CPS CENTER for PRODUCE SAFETY

CPS 2010 RFP
FINAL PROJECT REPORT

Project Title

The likelihood of cross contamination of head lettuce by *E. coli* O157:H7, *Salmonella* and norovirus during hand harvest and recommendations for glove sanitizing and use

Project Period

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Objectives

Objective 1. Develop and evaluate tools for characterizing and quantifying the accumulation of organic material (soil and lettuce latex) on the gloves during the process of harvesting head lettuce.

Objective 2. Conduct a field study in the Salinas Valley area whereby gloves (rubber and nitrile) used by harvesters/corers in the field at specific time intervals (0.5, 1, 2, and 4 hrs) during a typical day will be collected for subsequent measurement of organic material accumulation.

Objective 3. Using both field and laboratory analyses, determine the amount of organic material (ratio of soil and lettuce latex) that accumulated on gloves collecting in the field.

Objective 4. Create a mock soil/latex solution in the lab that, once applied to gloves, will mirror three levels (high, medium and low) of organic material (have the same ratios of soil and latex) on gloves collected during the field study.

Objective 5. Determine the likelihood and degree of pathogen (E. coli O157:H7, Salmonella and norovirus) transfer to "used" gloves (rubber or nitrile) coated with four levels (high, medium, low or none) organic matter after touching contaminated soil or lettuce.

Objective 6. Determine the likelihood and degree of pathogen (E. coli O157:H7, Salmonella and norovirus) transfer that occurs when contaminated gloves (rubber or nitrile), also treated with four degrees of organic matter (high, medium, low and none), touch a single head of lettuce and determine how many additional lettuce heads can become contaminated if touched following a single glove contamination event.

Objective 7. Optimize the formulation of a levulinic acid plus SDS sanitizing solution for maximal removal of organic material and inactivation of pathogens (E. coli O157:H7, Salmonella and norovirus) on one glove type (rubber or nitrile) and compare the efficacies to that of a bleach sanitizer for gloves.

Objective 8. Define the maximal organic/soil load that can be present on gloves or in sanitizing solution buckets (placed in the field for purpose of rinsing gloves) without diminishing efficacy of removing organic material or inactivating pathogens(E. coli O157:H7, Salmonella and norovirus).

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FINAL REPORT

Abstract

In response to growing concerns regarding the safety of raw produce, the mandatory use of gloves when handling raw produce and the practice of sanitizing gloves in chlorine glove-dunk buckets has become common in the leafy green industry. However, it is uncertain that these practices actually improve the safety of leafy greens. In this study, the impacts of glove use (glove type/composition) and disinfection (with traditional and novel sanitizers) on cross-contamination of raw head lettuce by bacterial (*E. coli* O157:H7, *Salmonella*) and viral (norovirus) pathogens during harvest was investigated. Re-creating field conditions in the laboratory was a priority from the start of this project. Field data was collected during a visit to the Central CA Valley, where UGA investigators followed iceberg lettuce-harvesting crews for one week. Gloves worn by lettuce harvesters (performing the coring operation) were shipped to UGA and analyzed for levels of accumulating soil, lettuce debris, lettuce sap and moisture. This information was the basis of protocols developed to re-create worst-case glove "soilage" in the lab. In disinfection studies, log reduction of viable bacterial and viral pathogens from the surface of gloves was investigated with a novel sanitizer (levulinic acid plus SDS), Purell hand sanitizer, and different concentrations of free chlorine (50 – 200 ppm). Different types of gloves were investigated (Uniseal Latex, Uniseal Nitrile, Ansell Canner's, Glove Plus Latex, and Fisherbrand Latex) using both clean and "soiled" conditions. Pathogen transfer to gloves during glove application and from gloves to heads of lettuce was also investigated with noroviruses (using murine norovirus, a surrogate for human norovirus). We demonstrated that high levels of virus could be transferred to gloves when gloves are applied. Our study also demonstrates that norovirus contamination on gloves can be reduced by disinfection of glove surfaces using waterless hand sanitizers (Purell or 5% levulinic acid plus 2% SDS foam) (reductions of ~1-2 log PFU) or using 50 ppm chlorine (pH 7) with a single glove-dunk bucket (~2 log PFU with latex gloves or 3-4 log PFU with nitrile gloves). Viable bacterial pathogens on gloves can also be significantly reduced after glove-dunk buckets containing chlorine concentrations of at least 50 ppm (pH 7). When contamination levels are low (≤ 3 CFU/glove), complete inactivation can be achieved; if high levels of glove contamination occur, we have demonstrated 4-6 log CFU reductions on gloves, but complete inactivation was not always achieved, especially in the case of nitrile gloves. Alternative sanitizers can also be used to reduce pathogen contamination, but the lower efficacy of Purell and the high cost of levulinic acid plus SDS make these alternatives less attractive. Taken together, we provide scientifically-based recommendations on glove use and disinfection during harvesting head lettuce. Communication and implementation of these recommendations will contribute to improving the safety of fresh produce.

Background

As produce consumption has increased in the United States, there has been a marked increase in reports of foodborne disease related to produce and a growing public concern over food safety. In addition, an emerging threat to food safety, human noroviruses (NoV) are now recognized as the leading cause of foodborne illnesses in the U.S. Heightened food safety awareness in the leafy green industry has driven important procedural changes in the way leafy greens are handled in the harvest environment. . One such change has been the widespread requirement that gloves be used by all persons coming into contact with pre- or post- harvest produce. However, it is unclear if this practice reduces the likelihood that pathogens are transferred from the harvest environment to gloves, with subsequent cross-contamination of produce. Also uncertain, is the impact of glove composition, frequency of glove changing, and efficacy of glove disinfection using glove-dunk buckets containing chlorine, which is a common practice in lettuce-harvesting operations.

Research Methods and Results

Objectives 1-4: Methods and Results

Overview: In the first four study objectives we sought information on glove use that could be considered "typical" for iceberg lettuce harvesting operations of scale in the Central California Valley region. In particular, we were interested in the types of gloves used, the frequency of glove changing, glove washing or sanitizing procedures, and how much soil, lettuce sap, moisture and lettuce debris typically accumulated on different glove types. The purpose for gathering this type of information was to ultimately reproduce these "typical" conditions in the lab where we could define variables that may be important for maximizing the disinfection of pathogens on gloves and for reducing the risk of pathogen cross-contamination occurring due to gloves. The field data we collected and the information we gleaned from various conversations with food safety specialists working directly with lettuce harvesting crews was invaluable for conducting the laboratory portion of this project.

Field Study: With the generous assistance of Thomas Mack, Nye Hardy, and especially Jenny Chavez (Dole Fresh Vegetables), individuals from "value-added" (VA) iceberg lettuce harvesting crews from the Central CA Valley were recruited as volunteer study participants. Approval by the UGA Institutional Review Board (IRB) for studies involving human subjects was obtained prior to recruiting volunteers or conducting the field portion of this study. Recruiting materials and consent documents were provided both in English and Spanish. Ms. Chavez also provided valuable information regarding the types and brands of gloves typically worn by harvesting crews. This allowed us to pre-order gloves we could pre-weigh and bring with us on during the field study. Ms. Chavez and her co-worker, Alexa Johnson, escorted us to the lettuce harvesting sites and assisted in English-Spanish translation of the instructions we provided to the study volunteers. Four different crews were followed over a 5 day period by UGA researchers (Cannon and Erickson). Pre-weighed gloves were provided to volunteer crew harvesters. Gloves were collected at specific time intervals (30 min to 1 hr, 2-3 hr, and 4-6 hr), with 16 glove pairs collected at each time point per glove type. After notating gloves (either "coring hand" or "other hand" during collection), glove weights were recorded before and after use, shipped to UGA and stored at -20°C. Soil and lettuce cores and/or field-extracted lettuce exudates were shipped to the lab and stored at -20°C. Observational data collected during the 5-day field study was critical to the later objectives of this project; although, this data is not quantifiable, our first-hand experiences following crews around from 4 am in the morning until the afternoon gave us a sense of the harvesting operation flow and what types of interventions may be practical.

Methods for determining soilage of field-worn gloves with soil, moisture, lettuce sap and lettuce debris. Total moisture, soil and lettuce accumulation on gloves was derived by weighing the gloves before and after use in the field. To determine the contribution of each component of the accumulated material, a series of separation steps (developed by Habteselassie) were performed and the weights of each component were determined. First, large lettuce pieces were removed with forceps. Then, smaller lettuce debris and soil was removed by elution with water. A slow-speed centrifugation step facilitated the separation of the lighter lettuce material from the more dense soil. Lettuce debris in the supernatant was collected by filtration and weighed with the larger bits of lettuce that were collected with forceps. The soil (pellet) was re-suspended, filtered, and oven-dried to determine the dry weight of the soil. The amount of moisture accumulation on the gloves was determined by subtracting the (wet) weight of the lettuce debris and the soil (dry) weight from the total weight of the accumulated material. The remaining elution buffer (filtered to remove lettuce debris) was submitted for analysis of percent total C and N to estimate the concentration of lettuce extract (containing lettuce latex) within the

liquid portion (moisture) of the accumulated material on the gloves. Total percent C and N was determined by dry combustion and detection with an elemental analyzer (Macro Elemental Analyzer). Water was determined to be the most appropriate elution buffer in preliminary recovery experiments, as it allowed sufficient recovery of lettuce sap from latex and nitrile gloves inoculated with field-extracted lettuce sap and did not interfere with % C and N analysis.

Measuring soilage of field-worn gloves: Analysis of the gloves collected in the field revealed the following: 1) no discernible trends were observed with regard to the accumulation of large lettuce debris when making comparisons between time points, glove types, or day of collection (range <0.001 g to 2.053 g per glove); 2) soil accumulation on gloves was also variable with no observable trends (range <0.001 g to 1.536 g per glove); 3) moisture accumulation was greater for gloves collected at time points ranging from 2-6 hr for latex gloves, and for gloves collected on day 2 for both latex and nitrile gloves (up to 7.557 g per glove). Negligible amounts of N were observed on all gloves tested. Percent C averaged 1.42 (± 3.36) for each glove tested (n = 123). Percent C in the field-extracted lettuce sap averaged 1.18 (± 0.17), indicating that the bulk of the moisture accumulating on the gloves was nearly identical in terms of C content to sap collected directly from iceberg lettuce cores (using the procedures we developed in-house). No significant differences were observed in % C of the moisture accumulated on the gloves collected from the field harvesters of this study, when comparing glove type, harvesting hand, or time the glove was worn (1, 2-3, or 4-6 hr); the average moisture accumulation levels of gloves worn by harvesters in the 2-3 hr or 4-6 hr time intervals (0.95 g and 1.27 g, respectively), and the moisture accumulation level at the <1 hr time interval was only slightly lower (0.54 g).

Recreating field-soiled gloves in the lab: Using the calculated average accumulation levels of lettuce sap, moisture, soil and lettuce observed on the gloves collected during the field study, a protocol was developed recreate these soilage levels in the lab. We chose to adopt a protocol for glove soilage that represented a "worst-case-scenario" for the majority of experiments in this study, reasoning that any glove treatments that resulted in significant pathogen reductions under these conditions would also work for cleaner gloves. Preliminary data giving support to this rationale will be explained later in the text. Soil obtained from lettuce fields in the San Joaquin Valley were adjusted to 40-60% water holding capacity. Approximately 2 g of soil was applied to each pair of gloves by manually rubbing gloved hands together, distributing the majority of the soil volume onto the palms of the gloves. Lettuce sap was extracted from heads of iceberg lettuce in the lab. The green outer leaves of iceberg lettuce were removed and the remaining material was homogenized (using a food processor) in DI water. Then, the water volume was reduced by evaporation to 1/3 of the original volume. Volumes of lettuce sap ranging from 100 µl to 1 ml were used on gloves or glove pieces, depending on the experimental objectives defined below. Lettuce sap was applied either alone, or with the pathogen dispersed in it and allowed to dry at ambient temperatures in the lab.

Objectives 5-6: Methods and Results

Overview: The purpose of conducting the experiments originally described in Objectives 5 & 6 was to derive information important for constructing/evaluating techniques for interrupting cross-contamination occurring in the harvest environment. It was thought that if accumulation of soilage (such lettuce debris, lettuce sap or soil) positively correlated with the duration of time that gloves were worn, *and* if pathogen transfer occurred more readily when gloves were more heavily soiled, then this would be a perfect opportunity for applying a simple intervention (washing gloves or changing gloves) that could minimize cross-contamination. Objectives 5 & 6 were written assuming that there would be detectable differences in soilage levels observable during the time course of a lettuce harvesting day. However, significant differences in soilage

levels were not apparent in our field study results. In addition, at the time we were beginning our work on pathogen transfer to and from gloves during lettuce harvesting, Dr. Linda Harris's lab at UC-Davis was working on a project very similar to what we had proposed. In her study examining *E. coli* O157:H7 transfer to and from Romaine lettuce and gloves worn by harvesters in the field, she found that even on clean gloves, cross-contamination occurred readily. Rather than duplicating efforts, we chose to focus our efforts on measuring the degree of cross-contamination that may be likely to occur for viral pathogens, which had not been previously investigated by the Harris lab. In addition, we examined if there were differences in transfer rates of viruses to/from different glove types (latex vs nitrile).

Transfer of norovirus from bare hands to gloves or gloves to lettuce. In conjunction with a study currently on-going in the Cannon lab, and in the scope of the tasks of Objective 6, the rate of pathogen transfer from contaminated gloves to lettuce was compared to pathogen transfer rates from porcine skins (serving as a model to bare human hands). Norovirus transfer from contaminated hands to gloves was performed to investigate the likelihood that gloves can become contaminated if they are handled by norovirus-contaminated hands during glove application. In addition, the extent to which sequentially touched lettuce leaves can become contaminated after they are touched by contaminated bare hands (porcine skins) or gloves was also determined. Transfer of a murine norovirus (MNV-1) surrogate was compared to the transfer of human norovirus (genotypes I [GI] and II [GII]). Porcine skin was used as a model for human fingertips. The latex and nitrile gloves (Uniseal) that have been used throughout this project were used and represented by the names, "blue latex" and "blue nitrile" to distinguish them from the laboratory latex gloves (Microflex) that were also used in the following experiments ("yellow latex"). Using a mixture of viruses (human GII and GI viruses and MNV-1) suspended in 10% stool as an inoculum, virus transfer from porcine skin to gloves (yellow latex, blue latex, and blue nitrile) (n=9 each) was measured after interfacing the two surfaces for 10 seconds using a mechanical transfer device (1,000 g/4.4 cm^2). A stool suspension of MNV-1 was used to quantify the extent of contamination after inoculating a donor surface (porcine skin or glove; n=6 each) and sequentially touching ten leaves of fresh iceberg lettuce. Viruses were eluted from recipient surfaces and real time RT-qPCR was used to determine virus recovery.

The transfer rates of human norovirus from porcine skins to gloves, in many cases, approached 50%, demonstrating the ease with which virus can transfer from contaminated hands to gloves during application (Figure 1). Rates of transfer of GII, GI, and MNV-1 from porcine skin to yellow latex were 38%, 33%, and 35%, respectively; to blue latex, 51, 52, and 41%, respectively; and to blue nitrile gloves, 35, 36 and 29%, respectively. Rates of norovirus transfer to the first iceberg lettuce leaf surface touched by contaminated blue latex gloves, blue nitrile gloves, and porcine skin were 54, 38, and 42%, respectively (Figure 2). To the second and third lettuce surfaces, rates of transfer ranged respectively from 20 to 23% and 8 to 13% for blue latex gloves, blue nitrile gloves, and porcine skins. After touching five lettuce leaves, transfer rates were 2 to 3% for all surface types. After touching ten lettuce leaf surfaces, norovirus was still detected on all replicate samples, but rates of transfer decreased to less than 1%. **Norovirus can easily transfer from bare hands to gloves during glove application. Transfer can occur regardless of glove type. Gloves that become contaminated with norovirus can contaminate ten or more subsequently touched iceberg lettuce surfaces. This set of experiments demonstrates the importance of good hand hygiene *before* gloves are applied.**

Objectives 7-8: Methods and Results

Overview: The bulk of our efforts were concentrated in the experiments of Objectives 7 & 8 which addressed pathogen survival on gloves under field-like conditions and their ability to be cleaned and decontaminated after application of practical glove-sanitizing treatments. Taken together, these studies provide quantitative data on the efficacy of promising and practical glove sanitizing procedures for reducing cross-contamination by bacterial (*E. coli* O157:H7 and *Salmonella*) and viral (norovirus) pathogens in the lettuce harvesting environment. We conclude with our recommendations for glove sanitation and use.

Pathogen Survival on Soiled and Unsoiled Gloves: Impact of lettuce sap on pathogen survival. Bacterial (*E. coli O157:H7, Salmonella)* or viral (norovirus) pathogens were suspended in either DI water or lettuce sap, inoculated onto nitrile gloves, and allowed to dry at ambient temperature. Both *E. coli O157:H7* and *Salmonella* were reduced by 2.10 and 2.45 log CFU, respectively after just a 75-min drying time (~58% RH) when suspended in DI water (data not shown). Similarly, when suspended in buffered peptone water, log CFU reductions of *E. coli* O157:H7 and *Salmonella* were 2.06 and 2.25. However, the respective bacterial pathogens were reduced by only 0.60 and 0.84 log CFU after 75 min when suspended in lettuce sap (data not shown). Survival on latex and nitrile glove fingertips was also compared (Table 1) and no significant differences in between pathogen log CFU reductions due to the type of pathogen or glove type were found when *E. coli* O157:H7 and *Salmonella* were suspended in lettuce sap.

While lettuce sap appeared to extend the survival of bacterial pathogens, pre-treatment of gloves with soil and/or lettuce sap did not negatively impact *E. coli* O157:H7 survival on gloves during drying (Table 2). Log CFU reductions ranged from 0.58 to 1.00, regardless of glove type (latex or nitrile) or pre-treatment (lettuce sap or soil) when the bacteria was suspended in lettuce sap and spot inoculated onto gloves. While these log CFU reductions were slightly higher than those presented for latex and nitrile gloves in Table 1, given the variability in the replicates, it is unlikely that such differences would differ significantly.

Expanding upon this data with alternative latex glove types (i.e. Canner's, Fisherbrand, GlovePlus), there were some differences in *E. coli* O157:H7 survival during drying on different glove types (all pretreated with soil and lettuce sap) (Table 3). Pathogen survival on Canner's and GlovePlus latex gloves (that have very different surface characteristics) was reduced when compared to other glove types, but CFU reductions did not exceed 2 logs.

At time intervals of 0, 1, 1.25, 3, 5 and 7 hr, pathogens were recovered from the glove surfaces and cultured. Pathogen survival at each time interval was calculated by comparison to the quantity of pathogen inoculated. *E. coli* O157:H7 and *Salmonella* log CFU reductions are reported in Table 4. Over the time course, *Salmonella* did not survive as well as did *E. coli* O157:H7, but there was no apparent difference in survival due to glove type. After 5 hrs, log CFU reductions ranged from 1.23 – 1.55 for *E. coli* O157:H7 and 1.95 – 2.34 for *Salmonella* when the inoculum was suspended in lettuce sap.

Norovirus was more resistant to drying, with 0.36 and 0.42 log PFU reductions of infectious virus after 1 hr of drying virus on nitrile gloves when the virus was suspended in water or lettuce sap, respectively. However, more dramatic differences were observed for norovirus survival after 7 hr of drying on nitrile gloves when the virus was first suspended in water versus lettuce sap. After 7 hr, infectious norovirus was reduced by 1.63 log PFU when the inoculum was suspended in water, and by only 0.64 log PFU when it was suspended in lettuce sap. Reasons for enhanced survival in lettuce sap as compared to DI water, may be explained by reduction of

osmotic stress occurring over time, or protection afforded by chemicals in the lettuce sap. **Pathogen survival on gloves is increased by the presence of lettuce sap on glove surfaces. These results are important because they suggest the simple act of rinsing gloves to remove lettuce sap can minimize pathogen survival on gloves.**

Comparison of the Bactericidal and Virucidal Efficacy of Common and Novel Glove Sanitizers: Gloves pieces (3" x 3") inoculated with *E. coli* O157:H7 were either immediately processed after the inoculum was dried, or first subjected to a 1-min treatment with (50 ml) of water, 200 ppm chlorine solution, or 5% levulinic acid plus 2% SDS. As indicated in Table 5, treatment with water alone resulted in minimal reductions of viable pathogen (~1.0 log CFU/glove piece). After glove treatment with the sanitizers, viable counts could no longer be determined and nearly all experimental samples were enrichment negative. Only after treatment with 200 ppm chlorine could *E. coli* O157:H7 be detected on nitrile gloves (2/10 samples). However, given the starting inoculum and the low limit of detection for enumeration (1.18 log CFU/glove piece), log CFU reductions were estimated to be > 4.6 CFU/glove. Similar results were obtained for *Salmonella* (data not shown). When inoculated with 6.7 log CFU *Salmonella*, greater than 4 log CFU could be recovered from gloves treated for 1 min with water alone, but no viable pathogens could be detected on latex or nitrile gloves treated with 5% levulinic acid plus 2% SDS solution. In addition, chlorine (200 ppm) treatment of latex gloves for 1 min resulted in no viable pathogens, and in 2/2 replicates with nitrile gloves, *Salmonella* could be detected by enrichment culture only.

Similar experiments were conducted with murine norovirus. Virus dried on the surface of latex glove pieces (2" x 2") was treated with water, water plus 2% SDS, 200 ppm chlorine, 200 ppm chlorine plus 2% SDS, or 5% levulinic acid plus SDS sanitizer for 1 min or 5 min. Log PFU reductions of viable murine norovirus were determined by comparison to untreated control latex and nitrile gloves (virus was dried on the surface of latex or nitrile glove pieces and immediately eluted from the surface). Log PFU reductions of viable MNV on gloves were < 2.0 log for 1 min and 5 min treatments with water or water plus 2% SDS (Figure 3). Significantly greater log PFU reductions were achieved after treatment with each of the disinfectants tested, but significant differences in efficacy between sanitizers was not found. Significantly greater log PFU reductions were revealed for murine norovirus on nitrile gloves when compared to latex gloves.

From this set of experiments it was clear that the 5% levulinic acid plus 2% SDS solution was at least as effective as 200 ppm chlorine for reducing populations of *E. coli* O157:H7, *Salmonella* and norovirus from latex and nitrile gloves. In addition, murine norovirus appeared to be more resistant to both the 200 ppm chlorine and 5% levulinic acid plus 2% SDS sanitizers than the bacterial pathogens. While this baseline information was helpful in directing our future experiments, the 1 min and 5 min sanitizer exposure times tested would not be practical for glove-dunk buckets used in the field. Also, the highly concentrated levulinic acid plus SDS sanitizer may be useful in some applications, but would be much too expensive for using in large volumes (such as in glove-dunk buckets). **Disinfection with 200 ppm chlorine and 5% levulinic acid plus 2% SDS is effective after 1 min for bacterial pathogens on gloves and 5 min for viral pathogens on gloves. Latex gloves were associated with greater log reductions of viable bacterial pathogens, while nitrile gloves were associated with greater log reductions of viable norovirus.**

Investigating the efficacy of waterless hand sanitizers on gloves: From conversations during our field study and through conversations with stakeholders at the Western Food Safety Summit (Spring 2012), we learned that some harvesting crews were using waterless hand sanitizers

either for their bare hands, on the outside of their gloves, or for both bare and gloved hands. We also learned that a different type of latex gloves (Canner's gloves) are commonly used. Testing first the more resistant murine norovirus, the commercially available Purell hand sanitizer was compared to using the 5% levulinic acid plus 2% SDS solution as a waterless (liquid or foam) sanitizer. Three glove types (latex, nitrile and Canner's) were evaluated in this set of experiments.

Eluting of noroviruses dried on the surface of intact gloves often results in poor recovery percentages (high percentages of loss during the elution procedure). Since enrichment culture is not possible, a reduction in the surface area being eluted is often necessary. For this reason, we devised a lab protocol that could be used to simulate, on a smaller scale, two gloves being rubbed together by a person wearing the gloves. The following procedure was adopted. Glove pieces (2" x 2") were held tauntly to autoclaved flat, wooden sticks (sticks used for stirring paint) by rubber bands. One pair of gloved wooden sticks represented a pair of gloved hands. One glove of the pair was inoculated with virus and allowed to dry. For waterless sanitizer (and water control) experiments, the virus-inoculated glove was treated with 100 µl of water, water plus 2% SDS, 5% levulinic acid plus 2% SDS (liquid), 5% levulinic acid plus 2% SDS (foam) or Purell and immediately (within 10 sec) interfaced with the uninoculated glove of the pair. The gloves were rubbed together for 10 seconds and allowed to air-dry for 1 min before placing in neutralizing/elution buffer (neut/elute buffer). Rubber bands were then cut with a sterile scalpel blade, releasing the glove pieces from the wooden sticks and into the neut/elute buffer. Each glove of the pair (the inoculated glove and the glove that was not inoculated but interfaced with the inoculated glove) was processed separately. Log PFU reductions in infectious virus were determined for each virus-inoculated glove. For the uninoculated gloves of each pair, log PFU virus transferred to and detected on the uninoculated gloves was reported. Log PFU reductions were calculated by comparison to inoculated but untreated control glove pieces.

Neither of the water or water plus 2% SDS controls resulted in average log reductions of viable murine norovirus that exceeded 1 log PFU/ml for any of the glove types tested (latex, nitrile, Canner's) (Figure 4). The foaming 5% levulinic acid plus 2% SDS sanitizer was significantly more effective than the liquid version of the solution when used as a waterless sanitizer, but neither solution reduced viable norovirus counts greater than 2 log PFU on average. The Purell hand sanitizer was the most effective of the waterless sanitizers tested ($p < 0.05$). Average log PFU reductions were greater for nitrile gloves than the two latex varieties of gloves ($p < 0.05$).

Viruses were detected on all of the gloves that were originally uninoculated, but interfaced for 10 sec with the virus-inoculated gloves in the presence of water, water plus 2% SDS, 5% levulinic acid plus 2% SDS (liquid), 5% levulinic acid plus 2% SDS (foam) or Purell (Figure 5). Significant reductions in the average log PFU counts were detected on the sanitizer-treated gloves when compared to the water or water plus SDS controls ($p < 0.05$). For all three glove types, the lowest log PFU counts were detected on the Purell-treated gloves. Previous published research has questioned the use of the murine norovirus surrogate when testing the efficacy of alcohol-based sanitizers *(5)*. When compared to feline calicivirus (an alternative surrogate for human norovirus), murine norovirus is recognized as at least as resistant to oxidative disinfection *(6)* and more resistant to organic acid-based disinfection (including the levulinic acid plus SDS sanitizer) *(3)*, but more sensitive to ethanol- and isopropanol-based sanitizers. For this reason, we performed additional studies with the ethanol-based Purell hand sanitizer.

In these studies, murine norovirus and feline calicivirus were co-inoculated onto latex or nitrile gloves. Using the same experimental set-up as described above, the virus-inoculated gloves were treated with 100 µl of Purell and immediately interfaced with the uninoculated glove of the pair. Residual sanitizer was allowed to evaporate for 30 sec (instead of 1 min) before neutralization/elution. Each glove piece of the pair was assayed separately for both infectious murine norovirus and infectious feline calicivirus.

Average log PFU reductions of viable MNV and FCV on latex and nitrile gloves were minimal after treatment with the Purell hand sanitizer (Figure 6). MNV log reductions were statistically greater for MNV than FCV, and log reductions were statistically greater for nitrile gloves ($p < 0.05$). Unexpectedly, when we compared the log PFU reductions observed in this set of experiments to the previous set of experiments, log PFU reductions of MNV were 1-2 log PFU/ml lower for the Purell sanitizer. It is likely that the decrease in sanitizer exposure time (1 min previously to 30 sec in this trial) contributed to this observation. Decreasing the exposure time to 30 sec was intended to better simulate how the sanitizer would be applied in practice. Interestingly, average log PFU counts of MNV transferred to the uninoculated gloves by the inoculated and Purell-treated gloves in this experiment were also lower than previously observed (Figure 7); although, high variability observed in the replicates of this set of experiments may be the reason for these differences. No differences in log PFU transferred to the gloves (both latex and nitrile) were observed when comparing MNV and FCV. These results are consistent with previous studies suggesting MNV is more sensitive to ethanol-based hand sanitizers than is FCV.

As a comparison, the efficacy of the Purell hand sanitizer in reducing populations of *E. coli* O157:H7 on pieces of latex and nitrile gloves was also investigated. Glove pieces (3 x 3") were inoculated with 4.48 ± 0.15 log CFU *E. coli* O157:H7, air-dried and exposed to either 50 ppm chlorine or Purell hand sanitizer for 15 sec before neutralization. Additional variables were also examined in this set of experiments; some gloves were pre-treated with lettuce sap (100 µl) or soil which was ground into the gloves in order to simulate field-soilage of gloves during regular wear in the lettuce harvesting environment. The numbers of latex and nitrile gloves testing enrichment-positive for *E. coli* O157:H7 over the total numbers tested for each disinfectant and pre-treatment variable are listed in Table 6.

Taken together, the Purell hand sanitizer did not perform as well as the 50 ppm chlorine disinfectant; although, log CFU reductions of the pathogen exceeded 3 log CFU/glove piece (given the inoculum concentration and the lower limit of detection for enumeration of 1.2 log CFU/glove piece). Soil and organic debris in the lettuce sap did appear to interfere with chlorine disinfection of bacteria on nitrile gloves, but no pathogens could be recovered from any of the latex gloves tested. These results suggest that even low concentrations of chlorine may be effective on certain glove types. **Of waterless sanitizers tested for efficacy agaist norovirus, only Purell and the foam application of levulinic acid plus SDS outperformed the water control; although, PFU reductions are unlikely to exceed 2 log. Purell was less effective against baterial pathogens than 50 ppm chlorine, particularly for latex gloves.**

Microbicidal effects of pre-treating gloves with levulinic acid plus SDS: While conducting studies with the levulinic acid plus SDS sanitizer, we noticed that after gloves were treated, a residue could be macroscopically seen on latex gloves. We questioned the impact this residue may have on pathogen survival on latex gloves. We investigated *E. coli* O157:H7 and *Salmonella* reductions due to inoculum drying on gloves that were either pre-treated with 5% levulinic acid plus 2% SDS, 200 ppm chlorine, or untreated. Glove pieces (3" x 3") were soaked in sanitizer

for 1 hr, rinsed under tap water, and dried, before pathogens were inoculated on the glove surfaces. The results presented in Table 7, indicate that significant losses in bacterial survival could be achieved when latex gloves were pre-treated with levulinic acid plus SDS. Subsequent experiments were performed where gloves were pre-treated with 2% SDS only and no significant reductions in *Salmonella* survival were found when comparted to controls that were not pre-treated (data not shown). In addition, reductions in murine norovirus survival (PFU/ml) were investigated with or without pretreatment of latex gloves with 5% levulinic acid plus 2% SDS, 2% SDS alone or 200 ppm chlorine for 1 hr prior to drying and virus inoculation. Log PFU reductions did not exceed 0.36 for any of the variables tested (data not shown), indicating glove pre-treatment is not an effective treatment for reducing norovirus survival on latex gloves.

Gloves that were pre-treated with 5% levulinic acid plus 2% SDS for 1 hr, rinsed, dried and then inoculated with bacterial pathogens, were next tested in combination with treatments of 50 ppm chlorine or Purell sanitizer. During pathogen inoculation, it was noted that the surface properties of the latex gloves had been altered by the levulinic acid plus SDS. The inoculum seemed to spread out over the glove surface better when gloves were pre-treated. The altered surface properties induced during glove pre-treatment variably impacted pathogen reductions achieved following treatment with chlorine or Purell (Table 8). *E. coli* O157:H7 was reduced to a greater extent on gloves that were pre-treated with 5% levulinic acid plus 2% SDS prior to treatment with Purell; however, only minimal differences were observed between pre-treated and non-pre-treated gloves when the 50 ppm chlorine disinfectant was applied. **Pre-treating gloves with levulinic acid plus SDS decreases pathogen survival on glove surfaces, but can interfere with disinfection of gloves with chlorine.**

Chlorine disinfection of clean or "soiled" gloves: Since chlorine seemed to perform as well as, and in some cases better than the other disinfectants tested, we began a series of experiments to determine the minimal effective concentration when used as a glove-dip sanitizer. To simulate field-soiled gloves, lettuce sap and soil were coated on the surface of the gloves (or glove pieces) for several of the experiments of this set.

Starting with 50 ppm chlorine, 3" x 3" glove pieces (latex, nitrile or canner's) were dipped into chlorine solution for 15 sec. Gloves were used either "clean", referring to no coating on the glove, or "soiled", in which soil was ground into the glove and then 50 µl of lettuce sap applied to same area prior to inoculation with *E. coli* O157:H7 (5.47 – 5.76 log CFU) or *Salmonella* spp. (5.63 - 6.08 log CFU). The results presented in Table 9, indicate that presence of soil and lettuce sap decreased the efficacy of the 50 ppm chlorine disinfectant for both *E. coli* O157:H7 and *Salmonella*. Recovering bacteria from untreated gloves (those inoculated but not treated with chlorine), resulted in similar levels of each bacteria recovered (range 3.60 – 4.15 log CFU/glove piece) (data not shown). For clean gloves, *Salmonella* was more resistant to chlorine disinfection than *E. coli* O157:H7; however, this difference was made mute when soil and sap were present. Considering the inoculum and the amount of virus that could be recovered from unwahsed gloves, log reductions were estimated to be ~4 log CFU/glove piece after 50 ppm treatment for both bacteria, on all glove types, with or without soilage.
Since the bactericidial activity of 50 ppm chlorine treatment was not completely effective, particularly for bacteria on nitrile gloves, we increased the concentration of chlorine in subsequent experiments and used only nitrile gloves to limit the number of variables when optimizing the chlorine concentration. *E. coli* O157:H7 or *Salmonella* detection on glove pieces after treatement of inoculated nitrile gloves with chlorine concentrations ranging from 0 to 175 ppm for 1 min are presented in Tables 10 and 11. Although the gloves were not soiled prior to inoculation and treatment, a higher than expected number of enrichment positive glove pieces

were detected. Even after treatment with 175 ppm chlorine, 30% of gloves tested were enrichment positive. It should, however, be noted that the inoculum was nearly 2 log CFU higher than in previous experiments with 50 ppm chlorine treatments (shown in Table 9). In this set of experiments, considering the inoculum and recovery from unwashed controls, log reductions were estimated to be ~6 log CFU/glove piece after 50 ppm treatment for both bacteria, on all glove types, with or without soilage.

Most likely, high levels of bacterial pathogens would not be present in the harvest environment. To better simulate pathogen contamination levels that may actually occur, nitrile gloves were inoculated with lower levels of *Salmonella* (3.12 log CFU/glove piece) and treated with 75 or 150 ppm of chlorine. In these experiments, the more practical exposure times of 15 or 30 sec were also used. No bacterial contamination could be detected by enrichment culture after treatment with either chlorine concentration for the shorter exposure times (Table 12). Taking the inoculum into consideration, these results indicate ~3 log CFU reductions due to disinfection. **When high levels of bacterial pathogen contamination was present on gloves, pathogens could be detected on gloves after treatment with chlorine (50-175 ppm) by enrichment only. In these experiments, viable cells were reduced by 4-6 log CFU. Only when the bacterial inoculum was ~3 log CFU, were gloves negative by enrichment culture.**

The inoculum concentration was a key variable effecting the outcome of chlorine disinfection. The lower ther inoculum, the lower the likelihood of detecting live bacterial cells on gloves after chlorine treatment. Increasing the concentration of chlorine used for glove treatment correlated with improvements in bactericidal activity, but the correlation was weaker than anticipated. Concentrations as high as 175 ppm did not completely reduce viable bacterial pathogen contamination. Glove type did seem to be an important factor, but more experiments were warranted.

Chlorine disinfection in glove-dunk buckets: In the next set of experiments, we continued to explore variables identified in prior experiments to be important in disinfection outcomes. Additionally, the experimental design was modified to include friction; we rubbed the gloves together during chlorine disinfection procedures to better simulate how glove-dunk buckets would be used in the field. As previously mentioned, it was not possible to use intact, whole gloves for experiments with noroviruses (due to the poor recovery efficiency of virus on untreated gloves). But for experiments with the bacterial pathogens, enrichment culture made it possible to use intact, whole gloves for the remaining glove disinfection experiments.

Glove pieces (2" x 2"; latex, nitrile or canner's) were attached to flat, wooden sticks by rubber bands as previously described (above). Murine norovirus was inoculated onto one glove piece of each pair. Glove pairs (on sticks) were immersed into glass beakers containing 200-400 ml of each chlorine solution (50 – 200 ppm) or water and rubbed together manually for 10 sec so that the flat part of the gloves, between the rubber-banded regions, were interfaced. The inoculated gloves were immediately transferred to neut/elute buffer and released from the sticks by cutting the rubber bands. Log reductions in infectious MNV were calculated by comparing to control samples that were inoculated with virus and eluted into neut/elute solution, but not treated with chlorine or water prior to analysis.

Figure 8 shows that there was no difference in MNV log PFU reductions due to the concentration of the chlorine treatment, even when compared to the control gloves treated with only water. There was a significant difference in mean log PFU reductions due to the glove

type, as virus reductions were greatest for nitrile gloves. Log PFU reductions ranged from 1.53 – 3.32 log for latex and canner's gloves regardless of the treatment. When treated with water, viruses inoculated onto nitrile gloves were reduced by 2.96 log PFU/ml, whereas after treatment with 50 ppm to 200 ppm chlorine, viruses were reduced by 3.82 - 3.89 log PFU/ml. Due to the lower limit of detection for the plaque assay and the inoculum concentration, maximal log reductions were ~4 log PFU/ml for this set of experiments.

Next, we investigated whether or not virus removal and inactivation could be improved if gloves were rubbed with a sponge while they were being subjected to the chlorinated glove-dunk bucket. For each experiment, 50 ppm chlorine was used. Gloves (latex, nitrile or canner's) attached to wooden sticks were rubbed against a sponge (Scotch Brite Non-Scratch Scour Pad) that was inserted into each chlorine beaker. First, an inoculated glove was immersed into the chlorine beaker and rubbed against the sponge using 10 back and forth motions (~6 sec). Then, an uninoculated glove was immersed into the same beaker and rubbed against the sponge in the same spot that was rubbed by the virus-inoculated glove. Each glove was immediately placed in neut/elute buffer after treatment. Experiments where no sponge was used were also repeated, this time rubbing the inoculated and uninoculated gloves together with 10 back and forth motions for ~ 6 sec. For each experimental replicate, inoculated but untreated gloves were included and used to calculate log reductions (PFU/ml) of virus due to treatment.

In experiments where no sponge was used, average log PFU reductions of infectious MNV were similar to those previously reported (above) (Figure 8). Log PFU/ml reductions of MNV from latex and canner's gloves were 2.56 and 3.22, respectively, whereas average log PFU/ml reductions of MNV were 3.89 when nitrile gloves were used. When gloves were rubbed against a sponge, log PFU reductions of MNV decreased significantly ($p < 0.05$) (Figure 9). When uninoculated gloves were analyzed, virus could be detected on all glove types, but only at low levels (Figure 10). While it did appear that more virus was transferred to uninoculated gloves in the experiments with sponges, the levels of virus detected did not significantly differ ($p < 0.05$).

Similar studies were conducted with *E. coli* O157:H7, except whole, intact gloves (latex and canner's) were used in glove-dunk buckets containing 50 ppm chlorine. As previously described, gloves were also pre-treated with soil and lettuce sap to better simulate field-soiled gloves in the lab. In addition to the sponge (sponge 1) used in the norovirus experiments, a different type of sponge (sponge 2) was also investigated (Scotch Brite Dobie). From two replicate experiments (n=28 gloves of each type) testing use of the sponges compared to simply rubbing gloves together (no sponge) during the chlorine treatment, we determined that bactericidal activity was not improved by using either sponge (Table 13). Although bacterial pathogens could only be detected by enrichment, more positives were detected on gloves when a sponge of either type was used. Log CFU/glove recoveries ranged from 3.43 to 5.37 for inoculated, but untreated gloves.

Due to these, as well as previous results with *E. coli* O157:H7-inoculated gloves pre-treated with soil, we investigated whether or not microscopic scratches introduced when soil is ground onto the glove surface may contribute to pathogen resistance to disinfection (by providing protection from disinfection by lodging into scratches). Since nitrile gloves consistently resulted in higher percentages of enrichment positive samples, we used these gloves to test this hypothesis. Gloves were either pre-treated by grinding soil onto the glove surface and inoculated with pathogen suspended in lettuce sap, or inoculated with pathogen suspended in lettuce sap without soil pre-treatment. After drying, gloves were washed in 50 ppm chlorine glove-dunk

buckets using the procedure previously described. In control experiments where bacterial pathogens were recovered from untreated gloves, an average of 4.62 to 4.93 log CFU/glove was recovered from nitrile gloves. After treatment, *E. coli* O157:H7 could only be detected by enrichment culture (Table 14); however, there was not a clear difference in enrichment positive ratios when the gloves pre-treated with soil were compared to the gloves that were not pre-treated prior to chlorine disinfection.

Contrary to the trends observed for nitrile gloves inoculated with bacterial pathogens, reviewing prior experiments conducted with latex gloves revealed a noticable trend toward relatively fewer enrichment-positive latex gloves after identical treatments. To further investigate this apparent trend and to see if different brands (and different textures) of latex gloves could yield similar results, a set of experiments were conducted with *E. coli* O157:H7 and *Salmonella*. Intact, whole gloves were pre-treated with soil ground into the glove palm and lettuce sap before inoculation, drying, and treatment in glove-dunk buckets containing 50 ppm chlorine. In all experiments to date, one lot of Uniseal latex gloves had been used. In experiments with *E. coli* O157:H7, these gloves were used and compared to Fisher brand latex gloves, GlovePlus latex gloves, and the nitrile and canner's gloves that had been used in previous experiments. Similarly, the same nitrile, Fisher brand latex and GlovePlus brand latex gloves were used in experiments with *Salmonella*, but the lot of Uniseal gloves that had been previously used were no longer available. A different lot of gloves was thus used. This lot of gloves was noticably different in color and texture when compared to the first lot. It is very likely that gloves from these two lots have different surface properties that may have impacted pathogen adsorption to the glove surface, removal from the surface, and/or response to disinfection.

In all experiments, the chlorine disinfection of bacterial pathogens on latex gloves resulted in the lowest ratios of positives for enrichment culture (Table 15). In many cases, no bacterial pathogens could be detected on latex gloves after treatment with just 50 ppm chlorine in glove-dunk buckets. These results were very promising. However, it should be noted that the lack of lot to lot consistency in glove appearance and texture may be problematic. Differences between the two lots of Uniseal latex gloves used in this set of experiments may be the cause of the differences in the enrichment-positive ratios observed when *E. coli* O157:H7 and *Salmonella* were compared. **Inability to control lot to lot variability is a cause for concern since surface properties which lead to greater protection of pathogens from disinfection or greater surface retention of pathogens may not be immediately apparent by visual inspection.**

Including a second chlorine glove-dunk bucket: Hoping to uncover glove disinfection procedures that could reduce levels of bacterial pathogens on nitrile and canner's gloves, we next investigated whether or not including a second glove-dunk bucket containing chlorine sanitizer could enhance pathogen removal and bactericidal activity. Furthermore, we questioned whether or not increasing the chlorine concentration in the two-bucket approach would further contribute to greater pathogen reductions. As in previous studies, gloves were pre-treated with soil and lettuce sap before inoculation with *E. coli* O157:H7. After the inoculum was dried, gloves were washed in the first bucket containing 50 or 100 ppm chlorine and immediately washed in the second bucket containing the same amount of chlorine disinfectant. After neutralization, bacterial pathogens on gloves were subjected to enrichment culture.

As shown in Table 16, fewer gloves were positive by enrichment for *E. coli* O157:H7 following disinfection in the 2 bucket set-up versus the 1 bucket set-up. The lack of complete inactivation of the bacterial pathogens after treatment of the gloves with a second chlorine glove-dunk

bucket suggests a second bucket may not be worth the extra measure. Also, increasing the concentration from 50 to 100 ppm did not lead to any noticeable improvement in the ability to ensure complete inactivation of pathogens on gloves. The ratio of enrichment-postive samples were similar for nitrile and Canner's gloves, but the log CFUs recovered from untreated controls were greater for nitrile gloves (5.23 log CFU) when compared to Canner's gloves (4.34 log CFU), suggesting that greater log CFU reductions were obtained in experiments using nitrile gloves.

During the experiments described above (Table 16), latex gloves (Uniseal lot 1) were also tested with one and two-bucket set-ups. As indicated in Table 15 above, no viable pathogens could be recovered from gloves treated with a single glove-dunk bucket containing 50 ppm chlorine. These results contrast the results for Canner's and nitrile gloves. To further investigate this phenomenon, we analyzed free chlorine and pH measurements which were collected at specific time intervals (0, 10, 30 and 50 min), while gloves were sequentially washed in glove-dunk buckets (see detailed methods section). Surprisingly, we found precipitous drops in both free chlorine levels and the pH of the disinfectant in the glove-dunk buckets when latex gloves (Uniseal lot 1) were used (example portrayed in Figures 11 & 12). Free chlorine levels dropped much more slowly over time when nitrile or canner's gloves were sequentially washed in glove-dunk buckets. Experiments with Canner's gloves trended toward greater declines in free chlorine and pH over time than did experiments nitrile gloves. Figures 11 & 12 are plots of the respective changes in free chlorine and pH over time for a set of experiments using just one glove-dunk bucket starting with 50 ppm chlorine. Other experiments demonstrated similar results (data not shown); although, in some cases, no changes in free chlorine or pH occurred throughout the experimental timecourse. In the second bucket of 2-bucket experiments, changes in free chlorine concentrations and pH were always less than the changes occuring in the first bucket.

A similar set of experiments were conducted with Uniseal lot 2 latex gloves. When compared to Canner's and nitrile gloves used in this set of experiments, reductions in free chlorine were similar for latex gloves (Figure 13). In addition, changes in pH were minimal for all three glove types (data not shown). Considering the data presented in Table 15, and comparing pathogen ratios of enrichment-positives for Uniseal lot 1 and lot 2 latex gloves, we again highlight our concerns regarding lot to lot variability of these gloves. **Taken together, bacterial pathogen disinfection by chlorine was greater on latex gloves than on nitrile gloves. *Salmonella* was generally survived better than *E. coli* O157:H7 in the chlorinated glove-dunk bucket treatment. Differences were noted between types of latex gloves; bacterial pathogens survived better on some latex gloves than others. It is likely that surface properties or residues on the surface of latex gloves make them more bactericidal than nitrile gloves when they are in the presence of the chlorine disinfectant.**

Outcomes and Accomplishments

Outcomes of this study are highlighted in bold font in the text above and are summarized in the text below. The major accomplishment of this study is providing recommendations for glove use and sanitation that are backed by scientific support. Communication of these findings to a broader audience and implementation of recommendations will be the next important step in achieving our ultimate goals of reducing pathogen cross-contamination in the harvest environment and improving the safety of fresh and minimally processed leafy greens.

Summary of Findings and Recommendations

Preventing cross-contamination by norovirus: Noroviruses are not known to be zoonotic pathogens. The control point for preventing cross-contamination is at the point where gloves are applied. Contaminated irrigation water is another potential concern, but unless the water is contaminated with raw human sewage, contamination of produce is suspected to occur with very little frequency and with very low levels of contamination. Alternatively, high levels of norovirus can be excreted from infected persons, both when symptomatic (up to 10^{12} viruses per gram of feces) and when asymptomatic (up to 10^{9} viruses per gram of feces) *(1)*. If strict hand hygiene is not followed after restroom use, we demonstrated that high levels of virus could be transferred to gloves when gloves are applied; the primary site of this contamination would be on the wrists of the gloves if gloves are applied properly. Our study also demonstrates that norovirus contamination on gloves can be reduced by disinfection of glove surfaces. Using waterless hand sanitizers (Purell or 5% levulinic acid plus 2% SDS foam) on gloves, infectious noroviruses can be reduced by ~1-2 log PFU. Using 50 ppm chlorine with a single glove-dunk bucket, similar levels of norovirus disinfection on latex and Canner's gloves could be achieved, but higher reductions in infectious virus (~3-4 log PFU) can be obtained when nitrile gloves are treated.

Preventing cross-contamination by bacterial pathogens: The primary route of cross-contamination by bacterial pathogens is likely to be in the field. Encounters of high levels of contamination in the harvest environment are low frequency occurrences, but if encountered, contamination of product can be amplified by handler cross-contamination. Our studies demonstrate that viable bacterial pathogens on gloves can be significantly reduced after glove-dunk buckets containing chlorine concentrations of at least 50 ppm (pH 7). When contamination levels are low (≤ 3 CFU/glove), complete inactivation can be achieved; if high levels of glove contamination occur, we have demonstrated 4-6 log CFU reductions on gloves, but complete inactivation was not always achieved, especially in the case of nitrile gloves. Alternative sanitizers can also be used to reduce pathogen contamination, but the lower efficacy of Purell and the high cost of levulinic acid plus SDS make these alternatives less attractive.

Taken together, we make the following recommendations: Strict hand hygiene is imperative prior to glove application. To minimize contamination by noroviruses we recommend disinfecting gloves in a chlorine (at least 50 ppm; pH 7) glove-dunk bucket after each time gloves are applied. Alternatively, or in addition, consider double-gloving or wearing glove liners. [Note: if non-disposable glove liners are worn, they should be laundered under sanitizing conditions (hot water) each day after use.] To minimize contamination by bacterial pathogens, gloves should be disinfected in chlorine (at least 50 ppm; pH 7) glove-dunk buckets before beginning work, before each break or lunch period, and when returning back to work after each break or lunch period. Gloves should be rubbed aggressively, palms-together for 5-10 seconds while gloved hands are immersed up to the wrist in the bucket. Sponges, brushes, or any other type of mildly abrasive cleaning aid should not be used with glove-dunk buckets. Free chlorine and pH should be measured and maintained above 50 ppm and neutral pH in the glove-dunk buckets. Measurements should be taken before use and the buckets should be covered when not in use to prevent chlorine dissipation. Buckets may need to be refreshed after each break period, especially when latex gloves are used or if lettuce or soil debris begins to accumulate. Gloves should not be taken home by employees (cannot control what the gloves will be used for at home). Disposable gloves should be discarded at the end of each day of use.

APPENDICES

Publications and Presentations (required)

Bulletins, Reports or Technical Communications

2013 **Cannon, JL.** and G. Kotwal. *Norovirus Cross-Contamination With/Without Gloves.* At-a-Glance. Vol. 22 No. 1. March 2013 issue. Center for Food Safety, University of Georgia publication.

Invited Presentations

2012 *Recommendations to Prevent Cross-contamination During Hand Harvest.* Western Food Safety Summit. Hartnell College, Salinas, CA. May 11, 2012.

Conference Abstracts/Proceedings

2013 Kotwal, G. and **JL Cannon.** Norovirus cross-contamination during produce handling with or without gloves. Center for Food Safety Annual Meeting, 2013. Atlanta, GA. Mar. 5, 2013.

2012 Kotwal, G., Q. Wang, and **J.L. Cannon.** Enteric Virus Contamination of Food Worker Gloves and Cross-Contamination during Produce Harvest or Preparation. USDA-AFRI Food Virology Collaborative (NoroCORE) Meeting. November 11-12, 2012.

2012 **Cannon, JL,** MC Erickson, and MY Habteselassie. The likelihood of cross-contamination of head lettuce by *E. coli* O157:H7, *Salmonella* and norovirus during hand harvest and recommendations for glove sanitizing and use. Western Food Safety Summit. Hartnell College, Salinas, CA. May 10, 2012.

2012 **Cannon, JL,** MC Erickson, and MY Habteselassie. The likelihood of cross-contamination of head lettuce by *E. coli* O157:H7, *Salmonella* and norovirus during hand harvest and recommendations for glove sanitizing and use. Center for Produce Safety; Produce Research Symposium. University of California-Davis. Sacramento, CA. June 27, 2012.

2011 **Cannon, JL,** MC Erickson, and MY Habteselassie. The likelihood of cross-contamination of head lettuce by *E. coli* O157:H7, *Salmonella* and norovirus during hand harvest and recommendations for glove sanitizing and use. Center for Produce Safety; Produce Research Symposium. Orlando, FL. June 28, 2011.

Budget Summary (required)

Necessary funds were allocated to complete the project as described in its completion in this report. The following is a brief summary of how funds were spent.

Travel: all travel monies except for the scheduled CPS meeting in June 2013 has been spent.
Operating: all funds designated for operating expenditures have been spent to our knowledge. This included payments to field study participants, chemical analysis, generation of virus stocks, survival, recovery and transfer, experiments with glove sanitizers, consumables, supplies needed for enumeration or enrichment of gfp-labeled *E. coli* O157:H7 or *Salmonella* spp. on gloves, supplies needed for enumeration of murine norovirus on gloves, equipment used in preparation of media for microbiological analyses, consumables such as gloves, collection bags, portable balance, funds for preliminary assays to differentiate amount of soil and lettuce latex accumulating or remaining on gloves (13C/15N isotopic ratios assays; this assay was deemed inadequate), assays to simulate amount of soil and lettuce latex accumulating or remaining on gloves for sanitizer studies (new assay was developed), shipping of field collected samples to the lab.
Personnel: All funds allocated to cover the salaries of employees working on the project have been spent to our knowledge.

Tables and Figures (optional)

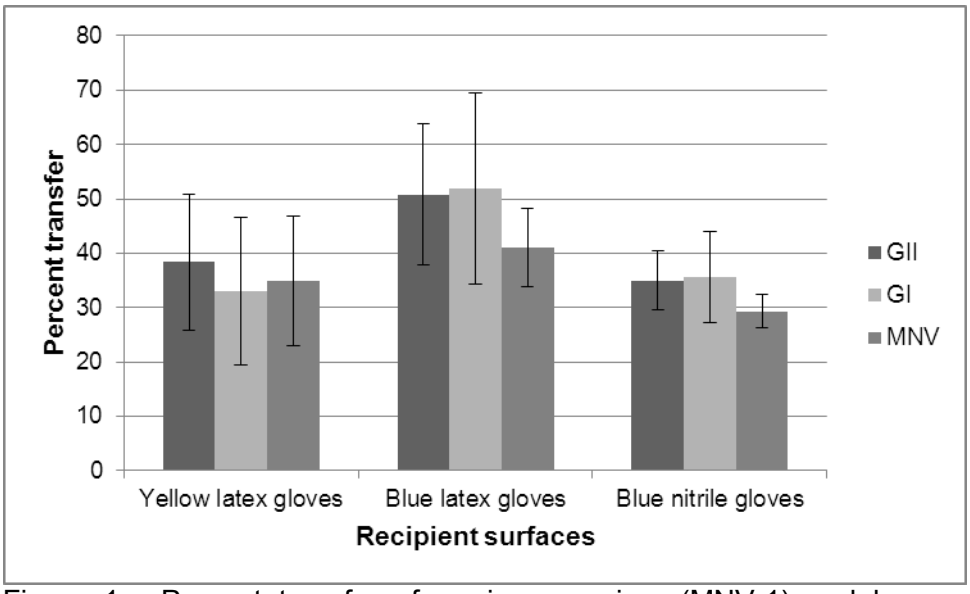

Figure 1: Percent transfer of murine norovirus (MNV-1) and human norovirus (GI and GII) from porcine skin sections (donor) to yellow latex gloves, blue latex gloves and blue nitrile gloves (recipients). Purpose is to quantify contamination of gloves if they are applied with norovirus-contaminated hands. *Transfer percentages are adjusted for assay recovery percentages as described in the text.*

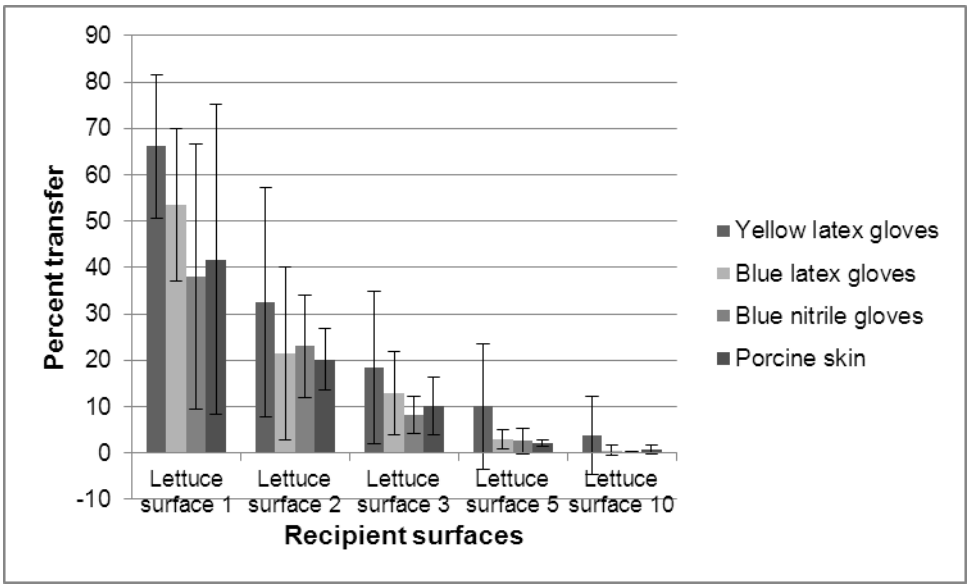

Figure 2: Percent transfer of murine norovirus (MNV-1) from porcine skin sections, yellow latex gloves, blue latex gloves and blue nitrile gloves (donors) to iceberg lettuce (recipient) surfaces through multiple transfers. Purpose is to quantify the extent of lettuce cross-contamination occurring after hands or gloves are contaminated with noroviruses. *Transfer percentages are adjusted for assay recovery percentages as described in the text.*

Table 1: Log CFU reduction of bacterial pathogens due to drying inoculum on latex and nitrile gloves in the presence of lettuce sap.

	Log CFU lost due to drying*	
Pathogen	UniSeal latex glove pieces	Nitrile glove pieces
Escherichia coli O157:H7	0.57 ± 0.18 a	0.42 ± 0.24 a
Salmonella	0.34 ± 0.09 a	0.28 ± 0.12 a

* Values followed by different letters are statistically different (P < 0.05)

Table 2: Log CFU reduction of *E. coli* O157:H7 due to drying of inoculum on pre-treated and untreated latex and nitrile glove pieces.

	Log CFU *E. coli* O157:H7/glove piece lost during drying*	
Pre-inoculation treatment**	Latex gloves	Nitrile gloves
None	0.86 ± 0.59	0.58 ± 0.59
Soil	0.78 ± 0.63	0.69 ± 0.63
Lettuce sap	1.00 ± 0.45	0.97 ± 0.54

* No significant differences in pathogen loss between individual glove treatments

Table 3: Log CFU reduction of *E. coli* O157:H7 due to drying of inoculum on the surface of different glove types.

Glove type	log CFU *E. coli* O157:H7/glove lost during drying*
Canner's	1.86 ± 0.54 c
Nitrile	1.06 ± 0.59 ab
Latex Fisher	0.72 ± 0.54 a
Latex GlovePlus	1.65 ± 0.25 c
Latex UniSeal	1.13 ± 0.45 b

*Values followed by different letters are statistically different (P < 0.05)

Table 4: Log CFU reduction of *E. coli* O157:H7 and *Salmonella* due to drying the inoculum on latex and nitrile glove surfaces over 5 hour period.

	Log CFU/glove piece inactivated			
	E. coli O157:H7		*Salmonella*	
Time after glove inoculation (h)	Latex	Nitrile	Latex	Nitrile
1	0.78	0.48	1.39	1.84
3	1.41	1.00	1.65	1.99
5	1.55	1.23	1.95	2.34

Table 5: Mean viable counts (log CFU) or results of enrichment culture (# positive/# tested) of *E. coli* O157:H7 recovered from latex or nitrile gloves after drying with or without a 1 min treatment with water, 200 ppm chlorine solution, or 5% levulinic acid plus 2% SDS.

Glove type	log CFU recovered/glove piece		# positive/# tested	
	No treatment	Water	200 ppm chlorine	5% Lev & 2% SDS
Latex	5.86 $\pm$ 0.11 a	4.82 $\pm$ 0.47 b	0/10	0/10
Nitrile	5.84 $\pm$ 0.17 a	4.86 $\pm$ 0.18 b	2/10	0/10

* Values followed by different letters indicate significant differences ($p < 0.05$).

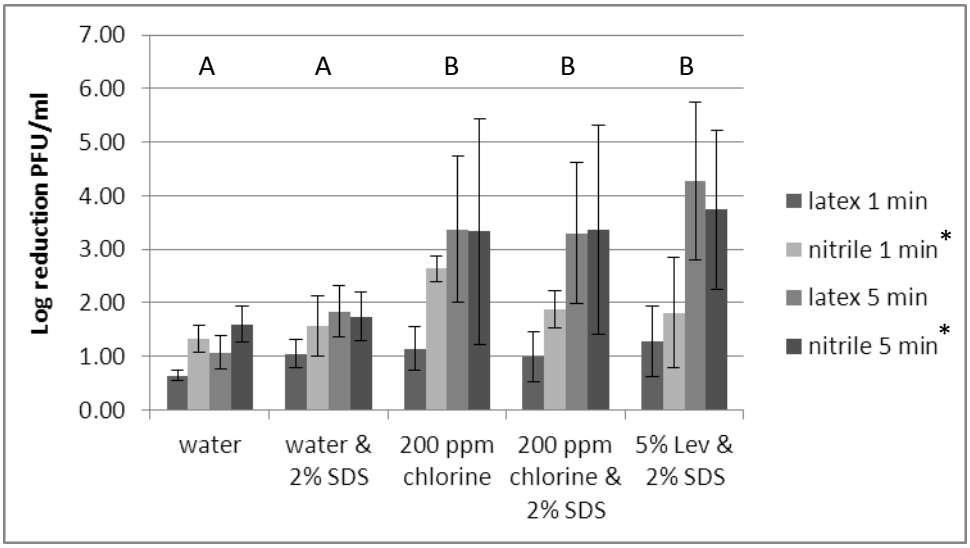

Figure 3: Log reductions of viable murine norovirus detected after virus inoculated latex or nitrile gloves were exposed to water, water plus 2% SDS, 200 ppm chlorine, 200 ppm chlorine plus 2% SDS, or 5% levulinic acid plus SDS sanitizer for 1 min or 5 min. Statistically significant differences in the means ($p < 0.05$) are indicated by different letters (A, B) or an astrick (*).

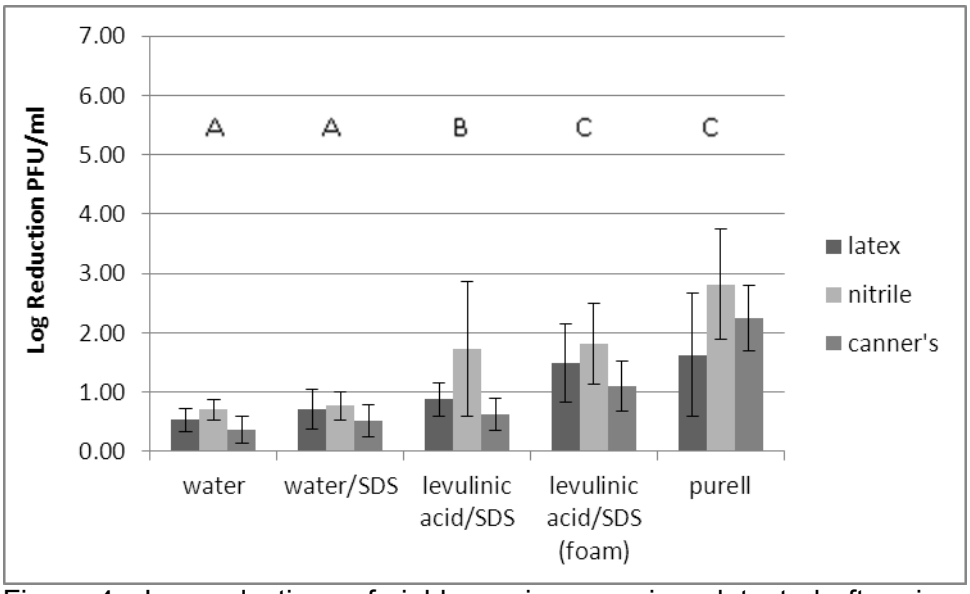

Figure 4: Log reductions of viable murine norovirus detected after virus inoculated latex, nitrile, or Canner's gloves were exposed to water, water plus 2% SDS, 5% levulinic acid plus 2% SDS (liquid), 5% levulinic acid plus 2% SDS (foam) or Purell for ~ 1 min. Statistically significant differences in the means ($p < 0.05$) are indicated by different letters (A, B, C) or an astrick (*).

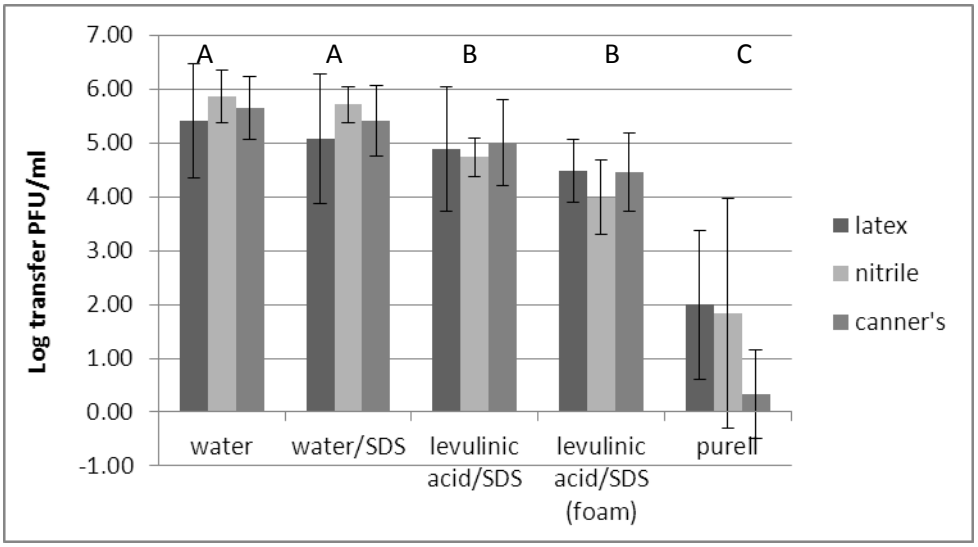

Figure 5: Log PFU of viable murine norovirus detected on uninoculated latex, nitrile, or Canner's gloves that were interfaced (~10 sec) with gloves that were inoculated with murine norovirus and treated with water, water plus 2% SDS, 5% levulinic acid plus 2% SDS (liquid), 5% levulinic acid plus 2% SDS (foam) or Purell. Statistically significant differences in the means ($p < 0.05$) are indicated by different letters (A, B, C).

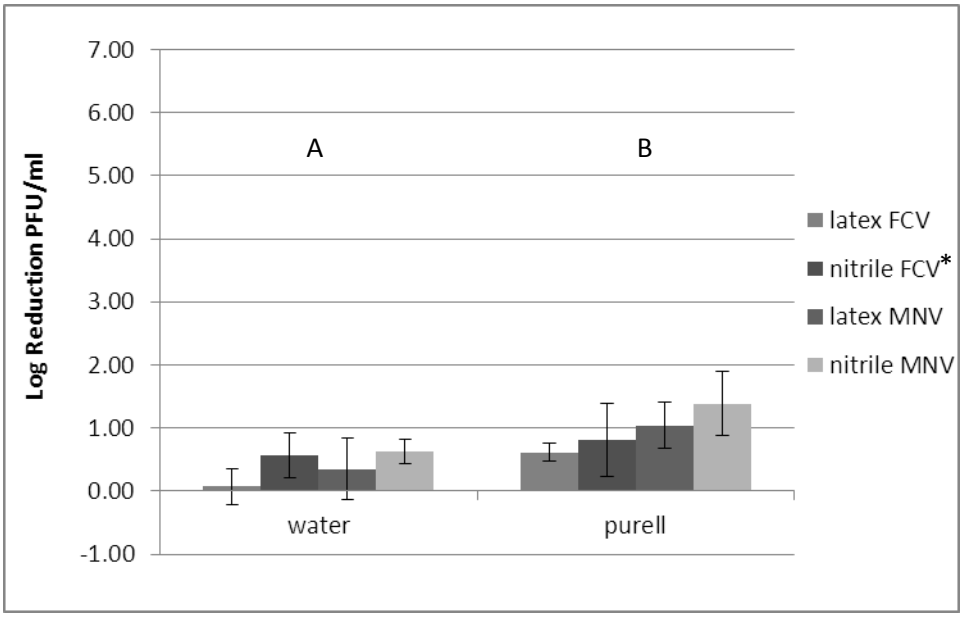

Figure 6: Log reductions of viable feline calicivirus (FCV) or murine norovirus (MNV) detected after virus inoculated latex or nitrile gloves were exposed to water or Purell for ~30 sec. Statistically significant differences in the means ($p < 0.05$) are indicated by different letters (A, B).

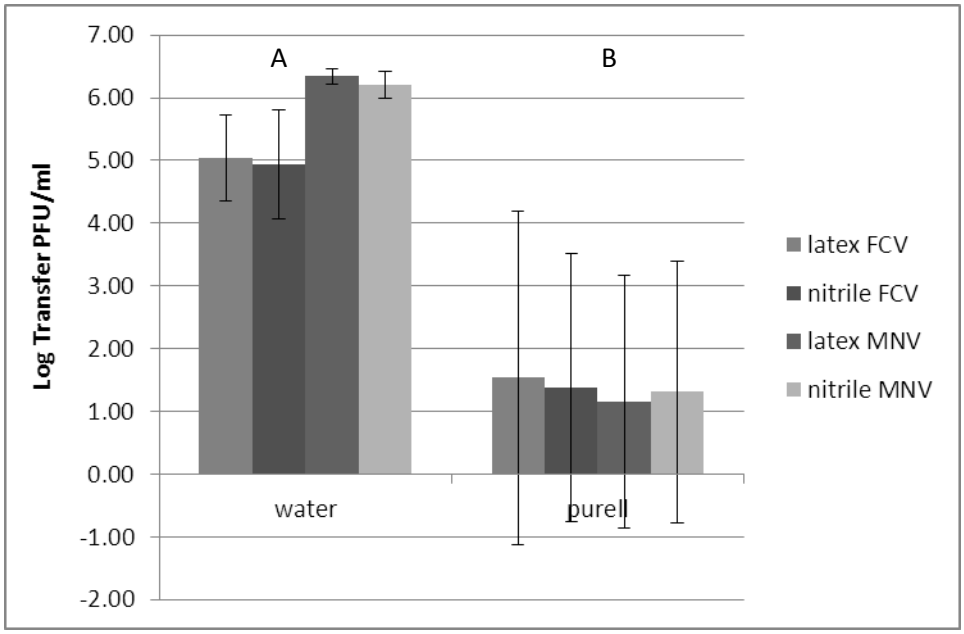

Figure 7: Log PFU of viable feline calicivirus (FCV) or murine norovirus (MNV) detected on uninoculated latex or nitrile gloves that were interfaced (~10 sec) with gloves that were inoculated with both viruses and treated with water or Purell for ~30 sec. Statistically significant differences in the means ($p < 0.05$) are indicated by different letters (A, B).

Table 6: Enrichment culture (# positive/# tested) of *E. coli* O157:H7 recovered from latex or nitrile gloves after exposure to 50 ppm chlorine or Purell hand sanitizer for 15 sec. Prior to pathogen inoculation, gloves were either left untreated (none) or coated with soil or lettuce sap.

	# positive for *E. coli* O157:H7 by enrichment / # glove pieces sampled			
	Chlorine (50 ppm) disinfection		Purell hand sanitizer	
Pre-inoculation treatment	Latex gloves	Nitrile gloves	Latex gloves	Nitrile gloves
None	0 / 6	0 / 6	3 / 6	6 / 6
Soil	0 / 6	5 / 6	3 / 6	5 / 6
Lettuce sap	0 / 6	3 / 6	3 / 3	3 / 3

Table 7: Log CFU reduction of *E. coli* O157:H7 and *Salmonella* due to drying on latex glove pieces that were either un-treated or pre-treated with 200 ppm chlorine or 5% Levulinic acid plus 2% SDS sanitizers prior to inoculation.

	Log CFU/latex glove piece lost during drying*	
Pre-inoculation treatment	*E. coli* O157:H7	*Salmonella*
Control	1.10 ± 0.38 a	1.73 ± 0.49 a
Chlorine, 200 ppm	1.67 ± 0.61 b	1.90 ± 0.51 a
Levulinic acid, 5%, and SDS, 2%	2.63 ± 0.62 c	2.98 ± 0.55 b

* Values within each column followed by a different letter are significantly different ($P < 0.05$)

Table 8: Log CFU reduction or enrichment culture (# positive/# tested) of *E. coli* O157:H7 recovered from latex glove pieces that were either un-treated or pre-treated with 5% levulinic acid plus 2% SDS, followed treatment with either 50 ppm chlorine, Purell, no sanitizer (none).

	Log CFU *E. coli* O157:H7 on latex glove pieces after drying* or # positive by enrichment / # samples	
Disinfection treatment	No pre-treatment	Pre-treatment
None	4.68 ± 0.18 b	3.67 ± 0.67 a
Chlorine, 50 ppm	0 / 8	2 / 8
Purell	8 / 8	1 / 8

* Values followed by different letters are statistically different ($P < 0.05$)

Table 9: Enrichment culture (# positive / # tested) of *E. coli* O157:H7 or *Salmonella* recovered from either clean or soiled latex, nitrile, or Canner's glove pieces that were disinfected with 50 ppm chlorine.

E. coli O157:H7			*Salmonella*		
	# positive by enrichment / # glove pieces disinfected with 50 ppm chlorine			# positive by enrichment / # glove pieces disinfected with 50 ppm chlorine	
Glove type	Clean glove	Soiled glove	Glove type	Clean glove	Soiled glove
Canners	0 / 10	4 / 10	Canners	2 / 10	5 / 10
Latex	0 / 10	2 / 10	Latex	3 / 10	1 / 10
Nitrile	1 / 10	6 / 10	Nitrile	1 / 10	6 / 10

Table 10: Log CFU reduction or enrichment culture (# positive / # tested) of *E. coli* O157:H7 or *Salmonella* spp. on nitrile gloves unwashed or washed for 1 minute with either 0 ppm, 75 ppm, or 150 ppm chlorine.

	log CFU or# positive / # tested	
Chlorine (ppm)	*E. coli* O157:H7	*Salmonella* spp.
Unwashed	5.98 ± 0.07 a	5.88 ± 0.11 a
0	4.42 ± 0.38 b	4.78 ± 0.28 b
75	6/10	9/10
150	5/10	5/10

Table 11: Log CFU reduction or enrichment culture (# positive / # tested) of *E. coli* O157:H7 on nitrile gloves unwashed or washed for 1 minute with either 0 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm or 150 ppm chlorine.

Chlorine (ppm)	log CFU *E. coli* O157:H7 or # positive/# tested
Unwashed	5.75 ± 0.36 a
0	4.66 ± 0.30 b
50	10/10
75	8/10
100	4/10
125	5/10
150	5/10
175	3/10

Table 12: Enrichment culture (# positive / # tested) of *E. coli* O157:H7 on nitrile gloves washed for either 15 or 30 seconds with 75 ppm or 150 ppm chlorine at lower inoculation level (3.12 log CFU/glove piece).

	# positive by enrichment / # nitrile glove pieces tested	
Exposure time (sec)	75 ppm chlorine	150 ppm chlorine
15	0 / 15	0 / 15
30	0 / 15	0 / 15

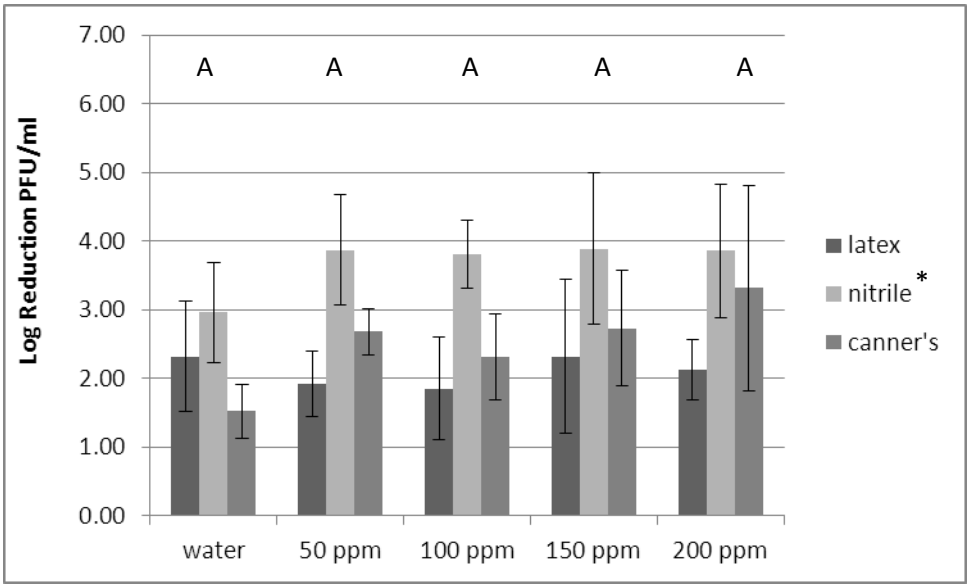

Figure 8: Log reduction of viable murine norovirus on latex, nitrile or canner's gloves after treatment with water or chlorine (50-200 ppm).

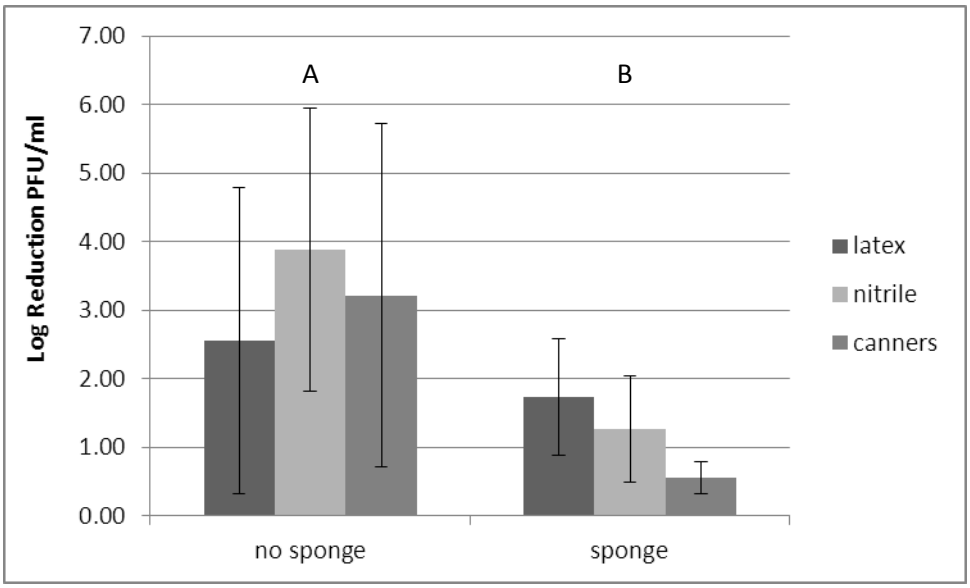

Figure 9: Log reduction of viable murine norovirus on inoculated latex, nitrile or canner's gloves after treatment with 50 ppm chlorine with or without rubbing with a sponge.

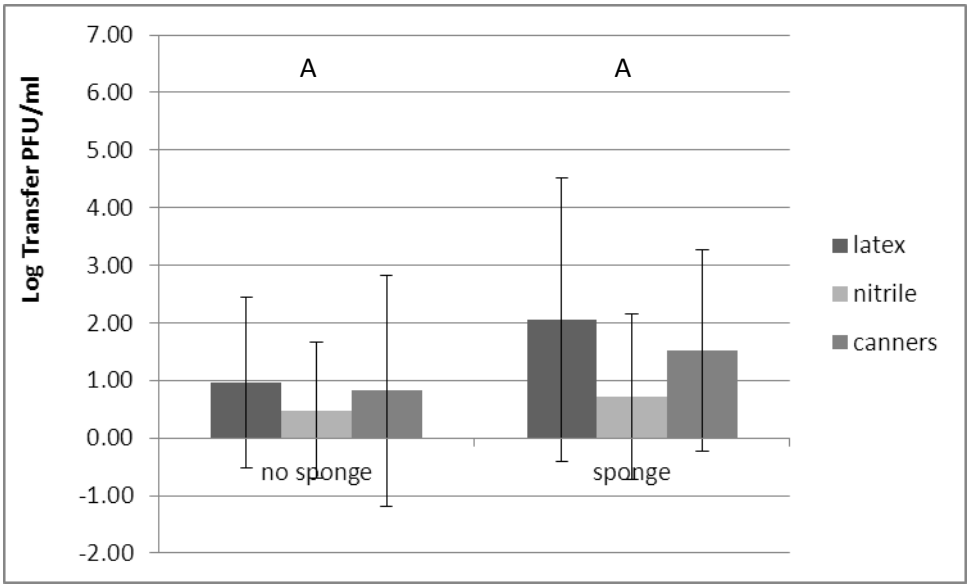

Figure 10: Log transfer of viable murine norovirus to clean latex, nitrile or canner's gloves from virus-inoculated glove pieces (no sponge) or sponges that were previously used on virus-inoculated glove pieces.

Table 13: Enrichment culture (# positive / # tested) of *E. coli* O157:H7 on canner's or nitrile gloves treated by rubbing gloves together or by rubbing gloves with a Scotch Brite Non-Scratch Scour Pad (sponge 1) or a Scotch Brite Dobie pad (sponge 2).

	# gloves positive for *E. coli* O157:H7 by enrichment / # glove samples disinfected		
Glove type	Rubbing	Sponge 1	Sponge 2
Canner's	9 / 28	17 / 28	19 / 28
Nitrile	16 / 28	23 / 28	21 / 28

Table 14: Enrichment cultures (# positive / # tested) of *E. coli* O157:H7 on nitrile glove surfaces either untreated or pre-treated by grinding soil on the surfaces then disinfected with 50 ppm chlorine.

Dirt	# positive by enrichment / # gloves disinfected
With	15 / 32
Without	13 / 32

Table 15: Log CFU recovery and enrichment cultures (# positive / # tested) of *E. coli* O157:H7 and *Salmonella* from various glove types (nitrile, canner's or 3 different latex brands) that were either untreated or disinfected with 50 ppm chlorine.

	E. coli O157:H7		*Salmonella*	
Glove type	Average log CFU/glove recovered from untreated controls	# positive by enrichment / # gloves disinfected	Average log CFU/glove recovered from untreated controls	# positive by enrichment / # gloves disinfected
Nitrile	5.00	37 / 84	4.17	23 / 32
Latex Fisher	5.18	2 / 32	4.90	1 / 32
Latex GlovePlus	4.25	0 / 32	3.61	0 / 32
Latex Uniseal Lot 1 or 2*	5.22	0 / 16	4.30	8 / 32
Canners	3.73	15 / 52	Not done	Not done

*Latex Uniseal Lot 1 gloves were used in experiments with *E. coli* O157:H7 and Lot 2 were used in experiments with *Salmonella.*

Table 16: Log CFU recovered or enrichment cultures (# positive tested / # tested) of *E. coli* O157:H7 on canner's or nitrile gloves that were untreated or disinfected with either 50 ppm or 100 ppm chlorine using a 1-bucket or 2bucket set-up.

		# gloves positive for *E. coli* O157:H7 by enrichment / # glove samples disinfected			
	Avg log CFU/glove	50 ppm chlorine		100 ppm chlorine	
Glove type	Untreated controls	1-bucket	2-bucket	1-bucket	2-bucket
Canner's	4.34	6 / 24	3 / 24	7 / 24	3 / 24
Nitrile	5.23	6 / 24	6 / 24	8 / 24	3 / 24

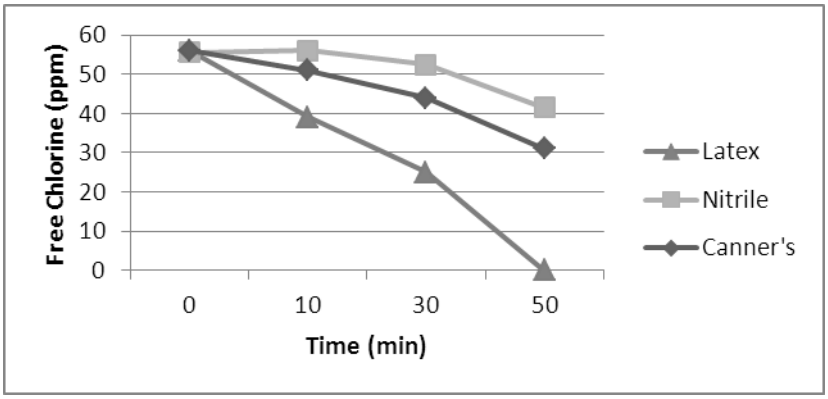

Figure 11: Free chlorine levels (ppm) taken over a 50 minute time course where latex (Uniseal lot 1), nitrile, and canner's gloves (20 pairs each) were sequentially dunked in 50 ppm chlorine.

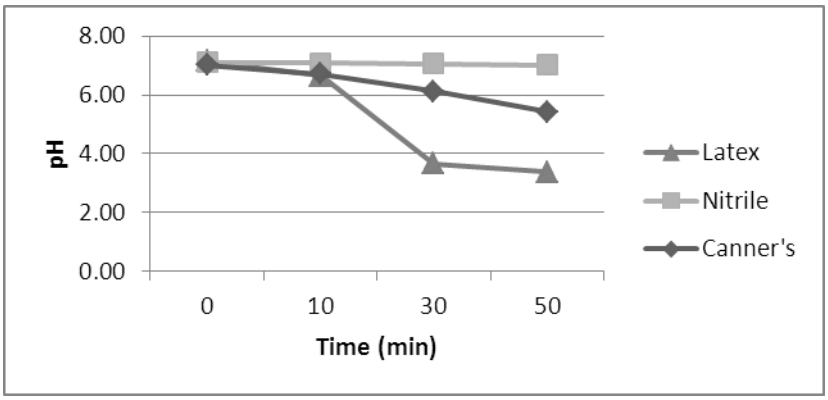

Figure 12: pH levels taken over a 50 minute time course where latex (Uniseal lot 1), nitrile, and canner's gloves (20 pairs each) were sequentially dunked in 50 ppm chlorine.

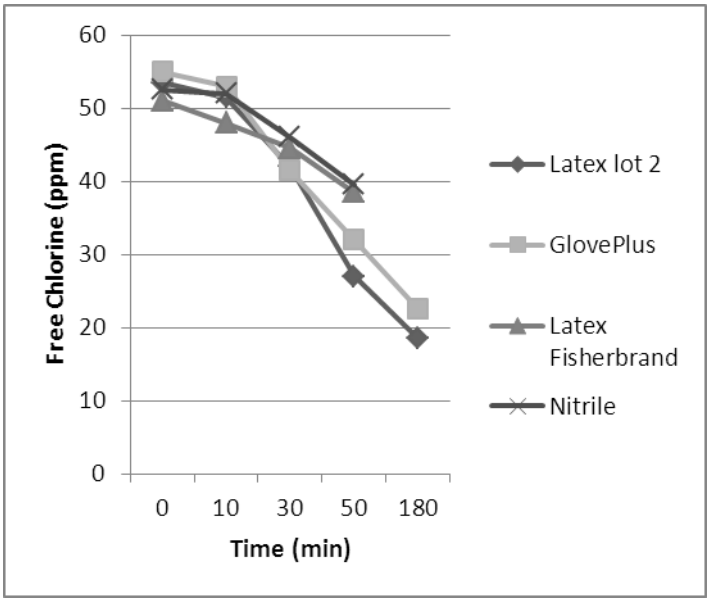

Figure 13: Free chlorine levels (ppm) taken over a 50 minute time course where latex (Uniseal lot 2), nitrile, and canner's gloves (20 pairs each) were sequentially dunked in 50 ppm chlorine.

Details on Methodologies

Methodologies developed to determine soilage of field-worn gloves (alternative method that was could not be used): As gloves collected in the field were visually screened, it became immediately apparent that very little soil could be found on the gloves. Instead, there was a considerable amount of lettuce debris (large chunks/shreds of cut lettuce) on many of the gloves. This observation was not one we had anticipated. To meet the objectives of this study, which is to recreate the levels of soil, lettuce debris, lettuce extract, and moisture accumulation occurring in the field, in the laboratory, a new approach was warranted.

In the first set of experiments, methodologies for characterizing the material accumulated on gloves by chemical analysis were explored. Specifically, we sought unique chemical signatures of soil and lettuce as determined by total percent carbon (C) and total percent nitrogen (N) content, and/or isotopic δ13C and δ13N content. We hypothesized that if unique chemical signatures could be determined for soil and lettuce, we could use this information for estimating the contents of a mixed sample (in the case of the harvesters' gloves, we would estimate the proportions of soil and lettuce material extracted from each glove). Soil was collected from 3 conventionally managed farms in the Central CA valley. Lettuce extracts were prepared by four different methodologies which generally consisted of either maceration and blending of lettuce cores or squeezing lettuce cores to release the liquid material. The impact of drying ground lettuce material on filter paper was also investigated. Total percent C and N of each soil and lettuce extract sample was determined by dry combustion and detection with an elemental analyzer (Macro Elemental Analyzer). The isotopic δ13C and δ13N content of soil was determined by gas isotopic ratio mass spectrometry. Total C:N ratios and δ13C:δ13N ratios were compared between the different soil samples and/or lettuce extracts to determine whether or not soil and lettuce material could be differentiated in a sample by chemical analysis. Percent total C and N differed slightly between soil and lettuce, but there was considerable variation in total percent C and N between soil samples collected from different farms. Isotopic C and N percentages also varied between soil samples. The percent C and N detectable in the lettuce extracts were impacted by the extraction procedure that was used. It was determined that the most reliable methods for obtaining lettuce extract consisted of either squeezing lettuce cores or macerating/blending lettuce cores in water, without including the drying step before analysis. This information is important for later studies that will recreate "field worn" gloves in the lab.

Bacterial pathogen isolates: Four *E. coli* O157:H7 isolates (1997 alfalfa sprout outbreak isolate; 2006 spinach outbreak isolate; 2006 Taco Bell lettuce outbreak isolate; and isolate from cattle feces) and four *Salmonella* isolates (*Salmonella* Enteritidis ME18; *Salmonella* Enteritidis H4717; *Salmonella* Newport 11590K; and *Salmonella* Enteritidis Benson 1) were used in this project. Using the method described by Sambrook et al. (1989), all isolates had previously been labeled with the green fluorescent plasmid that also contained an ampicillin-resistant marker.

Culture of bacterial isolates: Frozen cultures of each pathogen isolate were thawed and streaked individually onto tryptic soy agar plates containing 100 µg/ml ampicillin (TSA-Amp). After incubating the plates for 24 h at 37°C, individual colonies were transferred into tryptic soy broth containing 100 µg/ml ampicillin (TSB-Amp), grown at 37°C for 18-24 h with agitation (150 rpm) to give a culture in stationary phase, and then cells recovered by centrifugation (4,050 x g, 25 min, 4°C). The pellet was washed by suspending in sterile 0.1% peptone water and centrifuging three consecutive times. The final pellet was suspended in deionized water, 0.1% peptone water, or a lettuce sap mixture to give ca. 10^9 CFU/ml. Equal portions of each of the 4 *E. coli* O157:H7 isolates were then combined before diluting with the suspension solution to give the working *E. coli* O157:H7 inoculum. The *Salmonella* isolates were similarly mixed together

and diluted to give a working *Salmonella* inoculum. Working concentrations ranged from 4 to 7 log CFU/ml.

Alternative methods for culture of isolates were also employed to potentially generate isolates with different phenotypes that could respond differently to desiccation and disinfectant exposures. In one scenario, isolates were cultured to the stationary phase in M9 minimal media instead of TSB-Amp. Cells were recovered and resuspended in a similar manner to that used when cultured in TSB-Amp. In a second scenario, cells were cultured on TSA-Amp and the cells scraped off the solid media, washed in 0.1% peptone water, and then resuspended in a similar manner as to those cells cultured in liquid broth.

Lettuce sap preparation: To mimic the lettuce sap that is exuded during harvest of lettuce heads, different sections of the lettuce head (core, outer leaves, inner leaves) were mixed with sterile deionized water in a 1:2 w:v ratio and ground for 30 s in One-Touch Chopper, HC 306 (Black and Decker, Corp., Towson, MD). This homogenate was filtered through a double layer of cheesecloth (Mainstays™ Projects, imported by TUFCO, LP Manning, SC) with filtrate collected in a sterile Pyrex petri dish. This dish was placed in a 37°C incubator and the solution stirred slowly for three to five hours in order to evaporate the majority of the liquid. It was then brought back to the concentration it would be had it been exuded or squeezed from the plant material. Following carbon and nitrogen analysis on this concentrated extract and lettuce sap (latex) collected in the field from freshly harvested lettuce heads, it was confirmed that sap made from the cores and outer lettuce leaves had a similar base composition to field samples whereas that from the inner leaves of the head did not. The method of preparation described above was therefore followed and the material collected was frozen and later thawed prior to using it as the suspending medium for the pathogen inocula.

As browning developed if the lettuce sap was frozen and thawed multiple times, a small experiment was undertaken whereby lettuce saps varying in their degree of browning were generated by using different parts of the lettuce head as the source (outer leaves and inner leaves) and homogenizing the core in the presence of 50 mM NaCl (browning was accelerated). These lettuce saps were then used to suspend the pathogen and to determine if browning was associated with increased stability of the cells during drying on the gloves.

Glove types: Three major types of glove materials were used during this project. They were chemical susceptible latex (Uniseal textured latex gloves,12 mil, American Healthcare Products, Inc., Alhambra, CA; powder-free latex exam gloves, 4 mil, Safety Choice, distributed by Fisher Scientific, Pittsburgh; GlovePlus textured latex gloves, 12 mil, Barber Healthcare, Leyburn, England), chemical resistant latex (Ansell Canner's, 20 mil) and nitrile (Uniseal nitrile gloves, 7 mil,).

Glove preparation and pre-treatments: Both intact gloves and glove pieces were used for inoculation with pathogens and subsequent evaluation of pathogen response to desiccation and disinfectant treatments. Initial experiments cut the finger portions from each glove and inserted each over a stiff piece of cardboard for inoculation of the fingertip. In subsequent experiments, 75 mm x 75 mm glove pieces were cut from the palm of each glove, the outer side marked, and each piece pinned to a Styrofoam board to ensure the absence of wrinkles during inoculation in a 20 x 20 mm square in the center of the piece.

To mimic materials that also come into contact with gloves in the field and could potentially adhere, a sandy soil loam soil from California containing 4.5% organic matter was applied to the inoculation area of the 75 mm^2 piece, ground into the inoculation area using the back of a plastic spoon, and loose soil shaken off. Lettuce sap (50 µl) was also applied to 75 mm^2 glove pieces either in a clean inoculation area or to the inoculation area that had soil ground into it. Prior to drying overnight, the sap was spread over the inoculation zone by folding the piece over onto itself several times.

In selected experiments, soil and sap were also applied to intact gloves. In this case, gloves were donned and ca. 2 g of California sandy loam soil was scooped into the left glove hand. The two hands were then clamped together and the palms rubbed firmly together for 10 sec to grind the dirt into the gloves. The gloved hands were then patted against each other to remove any loosely adhering dirt before removing the gloves and placing them on a benchtop. Following this process, 100 µl of either non-inoculated or inoculated lettuce sap was applied to a pair of gloves in the palm area, the sap spread by folding over onto itself the palm area several times, and the sap dried at room temperature for a minimum of one hour.

A set of intact gloves (Ansell Canner's and GlovePlus latex) were also etched with a scalpel to mimic nicks that could occur on gloves of field workers while harvesting head lettuce with coring knives. The corners of a 25 mm^2 square were marked on the palm of each glove and then 5 lines lightly etched into the gloves, taking care not to penetrate the glove entirely.

To account for field workers who might use one set of gloves throughout the day and who could have washed their gloves in a disinfectant during breaks and hence, modified the glove's surface properties, several pre-treatments were applied to nitrile and Uniseal latex gloves. Gloves were stuffed with cotton pads and then immersed for 1 h either in 200 ppm chlorine, 2% sodium dodecyl sulfate (SDS), or 5% levulinic acid and 2% SDS. After removal from this solution, the gloves were dried and then 75 mm^2 pieces cut from the palm of each glove. With another set of Uniseal latex gloves, they were immersed in 50 ppm chlorine for 10 sec during which time the gloves were rubbed together in an up and down motion 10 times. These gloves were left intact to determine whether pathogens contaminating these gloves would respond to subsequent disinfectant treatments in a similar manner as gloves not having any prior exposure to disinfectant.

Bacterial pathogen inoculation of glove pieces and intact gloves: All glove pieces were inoculated with 20 µl of inoculum by spotting with as small of drops as possible in the inoculation zone. Intact gloves were inoculated with 100 µl of the pathogen inoculum in the palm area. Similar to non-inoculated gloves to which non-contaminated lettuce sap had been applied to intact gloves, the liquid inoculum was spread around the palm area by folding the area over onto itself several times. Intact gloves and glove pieces were inoculated at staggered times (two to five min separation) to ensure that each sample was dried for the same amount of time (75 min) prior to pathogen enumeration analysis, holding for additional time (2-4 h), or treatment with chemical disinfectant.

Disinfection of glove pieces and intact gloves: Glove pieces were exposed to disinfectant solutions (chlorine, 10-200 ppm; 5% levulinic acid/2% SDS) in a Pyrex Petri dish for periods ranging from 15 sec to 2 min. The piece was placed in the dish with the inoculation side facing down and submerged by rubbing over the piece with a sterile glass rod taking care to dislodge any bubbles that prevented contact between the solution and glove piece. After the designated contact time, the piece was removed from the solution using a sterile forceps and placed into a 4 oz.-Whirl-pak bag containing 15 ml of neutralizing solution (sodium thiosulfate [0.01 g/L] in 0.1% peptone water for chlorine disinfectant; sodium bicarbonate [40 g/L] in 0.1% peptone water or buffered 0.1% peptone water for levulinic acid/SDS disinfectant). The bag was immediately shaken for 5 sec to ensure that all portions of the glove piece were exposed to the neutralizing solution and then processed for pathogen enumeration and enrichment. In the case of pieces exposed to Purell disinfectant, 1.0 ml was applied and swirled in the inoculation area for 15 sec prior to placing the piece in a Whirl-Pak bag containing 15 ml of buffered 0.1% peptone water.

Four scenarios for disinfection of intact gloves were used. In scenario 1, 20 pairs of gloves with ground-in soil (SG) and 3 pairs of clean gloves (CG) were lined up on a counter. Non-contaminated lettuce sap was applied to SG pairs 1, 2, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 17,

and 18 and allowed to dry overnight whereas SG pairs 3, 4, 11, 12, 19, and 20 were contaminated with *E. coli* O157:H7 or *Salmonella* suspended in lettuce sap one hour prior to exposure to disinfectant solutions (50 or 100 ppm chlorine, 7L) held in buckets. Each pair of gloves were donned in sequence and inserted into one bucket of disinfectant solution upon which gloved hands were rubbed up and down 10 times (6-10 sec). Each gloved hand of those pairs that had been contaminated with pathogen was then inserted individually into a 52 oz. Whirl-pak bag containing 150 ml of sodium thiosulfate neutralizing solution to wash all surfaces exposed to the disinfectant. The gloves were removed while still in the Whirl-Pak bag for subsequent pathogen enumeration and enrichment. Prior to washing the next pair of gloves in the bucket of disinfectant, ca. 2 g of lettuce pieces were added to the bucket to serve as an additional organic load that could have been adhering to the gloves and been washed off. Subsequent to exposure of glove pairs 4, 12, and 20, a clean pair of gloves was donned and rubbed up and down in the disinfectant bucket to determine if cross-contamination from the solution occurred. These gloves were also rinsed in the neutralizing bag and analyzed for pathogen contamination by enumeration and enrichment. Water samples from the bucket were also taken after these clean gloves were washed in the disinfectant and active chlorine, pH, and ORP were measured.

In scenario two, a similar process of disinfection as was undertaken in scenario 1 was followed with the exception that two buckets of disinfectant solution were present and each pair of gloved hands was first inserted and rubbed in the first bucket and then immediately removed and inserted and rubbed in the second bucket before neutralizing and analysis of the contaminated gloves. In addition, after glove pairs 4, 12, and 20, one pair of clean gloves was rubbed in the first bucket, neutralized, and analyzed for pathogens while a second pair of clean gloves was rubbed in the second bucket of disinfectant solution to determine whether cross-contamination occurred.

In scenario three and four, only contaminated gloves (6-10 pairs) were washed in one bucket of disinfectant solution and no lettuce pieces were added. Scenario three consisted of rubbing either ground-in soil gloved hands up and down 10 times while in the disinfectant solution or contaminated gloves that had been etched with a scalpel. In scenario four, all contaminated ground-in soil glove surfaces were scrubbed with either a Scotch Brite Dobie cleaning pad (109 mm x 66 mm) or a Scotch Brite non-scratch scouring pad (152 mm x 76 mm) in the presence of the disinfectant. In both scenarios three and four, water samples were taken at the beginning and end of the disinfection treatments for measurement of active chlorine, pH, and ORP.

Enumeration and enrichment of glove samples: Glove pieces exposed to extraction solution (0.1% peptone water, 15 ml) or neutralizing/extraction solution (15 ml) were stomached for 60 sec at 260 rpm prior to enumeration and enrichment. Aliquots from non-disinfected gloves were removed for dilution and plate count enumeration on TSA-Amp (37°C, 18-24 h). Enrichment media (2x-TSB-Amp, 15 ml) was added to bags containing the disinfected glove pieces immersed in the neutralizing/extraction solution, incubated with shaking at 37°C overnight before streaking an aliquot on a TSA-Amp plate for overnight incubation at 37°C. Visualization of TSA-Amp plates under a fluorescent light (365 nm) was used to determine the presence or absence of fluorescent pathogens.

Intact gloves exposed to extraction solution (0.1% peptone water, 150 ml) or neutralizing/extraction solutions (150 ml) were also stomached for 60 sec at 260 rpm prior to enumeration and enrichment. Enumeration followed a similar protocol of that used for glove pieces but with enrichment, 150 ml of 2x-TSB-Amp was added to the extraction solution prior to incubating and streaking TSA-Amp plates.

Contaminated intact gloves that had been etched with a scalpel prior to washing in disinfectant solution were immediately placed on a cutting board and the area of etching cut out

with a sterile scalpel. This glove piece was then placed on a TSA-Amp plate, inoculated side in touch with the agar, and incubated for 90-120 min at 37°C. The glove piece was then removed after marking on the agar the area where the piece had been placed and where etched lines on the glove piece had been located. The plates were incubated for an additional 16 -22 h at 37° before placing under a fluorescent lamp for documentation of the presence and location of fluorescent colonies.

Chlorine, pH, and ORP measurement: The pH and oxidation-reduction potential of chlorine disinfectant solutions were measured using a dual channel ACCUMET meter (model AR50, Fisher Scientific, Pittsburgh, PA). Free active chlorine levels were determined using an iodometric method with digital titrator (Model 16900, Hach Co., Loveland, Colo., U.S.A.).

Statistical analyses: In experiments designed to address only one variable, data were subjected to Analysis of variance (ANOVA) using the StatGraphic Centurion XVI software package (StatPoint, Inc., Herndon, VA). When statistical differences were observed ($P < 0.05$) with ANOVA, differences among sample means were determined using the least significant difference (LSD) test.

In experiments designed to address multiple variables, the data was subjected to General Linear Model Analysis to determine both significance of individual variables and whether there was potential interaction among the variables. In the absence of any interactions, individual treatment data was then subjected to ANOVA and differences among sample means again differentiated using the LSD test.

Virus Cultivation and Plaque Assay

RAW 264.7 macrophage cells (ATCC # TIB-71) were maintained in complete DMEM [Dulbecco's modified eagles medium (Hyclone, Thermo Fisher Scientific, Inc., Logan, UT) containing 10% low endotoxin fetal bovine serum (HyClone FBS (#SH3007003), 1% penicillin (100 U/ml)/streptomycin (100 U/ml) (Hyclone), 1% 1 M HEPES buffer (Lonza, Biowhittaker, Alpharetta, GA), and 1% 100mM sodium pyruvate (Cellgro, Mediatech, Inc., Manassas, VA)], and passaged every 2-3 d. Confluent monolayers (80-90%) of cells were infected with MNV-1 (obtained from Dr. Herbert Virgin, Washington School of Medicine) for approximately 48 h at 37°C in a 5% CO_2 environment. For stock preparation, virus was harvested after complete cytopathic effect was apparent via three cycles of freeze-thawing. MNV-1 was centrifuged at 2,000 × *g* for 15 min at 20°C and filtered using a 0.2 µm membrane filter (Nalgene, Rochester, NY). To concentrate the virus stock, this partially-purified cell culture lysate was ultracentrifuged at 100,000 × *g* for 1 h at 4°C and the pellet was re-suspended overnight in 1/10 the original volume in phosphate buffered saline (PBS; pH adjusted to 7.4) containing 5% FBS (v/v). One ml portions of MNV-1 were stored at -70 until used.

Standard plaque assay was performed to quantify viral infectivity as previously described *(2,7)*. Briefly, cells were grown to 80-90% confluence on 60 mm x 15 mm tissue culture plates containing 5 ml complete DMEM. Media was replaced with 400 µl of infection media [1x modified eagle's medium (MEM) (Cellgro) containing 1% 1M HEPES buffer, 1% penicillin and streptomycin, 1% 100 mM sodium pyruvate, 5% HyClone FBS)] and 100 µl of either undiluted or serially diluted (in 1x MEM) sample, in duplicate. Plates were incubated for 1 h at 37°C with 5% CO_2, while rocking trays of cells every 15 min, to allow virus adsorption to cells. At the end of the hour, the liquid portion was aspirated, cells were overlaid with 3 ml of 1x MEM containing 0.5% agarose (SeaKem LE Agarose, Lonza, Rockland, ME), and incubated for 48 ±5 h at 37°C with 5% CO_2 to allow for virus infection. In some experiments, a second agarose overlay consisting of 3 ml of 0.75% agarose with 1.1% neutral red solution (from a 3.3 g/L stock solution, Sigma-Aldrich, Co. LLC, St. Louis, MO) was administered to each plate after 48 h and

plaques were counted 4-8 h later to determine infectious PFUs. In other experiments, pates were stained with crystal violet (10g crystal violet powder, 200mL methanol, 790mL mili-q water) for 24 hr prior to plaque visualization. Duplicate negative controls with and without agarose and duplicate positive controls with and without agarose were included to ensure plaque assay function.

Preparation of human norovirus and MNV stocks for transfer experiments. Human norovirus GI and GII stool samples were generous gifts from Dr. Jan Vinjé at the Centers for Disease Control and Prevention, Atlanta, GA. Ten grams of the solid material (GI) was mixed with 5 ml phosphate buffered saline (PBS) 5 ml of liquid stool sample (GII) was used directly. The samples were centrifuged at 10,000 x g for 15 min. The supernatant was aspirated and re-centrifuged at 10,000 x g for 30 min at 4°C. The supernatant was then filtered through a 0.2 µm syringe filter (Millipore), followed by ultracentrifugation at 100,000 x g for 1 hour at 4°C. The supernatant was removed and the pellet was re-suspended in 0.5 ml of PBS containing 5% fetal bovine serum (FBS) overnight. The ultra-centrifuged samples were stored at -80°C. The MNV-1 infected cell culture lysates described above were filtered through a 0.2 µm filter, followed by ultracentrifugation at 100,000 x g for 1 hr at 4°C. The pellet was re-suspended overnight in PBS and the stock was stored at -80°C. Two types of virus stocks were prepared to be used as the experiment inoculum. One was a cocktail of the three ultracentrifuged stocks prepared above, which were first tested individually by RT-PCR. Then calculated amounts were added in PBS, making the total amount 1 ml, followed by the addition of a norovirus free (prior tested) pea sized fecal sample. This mixed stock was then centrifuged at 10,000 x g for 2 min. The supernatant was removed and the stock was again tested for all three viruses. The final stock contained ~10^8 genomic copies per ml of each of the three viruses. A single virus stock was also prepared with MNV-1 ultracentrifuged sample, PBS and negative fecal sample following the above procedure. This stock contained ~10^9 genome copies per ml. The mixed stock was used as inoculum for single transfer experiments with porcine skin and gloves. The MNV-1 single stock was used as inoculum for multiple transfer experiments using fresh iceberg lettuce. Aliquots of 25, 50, 100, 200 µl were made for both inoculums and were stored at -80°C.

Virus Inoculation onto Glove Surfaces

Gloves (2" x 2") were spot-inoculated with ten 10-µl portions (100 µl total volume) of MNV-1 virus stock or PBS containing 5% FBS (v/v), serving as a negative control. The inoculum was then evenly spread over each coupon using a pipet tip to create a thin liquid surface layer. Gloves were allowed to dry in a biosafety level 2 hood for 40-60 min or until visibly dry. At least two inoculated gloves per experimental replicate were included as no treatment, recovery controls.

Porcine skin transfer device. A mechanical transfer device was constructed with stainless steel materials by Engineerable LLC. (Atlanta, GA). The device has both a donor and a recipient port. The donor block is detachable to allow easy attachment of donor surfaces (porcine skin and gloves), which can then be attached to the device with the help of magnets. The recipient surface has a base area where the recipient can be placed. The device is attached to a control box which can be used to set a particular pressure value to be used for the transfer process from donor to recipient surface. The pressure was kept at 1000 g for all the experiments. The control box can also control the time of contact for transfer. The contact time was kept 10 s for all the experiments.

Procurement and preparation of porcine skin. The porcine skin (cut and frozen) was obtained from Lampire® Biological Laboratories (Pipersville, PA). The obtained skin had been

removed from the back area of an adult pig (Yorkshire cross-breeds). The average weight of the pig at the time of slaughter is around 230 lbs, and the length and height are roughly 46 inches and 26 inches. The skin after removal is washed with potable water (approximate temperature 10-25°C). Then the skin is fleshed (hypodermis removed), split (run through a splitting machine to separate the dermis and epidermis) and cut into 4" x 4" size. The skin is also clean shaved to mimic the ventral surface of the hand. The skin is processed, packaged and frozen on the same day as slaughter. This end product was wrapped in an aluminum foil and shipped frozen to us. This skin was thawed on the day of the experiment followed by exposure to germicidal UV for 15 min on each side. The skin was used as donor for all three sets of experiments conducted; for this purpose the skin was cut into smaller sized squares with a scalpel and attached to dry cellulose material. This material was then attached to the donor block on the transfer device prior to inoculation.

Preparation of gloves and lettuce in transfer experiments. Three types of gloves were used for the experiments; yellow latex gloves (Diamond Grip Plus, powder-free latex exam gloves, Microflex, Reno, NV), blue latex gloves Uniseal and blue nitrile gloves (Uniseal). The gloves were cut into 2" x 2" size prior to the experiments. Then the gloves were subjected to UV rays for 15 min. Then the cut glove was attached to the donor block in experiments where it was used as donor (prior to inoculation) or recipient (experiments where transfer from porcine skin to gloves was being tested). Fresh iceberg lettuce heads were purchased from the grocery store. After the green outer leaves were removed, leaves were removed from the lettuce head and cut into 2 cm x 2 cm pieces, prior to the experiment. These were then exposed to UV light for 15 min on each side.

Inoculation and transfer process. The amount used as inoculum for all the experiments was 10 µl which was put in 10 drops of 1 µl each on the donor surface. After the donor surface was inoculated, the donor block was attached to the transfer device and the recipient was placed on the recipient port. The donor was then pressed on to the recipient surface at 1000 g pressure for 10 s. In multiple transfer experiments, involving lettuce, the donor was inoculated and pressed on the recipient lettuce for 10 sec at 1000 g, then the recipient was changed and the same process was repeated.

Elution from recipients. 1 M NaCl in 0.1 M PBS with 0.05% Tween 20 was used as the elution buffer for all transfer experiments. After transfer the recipient was placed in a petri-plate. 250 µl of the elution buffer was added on to the surface, followed by pipetting up and down ~ 25 times. The recovered liquid was transferred to a 1.5 ml centrifuge tube. For the first scenario 100 µl of the sample was used for RNA extraction, while for the second scenario the entire amount recovered (~ 250 µl) was used for further processing.

RNA extraction and RT-qPCR. RNA extraction was performed using viral RNA minikit (Qiagen, Valencia, CA). NoV-specific primers and probes, and a QuantiTect probe one-step RT-PCR kit (Qiagen) were used for amplification, through real time RT-PCR (StepOne, Applied Biosystems, Foster City, CA). The quantification of the RNA was done by comparing to the standard curve of NoV RNA transcripts of known concentration, for GI and GII. In case of MNV the quantification was done by comparing to the standard curve of purified RNA of MNV. The negative controls of all the experiments were processed similar to the samples, but here the donors were not inoculated with any of the three viruses

Calculations in transfer studies. The recovery controls for all transfer experiments were the quantities recovered from the recipients after direct inoculation of the recipient of that particular experiment. The calculations were performed as follows:

$$Percent\ recovery = \frac{Quantity\ recovery\ control}{Virus\ quantity} * 100\%$$

The quantity recovered from the samples is then corrected using the percent recovery calculated above:

$$Quantity\ corrected\ with\ percent\ recovery = \frac{Quantity\ recovered}{Percent\ recovery}$$

Finally the percent transfer was calculated:

$$Percent\ transfer\ corrected = \frac{Quantity\ corrected\ with\ percent\ recovery}{Virus\ quantity} * 100\%$$

Preparation of Sanitizers and Controls

Sodium Dodecyl Sulfate (SDS) (Sigma-Aldrich Co., St. Louis, MO), levulinic acid (LEV; 98% solution, Sigma) and sodium hypochlorite solution (5% available chlorine; Ricca chemical, Arlington, TX) were mixed with sterile DI water to formulate test liquids in this study. On each day of use, solutions of 5% levulinic acid plus 2% SDS and 50-200 ppm (free) chlorine were prepared. Free, available chlorine was measured using an iodometric method with digital titrator (Model 16900, Hach Co., Loveland, CO). Purell™ Advanced hand sanitizer (GoJo Industries Inc. Akron, OH; Active ingredient = 70% ethyl alcohol) was used undiluted for all experiments.

Neutralization of Sanitizer Liquids and Elution of Virus from Stainless Steel Coupons

A neut/elute buffer comprised of letheen broth with 5 g/L tween 80 (Neogen Corporation, Lansing, MI) plus 1 M NaCl, 0.02 g sodium thiosulfate/L, and 4.0 g sodium bicarbonate/L was used to neutralize all sanitizer liquids while simultaneously eluting virus from the glove surfaces. Post-treatment, each glove piece was immediately was placed into VWR clear sterlile sampling bags (24 oz) containing 15 ml of neut/elute buffer. Each bag was stomached for 1 min at 260 rpm. Samples were aliquoted into two 500 µl-portions for storage at -70°C for at least 24 h before plaque assay. The remaining (~13 ml) liquid from some samples was stored at -70°C until future use if necessary.

MNV-1 Concentrated by Polyethylene Glycol (PEG) Precipitation

For some experiments, where no infectious virus could be detected on the zero dilution plates, a PEG precipitation method was used to lower the assay limit of detection. In this procedure, 8% (w/w) polyethylene glycol (PEG 8000; Sigma-Aldrich, Co. LLC, St. Louis, MO) was added to each sample in 50 ml centrifuge tubes which were vortexed immediately for approximately 5 s. Tubes were placed in a shaking rack (200 rpm) and PEG was dissolved overnight at 4°C. The following day, samples were centrifuged at 9,000 $\times$ *g* for 30 min at 4°C. Subsequently, the supernatant was poured off, and the resulting pellet was suspended in 1 ml of 1 X MEM with vortexing. Samples were portioned into two 500 µl portions and frozen to -70 °C for at least 24 h before assaying.

Methods for Treating Glove Pieces with Disinfectants.
An adaptation to the U.S. EPA "Confirmatory Virucidal Effectiveness Test" *(4)* was employed to test the efficacy of each sanitizer solution against MNV-1 glove pieces. Briefly, test liquids (disinfectants and controls) were pipetted into (100 X 15 mm) glass petri dishes in 15-ml portions. Glove pieces inoculated with MNV-1 were floated on top of the test liquids so the inoculated area interfaced with the liquid solution for 1-5 min at room temperature. Gloves were then removed from the liquid and immediately neutralized using the neut/elute buffer.

Glove pieces (2" x 2") were attached to flat wooden (paint stirring) sticks (one glove piece per stick) using rubber bands along the top and bottom of the glove piece, keeping them flat and taunt against the stick. Gloves bound to sticks were exposed to germicidal UV for 15 min prior to inoculation. After the inoculum was dried, sticks with inoculated gloves and sticks with un-inoculated gloves of the same type (latex, nitrile or canner's) were selected as disinfection pairs. Individually, glove pairs were submerged into 200 ml of test solutions (200 ml) contained in 250-ml glass beakers, making sure the gloves are completely submerged throughout the disinfection procedure. The exposed portions of the gloves between the rubber bands were rubbed together for 6-10 sec (depending on the experiment), mimicking the palms of gloved hands rubbing together in a glove-dunk bucket. After each treatment, gloves were immediately transferred to neut/elute buffer to neutralize the sanitizer activity. Using a sterilized scalpel blade, the rubber bands were cut and the sticks removed from the neut/elute buffer, leaving the glove pieces behind for virus elution from the glove surfaces. For chlorine solutions, pH, ppm and ORP readings were taken during some experimental replicates. For levulinic acid-containing solutions, the pH was taken before and after each treatment. Neutralizaiton, cytotoxicity and recovery controls were performed with each experiment.

Calculation of Log Infectious Virus Reduction and Statistical Analysis
The lower limit of detection was 3.30 logs of virus, or 2,000 viral PFU/ml, for all replicates with that were not further concentrated by PEG precipitation. When PEG precipitation was employed, this detection limit was lowered to 1.10 log PFU/ml of virus. Average MNV-1 log reductions (PFU/ml) due to each treatment were calculated using the average log MNV-1 (PFU/ml) of recovery controls as the baseline for each experimental replicate. The log PFU MNV-1/ml recovered post-treatment for each sample in each replicate was subtracted from the average log PFU MNV-1/ml recovered from recovery controls. Data were statistically analyzed using a two-way analysis of variance (ANOVA) (SAS 9.3, Cary, NC). Differences between treatment log reduction means were considered significant when the P-value of the difference was less than 0.05.

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Suggestions to CPS (optional)

The help provided by CPS in locating collaborators in CA and industry resources was critical to this study, especially due to our location on the East coast. We thank you for that. Encouraging investigators to seek your help in this regard could be emphasized. I think this is very unique, so some investigators may not be aware you are available for this type of help, particularly new investigators.

Recommendations to Prevent Cross Contamination during Hand Harvest

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Center for Food Safety
University of Georgia

Western Food Safety Summit

May 10-11, 2012

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Cross-contamination during hand harvest

The importance of hand sanitation

Norovirus or "Cruise Ship virus"

Intestinal germ leaves trail of misery

The Boston Globe

City's ERs see more than 3,700 sufferers

By Stephen Smith, Globe Staff | January 17, 2007

More than 3,700 patients stricken with nausea, vomiting, and diarrhea have visited Boston's emergency rooms during the past six weeks in a wave of gastrointestinal illness that has swept cities across North America

A22 YNE THE NEW YORK TIMES **NATIONAL** FRIDAY, DECEMBER

Viruses Rattle the Cruise Industry, but Passe

Continued From Page A1

utive voyages, said that illness on ships may be a reflection of the many outbreaks now occurring on land. Scientists said the recent patterns of shipboard cases indicated that the illness was coming not from food or water on the ship, but from infected passengers or crew bringing the virus onto ships. "Any time you take people and put them together in close quarters and they have infection, it spreads," Dr. Murray said. "I think it's a fact of life. It will continue to happen."

The possibility of further outbreaks unnerves the cruise industry, an $11 billion a

Virus Rattles Cruise Industry and Health Officials

This article was reported by ***Joseph B. Treaster, Dana Canedy*** *and* ***Denise Grady*** *and was written by Ms. Grady.*

The reports from the Caribbean cruise ship Oceana began arriving at the Centers for Disease Control and Prevention late on Tuesday. By Wednesday night, the picture was disturbingly familiar: 117 on board were violently ill with vomiting and diarrhea, the same symptoms that had laid low passengers on three other ships in the last two months.

A common and highly contagious infection, caused by a germ known as a Norwalk-like virus, has been con-

SICK AT SEA

An Outbreak and a Mystery

health officials said they did not know why the recent outbreaks were occurring, and they said the recent burst of cases appeared to be an increase over previous years.

The nature of the viruses — they are common, hardy, highly contagious and hard to track — raises the possibility that periodic outbreaks on ships may be inevitable, one more risk that the traveling public must factor into the calculation of whether to book tickets or stay home. The tight quarters of a ship provide ideal conditions for contagious germs like Norwalk viruses to multiply.

Rapid turnaround times — often just hours between one cruise and the next — can make it difficult to eliminate stubborn organisms. Sick or recovering passengers, loath to spend their vacations in their cabins, may infect others, as may crew members who work while ill.

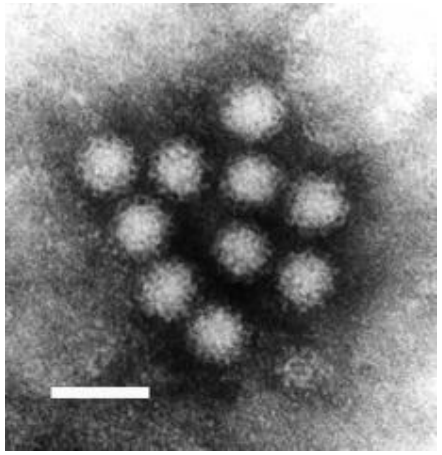
Dr. Megan Murray, a Harvard epidemiologist who was a consultant for the Holland America Line on disinfecting its cruise ship Amsterdam, which had outbreaks on four consec-

Continued on Page A22

Noroviruses are the leading cause of gastroenteritis in the United States

- Over 23 million illnesses annually in the United States
  - Leading cause of acute gastroenteritis in adults
  - Second most frequent cause of childhood diarrhea
- 5.5 million foodborne illnesses each year in the US
  - 58% of all cases with a known etiologic agent (Scallan et al, 2011)
- Improved diagnostics account for much of the increase over the last two decades
  - Although there is some evidence emergence of a more transmissible and perhaps virulent genotype (GII.4) is also responsible

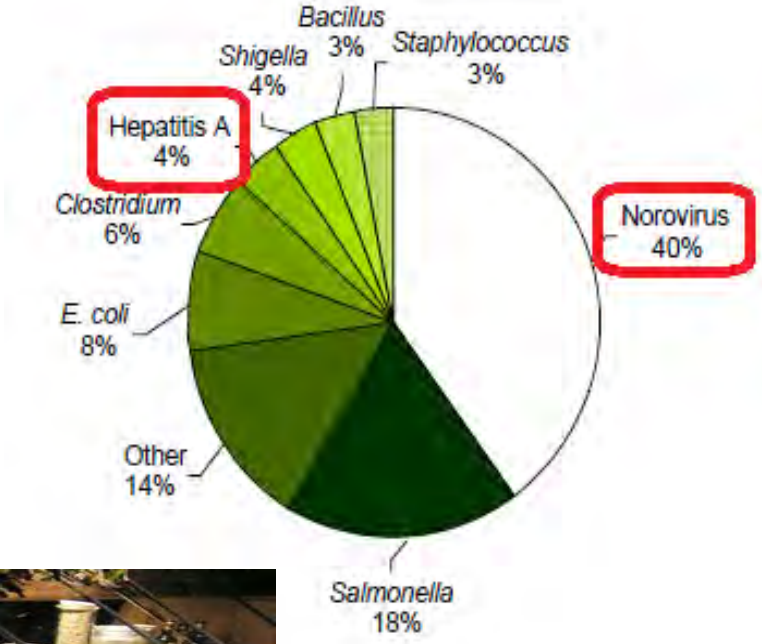
Human Noroviruses (HuNoV)



- Norwalk-like viruses
  - Norwalk, OH
  - 1968
- Infection
  - Incubation 12-48 hr
  - Duration 6-60 hr
  - Watery diarrhea, vomiting, nausea, abdominal cramps, low grade fever
  - Self limiting except in children, elderly and immuno-comprimised

Foods most often implicated in outbreaks with foodborne viruses

- Fresh produce consumed raw
  - Norovirus causes 40% of all produce-associated outbreaks
- Ready-to-eat, prepared foods
- Raw shell fish



(Dewaal and Bhuiya, 2007, Center for Science in the Public Interest)

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Contamination can occur pre-harvest or during food processing

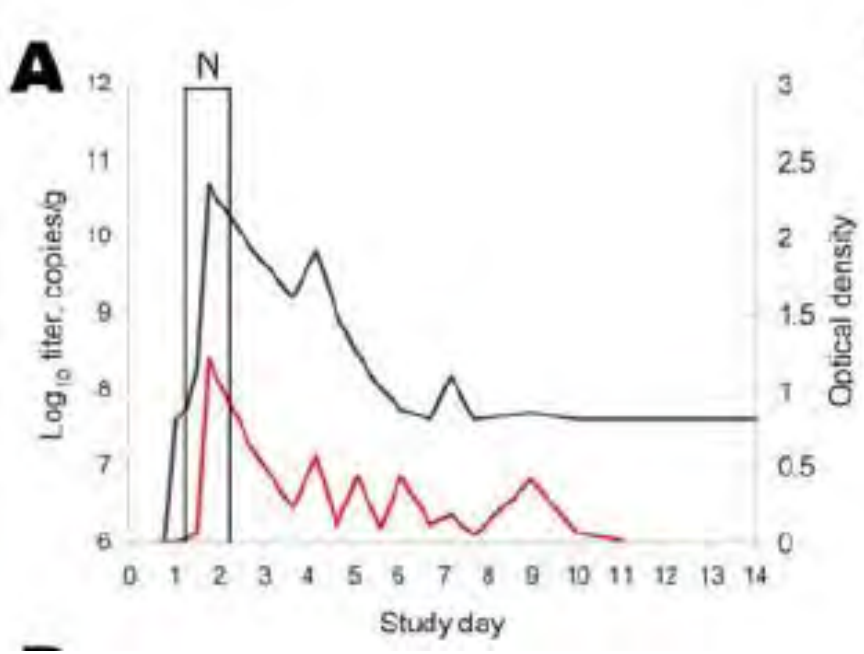
- Avenues for contamination
  - Shellfish are contaminated by harvest water
  - Irrigation water
  - Processing water or environmental surfaces
  - Handling during harvest or processing
- Multi-state and international outbreaks
  - Frozen raspberries (Falkenhorst et al., 2005; Ponka et al., 1999)
  - Lettuce (lollo bionda) imported from France to Demark: Jan 2010 (Eurosurveillance 2010:15(6))

Foodhandlers are responsible for the majority of foodborne virus outbreaks

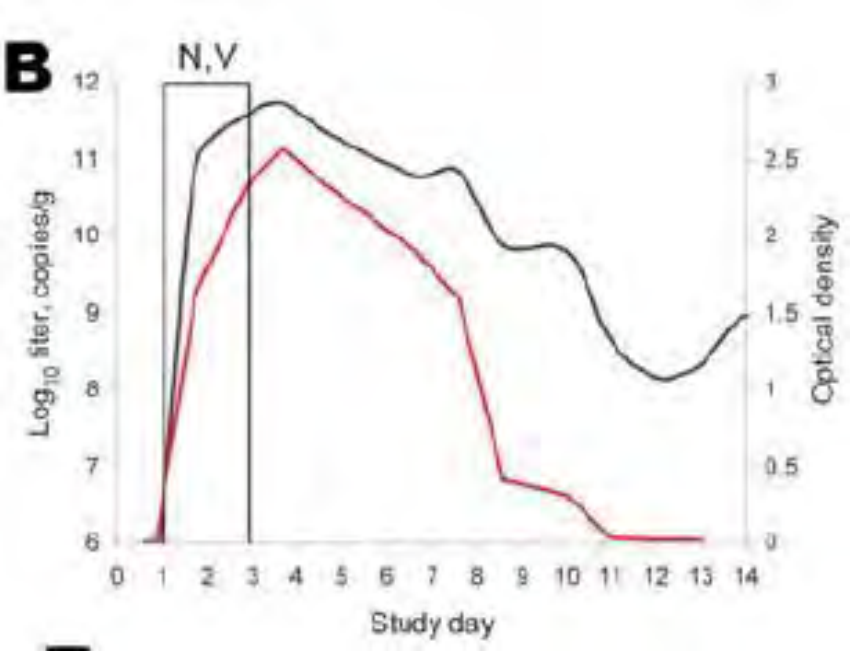
- Shed in very high numbers
  - Up to 10^6 to 10^{11} per g vomit or feces
- Asymptomatic shedding (1 day to several weeks)
  - Outbreaks caused by food handlers either without illness or post-illness (Lo et al, 1994; Patterson 1993)
- Low infectious dose (as few as 18 particles)
- Environmental persistence (days to months)
- Moderate resistance to disinfectants

Noroviruses can be shed at high concentrations asymptomatically

Asymptomatic patient



Symptomatic patient

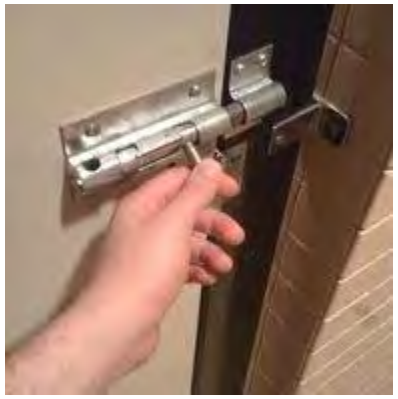


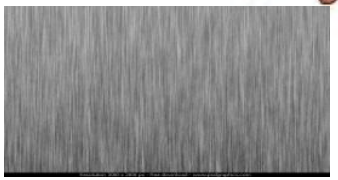
(Atmar et al, 2008 EID 14(10):1553-7)

Importance of hand sanitation

- 100 million to 10 billion viruses per gram of feces
- As much as 100 million viruses per 10 µl drop
- Coprostanol (fecal biomarker) detected on 6th (of 6) sheet of TP 15/24 times (63%) (Todd et al, 2009)
  - Avg: $10^{-5.6}$ g = 25,000 viruses
  - On one occasion: >0.001 g = 10,000,000 viruses from an ill person
    = 100,000 viruses from an asymptomatic person
- Handwashing reduces probability of hand transfer to foods by 25-100 fold (Bidawid et al, 2000)
- As few as 18 virus particles (Teunis et al, 2008)

Simplistic produce contamination scenario





Fomite

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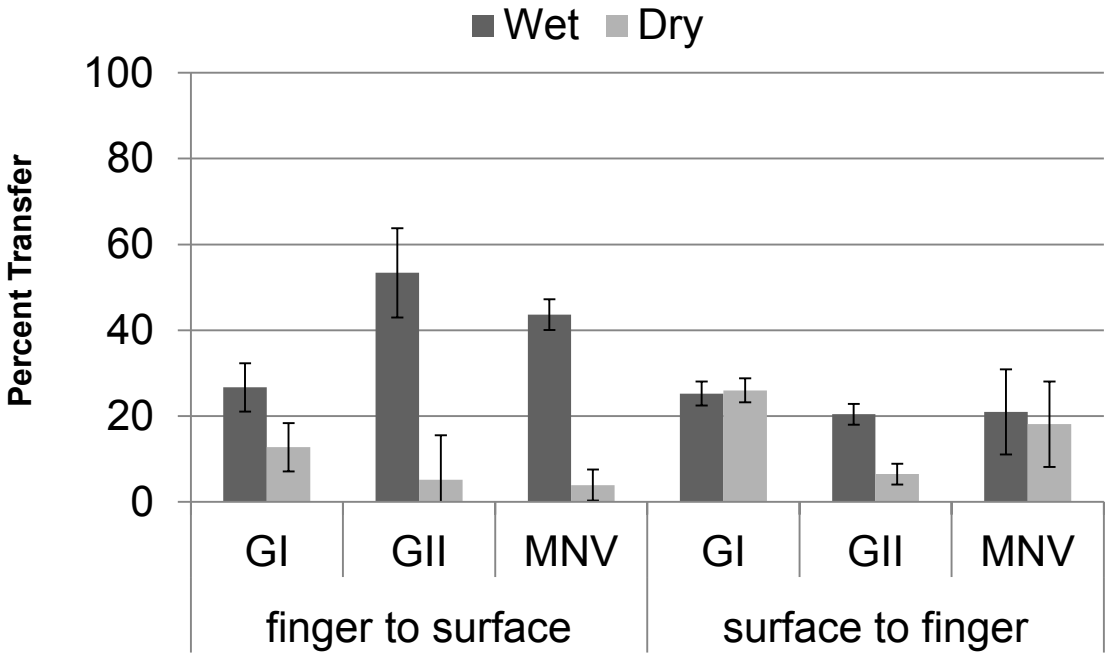
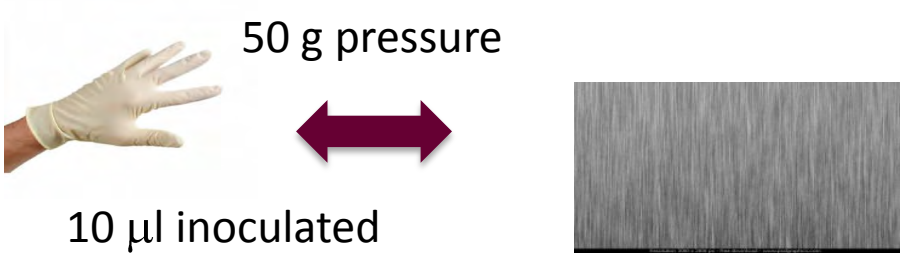
Norovirus transfer to food contact surfaces and foods during handling was previously demonstrated





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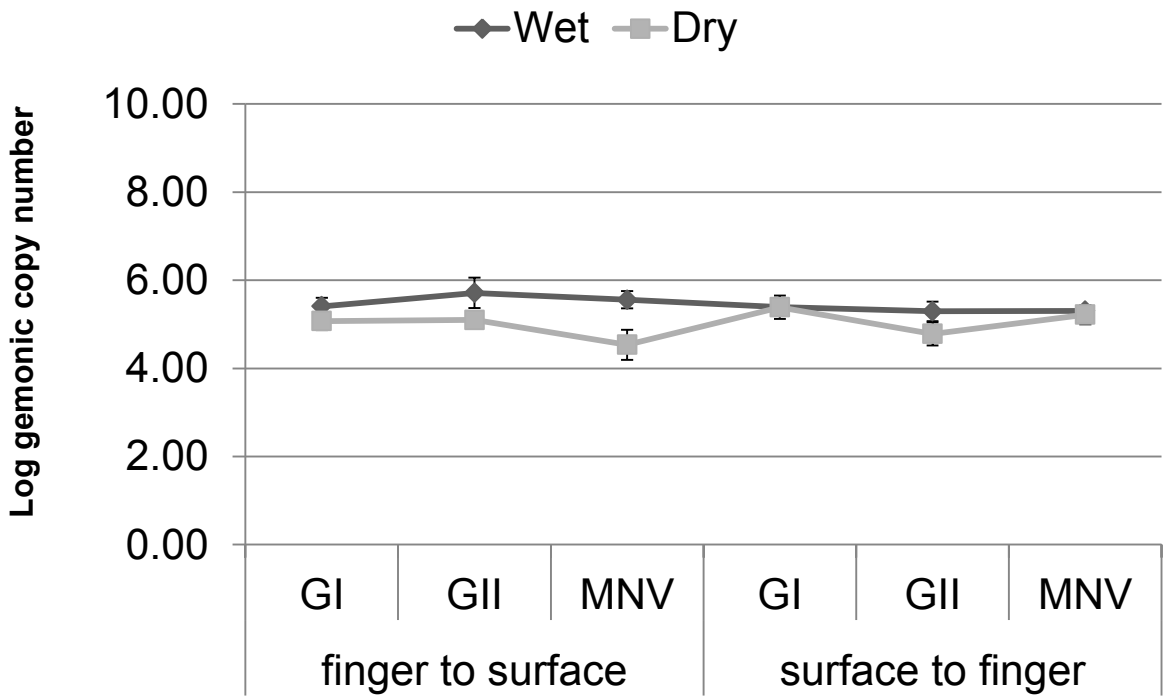
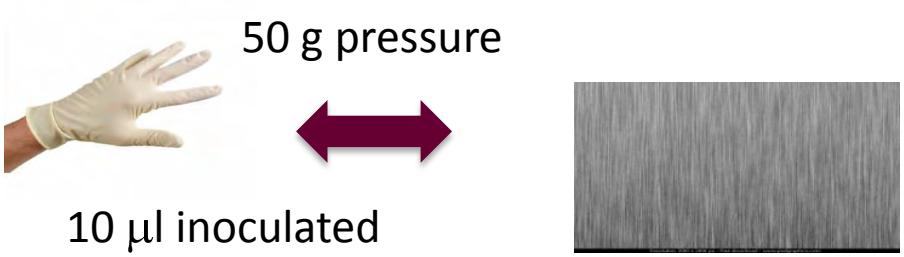
Norovirus transfer to food contact surfaces and foods during handling was previously demonstrated





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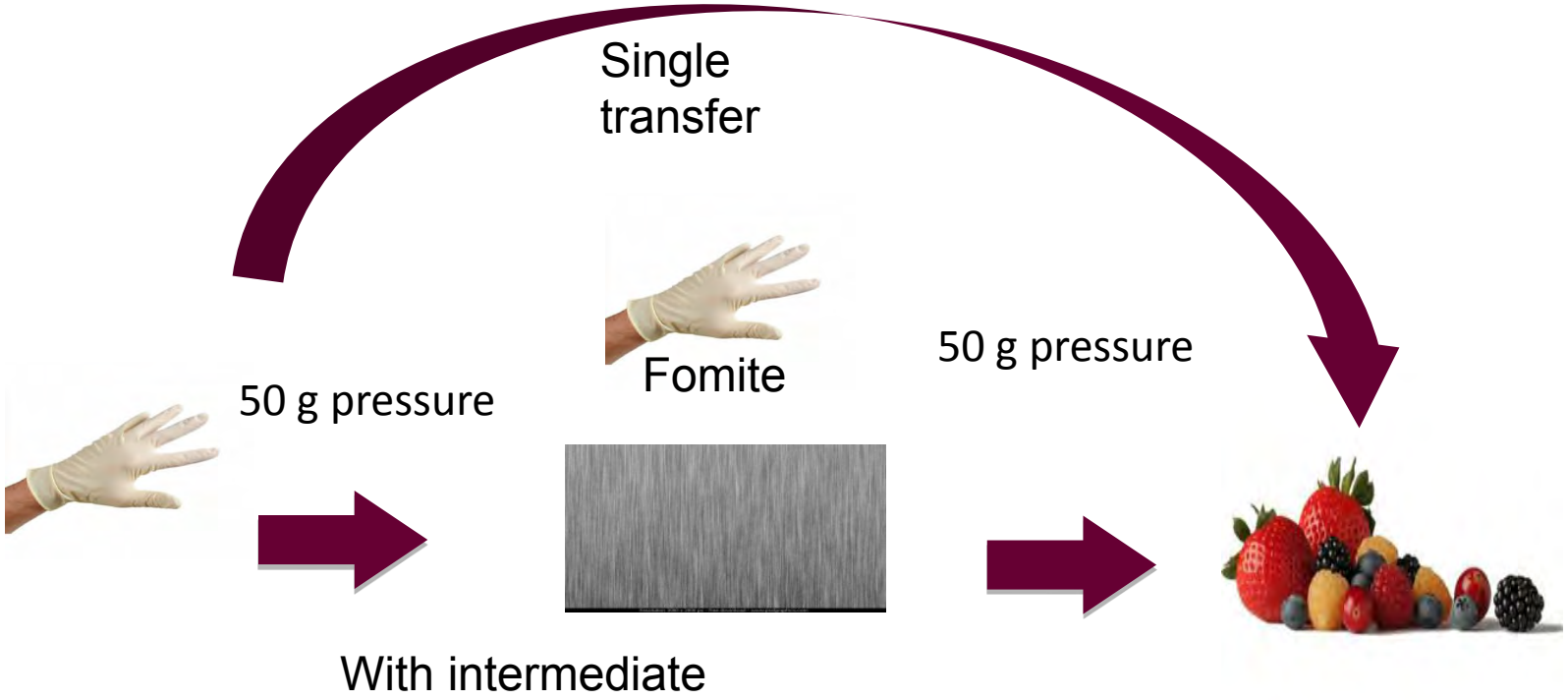
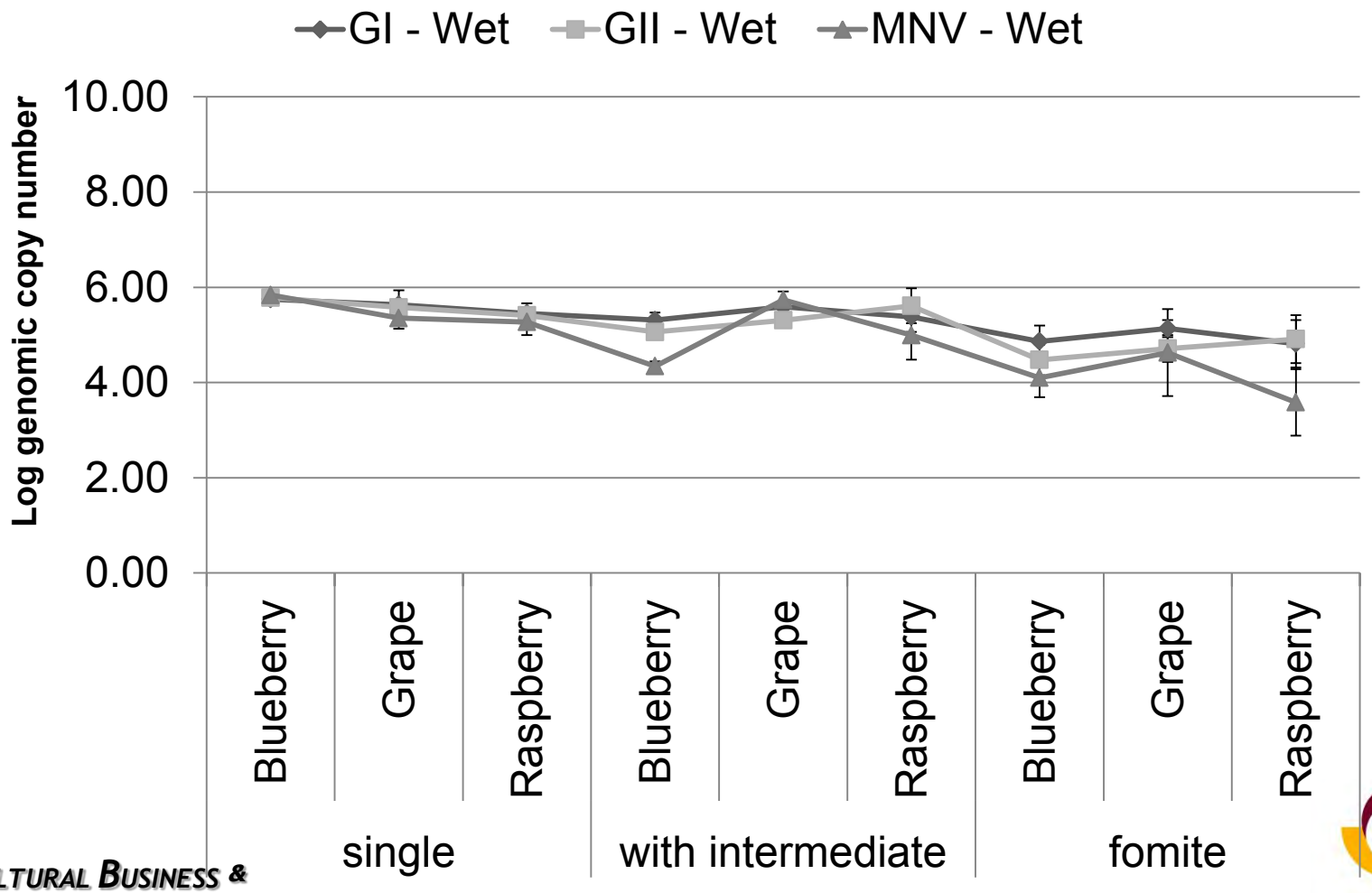
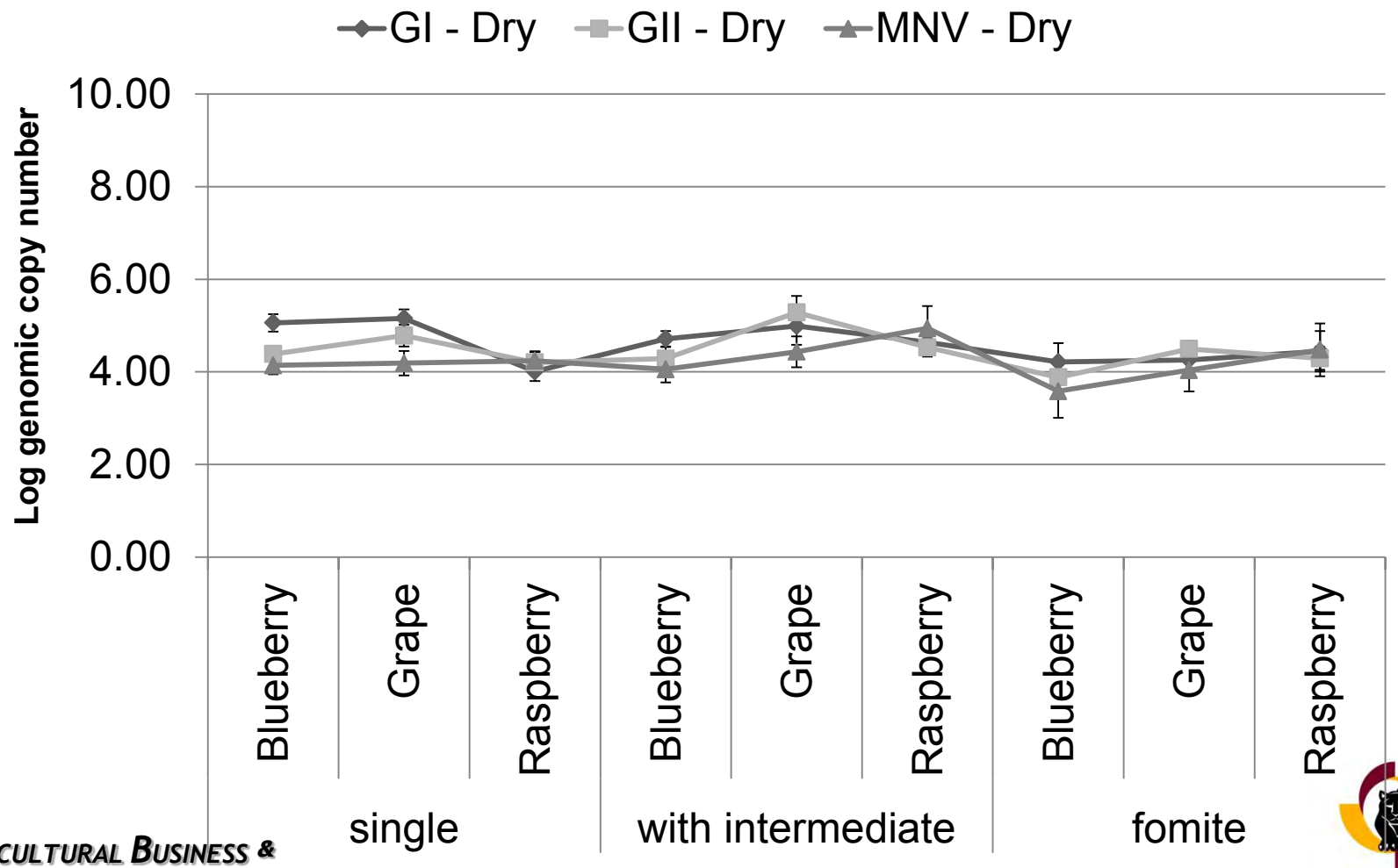
Direct or indirect contamination of hands before norovirus transfer to small fruits



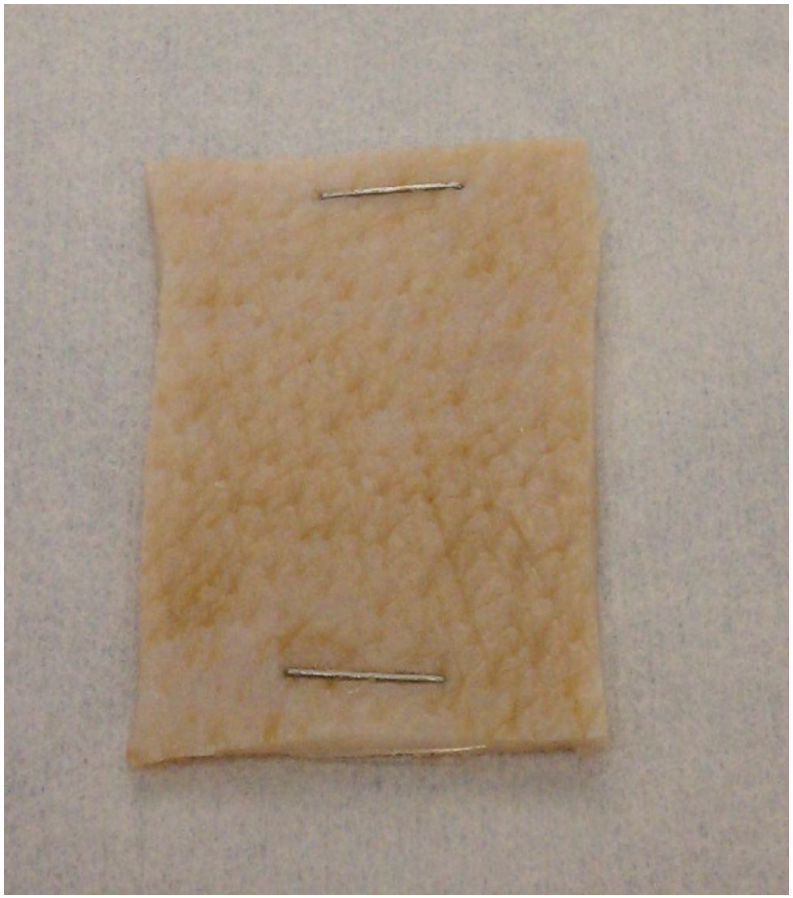
Direct or indirect contamination of hands before norovirus transfer to small fruits

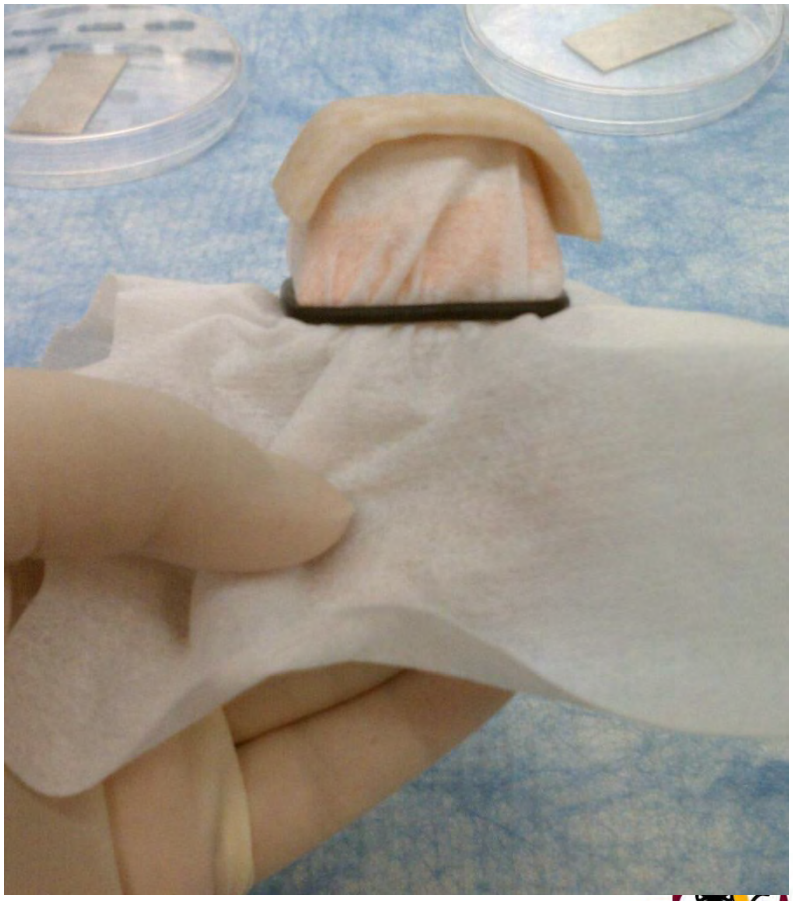


Direct or indirect contamination of hands before norovirus transfer to small fruits

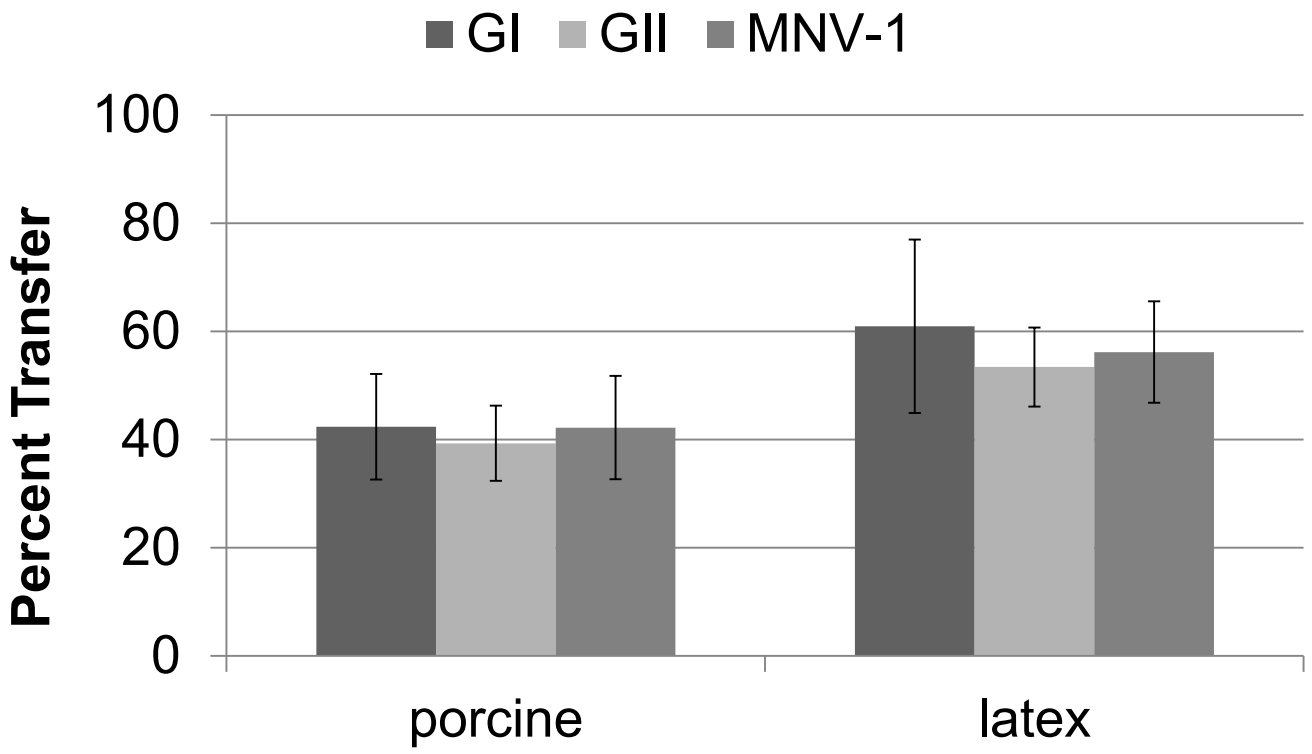


Porcine tissues to model bare hand contact and norovirus transfer

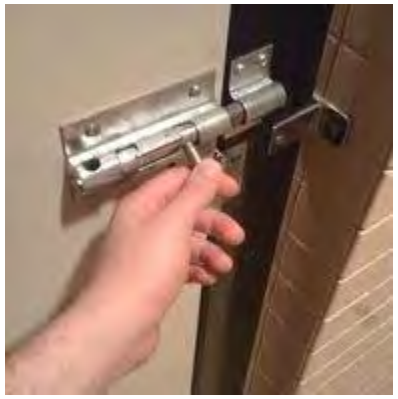




Norovirus transfer from latex gloves or porcine tissue sections to stainless steel



Simplistic contamination scenario



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Common sanitizers have limited efficacy against foodborne viruses

Limited efficacy

- Chlorination
  - If high amounts of organic matter is present
- QACs
- Phenolics
- Low pH
- Detergents
- Triclosan
- Ethanol-based sanitizers
  - Mixed data in the lab

What does work

- Hands: soap and water rinse for 20 sec (removal- not inactivation)
- Synergistic compounds*
  - Ethanol
  - Organic acid
- Strong oxidants with prior cleaning of surfaces
  - Chlorine dioxide
  - Ozone
  - Hydrogen peroxide
- Thermal processing (>62˚C)

* Macinga et al, 2008 AEM 74:5047-52

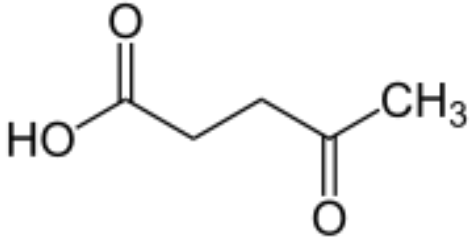
Surface disinfection

- CDC recommendations for disinfection of non-porous surfaces with bleach
  - 1,000 ppm if contact with ill
  - 5,000 ppm if visibly soiled

- List of EPA registered surface disinfectants
  - http://www.epa.gov/oppad001/list_g_norovirus.pdf

Novel sanitizer composed of levulinic acid plus sodium dodecyl sulfate

- Developed at UGA, Center for Food Safety
- Effective against *Salmonella* and *E. coli* O157:H7
  - Washing lettuce with 3% LVA with 1% SDS reduced populations by > 6 log cfu after 20 sec
  - Applications on poultry carcasses and cages
- Licensed as a produce wash solution
  - FDA Generally recognized as safe (GRAS)
  - Medical applications
- Exploring for other applications
  - Food contact surfaces
  - Hand/glove sanitizer



Levulinic acid and SDS do not inactivate human norovirus surrogates when used individually in solution

Log PFU reduction of infectious virus

	MNV (n=3)	FCV (n=5)	p value
0.05% SDS	-0.13 (±0.26)	-0.06 (±0.22)	0.71
0.5% SDS	0.13 (±0.20)	0.09 (±0.16)	0.78
1% SDS	0.03 (±0.12)	0.32 (±0.19)	0.06
2% SDS	-0.23 (±0.59)	0.51 (±0.34)	0.06
0.5% levulinic acid	0.09 (±0.38)	-0.09 (±0.27)	0.49
1% levulinic acid	-0.04 (±0.23)	0.05 (±0.18)	0.96
2% levulinic acid	-0.06 (±0.17)	0.05 (±0.22)	0.95
3% levulinic acid	-0.09 (±0.10)	-0.10 (±0.22)	0.13

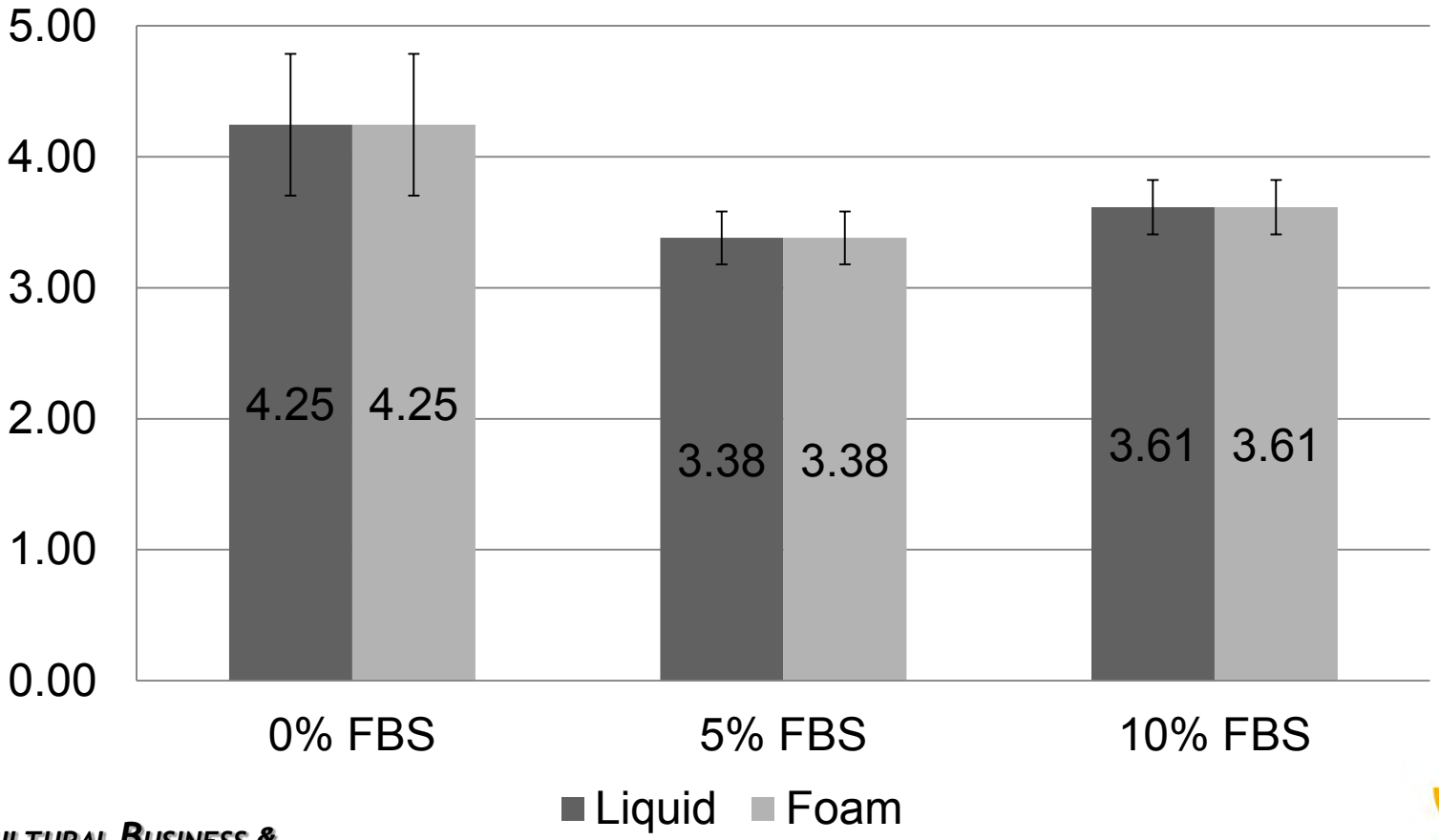
1 minute exposure time at room temperature

Sanitizer is effective against human norovirus surrogates at low concentrations in solution

	MNV	FCV	PEC
0.5% Lev/0.05% SDS	1.04 (± 0.17)	>3.6 (± 0.17)	>4.00 (± 0.0)
0.5% Lev/0.5% SDS	> 4.00 (± 0.34)	>3.6 (± 0.17)	>4.00 (± 0.0)
2% Lev/1% SDS	>3.08 (± 0.13)	ND	ND

1 minute exposure time at room temperature

Surface disinfection of MNV after treatment with liquid or foam levulinuic acid plus SDS with 0 to 10% organic material on stainless steel



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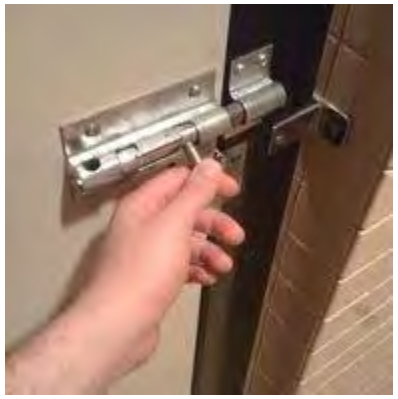
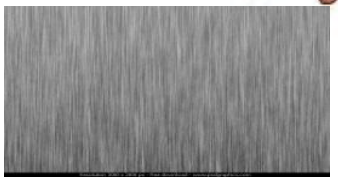
Simplistic contamination scenario





Fomite

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Recommendations for glove use and sanitation

How frequently should gloves be changed or washed?

- Do gloves accumulate soil, lettuce debris, lettuce sap, moisture over time?
- If so, how does this accumulation impact cross-contamination of lettuce with pathogens?
  - Bacterial (*E. coli* O157:H7, *Salmonella*)
  - Viral (Norovirus)

Determine accumulation levels on different glove types over time

- Gloves were collected from workers in field harvesting/coring operations
  - Latex
  - Nitrile
- Workers wore gloves for
  - 1, 2-3, or 4-6 hr time intervals
- Gloves were collected and analyzed in the lab
  - Soil, lettuce debris, moisture, lettuce sap
- Differences in time worn, hand worn on (coring, other), glove type

Results: Accumulation on gloves

- Very little amounts of soil
- Lettuce debris accumulation was random but no differences
- Moisture was greater for 2-3 hr and 4-6 hr time intervals, and on day 2
- Amount of lettuce sap per volume of moisture did not differ
- Moisture was mostly due to lettuce sap

What is the impact of lettuce sap on pathogen survival and transfer?

- Impact of moisture
  - Greater transfer
- Impact of organic material
  - Greater survival
  - Protection from disinfection or damage due to desiccation or UV
  - Creates chlorine demand making sanitizers less effective

How effective are glove sanitizers?

- Against *Salmonella*, *E. coli* O157:H7 and norovirus
- What type of sanitizers are typical?
  - How effective are they in the context of typical amounts of accumulation on the gloves
- Are there glove types that are more easily sanitized?
- Are there sanitizing methods that work better?

- So far in this study:
  - Chlorine buckets
  - Water-less hand/glove sanitizers
  - levulinic acid plus SDS sanitizer developed at UGA
- Types of gloves:
  - Different surface properties
    - Roughness, porosity, charge
- Sanitizing methods:
  - Wash bucket
  - Wipe or towellette

Other concerns?

Questions?

Jennifer L. Cannon

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The likelihood of cross-contamination of head lettuce by *E. coli* O157:H7, *Salmonella* and norovirus during hand harvest and recommendations for glove sanitizing and use

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Jan. 1, 2011 - Dec. 31, 2012

Leafy Greens

SUMMARY

In the harvesting environment, cross-contamination of food handler gloves with *E. coli* O157:H7, *Salmonella* or norovirus can occur after contact with contaminated soil, water, lettuce, and equipment or from the bare hands of workers while donning. Factors that impact cross-contamination of Iceberg lettuce by pathogens during handling, as well as sanitizing treatments for inactivating pathogens on contaminated gloves were investigated. Pathogens suspended in water or Iceberg lettuce sap (latex extract) were inoculated onto latex or nitrile gloves (Uniseal®). Pathogen survival was measured on untreated gloves, as well as gloves soaked in water, water containing 2% SDS (sodium dodecyl sulfate), chlorine (75-200 ppm) or a 5% levulinic acid plus 2% SDS sanitizer for time periods ranging from 15 sec to 5 min. Lettuce sap was protective of pathogens, resulting in greater survival on gloves. Bacterial pathogens on gloves were susceptible (>3.8 log CFU inactivation) to treatment with 75 ppm chlorine for 15 sec. Noroviruses on gloves were more resistant to treatment with sanitizers, requiring 5 min of exposure for similar levels of inactivation. Future studies seek to determine optimal glove sanitizing procedures so that recommendations on glove use and sanitation can be made.

OBJECTIVES

1) To investigate the impact of lettuce sap (latex extract) on survival of pathogens (*E. coli* O157:H7, *Salmonella* or norovirus) on latex and nitrile gloves worn by Iceberg lettuce harvesters.
2) To determine the efficacy of chlorine (75-200 ppm) and a 5% levulinic acid plus 2% SDS sanitizer in inactivating these pathogens on latex and nitrile gloves.

METHODS

Bacterial (*E. coli* O157:H7 and *Salmonella*) and viral (murine norovirus, a surrogate for human norovirus) pathogen cultures were prepared using standard techniques. Pathogens were suspended in water (sterile DI) or lettuce sap, inoculated on latex (15 mil, Uniseal®) or nitrile (8 mil, Uniseal®) glove pieces (at least 2"x2") and allowed to dry under a laminar flow hood. Sanitizers, prepared on the morning of each experimental trial, included chlorine (75-200 ppm), chlorine plus 2% SDS and 5% levulinic acid plus 2% SDS. Water (sterile DI) and water containing 2% SDS were included as non-microbicidal controls. Log CFU or PFU (plaque forming units) inactivation of bacterial and viral pathogen were calculated by comparison to untreated (recovery) controls. Three experimental replicates included with 2 to 5 samples per replicate.

RESULTS TO DATE

Lettuce sap (latex) protects pathogens on glove surfaces

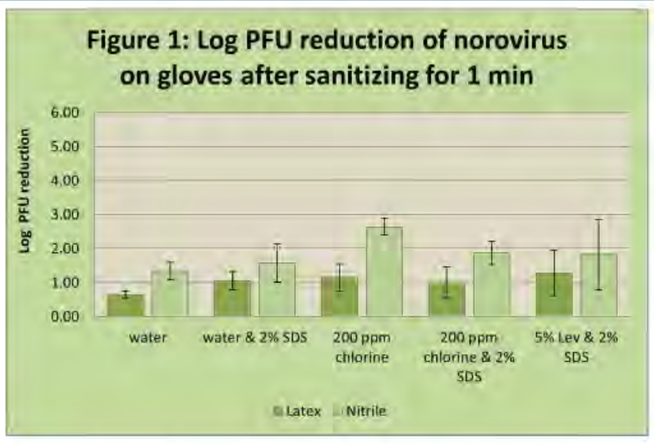
- Bacterial pathogens suspended in water or lettuce sap and inoculated on gloves were inactivated by 2.2-3.0 and 0.4-0.7 log CFU, respectively after 2 hr at room temperature.
- After 2 hr, there was little inactivation of norovirus, but after 7 hr, noroviruses were inactivated by 1.6 and 0.6 log PFU when suspended in water and lettuce sap prior to inoculation on gloves.

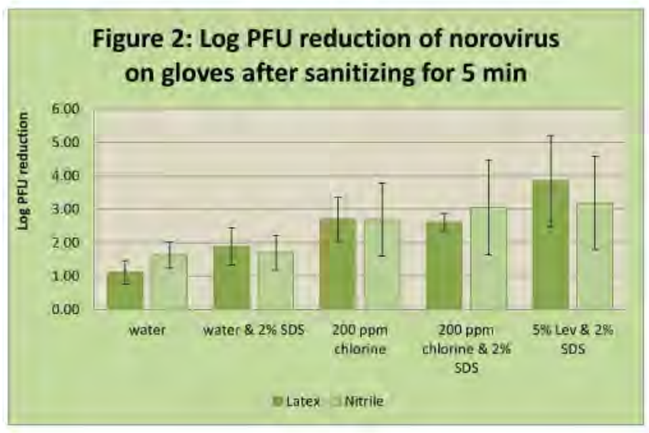
Inactivation of pathogens on gloves after treatment with sanitizers

- After a 15-sec exposure of nitrile glove pieces (inoculated with 3.8 log CFU *Salmonella*) to 75 ppm chlorine, the pathogen could not be detected by enrichment.
- At higher inoculum levels (~5.8 log CFU/glove piece), the pathogen could still be detected on glove pieces following a 1-min exposure to 75 ppm chlorine.
- *Salmonella* was also detected sporadically by enrichment when high -inoculum glove pieces were exposed to 150 ppm chlorine for 1 min.
- *Salmonella* was completely inactivated (>5.8 log CFU/glove piece) after 1 min exposure to 5% levulinic acid plus 2% SDS.
- Norovirus on latex and nitrile gloves was more difficult to inactivate as demonstrated in Figures 1 & 2.
- After 1 min exposure, inactivation was greater for norovirus on nitrile gloves than for norovirus on latex gloves (Figure 2).
- Greater than 3.0 log PFU inactivation of norovirus was only achieved after sanitizer exposure for 5 min (Figure 1).

BENEFITS TO THE INDUSTRY

Results indicate that lettuce sap enhances pathogen survival on food worker gloves. Including a glove washing/sanitizing procedure just before lunch breaks or other periods when employee gloves are stored may decrease the likelihood of pathogen survival on gloves if they become contaminated in the field. Sanitizing gloves with 75 ppm free chlorine (not consumed by demand from organic debris) is likely to inactivate bacterial pathogens on gloves (unless gross contamination occurs). However, noroviruses are more difficult to inactivate on nitrile gloves and especially latex gloves. Optimal sanitizing procedures need be established with consideration of glove composition as evidence of pathogen survival after treatment may correlate with the surfaces properties of gloves. The results of this study (and future plans of this project) will provide a scientific basis for making recommendations on glove use (frequency of changing and type) and sanitation during harvesting head lettuce and will contribute to improving the safety of fresh produce.





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University of Georgia

Acknowledgments: We thank Katie Roache, Chris Smith, Alison Payton, Jean Liao, Viyaya Mantri and Amy Mann for technical assistance in the laboratory. Dole Fresh Vegetables for assistance during the field portion of the study and the Center for Produce Safety at UC-Davis for their technical guidance and financial support (2010).

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The likelihood of cross-contamination of head lettuce by *E. coli* O157:H7, *Salmonella* and norovirus during hand harvest and recommendations for glove sanitizing and use

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Center for Food Safety[1]; Department of Crop and Soil Sciences[2] at The University of Georgia

ABSTRACT

The impact of glove use and sanitation on cross-contamination of raw head lettuce by bacterial (*E. coli* O157:H7, *Salmonella*) and viral (norovirus) pathogens during harvest is under investigation. Latex and nitrile gloves collected from iceberg lettuce harvesting crew members were analyzed for the amount of soil, moisture, and lettuce sap (latex) collected on the outer surface of the gloves at time points (1 hr., 2-3 hr., or 4-6 hr.) throughout the day. The impact of lettuce sap on pathogen survival and transfer to and from lettuce and gloves during harvest is under evaluation. Interruption of pathogen cross-contamination and removal of soil and organic debris from gloves by sanitizing rinses (dunk buckets containing chlorinated water or a novel sanitizer developed at UGA) is also being studied to identify the most effective sanitizing treatment, or combination of treatments for harvesters' gloves. To date, study results indicate that latex and nitrile gloves quickly (within 2 hr.) become saturated with moisture and lettuce sap. Lettuce sap increases pathogen survival on gloves but is readily washed off in sanitizing glove rinse buckets (consequently generating chlorine demand). Optimal sanitizing procedures need be established with consideration of glove composition as evidence of pathogen survival after treatment may correlate with the surfaces properties of gloves. The results of this study will provide a scientific basis for making recommendations on glove use (frequency of changing and type) and sanitation during harvesting head lettuce and will contribute to improving the safety of fresh produce.

1. Mimic glove cross-contamination events in the harvest environment to determine the likelihood of pathogen transfer to gloves and to lettuce

Bare hand contamination of gloves when putting gloves on; with or without adequate hand sanitation

From lettuce or soil that has been previously contaminated by irrigation water or animal manures; outer leaves to inner leaves with harvest

2. Determine efficacy of common and novel glove sanitizing solutions for inactivation and removal of *E. coli* O157:H7, *Salmonella* and norovirus

- 200 ppm free chlorine (pH 7)
- Levulinic acid plus SDS
- With or without 1M NaCl
- Purell hand sanitizer

Determine log removal and/or inactivation by culture and enrichment

3. Determine the impact of glove type and soilage on pathogen cross-contamination and removal/inactivation with glove sanitizers

Glove types: Latex (high risk) and Nitrile (plus) (UniSeal ®); Latex Canners gloves (Ansell)

Soilage: Lettuce sap (lettuce latex, lettuce debris, soil, and moisture

Examine pathogen survival, transfer and sanitation of soiled gloves of each type

Expected results based on preliminary findings.

- The duration that gloves are worn (> 2 hr) does not lead to more soilage by lettuce sap, soil, moisture, or lettuce debris; the gloves become saturated after 2 hr
- While glove type (latex or nitrile) doesn't impact glove soilage, glove type may impact removal/inactivation of pathogens with sanitizer application
- Lettuce sap increases pathogen survival on gloves, indicating rinsing gloves is important before and after breaks
- Sanitizer/glove combinations should be tested to ensure compatibility

ACKNOWLEDGEMENTS

We thank Katie Roache, Chris Smith, Alison Payton, Amy Mann, Jean Liao and Vijaya Mantri for technical assistance in the laboratory, Dole Fresh Vegetables for assistance during the field portion of the study and the Center for Produce Safety at UC-Davis for their technical guidance and financial support (2010).

Norovirus Cross-Contamination during Produce Handling With or Without Gloves

Grishma Kotwal and Jennifer L. Cannon
Center for Food Safety, The University of Georgia

INTRODUCTION

A norovirus infected food worker may harbor and shed up to 10^{11} genomic copies of norovirus/gram of feces when ill, and may also shed pathogens when asymptomatic. Glove use is recommended as a preventative barrier when handling produce. But care must be advised as gloves can serve as a vehicle for cross-contamination. Comparative data is needed regarding the degree of contamination due to bare hand contact or glove use, and the likelihood of cross-contamination between them.

PURPOSE

To quantify and compare contamination of food and food contact surfaces after bare-hand contact (simulated using porcine skin sections) or contact with gloves, and to quantify cross-contamination occurring between bare hands and gloves if gloves are incorrectly applied with contaminated hands.

MATERIALS AND METHODS

Preparation of inoculum

Human norovirus GI.3 and GII.12 stool suspensions (2g/ml PBS) were clarified by centrifugation and filtration (0.2 µm), followed by ultracentrifugation at 100,000 x *g* for 1 hour at 4°C. The supernatant was removed and the pellet was re-suspended in 0.5 ml of PBS containing 5% fetal bovine serum (FBS). MNV-1 cell culture lysates were clarified similarly, followed by ultracentrifugation at 100,000 x *g* for 1 hr at 4°C, and pellet re-suspension in PBS containing FBS. An 8 log genome copies per ml cocktail of human norovirus and MNV-1 was prepared and combined with a norovirus-negative stool sample to create the virus stock for transfer studies using porcine skin and gloves. For lettuce studies a single virus stock was prepared by combining MNV-1 and a negative stool specimen.

Mechanical Transfer Device

- Device has donor and recipient port.
- Set to consistently deliver 1000 (± 100) g force per 4.4 cm^2.
- Constructed by Engineerable LLC. (Scottdale, GA).

Porcine skin grafts

- 4" x 4" sections were washed, fleshed, split, clean shaven and stored at -20°C
- Provided by Lampire® Biological Laboratories (Pipersville, PA).
- 15 min UV treatment before each experiment.

Glove Thickness and Brands

- Yellow Latex = High Five (5 mil)
- Blue Latex = Uniseal Safety (10 mil)
- Nitrile = Uniseal High Risk (15 mil)

Scenario I : Impact of different donor surfaces (bare hands and gloves) on virus transfer to stainless steel surfaces.

Scenario II : Virus transfer from bare hands to gloves during application.

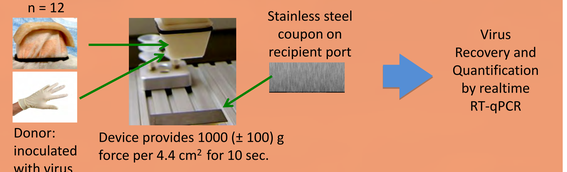
Scenario III : Virus transfer from contaminated hands and gloves to iceberg lettuce through multiple transfers.

ACKNOWLEDGEMENTS

This project was partially funded by a grant from the Center for Produce Safety at the University of CA-Davis (2010), the Center for Food Safety at the University of GA, and partially funded by NoroCORE, the United States Department of Agriculture-National Institute of Food and Agriculture, Food Virology Collaborative (2011-68003-30395).

RESULTS

Scenario I: Transfer from hands or gloves to food-contact surfaces



- Transfer rates of GII, GI and MNV-1 from porcine skin to stainless steel were 39.3%, 42.4% and 43.0%, respectively.
- Transfer rates were **higher** from gloves to stainless steel (53.4%, 61.0% and 56.9%) ($p<0.05$).
- Contaminated gloves can lead to more contamination of food or food contact surfaces than contaminated bare hands.

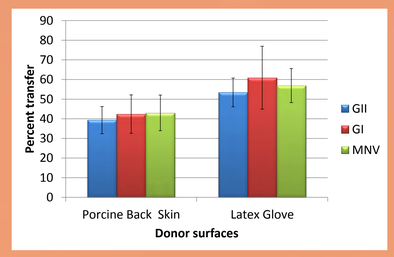


Figure 1. Percent transfer of human norovirus and MNV **from porcine skin or latex gloves (High 5) to stainless steel** after contact. Error bars = standard deviation.

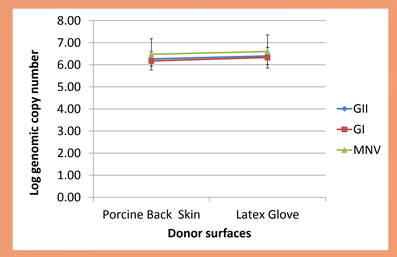
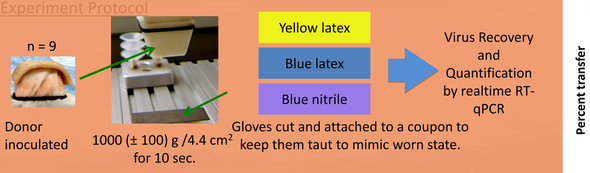


Figure 2. Log transfer of human norovirus and MNV-1 from **porcine skin or gloves (High 5) to stainless steel.** Error bars indicate standard deviation of the means.

Scenario II: Glove contamination by hands during application

Experiment Protocol



- Transfer rates of norovirus from porcine skin to gloves were found to be as high as 50%.
- Transfer rates of GII, GI, and MNV-1,from porcine skin to yellow latex (38.4%, 33.1% and 34.9%), to blue latex (50.8%, 51.9% and 41.0%), and to nitrile gloves (35.0%, 35.6% and 29.3%).
- Virus can easily transfer from contaminated hands to gloves during application.

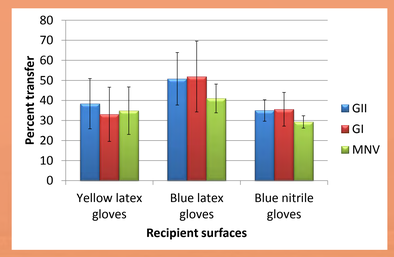


Figure 3. Percent transfer of human norovirus or MNV **from porcine skin to gloves** after contact to simulate contamination when putting gloves on. Error bars = standard deviation.

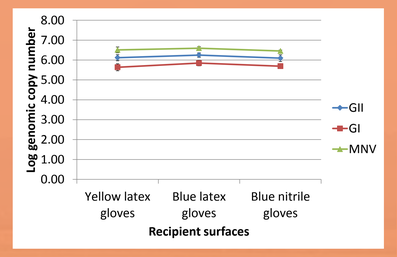


Figure 4. Log transfer of human norovirus and MNV-1 from **porcine skin to gloves.** Error bars indicate standard deviation of the means.

Scenario III: Sequential contamination of lettuce after touching with a contaminated hand or glove

- Viruses were recovered from recipient surfaces.
- Quantification of genome copy numbers by realtime RT-qPCR and comparison to RNA standard curve.
- Calculation of percent recovery by comparison to a positive control (recovery control).

- Virus transfer from a single donor to sequential lettuce surfaces was observed at the 10th lettuce surface atleast, for all donors tested.
- Percent transfers to the first iceberg lettuce surface touched by contaminated yellow latex, blue latex gloves, nitrile gloves and porcine skins were 66%, 54%, 38% and 42%, respectively.
- After touching 10 lettuce surfaces, norovirus was still detected on all replicate samples, but rates of transfer dropped to below 1% for blue latex gloves, nitrile gloves and porcine skins.
- Contaminated hands and gloves can contaminate large quantities of produce during harvesting, processing, packaging or distribution.

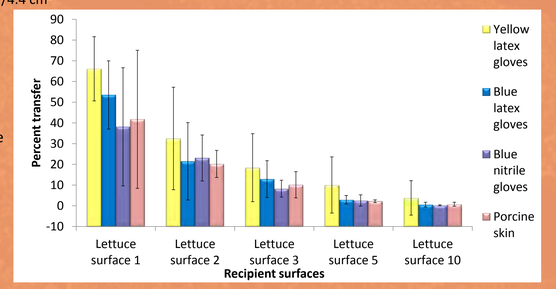


Figure 5. Percent transfer of MNV-1 from **porcine skin or gloves to sequentially touched pieces of iceberg lettuce** (up to 10 lettuce surfaces).

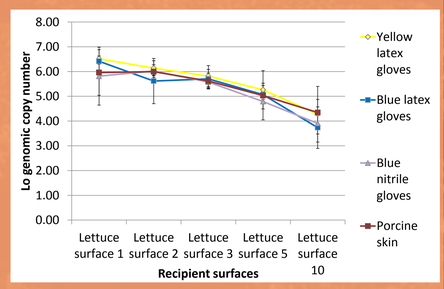


Figure 6. Log transfer of MNV-1 from **porcine skin or gloves to sequentially touched pieces of iceberg lettuce** (up to 10 lettuce surfaces).

SUMMARY AND CONCLUSIONS

The data obtained from this study help in developing quantitative models for estimating the risk of human norovirus contamination of foods and food-contact surfaces by food handlers if hand hygiene is inadequate. The study also supports the use of MNV-1 as a human norovirus surrogate for transfer studies, since there was no significant difference in transfer rates when compared to GI or GII human norovirus. As observed, gloves transfer more virus than bare hands, when contaminated. This shows hand sanitation is very important 'even' if gloves are being used when handling produce, as contaminated hands can easily contaminate gloves during application or wear, making glove use pointless. Results of the lettuce experiments showed that although the transfer percent goes down with consecutive touch, since the viral infective dose is very low, a little negligence can easily cause contamination and possible illness in the consumer of the product. Similarities between porcine skins and human hands will be investigated in the future when human volunteers are recruited for conducting similar norovirus transfer experiments.