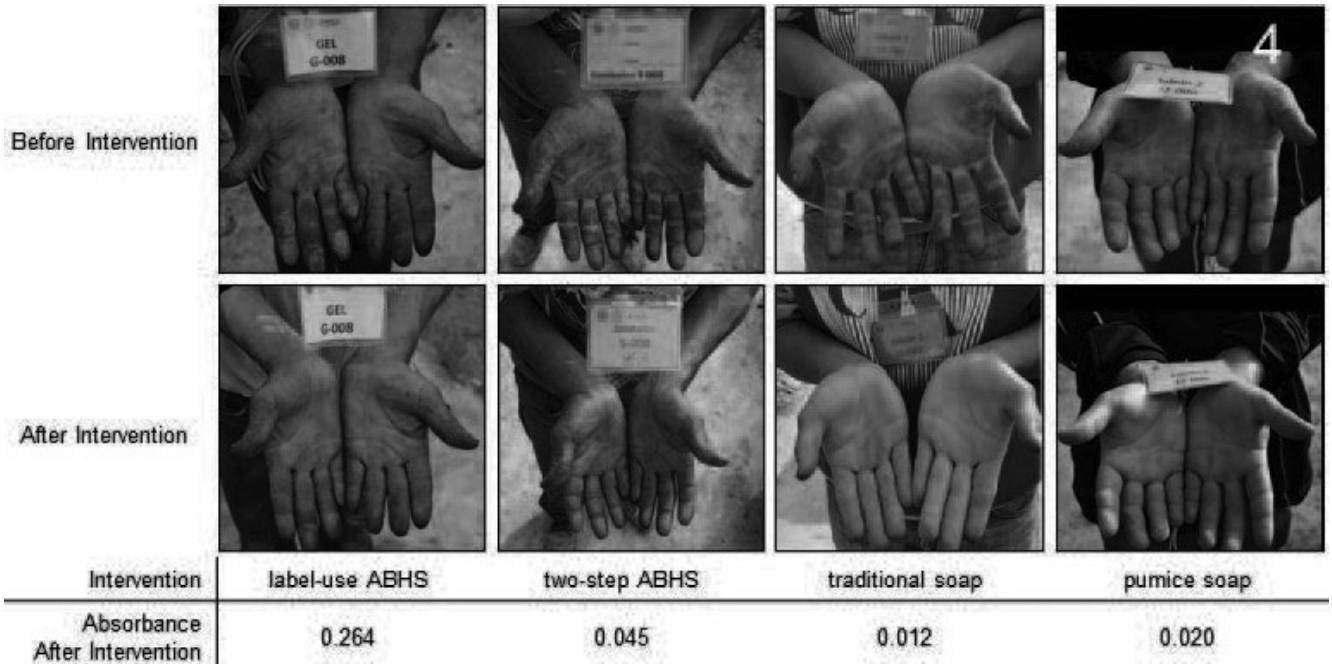


FIGURE 4. *Photographs of hands and corresponding individual hand rinsate absorbance readings from samples collected after intervention from study participants—workers harvesting tomatoes on a farm in Mexico. Photographs were taken immediately before and after each worker performed hand hygiene.*

concentration compared with the control group. In a previous study comparing two-step ABHS and foam soap to a control group, only two-step ABHS had significantly lower levels of coliforms (~2 log *(13)*) than the control group. These results suggest that coliforms may be more persistent on hands than *E. coli* and *Enterococcus* spp. after hand washing or ABHS use. Given that total coliforms are poor indicators of fecal contamination in an environmental setting *(36),* it is unclear whether this result has a practical application in hand hygiene techniques.

All four interventions significantly removed soil from hands, soap-based interventions more so than ABHS-based interventions. It was expected that soap-based interventions would be the most efficacious at soil removal, given soap's emulsion properties described above. The removal of soil from hands with label-use of ABHS was a somewhat unexpected result, as the intervention does not involve wiping or removing anything from the hands. This result contradicts previous research on alcohol-based gels *(21, 34)*. However, study participants' hands were quite heavily soiled, and particles may have been solubilized in the ABHS and then dropped to the ground as the liquid portion evaporated. The two-step ABHS intervention uses paper towels to remove excess ABHS *(11)*; it is likely that additional soil particles were also removed by the paper towel when wiping dry.

The label-use ABHS and pumice soap interventions were similar to the traditional soap intervention in their effectiveness at reducing the microbial load on farmworker hands. However, the two-step ABHS intervention was more efficacious than the label-use ABHS and pumice soap interventions and was at least as efficacious as traditional soap at reducing microbes on soiled farmworker hands. The two-step ABHS intervention resulted in significantly lower percentages of positive samples and lower geometric mean concentrations of all indicators than did the label-use ABHS intervention (concentrations of coliforms and *Enterococcus* bacteria) (Fig. 2) and pumice soap intervention (prevalence and concentrations of coliforms and concentrations of *Enterococcus* bacteria) (Table 1 and Fig. 2). These results confirmed the results in a previous study of hand hygiene interventions with farmworkers harvesting jalapeños, where the same two-step ABHS intervention resulted in 1 to 2 log CFU fewer bacteria per hand than were found for the control group and performed better at eliminating indicator bacteria than hand washing with foam soap *(13)*. The results suggest that the most efficacious hand hygiene intervention in the agricultural environment may be a dual-mechanism intervention, such as the two-step ABHS, that combines physical removal from hands (e.g., with paper towels) with inactivation of indicator bacteria (e.g., by ethanol, the active ingredient in the ABHS and an effective antimicrobial agent *(3, 24)*).

This study has several strengths and limitations. It addresses a gap in the hand hygiene literature by evaluating the efficacy of hygiene interventions in an agricultural environment under real-use conditions. The study also compares an array of hygiene interventions, both soap based and ABHS based. Although the study was conducted on only one farm with participants harvesting only one type of produce, the similarity of the results to those of a previous pilot study evaluating foam soap and two-step ABHS on a different farm with different produce *(13)* suggests that these results may be broadly applicable to the agricultural field environment during produce harvest.

The results of this field evaluation of hand hygiene techniques have several implications. Hands may be a source of produce contamination if a farmworker is ill, and

hands may also contribute to produce contamination by transferring indicator bacteria from the environment (e.g., soil, water, or produce bins) to the produce during harvest. These results show that the performance of hand hygiene interventions can vary with the hygiene product and technique, and hand hygiene recommendations may need to be tailored to meet the environment and availability of hygiene resources. Hand hygiene performed incorrectly or with an ineffective product may not improve the microbial quality of hands even if they appear cleaner after hygiene. Although they did not remove soil as well as soap-based interventions, the ABHS-based interventions reduced the concentrations of indicator bacteria similarly to the soap-based interventions and can be viewed as efficacious hand hygiene solutions even on soiled hands.

## ACKNOWLEDGMENTS

We thank the farmers for their collaboration. We also thank Nereida Rivera, Roberto Blancas, and Aldo Galván from Universidad Autónoma de Nuevo León for the collection and processing of samples and Sanemba Aya Fanny, Valerie Morrill, Carol Ochoa, Vidisha Singh, and Amelia Van Pelt at Emory University for data entry. This project was financially sponsored by GOJO Industries, Inc., through an unrestricted research grant to cover partial salary for the effort of the study team, study supplies, and communication of results. Both Emory University and UANL covered the remainder of staff salary through internal funding and government fellowships and subsidies. UANL-affiliated authors provided significant input into the overall study design, data collection, and editing of the manuscript. Emory-affiliated authors provided significant input into the overall study design, data analysis, and writing of the manuscript. GOJO-affiliated authors provided significant input into the overall study design, choice of hygiene products evaluated, creation of Figure 1, and editing of the manuscript. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

## REFERENCES

1. Aiello, A. E., R. M. Coulborn, V. Perez, and E. L. Larson. 2008. Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis. *Am. J. Public Health* 98:1372–1381.
2. Ailes, E. C., J. S. Leon, L. A. Jaykus, L. M. Johnston, H. A. Clayton, S. Blanding, D. G. Kleinbaum, L. C. Backer, and C. L. Moe. 2008. Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. *J. Food Prot.* 71:2389–2397.
3. Ali, Y., M. J. Dolan, E. J. Fendler, and E. L. Larson. 2001. Alcohols, p. 229–254. *In* S. S. Block (ed.), Disinfection sterilization and preservation. Lippincott Williams & Wilkins, Philadelphia.
4. Bloomfield, S. F., A. E. Aiello, B. Cookson, C. O'Boyle, and E. L. Larson. 2007. The effectiveness of hand hygiene procedures in reducing the risks of infections in home and community settings including handwashing and alcohol-based hand sanitizers. *Am. J. Infect. Control* 35:S27–S64.
5. Boehm, A. B., and L. M. Sassoubre. 2014. Enterococci as indicators of environmental fecal contamination. *In* M. S. Gilmore, D. B. Clewel, Y. Ike, and N. Shankar (ed.), Enterococci: from commensals to leading causes of drug resistant infection. Massachusetts Eye and Ear Infirmary, Boston.
6. Boyce, J. M., and D. Pittet. 2002. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Society for Healthcare Epidemiology of America/Association for Professionals in Infection Control/Infectious Diseases Society of America. *Morb. Mortal. Wkly. Rep. Recommend. Rep.* 51(RR-16):1–45.
7. Calvin, L., and P. Martin. 2010. The U.S. produce industry and labor: facing the future in a global economy. Economic research report number 106. U.S. Department of Agriculture, Economic Research Service, Washington, DC. Available at: http://www.ers.usda.gov/media/135123/err106.pdf.
8. Critchlow, D. E., and M. A. Fligner. 1991. On distribution-free multiple comparisons in the one-way analysis of variance. *Commun. Stat. Theory Methods* 20:127–139.
9. Daniel, W. W. 1999. Biostatistics: a foundation for analysis in the health sciences, 7th ed., p. 571–606. John Wiley and Sons, Inc., Hoboken, NJ.
10. Davis, M. A., H. Sheng, J. Newman, D. D. Hancock, and C. J. Hovde. 2006. Comparison of a waterless hand-hygiene preparation and soap-and-water hand washing to reduce coliforms on hands in animal exhibit settings. *Epidemiol. Infect.* 134:1024–1028.
11. Edmonds, S. L., J. Mann, R. R. McCormack, D. R. Macinga, C. M. Fricker, J. W. Arbogast, and M. J. Dolan. 2010. SaniTwice: a novel approach to hand hygiene for reducing bacterial contamination on hands when soap and water are unavailable. *J. Food Prot.* 73:2296–2300.
12. Edmonds, S. L., R. R. McCormack, S. S. Zhou, D. R. Macinga, and C. M. Fricker. 2012. Hand hygiene regimens for the reduction of risk in food service environments. *J. Food Prot.* 75:1303–1309.
13. Fabiszewski de Aceituno, A., N. Heredia, A. Stern, F. Bartz, F. Venegas, L. Solis-Soto, J. Gentry-Shields, J. Leon, L.-A. Jaykus, and S. Garcia. 2016. Efficacy of two hygiene methods to reduce soil and microbial contamination on farmworker hands during harvest. *Food Control* 59:787–792.
14. Falkenhorst, G., L. Krusell, M. Lisby, S. B. Madsen, B. Bottiger, and K. Molbak. 2005. Imported frozen raspberries cause a series of norovirus outbreaks in Denmark, 2005. *Euro Surveill.* 10:E050922.2.
15. Heredia, N., L. Solis-Soto, F. Venegas, F. E. Bartz, A. M. Fabiszewski de Aceituno, L.-A. Jaykus, J. S. Leon, and S. Garcia. 2015. Validation of a novel rinse and filtration method for efficient processing of fresh produce samples for microbiological indicator enumeration. *J. Food Prot.* 78:525–530.
16. Hjertqvist, M., A. Johansson, N. Svensson, P. E. Abom, C. Magnusson, M. Olsson, K. O. Hedlund, and Y. Andersson. 2006. Four outbreaks of norovirus gastroenteritis after consuming raspberries, Sweden, June-August 2006. *Euro Surveill.* 11:E060907.1.
17. Jin, M., and B. Wang. 2014. Implementing multiple comparisons on Pearson chi-square test for an RxC contingency table in SAS. SAS technical paper 1544-2014. Proc. SAS Global Forum. SAS Institute, Cary, NC.
18. Johnston, L. M., L. A. Jaykus, D. Moll, J. Anciso, B. Mora, and C. L. Moe. 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *Int. J. Food Microbiol.* 112:83–95.
19. Johnston, L. M., C. Moe, D. Moll, and L. A. Jaykus. 2006. The epidemiology of produce-associated outbreaks for foodborne disease, p. 37–72. *In* J. L. James (ed.), Microbial hazard identification of fresh fruit and vegetables. John Wiley and Sons, Inc., Hoboken, NJ.
20. Kruskal, W. H. 1952. A nonparametric test for the several sample problem. *Ann. Math. Stat.* 23:525–540.
21. Larson, E., and L. Bobo. 1992. Effective hand degerming in the presence of blood. *J. Emerg. Med.* 10:7–11.
22. Leon-Felix, J., R. A. Martinez-Bustillos, M. Baez-Sanudo, F. Peraza-Garay, and C. Chaidez. 2010. Norovirus contamination of bell pepper from handling during harvesting and packing. *Food Environ. Virol.* 2:211–217.
23. Lickness, J. 2014. Assessing microbial contamination on produce, environmental sources, and farm worker hands throughout the production process on farms and packing sheds in northern Mexico. M.S. thesis. Emory University, Atlanta.
24. Morton, H. E. 1950. The relationship of concentration and germicidal efficiency of ethyl alcohol. *Ann. N. Y. Acad. Sci.* 53:191–196.
25. Pickering, A. J., A. B. Boehm, M. Mwanjali, and J. Davis. 2010. Efficacy of waterless hand hygiene compared with handwashing with soap: a field study in Dar es Salaam, Tanzania. *Am. J. Trop. Med. Hyg.* 82:270–278.
26. Pickering, A. J., J. Davis, and A. B. Boehm. 2011. Efficacy of alcohol-based hand sanitizer on hands soiled with dirt and cooking oil. *J. Water Health* 9:429–433.

27. Pickering, A. J., T. R. Julian, S. Mamuya, A. B. Boehm, and J. Davis. 2011. Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. *Trop. Med. Int. Health* 16:233–239.
28. Racicot, M., A. Kocher, G. Beauchamp, A. Letellier, and J. P. Vaillancourt. 2013. Assessing most practical and effective protocols to sanitize hands of poultry catching crew members. *Prev. Vet. Med.* 111:92–99.
29. Ravaliya, K., J. Gentry-Shields, S. Garcia, N. Heredia, A. F. de Aceituno, F. E. Bartz, J. S. Leon, and L. A. Jaykus. 2014. Use of *Bacteroidales* microbial source tracking to monitor fecal contamination in fresh produce production. *Appl. Environ. Microbiol.* 80:612–617.
30. Schaffner, D. W., and K. M. Schaffner. 2007. Management of risk of microbial cross-contamination from uncooked frozen hamburgers by alcohol-based hand sanitizer. *J. Food Prot.* 70:109–113.
31. Seymour, I. J., and H. Appleton. 2001. Foodborne viruses and fresh produce. *J Appl. Microbiol.* 91:759–73.
32. Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52:591–611.
33. Smith DeWaal, C., C. Roberts, and C. Catella. 2012. Outbreak alert! 1999-2008. Center for Science in the Public Interest, Washington, DC.
34. Todd, E. C. D., B. S. Michaels, J. Holah, D. Smith, J. D. Greig, and C. A. Bartleson. 2010. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 10. Alcohol-based antiseptics for hand disinfection and a comparison of their effectiveness with soaps. *J. Food Prot.* 73:2128–2140.
35. Traub-Dargatz, J. L., J. S. Weese, J. D. Rousseau, M. Dunowska, P. S. Morley, and D. A. Dargatz. 2006. Pilot study to evaluate 3 hygiene protocols on the reduction of bacterial load on the hands of veterinary staff performing routine equine physical examinations. *Can. Vet. J.* 47:671–676.
36. U.S. Environmental Protection Agency. 1997. Water: monitoring & assessment. 5.11. Fecal bacteria. Available at: http://water.epa.gov/type/rsl/monitoring/vms511.cfm. Accessed 1 December 2014.
37. U.S. Environmental Protection Agency. 2000. Guidance for data quality assessment. practical methods for data analysis. EPA QA/G-9. U.S. Environmental Protection Agency, Washington, DC.
38. U.S. Food and Drug Administration. 2013. Food Code 2013, chap. 2, Management and personnel; part 2-3, Personal cleanliness. U.S. Food and Drug Administration, Public Health Service, College Park, MD. Available at: http://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm181242.htm#part2-3. Accessed September 2015.
39. U.S. Food and Drug Administration. 2013. Food Safety Modernization Act proposed rule for produce safety. Standards for the growing, harvesting, packing, and holding of produce for human consumption (corrected version). 21 CFR Parts 16 and 112. FDA-2011-N-0921. U.S. Food and Drug Administration, Washington, DC.
40. U.S. Food and Drug Administration. 2013. Statistics applied to microbiological analysis, section 4.10. *In* ORA laboratory manual, vol. III. U.S. Food and Drug Administration, Office of Regulatory Affairs, Washington, DC.
41. van Elsas, J. D., A. V. Semenov, R. Costa, and J. T. Trevors. 2011. Survival of Escherichia coli in the environment: fundamental and public health aspects. *ISME J.* 5:173–183.
42. Wheeler, C., T. M. Vogt, G. L. Armstrong, G. Vaughan, A. Weltman, O. V. Nainan, V. Dato, G. L. Xia, K. Waller, J. Amon, T. M. Lee, A. Highbaugh-Battle, C. Hembree, S. Evenson, M. A. Ruta, I. T. Williams, A. E. Fiore, and B. P. Bell. 2005. An outbreak of hepatitis A associated with green onions. *N. Engl. J. Med.* 353:890–897.
43. World Health Organization. 2009. WHO guidelines on hand hygiene in health care. WHO Press, Geneva.