**Distribution Agreement**

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Alexandra Stern Date

Efficacy of Two Hand-Hygiene Methods to Reduce Organic Matter and Fecal Contamination on Farmworker Hands During Harvest

By

Alexandra Stern
Master of Public Health

Environmental Health

_____
Juan Leon, PhD, MPH
Committee Chair

_____
Faith Bartz, PhD
Committee Member

_____
Paige Tolbert, PhD
Committee Member

# Efficacy of Two Hand-Hygiene Methods to Reduce Organic Matter and Fecal Contamination on Farmworker Hands During Harvest

By

Alexandra Stern

Bachelors of Science
Emory University
2013

Thesis Committee Chair: Juan Leon, PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2014

**Abstract**

Efficacy of Two Hand-Hygiene Methods to Reduce Organic Matter and Fecal Contamination on Farmworker Hands During Harvest
By Alexandra Stern

Harvesters' hands have been repeatedly implicated in the contamination of produce and are thus a serious risk factor for foodborne illness. Improvements in the hygiene of harvesters are needed to reduce the risk associated with produce handling. This study evaluated two hand-hygiene methods in their ability to reduce both fecal indicator and dirt levels on farm workers' hands during harvest. Hand rinse samples were collected from 159 individuals performing various hygiene techniques: SaniTwice, hand washing, SaniTwice + harvest, hand washing + harvest, or no hygiene (control). Individuals in the SaniTwice group used an ethanol-based hand sanitizer and the hand washing group used water and a foam cleanser. Intervention groups submitted their hands for rinses immediately after performing the intervention. Intervention + harvest groups performed the intervention and then continued harvesting three five-gallon buckets of produce (for approximately 30 minutes) before submitting their hands for samples. Effects were measured using absorbance of hand rinses at 600nm (related to organic matter), and concentration and prevalence of fecal indicators ($\log_{10}$ CFU fecal coliforms, *Enterococcus*, and *E. coli*). Both intervention groups had a significantly lower mean absorbance than the control group (hand washing 0.01, SaniTwice 0.10, control 0.24) ($p<0.01$). The hand washing group had a significantly lower absorbance, than the SaniTwice group ($p<0.01$). The SaniTwice group had significantly lower concentrations of fecal coliforms and *Enterococcus* compared to the control group (SaniTwice 1.47 fecal coliforms, 3.11 *Enterococcus*, control 3.28 fecal coliforms, 4.09 *Enterococcus*) ($p<0.01$). There was no significant difference between concentrations of fecal coliforms, and *Enterococcus*, in the control and hand washing groups (hand washing 2.77 fecal coliforms, $p=0.05$, 3.99 *Enterococcus*, $p=0.99$). *E. coli* prevalence were very low for all groups (12.5-20% samples positive). The SaniTwice method was superior to hand washing in reducing fecal indicators and significantly reduced particulate matter compared to the control group on hands of harvesters, therefore this method might be a viable alternative to hand washing when soap and water are not available.

# Efficacy of Two Hand-Hygiene Methods to Reduce Organic Matter and Fecal Contamination on Farmworker Hands During Harvest

By

Alexandra Stern

Bachelors of Science
Emory University
2013

Thesis Committee Chair: Juan Leon, PhD, MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Environmental Health
2014

**TABLE OF CONTENTS**

**LITERATURE REVIEW ……...……1**

**INTRODUCTION ……...……...15**

**METHODS ……...……...21**

**RESULTS……...…….....28**

**DISCUSSION……...…….....34**

**CONCLUSION ……...…….....37**

**REFERENCES ……...……42**

**TABLE 1 ……...…….....47**

**TABLE 2 ……...……...47**

**TABLE 3 ……...…….....48**

**TABLE 4 ……...…….....48**

**TABLE 5 ……...……...49**

**TABLE 6 ……...……...49**

**TABLE 7 ……...……...50**

**TABLE 8 ……...……...50**

**FIGURE 1 ……...……..……...52**

**FIGURE 2 ……...……..……..53**

# LITERATURE REVIEW—PRODUCE CONTAMINATION AND PREVENTION

## BURDEN OF FOODBORNE DISEASE

It is estimated that one in every six Americans will contract a foodborne illness this year—emphasizing the seriousness of foodborne diseases (1). One in six amasses to nearly 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths, all directly attributable to the consumption of contaminated food (2). The actual numbers may be greater as estimates calculated by surveillance systems miss cases due to lack of reporting to medical professionals and inadequate test methods for identifying pathogens (3). Foodborne illness plagues millions of Americans; therefore it should be a high priority for public health officials.

While not a noteworthy contributor historically, contaminated produce has become progressively more important in the proliferation of foodborne illness (4, 5). This is clear in reports by the U.S. Foodborne Outbreak Surveillance System; produce-associated outbreaks have increased from less than 1% in the 1970s to 6% in the 1990s (5). This is equivalent to two outbreaks per year in the 1970s, increasing to 16 outbreaks per year in the 1990s (4). More recently, from 1999 to 2008, produce related illness constituted 23% of all foodborne disease outbreaks, causing the largest number of illnesses associated with outbreaks in the United States (6). This trend is very much the result of transitions in diet, wealth, and transportation. As globalization supplies more diverse produce to a wealthier and more health conscious population, produce consumption increases (4, 7). Moreover, a widened food distribution means that contaminated produce can reach a variety of populations, and new strains of pathogens

may be introduced to vulnerable environments. Due to 717 outbreaks and 27,849 illnesses from 1999 to 2008, the U.S. Food and Drug Administration (FDA) now regulates produce as a high-risk product (6).

## ETIOLOGY—PATHOGENS AND MECHANISMS

Pathogens related to foodborne illness fall within three main categories: viruses, bacteria, and parasites (4). Available data do not always report the etiology of disease, but in the cases where the causal agent can be identified the top pathogens causing foodborne illness are norovirus at 48%, Salmonella at 11% and *Clostridium perfringens* at 10% of disease cases (1). The most deadly pathogens between 1998 and 2008, were *Salmonella,* accounting for 30% of deaths, *Listeria*, accounting for 24% of deaths, and Shiga toxin-producing *Escherichia coli*, accounting for 11% of deaths (2). Among all produce associated outbreaks between 1990 and 2004, the majority of disease was caused by *Salmonella* spp. and norovirus (8). It is of the utmost importance that food does not come in contact with these pathogens, because very low concentrations of these pathogens can result in illness.

Reducing the risk of contamination is a fundamental step in lessening disease cases. Food contamination results from pathogens depositing onto and potentially binding to the surface of plants, and once bound they are difficult to remove. In a study of *E. coli* contamination of sprouts and tomatoes it was found that pathogenic *E. coli* might have multiple mechanisms by which they attach to produce (9). One distinct mechanism is the production of a biofilm, or a matrix associated with the plant cell wall that accommodates the growth of bacteria, yeast, and mold (10, 11). In a study of *Listeria*

*moncytogenes* in a multispecies biofilm with *Pseudomonas fragi* and *Staphylococcus xylosus*, bacteria were unaffected by sanitizing treatments of 500 ppm free chlorine (12). This is a problem because vegetables are commonly eaten raw, and salad has repeatedly been implicated in foodborne illness outbreaks (8). Without any heat or other process to remove or inactivate microbes, pathogens remain on food and can cause disease. This highlights how imperative it is to stop contamination at the source before the produce is exposed to the pathogen. Prevention of contamination is preferable to remediation because of difficulties in removing pathogens from raw produce.

## INDICATOR VALIDATION

Foodborne illness and outbreaks result from ingestion of pathogenic viruses, bacteria, or parasites. These pathogens take residence in the intestinal tract of warm-blooded animals and generally contaminate food through direct or indirect contact with animal, including human, feces (13). It is costly to measure pathogens in the environment and on produce as there are many varieties and they are present in miniscule concentrations (14). A more feasible alternative to the difficult task of detecting pathogens is the detection of fecal indicators, which are normally more abundant (14). Indicator organisms thrive in a similar environment or share a similar ecology as most foodborne pathogens, this is the feces of warm-blooded animals, hence the name fecal indicator. Indicator presence and survival is significantly associated with the presence and survival of pathogens (15). Therefore, the presence of fecal indicators implies a greater risk of the presence of pathogens, and indicators are often studied as a substitute for pathogens.

When choosing fecal indicators to act as a proxy for pathogen contamination, the indicators must assume certain characteristics. General standards for indicator use include: the indicator should be present whenever the enteric pathogen is present, there should be a relationship between the concentration of the indicator and the pathogen, the organism should have a longer survival time than the enteric pathogen; and enumeration and testing should be inexpensive and simple (14, 16). By assuring that these characteristics are met there is an enhanced probability of indicator detection even after adverse conditions, making testing for indicators more conservative than testing for pathogens (the probability of a false positive is greater than that of a false negative.) Although there is no single best indicator to represent all foodborne pathogens: total coliform bacteria, fecal coliform bacteria, generic *E. coli*, and total *Enterococcus* spp are repeatedly used as indicators in food contamination research (17-21).

**CONTAMINATION ATTRIBUTION—FOOD WORKERS**

Feces can indirectly contaminate produce through vehicles such as human hands, water, soil, tools, and equipment (4). Specifically, human hands and poor hygiene of food handlers have been implicated in numerous reports and studies as a risk for food contamination (22-25). The FDA estimates that this is one of the five leading causes for outbreaks of foodborne disease in the United States (26). In a study of produce related outbreaks between 1973 and 1997, fifty separate outbreaks were potentially caused by insufficient hygiene of food workers (5). Furthermore, in multiple norovirus and hepatitis A outbreaks, hands of infected workers were positively identified as the cause of food contamination (27). In Pickering et al. (2011) food preparation increased

*Enterococci* levels on hands significantly more than other household tasks, including changing of diapers—evidence that hands of food workers are unique, and food handling itself contaminates hands (28). These findings illustrate how substandard hygiene can transform hands into a source and a vehicle of foodborne disease.

Although there are multiple routes for contamination of food on farms involving environmental and management factors, research repeatedly confirms that harvesting is a major contributor to contamination. Harvesting is labor intensive, and research has shown that human contact increased levels of produce contamination on farms. Looking at plants during pre- and post-harvest, levels of produce contamination grew significantly from field to packing environments (21, 29, 30). A link between workers hands, processing, and food microbial quality is further suggested by a positive correlation amid universal *Bacteroidales* concentrations on samples of produce and harvesters hands (31). These findings support the idea that on farms soiled hands play a substantial role in the transfer of disease-causing microbes to produce.

A clear solution, to minimize the risk of produce contamination, is proper hygiene among workers during agricultural processing. This requires both clean water and soap, yet many farms lack these amenities (32). In a study testing for *E. coli* on spinach, the odds of contamination were significantly reduced for farms with proper toilet and washing facilities as compared to farms without such facilities (33). This conclusion offers insight on risk factors; a lack of access to hygienic amenities increases the risk of produce contamination. While these findings were informative, there were many confounding variables in this research including, if fields were tilled and use of reservoir water. Due to confounding effects, and a general shortage of comparable literature, more

research in similar settings should address whether lack of access to sanitary facilities is a major contributor to contamination. If this is the case, then hygiene methods can be tailored to environments lacking in facilities. Hygiene interventions in the production and processing environments of fruits and vegetables can be individualized for a specific setting and implemented to reduce hand contamination and prevent transfer of pathogens to produce.

## HYGIENE PRACTICES

Hand washing with soap is the standard hygiene practice for food handlers (34). In 2009, the FDA developed "harmonized" standards for the production of fruits and vegetables, which provide information on Good Agricultural Practices (GAPs) and require that workers be trained on hygiene routines and provided with ample facilities (13, 34). The FDA's rule for hand washing is as follows: 1) personnel engaged in hand-labor on farms must wash hands thoroughly, i.e. scrubbing with soap and water (35). 2) The water must be tested and determined to be free of generic *E. coli*, or drinking water quality (34). Specifically, the water must meet OSHA requirements in 29 CFR 1928.110 (36), which specify the maximum microbial contaminant level in the National Primary Drinking Water Regulations. 3) Drying of hands must be done with single use towels or "clean" cloth towels. Requirements on when hand washing should take place include: before starting work and putting on gloves, after a break, after touching animals or waste, and after using the toilet. Hand washing is also required at any point when workers feel as though their hands might reasonably contaminate produce. The FDA recognizes that there is no specific point in production where produce is sterilized of all pathogens, and

therefore enforces hand washing as the key control measure in prevention of produce contamination.

Numerous studies support the FDA's recommendations. Research shows hand washing with soap and water removes dirt, and both resident and transient microbes (37-39). Antimicrobial washing agents are shown to kill pathogens and continue to provide a residual effect for up to three hours (40). Hand washing is considered effective at removing bacteria, however it is not always effective in reducing parasitic protozoa and viruses (41, 42). Mbithi et al. (1993) found that hepatitis A virus and poliovirus were transferred from finger pads to clean metal disks, even after hands were treated with various washing products (41). This pressures researchers to develop and define hand sanitizers and hygiene techniques, which are more effective than standard hand washing.

Alcohol based sanitizers have been found to significantly reduce the risk of illness. In a U.S. Army basic training setting, researchers found that there were 40% less respiratory illnesses, 48% less gastrointestinal illness, 44% less lost training time, and 31% fewer health care encounters in trainees that were given personal alcohol-based hand sanitizer, health hygiene education, and easy access to sanitizing stations as compared to a control group (43). Purell VF481 gel sanitizer with 70% ethanol significantly reduces norovirus RNA over several commercial alcohol-based rubs and standard washing (44). This is evidence of the advantages of the regular use of instant hand sanitizer in reducing the risk of disease.

In order to increase compliance and effectiveness, the hygiene industry strives to develop product formulations and techniques best suited for food service and farming. One such innovation is the SaniTwice technique, which incorporates a washing like

procedure with a sanitizing gel (45). In a study comparing standard hand washing with this new integrated washing and sanitizing approach, researchers found that this waterless method with a 70% ethanol was the most effective sanitizing system. This process significantly outperformed all other sanitizing configurations on heavily soiled hands (45). SaniTwice was equally or more effective at reducing fecal indicators on hands than standard Food Code hand washing. In the "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Proposed Rule," the FDA does support sanitizing after hand washing, however, the rule is insistent upon hand washing with water as the primary practice. For farms with insufficient facilities, compliance with such standards might be difficult, and a waterless method such as SaniTwice may be more practical.

Gloves are another widely used practice in the field of medical and food safety. In a review of gloving compared to hand washing and sanitizing it was revealed that gloves alone are never effective; they must always be used in conjunction with, and not as a substitute for, hand hygiene (46). Contaminated gloves and hands act an important vector for disease. Whether gloves are intact or not the surface of the gloves can become contaminated with bacteria. This was observed in both active and inactive workers, suggesting that gloves are not an effective barrier or a viable alternative to hand hygiene in the food industry (46). When workers were dealing with inoculated meat barehanded, hourly washing and hand sanitizing significantly reduced $\log_{10}$ mean bacterial colonies on hands as compared to other methods. Washing and sanitizing hands significantly reduced risk of health hazards as compared to changing gloves and just washing hourly

(40). This suggests that gloves might give workers a false sense of security, and proper sanitizing and washing protocol, without gloves, should be established for food safety.

## HAND HYGIENE INTERVENTIONS: COMPARING HYGIENE METHODS IN DIFFERENT SETTINGS

Hand hygiene interventions are an excellent means to determine superior practices for food workers. Finding alternatives to the standard is necessary in situations when compliance, access, and effectiveness are poor. In a meta-analysis comparing numerous techniques, non-antibacterial soap combined with education showed the strongest protective effect against gastrointestinal illness (47). Alcohol-based sanitizer only showed moderate reductions in illness as compared with control groups (47). This research supports hand washing, however these studies were not representative of actual working conditions of farm workers.

While hand washing has been shown to be the most effective routine in experimental interventions, it is not always successful in actual food handling settings. This has been shown when assessing hospital kitchens in Brazil (48). Kitchens were rated with a food-safety checklist and swab testing for microbial contaminants revealed that checklist inspections were not sufficiently sensitive. Kitchens received passing ratings because workers preformed "good practices," e.g., hand washing, even though workers' hands had coliform contamination. Food workers had levels greater than 100 colony forming units per handler of coagulase-positive staphylococcus on hands even though they had been trained on personal hygiene on a regular basis (48). This observational study implied that hand washing was not an effective hygiene technique,

and that current review systems which had rated these kitchens as "satisfactory" are not sufficiently stringent. Standard systems should be conducive to worker access and compliance, as well as reduce fecal indicators on hands of food workers. Alternatives to hand washing, which increase compliance and are highly effective, should be considered.

Several intervention studies support the use of alcohol-based hand sanitizer, and illustrate that hand sanitizer is either equally or more effective at improving microbial quality of hands than standard hand washing with soap and water in the food industry (38, 49, 50). For example, alcohol based hand sanitizer was significantly more effective than antiseptic detergents in reducing mean $\log_{10}$ counts of *Staphylococcus auerus*, *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella* spp. (51, 52). In Schaffner & Schaffner (2007) the minimum reduction using sanitizer was two logs greater than that of either no intervention or hand washing. This was the case even when hands were perceived as dirty by researchers and participants (53). *In vitro* and *in vivo* experiments have shown that hand disinfectants are significantly better at killing bacteria than hand washing (54). Research further shows that alcohol-based hand sanitizers can increase compliance—potentially making them a more suitable hygiene technique for food cultivation environments (49, 55, 56). Due to discrepancies in research findings and standards, further studies must ensure that the current requirements are the most efficient and effective method for hand disinfection in the food industry.

Information from hygiene interventions in the field of animal husbandry can be informative for all farms, as numerous produce farms contain livestock. Hands become highly contaminated on farms with livestock (18). Research supports the use of alcohol-based hand sanitizing either alone or in conjunction with a specific protocol when hands

are contaminated from livestock (18, 19, 57). Evidence suggests that hand sanitizer is either as or more effective at improving microbial quality of hands than standard hand washing with soap and water when handling animals. In an animal exhibit showcase, researchers found that participants with *E.* coli contamination on hands, had no detectable *E. coli* after using a sanitizing gel, while those who used soap and water had low counts of *E. coli* detected on their hands (19). Similar conclusions were made with veterinary staff; groups that used alcohol based hand sanitizers after routine equine checkups had significantly greater reduction as compared with hand washing groups (57). When working with poultry catching crews, researchers found that hygiene intervention effectiveness and initial hand contamination levels were significantly related. Regardless of the intervention, its efficacy was affected by the prior dirtiness of the hands. Due to this finding, researchers decided to split participants into categories of high, medium, and low contamination. When contamination was considered high, degreasing cream combined with an alcohol-based gel was significantly more effective than alcohol-based gel alone. For reduction of total aerobic bacteria counts, water, soap, and an alcohol-based gel protocol was more effective than scrubbing wipes and alcohol-based gel (18). At petting zoos compliance with hand hygiene increases when interventions are active rather than passive. Researchers have found a significant increase in compliance when hand sanitizers are available (58, 59). This information can be applied to the design of hygiene strategies for farmers working with produce.

Few hygiene interventions look specifically at harvesters. In order to better understand microbial quality of farm worker hands before and after any hand hygiene

method more research is required. This will provide vital information on how to reduce contamination of produce at the source.

## CURRENT REGULATIONS AND REQUIREMENTS

As part of the U.S. Food and Drug Administration Food Safety Modernization Act (FSMA), standards regarding farm worker hygiene behavior have been established (60). According to subpart D—Standards directed to health and hygiene, proposed § 112.32(b) (3), workers that touch produce with bare hands are expected to have sufficient training to know how and when to wash their hands with soap and water. Hand sanitizer is not considered a viable alternative to hand washing. While sanitizer is not prohibited, the rule states that hand washing with "soap and water are far more effective than sanitizers in removing pathogens." It states that "dirt, grease, or soil significantly reduces [sanitizer] effectiveness in eliminating bacteria on hands" (34). The regulations are supported by research; however, compliance with standards is necessary to reduce the risk of produce contamination.

While compliance with such standards reduces the risk of transmission of disease, observance of guidelines is difficult to measure (61). In the United States, third party auditors inspect farms to make sure they are maintaining GAPs, but each firm has its own verification system for monitoring (62). Studies have reported less than 40% compliance in the health care setting where hand washing is regarded as obligatory (55, 56, 63-65). In a survey of migrant farm workers in the United States, only about two-thirds were provided with toilet facilities and water for hand washing; workers were even concerned with skin irritation due to lack of access to hand washing facilities (32). This means that

it is crucial to accompany any requirements, such as hand washing, with training, adequate equipment, and oversight. Without the aforementioned, compliance will be low and risk of contamination is high.

## GOAL

It is essential to reduce the cases of illness associated with contaminated produce. Farm workers' hands contribute to the contamination of produce. Reducing fecal contamination on workers' hands during the workday is a necessary step in diminishing the risk of produce related illness. These topics, associated with hand hygiene on farms, highlight the dire need for research on the efficacy of and compliance with existing standards. With the knowledge that proper facilities might not be available and waterless alcohol-based hand sanitizers can function to enhance microbial quality of hands, alternatives to the standard should be tested.

This study's goal is to assess the effectiveness of two hand hygiene interventions, standard hand washing and SaniTwice, in reducing dirtiness and fecal contamination of field workers' hands, and whether the effects are sustained throughout the workday. Interventions will be compared to one another and a control. A comparison of any sustained effects of the hygiene practices will also be reported. This research will facilitate improvements and innovations in hand hygiene procedures on produce farms.

## SIGNIFICANCE

By defining the best hygienic practice suited for field workers, compliance and overall hand quality will improve immensely. This will decrease the risk of contaminated

produce cultivated on farms. By addressing and important the source of produce contamination, produce-related outbreaks will begin to dwindle throughout the United States. This small change in hand hygiene on farms will greatly impact the overall wellbeing of United States citizens.

# INTRODUCTION

## BURDEN OF FOODBORNE DISEASE ASSOCIATED WITH PRODUCE

Every year nearly 48 million Americans are plagued by foodborne illness as a result of pathogen contamination of food (1). Fruits and vegetables have been the largest contributing commodity to these diseases over the past ten years (8). Between 1999 and 2008, fruits caused over 4,000 illnesses, vegetables caused almost 12,000 illnesses, and produce dishes caused more than 11,000 illnesses (6); however, the actual number of ilnesses may be greater due to lack of reporting to medical professionals and inadequate test methods for identifying pathogens (3). The frequency of produce-associated outbreaks has gradually increased from the 1970s because of a greater demand for and consumption of fruits and vegetables (5). This highlights how imperative it is to reduce the risk of produce contamination in order to prevent foodborne illness.

As globalization supplies more diverse produce to a wealthier and more health conscious populous, contaminated produce can reach a variety of populations, introducing new strains of pathogens to vulnerable environments (4, 7). In the past ten years there have been two serious outbreaks related to jalapeño peppers in North America. (66, 67). The most recent outbreak spread to 43 states, the District of Colombia, and Canada, and caused nearly 1,500 illnesses (67). Investigators traced the source of the *Salmonella* Saintpaul to jalapeño pepper distributors in the United States that received produce grown and packed in Mexico. Attention to global farming practices is vital as America imports nearly seven billion dollars of produce each year (68).

## PRODUCE-ASSOCIATED PATHOGENS

Pathogens related to foodborne illness fall within three main categories: virus, bacteria, or parasite (4). Available data do not always report the etiology of disease, but in the cases where the causal agent can be identified, the top pathogens related to foodborne illness are Norovirus at 48%, Salmonella at 11% and *Clostridium perfringens* at 10% of disease cases (1). The most deadly pathogens between 1998 and 2008, were *Salmonella,* accounting for 30% of deaths, *Listeria*, accounting for 24% of deaths, and Shiga toxin-producing *Escherichia coli*, accounting for 11% of deaths (2). Among all produce associated outbreaks between 1990 and 2004, the majority of disease was caused by *Salmonella* spp. and norovirus (8). Consumption of very low concentrations of pathogens can result in disease, therefore it is imperative to reduce the risk of produce contact with such pathogens (4).

## QUANITFICATION OF MICROBIAL INDICATORS

Foodborne illness and outbreaks result from ingestion of pathogenic viruses, bacteria, or parasites. These pathogens take residence in the intestinal tract of warm-blooded animals and generally contaminate food through direct or indirect contact with animal, including human, feces (13). It is costly to measure pathogens in the environment and on produce as there are many varieties and they are present in minute concentrations (14). A more feasible alternative to the difficult task of detecting pathogens is the detection of fecal indicators, which are normally more abundant (14). Indicator organisms thrive in a similar environment or share a similar ecology as most foodborne

pathogens, this is the feces of warm-blooded animals, hence the name fecal indicator. Indicator presence and survival is significantly associated with the presence and survival of pathogens (15). Therefore, the presence of fecal indicators implies a greater risk of the presence of pathogens, and indicators are often studied as a substitute for pathogens.

When choosing fecal indicators to act as a proxy for pathogen contamination, the indicators must assume certain characteristics. General standards for indicator use include: the indicator should be present whenever the enteric pathogen is present; there should be a relationship between the concentration of the indicator and the pathogen; the organism should have a longer survival time than the enteric pathogen; and enumeration and testing should be inexpensive and simple (14, 16). By assuring that these characteristics are met there is an enhanced probability of detection even after adverse conditions. Testing for indicators is therefore more conservative than testing for pathogens, because the probability of a false positive is greater than that of a false negative. Although there is no single best indicator to represent all foodborne pathogens: total coliform bacteria, fecal coliform bacteria, generic *E. coli*, and total *Enterococcus* spp. are repeatedly used as indicators in food contamination research (17-21).

## WORKER HYGIENE AND PRODUCE QUALITY

Numerous reports implicate poor hygiene of food workers in the contamination of produce (22-25). The Food and Drug Administration estimates this is one of the five leading causes for outbreaks of foodborne disease in the United States (26). Worker hygiene can be an issue as proper sanitary facilities, including running water, are not always available, particularly on farms (32, 33).

Feces and foodborne pathogens can indirectly contaminate produce through vehicles such as human hands, water, soil, tools, and equipment in the environment in which produce is grown (4). Evidence for microbial transfer between produce and produce farm worker hands includes a positive correlation observed amid universal *Bacteroidales* concentrations on samples of produce and harvesters hands (31). In the same study, 34% of *Bacteroidales* contamination on produce farms was from a human source, further implicating human handling as a risk factor for produce contamination. Given this evidence, it is expected that produce contamination should increase from field to packing because of the amount of human handling involved in harvesting. This is supported by research showing that levels of indicators on produce increase significantly from field to packing environments in which human handling generally intensifies, once again implicating human contact as a major culprit of contamination (21, 29, 30).

A vital reason why hands contribute to contamination is the lack of effective and accessible hygiene practices (33). Hand washing with soap is the standard required hygiene practice for food handlers (34). This method involves water that is determined free of generic *E. coli*, an amenity that all farms may not have (32, 34). It also calls for workers to wash their hands before starting work and putting on gloves, after a break, after touching animals or waste, and after using the toilet. Compliance with standards is determined by third party auditors and rarely revealed; often when these levels of compliance are released they are found to be subpar (32, 55, 56, 61-64). Research has shown increases in compliance when workers can use alcohol-based rubs, as they require less time and no water (55, 56, 58, 59). Although hand washing is the current standard,

other practices that are more effective and methods to increase compliance should be considered for a farm environment.

## HYGIENE INTERVENTIONS

While numerous studies compare the effectiveness of hand washing and alcohol-based sanitizers, few have focused on a farm setting (47, 49-52). Research has not reached a consensus on which practice is better and whether this varies in different settings (47, 49, 69). Hygiene intervention studies performed on farms primarily deal with animal husbandry operations and not harvesting of produce (18, 19, 57). These interventions also do not concentrate on the SaniTwice or other waterless methods. More research on effective hygiene methods for produce harvesters is important to reduce risk of produce contamination.

## GOAL

It is essential to reduce the cases of illness associated with contaminated produce. Farm workers' hands contribute to the contamination of produce; therefore, reducing fecal contamination on workers' hands during the workday is a necessary step in diminishing the risk of produce-related illness. There is a dire need for research on the efficacy of hand hygiene on produce farms and methods to improve compliance with existing standards. With the knowledge that potable water might not be available, waterless alternatives to the standard should be tested (32). Alcohol-based hand sanitizers can be used without water to enhance microbial quality of hands, and may be an appropriate alternative to the standard hand hygiene method on produce farms.

Our goals are to assess the effectiveness of two hand hygiene interventions, standard hand washing and SaniTwice, in reducing fecal indicator contamination and dirtiness of harvesters' hands, and determine whether these effects are sustained throughout the workday. This research will facilitate improvements and innovations in hand hygiene procedures on farms. By identifying effective and innovative hand hygiene procedures on farms, we aim to improve the microbial safety of fresh produce and reduce the burden of foodborne disease.

# METHODS

## SAMPLE COLLECTION

In May of 2013, data was collected over four days on two separate produce farms in Nuevo León Mexico in order to evaluate two hygiene methods. The farms hire migrant workers who harvest fruit manually without gloves and have limited access to sanitary facilities. Research focused on workers harvesting jalapeños. Study design, protocols, and a waiver of written consent were reviewed and approved by the Institutional Review Board of Emory University, Atlanta, Georgia, USA (IRB # IRB00035460). On each day of the experiment, workers carried out harvesting activities all morning and were stopped at midday, when explanations of the project and recruitment of volunteers occurred. Oral consent was obtained, per approved IRB protocol, after benefits and risks of participation in the study were described.

On each day, approximately forty workers were randomly assigned to one of three groups depending on the hygiene method they would practice, no-hygiene control (10 workers), hand washing, (15 workers) or SaniTwice (15 workers). Each group practicing a hygiene method received a demonstration of proper technique and then executed a guided practice. Individuals enrolled in the control group did not perform any hygiene intervention; they continued normal activities after recruitment until hand rinse samples were collected.

The SaniTwice technique is a hybrid waterless hand washing and sanitizing system (45). SaniTwice was performed as described by Edmonds et al. (2010) with minor modifications. First, five milliliters (mL) (two pumps of the sanitizer bottle dispenser) of

an alcohol-based sanitizer was dispensed into a study participant's hands. After rubbing vigorously for 15 seconds, participants wiped their hands with paper towels to remove excess sanitizer. An additional 2.5 mL aliquot of sanitizer (one dispenser pump) was dispensed and hands were rubbed until dry. The alcohol-based hand sanitizer used in this study had an active ingredient of 70% Ethyl Alcohol and was produced by Desinfectantes y Aromatizantes, Sociedad Anónima (DYA S.A.) (Monterrey, Mexico).

The hand washing procedure followed as closely to FDA protocol as possible (34). First, hands were rinsed under running, potable, ambient temperature water, which had previously been tested by the study laboratory and found to be free of coliform, *Enterococcus*, and *E. coli* bacteria. Then, two mL (one pump of the cleanser bottle) of non-antimicrobial foam hand soap was dispensed into a study participant's hands. After rubbing vigorously for 20 seconds, participants rinsed their hands again with about 750ml of running, potable, ambient temperature water, and dried them with paper towels. The foam cleanser used in this study was GOJO® Green Certified Foam Hand Cleanser (GOJO Industries, Akron, OH).

After providing consent to participate, each worker observed a demonstration of proper technique for their hygiene intervention method, then preformed the intervention with researcher supervision and feedback to ensure understanding and compliance with technique. At this time, all groups then returned to the field to harvest three five-gallon buckets of jalapeños. Once this was completed, hand rinses were taken from the entire control group, five individuals from the SaniTwice group, and five individuals from the hand washing group. The remaining workers in the SaniTwice (10) and hand washing

(10) groups repeated the intervention before submitting their hands for rinse sample collection.

A hand rinse involved workers placing one hand into a single Whirl-Pak bag containing 750ml of 0.15% peptone water (rinsate). With their hand in the bag, workers shook their hand vigorously for thirty seconds, received a hand massage from researchers from outside of the bag, for thirty seconds, and then shook their hand for another fifteen seconds. This procedure was repeated with their other hand using the same bag of peptone water. This generated hand rinse samples from five different groups: control (10), SaniTwice (10), SaniTwice + harvest (5), hand washing (10), and hand washing + harvest (5). In total, 159 samples were collected over four days. After collection, samples were immediately placed on ice, and transported to the Laboratory of Microbial Biochemistry and Genetics at Universidad Autónoma de Nuevo León (UANL), where they were stored at four degrees Celsius until analysis.

Information on the participant's gender, age, and the time that elapsed between intervention and sample collection were collected at the time of sampling. Participants were also asked to respond to a voluntary, verbal, qualitative survey. Survey questions were designed to provide information on the perceived benefits and drawbacks to each hand hygiene method, the feasibility of continuing hand hygiene outside of the study, and suggestions for improvements to either of the hand hygiene methods. All study participants were compensated for their time with a gift (e.g. soda and chips).

## MICROBIAL ANALYSIS

Samples were transported to the lab at UANL and were tested for absorbance at 600 nm using a spectrophotometer. Fecal indicators (fecal coliforms, *Enterococcus*, and generic *E. coli*) were enumerated on media for their respective assessments. Initiation of microbial analysis took place within 24 hours of the sample collection. Fecal indicators were concentrated from the samples by membrane filtration. Different volumes of each sample ranging from 0.01 to 50ml were vacuum filtered through a 47nm, 0.45 μm pore size, S-Pack filter (Millipore, Billerica, MA). Filters were then placed on media for enumeration of microbes. *Enterococcus spp.* were quantified using KF Streptococcus agar (Oxoid Limited, Basingstoke, Hampshire, UK) incubated at 37°C for 48 hours. Generic *E. coli* and fecal coliforms were enumerated on RAPID'E. coli 2 agar (Bio-Rad Laboratories, Inc., Hercules, CA) incubated at 44°C for 24 hours.

## DATA ENTRY

All data, including participant demographic, survey, and sample data were first recorded on hard copy forms and later entered in duplicate into Microsoft Excel (Seattle, WA). Duplicate entries were compared to identify and correct any transcription errors. Lastly, 5% of all data were randomly selected and compared to the hard copy data– no further errors were found.

## MICROBIAL QUANTIFICATION

Duplicate plates were prepared for each sample volume processed, resulting in up to sixteen individual plate counts from each sample per indicator. Total colony forming

units (CFU) and effective sample volume delivered to each plate were counted and recorded. The lower limit of detection was one colony forming unit per 50mL sample (equivalent to 0.88 $\log_{10}$ CFU per hand) and the upper limit of quantification was 250 CFU per 0.01ml sample (equivalent to 6.97 $\log_{10}$ CFU per hand). An algorithm was employed in order to calculate the mean concentration of each indicator in a given sample across replicate assays (Table 2). The quantifiable range for CFU was designated as 25 to 250 CFU per plate. For samples with CFU values that fell within the quantifiable range, an arithmetic mean of these values was calculated. For some samples, CFU values from all plates were outside of this range, therefore the concentration of indicators in these samples was estimated or imputed.

Indicator concentrations were estimated when CFU data were available, but values were outside the range of 25 to 250. For samples with all CFU values below 25, data from the assays using the largest effective volumes were used to estimate indicator concentrations. This approach was also used to estimate indicator concentrations from samples with CFU values both above and below, but not within the quantifiable range. Values from assays with the smallest effective volume were used to estimate indicator concentrations from samples that had all CFU values above 250.

Indicator concentrations were imputed when CFU data were not available. In cases were all CFU values were zero, a value of half the limit of detection was imputed (0.5 CFU divided by the maximum effective volume assayed). In cases were all CFU were too numerous to count (TNTC), a value of twice the upper limit of quantification was imputed (500 CFU divided by the minimum effective volume assayed). In odd cases

where all CFU values were either 0 or TNTC, a value of twice the upper limit of quantification was imputed.

**STATISTICAL ANALYSIS**

Analyses were performed using JMP Pro 10 (SAS Institute Inc., Cary, NC) and SAS 9.3 (SAS Institute Inc., Cary, NC). Data obtained from assays that were not conducted within four days of sample collection were not included in analyses. All concentration data were $\log_{10}$ transformed prior to analysis. Descriptive statistics such as geometric mean, confidence intervals, prevalence, skewness, and kurtosis were obtained using SAS. Prevalence was defined as the percent of samples that had detectable levels of a given indicator. In order to assess normality, a Shapiro-Wilk test was employed (70) (data no shown). Due to non-normal distributions, all further tests were nonparametric.

Significant differences between concentrations of fecal indicators in control and intervention groups were assessed using a means separations test known as Kruskal-Wallis (71). Prevalence of fecal indicators were compared using chi-square tests with $\alpha = 0.05$. Kruskal-Wallis does not report which variable is significantly different; therefore post-hoc procedures were performed. Individual pairs were tested using repeated Kruskal-Wallis tests and a Steel Dwass multiple comparison (72-74). Steel Dwass and primary Kruskal-Wallis tests were conducted with $\alpha = 0.05$. Repeated individual pair Kruskal-Wallis tests were conducted with $\alpha = 0.025$.

Absorbance data were treated differently than microbial concentration data. First, they were not $\log_{10}$ transformed. Second, the data were analyzed using a Tukey-Kramer

HSD test instead of a non-parametric test. Tukey-Kramer HSD tests were conducted with $\alpha = 0.05$.

**SAMPLE SIZE**

With the given resources, a sample size near 40 was obtained for each comparison group. Depending on the indicator, the ability to detect a meaningful significant difference varied. Using the most conservative estimates for fecal coliforms, we had the ability to detect a significant difference if the mean concentration was at least 2.4 $\log_{10}$ CFU/hand greater or lower than the comparison mean concentration (Table 3). A similar level was found for our most conservative estimates for *Enterococcus*, 2.03 $\log_{10}$ CFU/hand (Table 3) or greater. Using the most conservative estimates, we were able to detect a significant difference if the difference in mean concentration of *E. coli* was 1.15 $\log_{10}$ CFU/hand or greater. The capability to detect small differences in mean concentration lends to the value of this study.

# RESULTS

## NORMALITY ASSESSMENT

The distribution of each indicator was assessed in order to determine the most appropriate statistical tests. Even after $\log_{10}$ transformation, the distributions of the concentrations for each indicator appeared non-normal as displayed in histograms (data not shown). This observation was reaffirmed with significant p-values from Shapiro-Wilks tests, which statistically assess normality (data not shown). Due to non-normal distributions all further statistical tests of indicator concentration data were nonparametric.

## STUDY POPULATION DEMOGRAPHICS

An assessment of the population demographics was necessary in order to identify any potential confounders. If there were differences in the demographics of intervention or control groups, then other factors besides interventions might have altered levels of indicators or dirt on hands. Participants were generally homogenous among intervention and control groups. Most workers were male and about thirty years old (Table 4). Time between recruitment and sample collection was generally 27.5 minutes (Table 4). Population demographics did not confound findings because characteristics were largely the same between intervention and control groups.

## COMPARISON OF HYGIENE INTERVENTIONS TO NO-HYGIENE CONTROL

In order to understand if either intervention is an effective method for improving microbial quality of workers' hands, microbial concentration and prevalence on hands were compared between workers in each intervention group and the control using Kruskal-Wallis and Steel-Dwass tests for concentration and a chi-square test for prevalence. There was a significantly lower concentration of fecal coliforms on workers' hands in the SaniTwice compared to the control group ($p<0.01$) (Table 5). The geometric mean concentration of fecal coliforms on workers' hands in the SaniTwice group was about 2 $\log_{10}$ CFU per hand lower than in the control group (Figure 1). This result is paralleled by prevalence data; there was a significantly lower prevalence of fecal coliforms on workers' hands in the SaniTwice compared to control group (Table 5). Although geometric mean fecal coliform concentrations were 0.51 $\log_{10}$ CFU per hand lower in the hand washing group compared to the control group (Figure 1) this difference was not found to be significant ($p=0.05$) (Table 5). This finding was similarly mirrored by prevalence results; both hand washing and control groups had 100% prevalence of fecal coliforms (Table 6). In summary, SaniTwice was effective at reducing concentration and prevalence of fecal coliforms compared to the control, and hand washing was not.

Similar findings were made for *Enterococcus*. There was a significant difference between concentrations of *Enteroccocus* on hands of workers performing the SaniTwice intervention compared to the workers in the control group ($p<0.01$) (Table 5) The geometric mean concentration of *Enteroccous* for workers' hands in the SaniTwice group

was about 1 $\log_{10}$ CFU per hand lower than in the control group (Figure 1). Although geometric mean *Enterococcus* concentrations were 0.1 $\log_{10}$ CFU per hand lower in the hand washing group compared to the control group (Figure 1) this difference was not found to be significant (p=0.99) (Table 5). Prevalence data were not informative for comparisons across groups for *Enterococcus*, all groups had 100% prevalence (Table 6). In summary, SaniTwice was effective at reducing concentration of *Enterococcus* compared to the control, and hand washing was not.

Levels of *E. coli* were very low for all three groups. Statistical analyses were still performed even though many values were imputed. Converse to the results for fecal coliforms and *Enterococcus*; there was a significant difference between *E. coli* concentrations on hands in the hand washing and control groups (p=0.04) (Figure 1) (Table 5). The geometric mean concentration of *E. coli* for workers' hands in the hand washing group was about 0.12 $\log_{10}$ CFU per hand lower than in the control group (Figure 1). There was no significant difference between *E. coli* concentrations in SaniTwice and control groups (Table 5). There were no significant differences in the prevalence of *E. coli* in the hand washing or SaniTwice groups compared to the control (Table 5). These are the only results that suggest that hand washing significantly reduces mean fecal indicator concentration compared to the control.

Absorbance was compared using a Tukey-Kramer HSD test to determine which hygiene process removed the most dirt and debris from hands. The groups with the smallest mean absorbance values had the least amount of dirt remaining on hands. There was a significant difference between the mean absorbance for control and each intervention group (hand washing $p<0.01$) (SaniTwice $p<0.01$) (Figure 2). Both hand

washing and SaniTwice procedures removed significantly more dirt from workers' hands than the control group.

**COMPARISON OF SANITWICE AND HAND WASHING INTERVENTIONS**

In order to determine which intervention is most effective at improving microbial quality of workers hands, a comparison was made between intervention groups using Kruskal-Wallis and Steel Dwass tests for concentration and chi-square tests for prevalence. The groups compared in this section are solely SaniTwice and hand washing groups. This does not include SaniTwice + harvest or hand washing + harvest groups. There was a significant difference between the mean concentration of fecal coliforms and *Enterococcus* on hands of workers performing the SaniTwice intervention compared to the hand washing intervention ($p<0.01$) (Table 5). The geometric mean concentration of the indicators for workers' hands in the SaniTwice group was approximately 1 $\log_{10}$ CFU per hand lower than in the hand washing group (Figure 1). This is paralleled by a significantly lower prevalence of fecal coliforms on hands' of workers in the SaniTwice compared to hand washing group (Table 5) (Figure 1). There was no significant difference between mean concentration of *E. coli* between intervention groups ($p=0.24$) (Figure 1). There was also no significant difference between prevalence of *Enterococcus* or *E. coli* in SaniTwice and hand washing groups (Table 5). These findings show that SaniTwice is more effective at reducing prevalence and concentration of fecal indicators compared to hand washing.

Absorbance was measured as a proxy for dirtiness. The mean absorbance was significantly higher in the SaniTwice group compared to the hand washing group based

on a Tukey HSD test (Figure 2). This suggests that a substantial amount of dirt remains on hands even after practicing the SaniTwice method.

**DURATION OF HYGIENE INTERVENTION EFFECTS**

A comparison of concentrations of fecal indicators on hands of individuals in intervention and intervention + harvest groups reveals if the effects of interventions were maintained after a short period of work harvesting three five-gallon buckets of jalapeños. No significant differences were found between hand washing and hand washing + harvest for mean fecal coliform ($p=0.41$), *Enterococcus* ($p=1.0$), and *E. coli* concentrations ($p=0.07$) (Table 7). There was a significantly lower concentration of mean fecal coliforms ($p<0.01$) and *Enterococcus* (0.01) in the SaniTwice group compared to the SaniTwice + harvest group (Table 7). There was approximately a 1 $\log_{10}$ CFU difference in means between SaniTwice and SaniTwice + harvest for both fecal coliforms and *Enterococcus*. There was no significant difference between *E. coli* concentrations in SaniTwice and SaniTwice + harvest groups (Table 7). Results suggest that reductions in fecal indicator concentration remained the same for groups that did or did not return to work after hand washing.

To determine whether hands became dirty while harvesting, differences in the mean absorbance in intervention and intervention + harvest were identified by a Tukey HSD test. There was no significant difference between the mean absorbance of SaniTwice and SaniTwice + harvest ($p=1.0$) (Table 8). There was a significant difference between hand washing and hand washing + harvest for mean absorbance ($p<0.01$) (Table 8). The hand washing group had a significantly lower mean absorbance compared to the

hand washing + harvest group. Results suggest that reductions in mean absorbance remained the same for groups that did or did not return to work after practicing the SaniTwice intervention.

## DISCUSSION

Numerous comparisons were made in order to critically assess the two hand-hygiene methods in their ability to reduce both fecal indicator and dirt levels on farm workers' hands during harvest. The effects were measured by absorbance of hand rinses, as well as concentration and prevalence of fecal indicators. Absorbance results quantify dirt on the hands of workers, while indicator concentration and prevalence quantify fecal contamination on hands. Each intervention was compared to the no-hygiene control to determine if either hygiene practice was able to reduce contamination to below typical levels on produce farm worker hands. SaniTwice was compared to hand washing to determine which hygiene method was more powerful in eliminating indicators or dirt. Comparison of intervention and intervention + harvest was undertaken to determine if outcomes of the interventions were sustained over time, and if hands are more or less prone to re-contamination after performing an intervention.

### HAND WASHING MOST EFFECTIVELY REDUCES DIRT

Both hygiene intervention methods led to significantly lower amounts of dirt on workers' hands compared to the control group that did not practice hand hygiene. Previous research shows that hand washing removes dirt and debris, therefore it is recommended in a farm environment where hands are heavily soiled (34, 37-39, 75). Our data on absorbance agrees with these findings and support current standards. Hand washing groups had less particulate matter, as evidenced by lower mean absorbance, than control groups. This is most likely due to soap acting as an emulsifier to suspend dirt particles, which are removed with soap during hand rinsing (37). The SaniTwice group

had a significantly lower mean absorbance than the control group as well. This is contrary to research supporting hand washing as the only effective practice to remove dirt from hands. Most research testing alcohol-based gels find that sanitizers do not remove dirt from hands (37, 38). Friction created during wiping with a towel is the most probable reason for the observed effectiveness of the SaniTwice method at removing dirt from hands (37). In summary, both hygiene methods were effective at removing dirt and debris from hands.

The SaniTwice method was less effective than hand washing at reducing dirt on hands. Other research has had similar findings with alcohol-based rubs—this is the main reason why the FDA supports hand washing over hand sanitizing for farm hygiene practices (34, 38, 39, 69, 75). While workers in the SaniTwice group of this study had more particulates on their hands than those in the hand washing group, the levels of fecal indicators do not follow the same pattern as dirt. Fecal indicator concentration was significantly lower for the SaniTwice group compared to both hand washing and control groups. This provides a serious question to consider: which is more important to eliminate from workers' hands, dirt or pathogens? Removal of soil from hands does not imply removal or inactivation of microbes—reducing pathogens should be the main focus of hygiene practices.

**SANITWICE EFFECTIVELY REDUCES FECAL INDICATORS**

Our findings support those of previous studies that have shown that hand washing removes dirt, but does not effectively eliminate fecal indicators (38, 45, 49, 51-53, 76, 77). Non-significant differences in the mean concentration of fecal indicators between

control and hand washing groups suggests that the FDA recommended standard hand washing is not an acceptable means for hand disinfection in a produce harvesting environment. Bland soap lacks an extermination mechanism; therefore concentrations of fecal coliforms and *Enterococcus* did not differ between control and hand washing groups in the current study (69). In contrast, the ethanol in the sanitizer used for the SaniTwice method effectively kills bacteria (69). There was a significant difference between fecal indicator concentrations and prevalence of fecal coliforms in the SaniTwice group compared to the control in the current study, indicating that SaniTwice is an effective means for hand disinfection. Larson et al. (1992) had similar conclusions: 70% isopropyl alcohol use led to significantly greater reductions in colony forming units on hands of medical workers than non-antimicrobial soap (38). In conclusion, SaniTwice is an effective method for reducing fecal indicators on produce harvesters' hands, while hand washing is not.

Our findings contradict those of Larmer et al. (2008) and Todd et. al (2010): both found that hand washing is equivalent or more effective at reducing fecal indicator contamination than alcohol-based hand rubs (37, 78). This difference might be due to the large amount of methodological limitations expressed in Larmer et al (2008), including no assessor blinding or difficulty in creating experimental conditions. Our finding, that alcohol-based hand hygiene increases microbial quality of workers hands, agrees with data collected in the food industry, medical settings, and in animal husbandry (38, 44, 49-54, 79-81). In cases when hands are not visibly soiled, alcohol-based hand sanitizers are used as an alternative to hand washing in the medical field (82, 83). Our data indicate that the SaniTwice method can act as an effective alternative system for hand hygiene on

farms even when hands are visibly soiled. Effective alternative systems to the use of soap and water, as recommended by FDA, may be incredibly useful to improve the safety of produce grown under conditions of limited availability of potable water for hand washing (34).

**NEITHER INTERVENTION HAS LASTING EFFECTS**

Findings for the comparison of intervention and intervention + harvest are consistent with my maximum load hypothesis and previous results for each intervention. My hypothesis postulates that hands can only hold a limited amount of organic matter, including dirt and fecal indicators. Once a maximum load is reached no further matter can accumulate on hands even if they are exposed to further contamination, e.g. through harvesting. Study results show that there was a significantly lower mean absorbance for hand washing as compared to hand washing + harvest, but there was no significant difference in the mean absorbance of SaniTwice and SaniTwice + harvest. Opposite results were found for concentration of fecal indicators. There was a significantly lower mean concentration of indicators for SaniTwice as compared to SaniTwice + harvest, but there was no significant difference in the mean concentration of indicators between hand washing and hand washing + harvest. By incorporating the knowledge that hand washing is significantly better at removing dirt than SaniTwice, and that SaniTwice is significantly better at reducing fecal indicators than hand washing, the maximum load hypothesis helps explain the findings. Consider the scenario in which the SaniTwice method reduced levels of dirt to slightly below the maximum load, and hand washing reduces levels of indicators to slightly below the maximum load. No significant

differences in absorbance between SaniTwice and SaniTwice + harvest may have been observed because hands were near the maximum threshold of dirt prior to harvesting. In this situation, subsequent harvesting would only allow for the accumulation of a non-significant amount of dirt. No significant difference in concentration of indicators between hand washing and hand washing + harvest, may have been observed because hands were near the maximum threshold of fecal contamination prior to harvesting. In this situation, subsequent harvesting would only allow for the accumulation of a non-significant amount of fecal indicators. Comparison of absorbance and concentration results for groups that did or did not return to work after practicing hygiene show that hands can still collect large quantities of dirt after hand washing and indicators after SaniTwice. This suggests that in order to ensure sustained beneficial effects of these specific hygiene interventions on produce farms, field workers must practice the interventions more frequently. If this is not feasible, more effective hygiene methods should be identified.

## LIMITATIONS AND FUTURE DIRECTIONS

While we highlight numerous critical implications for the field of farm hygiene, there were several limitations to this study. First, only two interventions were compared. The hand washing intervention in this study represented standard methods as proposed by FDA, but typical methods may vary on different farms. The SaniTwice method has been supported in the literature, however there is a copious amount of formulations and methods for hand sanitizing (45, 69). Another limitation was the harvesting season. Samples were only collected from jalapeños harvesters because of a storm, which halted

planned research on cantaloupe farms. This reduces the generalizability of our findings. Overall, limitations did not affect the major findings of the study.

Prevalence of *E. coli* was low across all comparison groups, making this indicator uninformative for statistical analyses. The dataset for this indicator contained many imputed values, because values were assigned to all samples that did not contain *E. coli* at a detectable level. Standard deviation estimates, calculated from imputed values, were therefore unreliable estimates of the true variability in *E. coli* concentrations on field worker hands. We therefore have limited confidence in all additional calculations using these standard deviation estimates (Tables 3 and 9). Davis et al. (2006) experienced similar difficulties in detecting *E. coli* on hands (19). We lost statistical power for valid comparisons due to these low concentrations, therefore *E. coli* results should be understood with discretion.

Future farm hygiene research should focus on applying similar methods to determine hygiene practices best suited for differing farm environments. Our research concentrated solely on jalapeños, however other produce, such as cantaloupe, tend to be more highly contaminated and also involve more human handling. Selecting different types of produce as well as different types of hygiene techniques will allow for the optimum practice to be defined for a given farm setting. Current standards should continually be examined against new innovative practices. Assessment of whether dirt is related to both fecal indicators and pathogens is also vital to understanding what methods of hygiene are most effective. If the level of dirt on workers' hands is not significantly correlated with the risk of fecal contamination of produce, then hygiene evaluation techniques can focus singularly on fecal indicators. There are countless future steps for

the field of farm hygiene research, however all undertakings should consider the findings of this research in order to develop a broader picture of hand hygiene practices on farms.

## STRENGTHS

Several studies have evaluated the effectiveness of hand hygiene interventions among workers in the medical, food retail, or animal husbandry field, however none have focused on reducing fecal indicators on harvesters' hands in a farm environment (18, 20, 47). Our study evaluated interventions to find innovative hygiene methods in a field with limited research. Our findings support the use of a waterless hand hygiene method, SaniTwice, on farms. This is vital for farms without running water, sinks, and other adequate sanitation facilities. Our results are corroborated by similar studies in other fields (18, 57). Other strengths include a limited amount of bias: bias was diminished by randomization and similarities in characteristics of each comparison group. Lastly our research had the ability to detect small differences between intervention and control groups (Table 3). These differences were approximately equal to the standard difference of 2 $\log_{10}$ CFU used to test effectiveness of hygiene practices in the medical field (84). This was a result of our sample size (Table 9).

## CONCLUSION AND IMPLICATIONS

Hand hygiene is an essential practice used to reduce the risk of food contamination on farms. Specifically, performing the SaniTwice method resulted in the lowest mean concentration of fecal indicators on harvesters' hands compared to hand washing and control; however hand washing was superior at removing dirt and debris

from hands compared to SaniTwice. Lasting reductions of indicators and dirt were not observed from either intervention. This study design may be applied to future investigations of other hygiene methods on different types of produce in order to determine the best practices for workers on a diversity of farms.

Our research demonstrates the effectiveness of a hygienic practice for field workers harvesting jalapeños. The SaniTwice method was superior in reducing fecal indicator concentration and prevalence compared to the FDA required standard, while also removing significant amounts of dirt, compared to the control group. This waterless hygiene practice should increase compliance and overall hand quality on farms. With the installation of this practice, farms will likely see a decrease in the level of contaminated produce and an increase in overall working conditions. By reducing the risk of contamination at the source, produce-related outbreaks will begin to dwindle throughout the United States. This small change in hand hygiene on farms will greatly impact the overall wellbeing of United States citizens.

**REFERENCES**

1. CDC. CDC Estimates of Foodborne Illness in the United States. In: National Center for Emerging & Zoonotic Infectious Disease Division of Foodborne W, and Environmental Diseases, editor. Atlanta; 2011.
2. Gould LH, Walsh KA, Vieira AR, Herman K, Williams IT, Hall AJ, et al. Surveillance for foodborne disease outbreaks - United States, 1998-2008. MMWR Surveill Summ 2013;62(2):1-34.
3. Mead PS, Slutsker L, Griffin PM, Tauxe RV. Food-related illness and death in the United States reply to Dr. Hedberg. Emerg Infect Dis 1999;5(6):841-2.
4. Leon JS, Jaykus L., et al. . Food safety issues and the microbiolgy of fruits and vegetables. Hoboken, NJ: John Wiley and Sons 2007.
5. Sivaplaasingam SF, Cindy R.; Cohen, Linda; Tauxe, Robert V. Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997. Journal of Food Production 2004;67(10):2337-2341.
6. De Waal CS, Roberts, C., and Catella, C. . Outbreak Alert! 1999-2008. Washington DC: Center for Science in the Public Interest; 2012.
7. Pollack SL. Consumer Demand for Fruit and Vegetables: The U.S. Example. In: Service/USDA ER, editor. Washington, D.C. USA: United States Department of Agriculture; 2001.
8. De Waal CS, Johnson, K., Bhuiya, F. Outbreak alert! Closing the gaps in our food-safety. In: Interest CfSitP, editor.; 2006.
9. Jeter C, Matthysse AG. Characterization of the Binding of Diarrheagenic Strains of E. coli to Plant Surfaces and the Role of Curli in the Interaction of the Bacteria with Alfalfa Sprouts. Molecular Plant-Microbe Interactions 2005;18(11):1235-1242.
10. Morris CE, Monier, J., Jacques, M. Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms. Applied Environmental Microbiology 1997;63(4):1570-1576.
11. Leigh JA, Coplin, D. L. Exopolysaccharides in plant-bacterial interactions. Annual Review of Microbiology 1992;46:307-46.
12. Norwood DE, Gilmour, A. The growth and resistance to sodium hypochlorite of Listeria monocytogenes in a steady-state multispecies biofilm. Journal of Applied Microbiology 2000;88(3):512-20.
13. James J. Pathogens and Outbreaks Associated with the Fresh Produce Industry In: J J, editor. Microbial Hazard Identification of Fresh Fruit and Vegetables. Hoboken, New Jersey John Wiley & Sons 2006.
14. EPA. Bacteria Indicators of Potential Pathogens. In: EPA, editor.; 2006.
15. Natvig EE, Ingham S.C., Ingham, B. H., Cooperband, L.R., Roper, T.R. Salmonella enterica serovar Typhimurium and Escherichia coli contamination of root and leaf vegetables grown in soils with incorporated bovine manure. Applied Environmental Microbiology 2002;68(6):2737-44.
16. Gerba C. Indicator Microorganisms. New York: Academic Press; 2000.
17. Human IS, Lues RYK. Assessing relationships between microbiota and food handler practices in delicatessen sections: An interdisciplinary approach. Journal of Food Safety 2012;32(1):122-128.

18. Racicot M, Kocher A, Beauchamp G, Letellier A, Vaillancourt JP. Assessing most practical and effective protocols to sanitize hands of poultry catching crew members. Preventive Veterinary Medicine 2013;111(1-2):92-99.
19. Davis MA, Sheng H, Newman J, Hancock DD, Hovde CJ. Comparison of a waterless hand-hygiene preparation and soap-and-water hand washing to reduce coliforms on hands in animal exhibit settings. Epidemiol Infect 2006;134(5):1024-8.
20. Pickering AJ, Boehm, A.B., Mwanjali, M., Davis, J. Efficacy of waterless hand hygiene compared with handwashing with soap: a field study in Dar es Salaam, Tanzania. Am J Trop Med Hyg 2010;82(2):270-8.
21. Ailes EC, Leon JS, Jaykus LA, Johnston LM, Clayton HA, Blanding S, et al. Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. Journal of Food Protection 2008;71(12):2389-97.
22. Bryan FL. Risks of practices, procedures and processes that lead to outbreaks of food-borne diseases. Journal of Food Protection 1988;51:663-673.
23. Altekruse SE, Timbo, B.B., Mowbray, J.C., Bean, N.H. and Potter, E. Cheese-associated outbreaks of human illness in the United States, 1973 to 1992: Sanitary manufacturing practices protect consumers. . Journal of Food Protection 1998;61:1405-1407.
24. Parish ME. Coliforms, Escherichia coli and Salmonella serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. Journal of Food Protection 1998;61:280-284.
25. Van Tonder IL, J.F.R. and Theron, M.M. The personal and general hygiene practices of food handlers in the delicatessen sections of retail outlets in South Africa. The Journal of Environmental Health 2007;70(33-38).
26. Report of the FDA Retail Food Program Database of Foodborne Illness Risk Factors. In. Aug 10, 2000 ed: FDA; 2000.
27. Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P, et al. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ Microbiol 2010;12(9):2385-97.
28. Pickering AJ, Julian TR, Mamuya S, Boehm AB, Davis J. Bacterial hand contamination among Tanzanian mothers varies temporally and following household activities. Trop Med Int Health 2011;16(2):233-9.
29. Johnston LM, Jaykus L-A, Moll D, Anciso J, Mora B, Moe CL. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. International Journal of Food Microbiology 2006;112(2):83-95.
30. Leon-Felix J, et al. Norovirus Contamination of Bell Pepper from Handling During Harvesting and Packing. Food Environmental Virology 2010;2:211-217.
31. Ravaliya K, Gentry-Shields J, Garcia S, Heredia N, Fabiszewski de Aceituno A, Bartz FE, et al. Use of bacteroidales microbial source tracking to monitor fecal contamination in fresh produce production. Appl Environ Microbiol 2014;80(2):612-7.
32. Cameron L, Lalich, N., Bauer, S., Booker, V., Bogue, H. O., Samuels, S., Steege, A. L. Occupational health survey of farm workers by camp health aides. J Agric Saf Health 2006;12(2):139-53.
33. Park S, Navratil S, Gregory A, Bauer A, Srinath I, Jun M, et al. Generic Escherichia coli Contamination of Spinach at the Preharvest Stage: Effects of Farm

Management and Environmental Factors. Appl Environ Microbiol 2013;79(14):4347-4358.
34. FDA. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Proposed Rule. In: D. Subpart D – Standards directed to health and hygiene. Rockville, MD; 2013.
35. James J. Overview of Microbial Hazards. In: J J, editor. Microbial Hazard Identification of Fresh Fruit and Vegetables. Hoboken, New Jersey John Wiley & Sons 2006.
36. Part 1928 - OCCUPATIONAL SAFETY AND HEALTH STANDARDS FOR AGRICULTURE Subpart I - General Environmental Controls Section 1928.110 - Field sanitation. In: OSHA, editor. 29 CFR 1928.110. Washington, D.C.: GPO; 2009.
37. Todd ECD, Michaels, B. S., Smith, D. et al. . Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease. Part 9. Washing and Drying of Hands To Reduce Microbial Contamination. Journal of Food Production 2010;73(10):1937-55.
38. Larson E, Bobo L. Effective hand degerming in the presence of blood. J Emerg Med 1992;10(1):7-11.
39. Gravani RB. Produce Safety Project Issue Brief: Farm Worker Health and Hygiene: Department of Food Science, Cornell University 2010.
40. Fendler E. J. DMJ, Williams R. A. Handwashing and Gloving for Food Protection Part II: Exmination of Evidence. Dairy, Food and Environmental Sanitation 1998;18(12):824-829.
41. Mbithi JN, Springthorpe, V.S., Sattar, S.A. Comparative in vivo efficiencies of hand-washing agents against hepatitis A virus (HM-175) and poliovirus type 1 (Sabin). Appl Environ Microbiol 1993;59(10):3463-3469.
42. Weir SC, Pokorny, N.J., Carreno, R.A., Trevors, J.T., Lee, H. . Efficacy of common laboratory disinfectants on the infectivity of Cryptosporidium parvum oocysts in cell culture. Appl Environ Microbiol 2002;68:2576-2579.
43. Mott PJ, Sisk BW, Arbogast JW, Ferrazzano-Yaussy C, Bondi CA, Sheehan JJ. Alcohol-based instant hand sanitizer use in military settings: a prospective cohort study of Army basic trainees. Mil Med 2007;172(11):1170-6.
44. Liu P, Macinga D, Fernandez M, Zapka C, Hsiao H-M, Berger B, et al. Comparison of the Activity of Alcohol-Based Handrubs Against Human Noroviruses Using the Fingerpad Method and Quantitative Real-Time PCR. Food and Environmental Virology 2011;3(1):35-42.
45. Edmonds SL, Mann J, McCormack RR, Macinga DR, Fricker CM, Arbogast JW, et al. SaniTwice: a novel approach to hand hygiene for reducing bacterial contamination on hands when soap and water are unavailable. J Food Prot 2010;73(12):2296-300.
46. Fendler E. J. DMJ, Williams R. A. Handwashing and Gloving for Food Protection Part I: Examination of the Evidence. Dairy, Food and Environmental Sanitation 1998;18(12):814-823.
47. Fewtrell L KR, Kay D, Enanoria W, Haller L, and Colford JM. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. . Lancet Infectious Diseases 2005;5(1):42-52.

48. Gonçalves JM, Rodrigues KL, Demoliner F, Rossales R, Almeida ÂTS, Buchweitz MRD. Hygienic and Sanitary Conditions in the Hospital Foodservice: Relationship between Good Practices and Microbiological Quality. Journal of Food Safety 2013;33(4):418-422.
49. Widmer AF. Replace Hand Washing with Use of a Waterless Alcohol Hand Rub? Clinical Infectious Disease 2000;31(1):136-143.
50. Ehrenkranz NJ, Alfonso, B.C. Failure of bland soap handwash to prevent hand transfer of patient bacteria to urethral catheters. Infect Control Hosp Epidemiol 1991;12(11):654-62.
51. Ayliffe GF, Babb, J. R., Quoraishi, A. H. A test for 'hygienic' hand disinfection. J Clin Pathol 1978;31(10):923-8.
52. Kampf G, Ostermeyer C. Efficacy of alcohol-based gels compared with simple hand wash and hygienic hand disinfection. J Hosp Infect 2004;56(2):S13-5.
53. Schaffner DW, Schaffner KM. Management of risk of microbial cross-contamination from uncooked frozen hamburgers by alcohol-based hand sanitizer. J Food Prot 2007;70(1):109-13.
54. Wewalk G RM, Koller W, et al. Comparison of efficacy of 14 procedures for the hygienic disinfection of hands. Zentralbl Bakteriol [Orig B] 1977;165:242-249.
55. Bischoff WE, Reynolds, T.M., Sessler, C.N., et al. Handwashing compliance by health care workers: the impact of introducing an accessible, alcohol-based hand antiseptic. Arch Intern Med 2000;160:1017-21.
56. Pittet D, Mourouga, P., Perneger, T.V. Compliance with handwashing in a teaching hospital. Ann Intern Med 1999;130:126-30.
57. Traub-Dargatz JL, Weese JS, Rousseau JD, Dunowska M, Morley PS, Dargatz DA. 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 2006;47(7):671-6.
58. Anderson ME, Weese, J.S. Video observation of hand hygiene practices at a petting zoo and the impact of hand hygiene interventions. Epidemiol Infect 2012;140(1):182-90.
59. Erdozain G, KuKanich, K.F., Chapman, B., Powell, D. Observation of public health risk behaviours, risk communication and hand hygiene at Kansas and Missouri petting zoos--2010-2011. Zoonoses Public Health 2013;60(4):304-10.
60. FDA. Food Safety Modernization Act. In: Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Proposed Rule; 2013.
61. Mokhtari A, Jaykus LA. Quantitative exposure model for the transmission of norovirus in retail food preparation. Int J Food Microbiol 2009;133(1-2):38-47.
62. James J. 1.4.4 Effectiveness of Auditing. In: J J, editor. Microbial Hazard Identification of Fresh Fruit and Vegetables. Hoboken, New Jersey John Wiley & Sons 2006.
63. Albert RF, Condie, F. Hand-washing patterns in medical intensive-care units. N Engl J Med 1981;304(24):1465-6.
64. Conly JM, Hill, S., Ross, J. Lertzman, J., Louie, T.J. Handwashing practices in an intensive care unit: the effects of an educational program and its relationship to infection rates. Am J Infect Control 1989;17(6):330-9.

65. Raju TN, Kobler, C. Improving handwashing habits in the newborn nurseries. Am J Med Sci 1991;302(6):355-8.
66. CDC. MMWR: Multisite Outbreak of Norovirus Associated with a Franchise Restaurant --- Kent County, Michigan, May 2005. In: D.H.H.S., editor. Atlanta, GA; 2006.
67. CDC. Investigation of Outbreak of Infections Caused by *Salmonella* Saintpaul. In: D.H.H.S., editor. Atlanta, GA; 2008.
68. Huang SW. Imports Contribute to Year-Round Fresh Fruit Availability. In: USDA, editor.; 2013.
69. Todd ECD, Michaels, B. S., Smith, D. et al. . 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. Journal of Food Production 2010;73(11):2128-2140.
70. Shapiro SS, Wilk MB. An Analysis of Variance Test for Normality (Complete Samples). Biometrika 1965;52:591-&.
71. Kruskal WH. A Nonparametric Test for the Several Sample Problem. Annals of Mathematical Statistics 1952;23(4):525-540.
72. Critchlow DE, Fligner, M.A. On distribution-free multiple comparisons in the one-way analysis of variance. Communications in Statistics - Theory and Methods 1991;20(1):127-139.
73. Dwaas M. Some k-sample rank order tests. In: Contributions to probability and statistics. Stanford: Stanford University Press; 1960.
74. Steel RG. A rank sum test for comparing all pairs of treatments. Technometrics 1960;2:197-207.
75. Michaels BS, and Todd, E.C.D. Microbial Hazard Identification in Fresh Fruits and Vegetables. Hoboken, NJ: John Wiley & Sons, Inc.; 2006.
76. Girou E, Loyeau S, Legrand P, Oppein F, Brun-Buisson C. Efficacy of handrubbing with alcohol based solution versus standard handwashing with antiseptic soap: randomised clinical trial. BMJ 2002;325(7360):362.
77. Winnefeld M, Richard MA, Drancourt M, Grob JJ. Skin tolerance and effectiveness of two hand decontamination procedures in everyday hospital use. Br J Dermatol 2000;143(3):546-50.
78. Larmer PJ, T. M. Tillson, F.M. Scown, P.M. Grant, and J. Exton. Evidence-based recommendations for hand hygiene for health
care workers in New Zealand. N. Z. Med. J. 2008;121:69-81.
79. Gruendemann BJ, Bjerke, N. B. Is it time for brushless scrubbing with an alcohol-based agent? AORN J. 2001;74:859-873.
80. Cardoso CL, H. H. Pereira, J. C. Zequim, and M. Guilhermetti. Effectiveness of hand-cleansing agents for removing Acinetobacter baumannii strain from contaminated hands. . Am. J. Infect. Control 1999;27:327-331.
81. Butz AM, B. E. Laughon, D. L. Gullette, and E. L. Larson. . Alcohol-impregnated wipes as an alternative in hand hygiene. Am. J. Infect. Control 1990;18:70-76.
82. WHO. Surgical hand preparation: state-of-the-art. Geneva, Switzerland; 2009.
83. WHO. WHO-recommended handrub formulation. Geneva, Switzerland; 2009.
84. ASTM. Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations. In; 1996.

**Table 1. Hand hygiene intervention procedures**

| Procedural step | SaniTwice | Hand Washing |
|---|---|---|
| 1 | Dispense 5ml product into cupped hands | Wet hands with 40C water |
| 2 | Rub 15s | 1.5ml of product |
| 3 | Clean with towels | Lather 20s |
| 4 | Dispense 2.5ml product into cupped hands | Rinse in water for 10s |
| 5 | Rub until dry | Pat dry with towels |
| Sample Size | 40 | 40 |

**Table 2. Average type classification**

| Average type | CFU/assay values represented | | | | | CFU/mL calculation across replicate assays | |
|---|---|---|---|---|---|---|---|
| | 0 | 1-24 | 25-250 | >250 | TNTC | Numerator | Denominator |
| 1 | ✓ | x | x | x | x | 0.5 | Maximum EV assayed |
| 2 | | ✓ | x | x | x | ∑CFU values from assays with largest EV | ∑corresponding EV |
| 3 | | | ✓ | | | ∑CFU values between 25-250 | ∑corresponding EV |
| 4 | x | x | x | ✓ | | ∑CFU values from assays with smallest EV | ∑corresponding EV |
| 5 | | ✓ | x | ✓ | | ∑CFU values from assays with largest EV | ∑corresponding EV |
| 6 | x | x | x | x | ✓ | 500 | Minimum EV assayed |
| 7 | ✓ | x | x | x | ✓ | 500 | Minimum EV assayed |

CFU = colony forming unit
EV = effective volume
TNTC = too numerous to count
✓ = Must include
x = Must not include

**Table 3. Difference detected in concentrations of fecal coliforms, *Enterococcus*, and *E. coli* with a sample size of 40 in each comparison group**

| Indicator | Difference detected ($log_{10}$ CFU/hand) | |
|---|---|---|
| | Least Conservative[a] | Most Conservative[b] |
| Fecal coliform | 1.25 | 2.4 |
| *Enterococcus* | 1.13 | 2.03 |
| *E. coli* | 0.18 | 1.16 |

[a] Assumes standard deviation equal to the smallest observed in this study
[b] Assumes standard deviation equal to the largest observed in this study

**Table 4. Characteristics of jalapeño harvesters in control and intervention groups (standard deviation)**

| Variable | Control | Hand Washing+ Harvest | Hand Washing | SanitTwice+ Harvest | SaniTwice |
|---|---|---|---|---|---|
| Male % | 82.5 | 100 | 97.5 | 95 | 82.5 |
| Age (yr) | 30 (12) | 30 (13) | 31 (12) | 30 (13) | 30 (12) |
| Time Elapse (min) | 28 (14) | 27 (14) | 28 (14) | 27 (15) | 28 (15) |
| Sample Size | 40 | 19 | 40 | 20 | 40 |

**Table 5. P-values for differences in the concentration and prevalence of fecal indicators between intervention and control groups**

| Indicator | Comparison | p-value Concentration | p-value Prevalence |
|---|---|---|---|
| Fecal Coliforms | | | |
| | Control by Hand Washing | 0.05 | NA[C] |
| | Control by SaniTwice | <0.01[A] | <0.01[A] |
| | SaniTwice by Hand Washing | <0.01[B] | <0.01[B] |
| *Enterococcus* | | | |
| | Control by Hand Washing | 0.99 | NA |
| | Control by SaniTwice | <0.01[A] | NA |
| | SaniTwice by Hand Washing | 0.03[B] | NA |
| *E. coli* | | | |
| | Control by Hand Washing | 0.04[A] | 0.75 |
| | Control by SaniTwice | 1.00 | NA |
| | SaniTwice by Hand Washing | 0.13 | 1.00 |

[A] P<0.05 compared with control
[B]P<0.05 compared with SaniTwice
[C] When prevalence values were equal statistical analysis was not applicable

**Table 6. Prevalence of fecal indicators between intervention and control groups**

| Indicator | Control | Hand washing | SaniTwice |
|---|---|---|---|
| Fecal Coliforms | 100% | 100% | 47.5% |
| *Enterococcus* | 100% | 100% | 100% |
| *E. coli* | 12.5% | 20% | 12.5% |

**Table 7. Comparison of concentrations of fecal indicators in intervention and intervention + harvest groups for each intervention**

| Indicator | Comparison | p-value |
|---|---|---|
| Fecal Coliforms | | |
| | Hand washing by Hand Washing+ Harvest | 0.41 |
| | SaniTwice by SaniTwice+ Harvest | <0.01[A] |
| *Enterococcus* | | |
| | Hand washing by Hand Washing+ Harvest | 1.00 |
| | SaniTwice by SaniTwice+ Harvest | <0.01[A] |
| *E. coli* | | |
| | Hand washing by Hand Washing+ Harvest | 0.07 |
| | SaniTwice by SaniTwice+ Harvest | 0.96 |

[A] P<0.05 compared with SaniTwice

**Table 8. Comparison of absorbance in intervention and intervention + harvest groups for each intervention**

| Comparison | p-value |
|---|---|
| Hand washing by Hand Washing+ Harvest | <0.01[A] |
| SaniTwice by SaniTwice+ Harvest | 0.13 |

[A] P<0.05 compared with Hand Washing

**Table 9. Sample size required to detect a 2 $\log_{10}$ CFU/hand difference in concentration of fecal coliforms, *Enterococcus*, and *E. coli*, between comparison groups**

| Indicator | Least Conservative[a] | | Most Conservative[b] | |
|---|---|---|---|---|
| | Standard deviation ($\log_{10}$ CFU/hand) | Sample Size (per group) | Standard deviation ($\log_{10}$ CFU/hand) | Sample Size (per group) |
| Fecal coliform | 2.58 | 27 | 4.31 | 73 |
| *Enterococcus* | 2.37 | 23 | 3.17 | 40 |
| *E. coli* | 0.71 | 2 | 1.26 | 7 |

[a] Assumes standard deviation equal to the smallest observed in this study
[b] Assumes standard deviation equal to the largest observed in this study

**Figure 1. Concentrations of fecal indicators on hands of jalapeño harvesters in control and hygiene intervention groups on farms in Nuevo León, Mexico.** Boxes display the quartiles (25th, 50th,and 75th) and whiskers display the minimum and maximum $\log_{10}$ colony forming units per hand. Diamonds display arithmetic mean. Values above boxes are the geometric mean with 95% confidence intervals in parenthesis. Letter superscripts indicate $p<0.05$. "A" indicates significant difference from control, "B" indicates significant difference from SaniTwice.

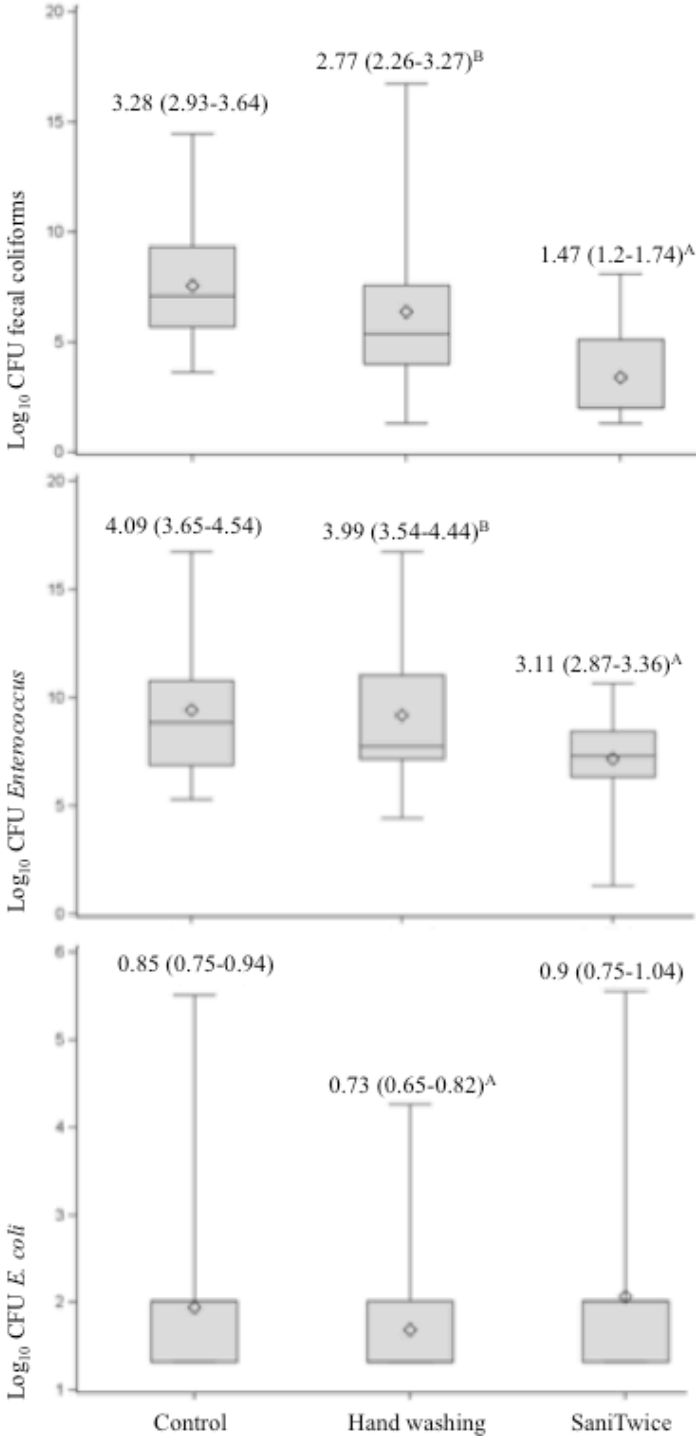

**Figure 2. Absorbance of hand rinsates received from jalapeño harvesters in control and hygiene intervention groups on farms in Nuevo León, Mexico.** Boxes display the quartiles (25th, 50th,and 75th) and whiskers display the minimum and maximum absorbance of rinsate per hand of harvesters. Diamonds display arithmetic mean. Values above boxes are the geometric mean with 95% confidence intervals in parenthesis. Letter superscripts indicate $p<0.05$. "A" indicates significant difference from control, "B" indicates significant difference from SaniTwice.