THE LIKELIHOOD OF HUMAN NOROVIRUS CONTAMINATION OF PRODUCE DURING HANDLING AND THE REMOVAL OF FOODBORNE PATHOGENS FROM SURFACES AND HANDS USING A NOVEL CHARGED SANITIZING WIPE

by

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(Under the Direction of Jennifer Cannon)

ABSTRACT

Food workers have been implicated in recent foodborne outbreaks associated with ready-to-eat foods. Few sanitation measures exist that are effective in removing and inactivating a broad range of pathogens from hands and surfaces, including enteric, non-enveloped viruses. This study was designed to quantitatively investigate the level of human norovirus contamination that can occur during handling of small fruits. Positively-charged, sanitizing wipes were also investigated for their ability to remove murine norovirus, Hepatitis A, and *Salmonella enterica* from stainless steel and gloved hands, with variations in wipe charge, number of swipes, and concentration of a novel levulinic acid plus sodium dodecyl sanitizer. The data from these studies indicate that norovirus transfer to fruits during harvest can occur readily in the absence of hand sanitation, and the sanitizing wipes used in this study can reduce, but not completely eliminate pathogens from hands and food contact surfaces.

INDEX WORDS: Human Norovirus, Murine Norovirus, Hepatitis A, *Salmonella enterica*, Hand Wipes, Pathogen Transfer, Levulinic Acid, Sodium Dodecyl Sulfate

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DEDICATION

I would like to dedicate this thesis to my family and friends for their never ending love, support, and encouragement.

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CHAPTER 1

Introduction

Reducing foodborne illness requires implementation of preventative and control measures to reduce the risk of contaminating food. As human norovirus (NoV), hepatitis A virus (HAV), and *Salmonella* have been linked with foodborne disease outbreaks related to the consumption of contaminated ready-to-eat (RTE) foods via the fecal-oral route, they serve as model pathogens for testing the efficacy of hand and surface sanitizing solutions. Human noroviruses are the most common cause of nonbacterial acute gastroenteritis, responsible for approximately 81% of outbreaks in the United States *(12)*. The CDC estimates that nearly 23 million individuals are sickened each year as a result of human norovirus with 9.2 million of the illnesses considered foodborne *(66)*. HAV causes over 83,000 illnesses a year with greater than 4,000 of them considered foodborne *(66)*. *Salmonella* is responsible for ~1.4 million illnesses and greater than 1.3 million cases are foodborne, *(66)* where humans are thought to be a significant source of contamination. These foodborne pathogens can be found in contaminated water and food, on hands, and food contact surfaces. Transfer to food readily occurs by contact with contaminated surfaces, but the microorganisms, especially human NoV, are also spread directly from person to person *(12, 46)*. Hands are thought to be an important vehicle in transfer and contamination. Of foods commonly contaminated with foodborne pathogens, RTE foods are often implicated because they lack a thermal processing step during production and may be eaten directly out of packaging. Among RTE foods, produce has been increasingly responsible for outbreaks of human NoV, *Salmonella* and HAV. Recent government and industry campaigns to

increase consumption of fresh fruits and vegetables in the United States have been met with improvements in agricultural practices, processing, storage, and transport. This in turn, has increased the availability and demand for many RTE products, including produce *(4, 9)*.

Hand-picked, RTE fruits and vegetables are highly susceptible to contamination, especially by human NoV as they are often harvested directly by hands, which may have high levels of virus due to fecal contamination. Previous studies assessing the transfer rate of norovirus from hands to lettuce and stainless steel have yielded as high as 47% transfer *(11, 26)*. Additionally, a low infectious dose (1-10 infectious viruses), the ability to persist on inanimate objects, and survival during low temperatures means that norovirus is likely to remain infectious and cause infection when the contaminated produce reaches the consumer *(8, 18, 90)*. While it is evident that fruit and vegetables are vehicles for NoV and contamination can directly follow handling by food workers, less is known about the likelihood of contamination from the field worker to hand-picked produce such as small berries during harvest and little quantitative data exists for risk assessment modeling. Few studies exist that replicate real-life situations which could cause contamination of produce during handling, and even fewer use starting contamination levels representative of the amount shed by persons infected with norovirus.

Hand and surface sanitation, including sanitizing wipes, remain an important intervention step in the transfer and contamination of pathogens to food and food contact surfaces. While handwashing is considered to be effective in reducing transfer of pathogens from hands to food *(6, 11, 72)*, it may not always result in a significant reduction of pathogens. A lack of proper washing facilities, improper washing techniques, or complete neglect of washing all contribute to increased chances of food contamination. Hand wipes are appealing both as alternative to or in conjunction with handwashing because they are simple to use and portable. Cleansing wipes

have been used for many applications ranging from hand and baby wipes to electrostatic wipes that attract dust. Many of the moist wipes available include sanitizers that are ethanol based, providing protection against bacteria but not from non-enveloped viruses, which are commonly resistant to ethanol *(59)*. Enteric viruses and bacteria including norovirus, Hepatitis A, and *Salmonella* possess a net negative surface charge when their surrounding environment is at neutral pH. Positively charged filters have been employed as a means of pathogen collection when filtering water samples *(56, 57)*. However, the ability of positively charged dry hand wipes to attract viruses and bacteria have not been examined. Additionally, as many commercially available wet wipes containing an ethanol based hand sanitizer are ineffective in the inactivation of enteric viruses, a levulinic acid plus sodium dodecyl sulfate (SDS) sanitizer recently developed at the Center for Food Safety, University of Georgia (UGA) may serve as a viable alternative for improving sanitizing wipe efficacy. Combinations of this sanitizer have proven to be effective against *Salmonella* and *E. coli* O157:H7 as seen by >7 log reduction within 10 seconds *(95)* and against murine norovirus as evidenced by a >4 log reduction within 1 min when used in a liquid solution *(17)*. While a combination of levulinic acid and SDS has been tested in solution and on food surfaces, the sanitizer has not been tested for its efficacy on pathogen removal and/or inactivation on hands and food contact surfaces when used as a sanitizing wipe.

CHAPTER 2

Literature Review

Public Health Impact of Foodborne Disease

The health impact from foodborne illness in the United States is evidenced by 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths estimated to occur annually *(66)*. Of the 13.8 million illnesses due to a known pathogen as the etiologic agent an estimated 4.2 million are due to bacteria, 350,000 due are to parasites, and 9.2 million are due to viruses *(66)*. The annual economic impact of foodborne illness in the United States caused from 6 bacterial and 1 parasitic agent is estimated to cost between 6.5 and 34.9 billion dollars *(16)*. The scope of the damage caused by foodborne illness stretches far beyond the morbidity and mortality of humans to include lost wages, medical expenditures, decreased work productivity, and the deterioration of a company's image *(16)*. Currently, there are over 250 etiologic agents, several of which create health and economic damage related to foodborne illness and merit further investigation to ensure the continuing safety of food *(3)*.

Foodborne illnesses impact all segments of the food supply chain including processing, distribution, and retail with potential contaminants including toxins, heavy metals, and infectious agents such as bacteria, viruses, and parasites *(66)*. Infectious agents are responsible for the majority of cases of foodborne illness and several recent high profile outbreaks highlight their potential severity. For example, a 2006 outbreak of *E. coli* O157:H7 spanning 26 states and sickening over 200 people was traced back to bagged spinach *(3, 93)*. Between 2002 and 2005, a recurrent outbreak of *Salmonella* sickened people in sixteen states due to contaminated raw

tomatoes *(83)*. In 1997, an outbreak of norovirus in Quebec stemming from consumption of raspberries sickened over 200 people *(34)*. During 2008-2009, over 500 people in the United States and Canada were sickened after consumption of *Salmonella* tainted peanut butter *(67)*. For decades, food safety has focused on inactivating or inhibiting growth of pathogens that may be present on food, therefore much emphasis in fighting foodborne illness has depended on prevention. In determining how to best implement preventative measures such as Hazard Analysis and Critical Control Points (HACCP) and employee education programs to reduce the risk of contamination, it is essential to understand how food becomes tainted with pathogens including what the sources are, modes of contamination, stability on food, and the role of the food handler both in terms of cause and prevention.

Pathogens Known to Contaminate Produce

Salmonella is the second most frequent cause of bacterial foodborne illness *(66)*. Annual sickness in the U.S. from *Salmonella* is estimated at 1.4 million illnesses, 200,000 hospitalizations, and 500 deaths *(66)*. It is a gram-negative non-sporeforming enterobacter with a majority of human illness cases caused by *S. enterica (75)*. Both wild and domestic animals including poultry and their eggs can naturally harbor the bacteria and consumption of improperly cooked meat has been linked to *Salmonella* illness. Reptiles, including snakes and turtles, can also carry the bacteria. Many varieties of produce may be contaminated, either from soil, irrigation water, animal feces (from animal manures or wild-life), or contact with contaminated equipment. There are over 2,700 serotypes of *Salmonella*, many of which have been associated with multi-state outbreaks stemming from contaminated produce *(38)*. Produce previously implicated in outbreaks of salmonellosis includes tomatoes, melons, and peppers *(38, 69)*. A study monitoring *Salmonella* presence on produce found that the pathogen could persist on

tomatoes and peppers at 12°C for up to 7 days, and on chopped peppers and tomatoes growth was evident when produce was stored at 12 and 21°C *(63)*. *Salmonella* can also persist in soil surrounding tomato crops for more than 45 days and on tomato surface for 14 days *(38)*. After contamination of produce, pathogens may continue to multiply, as evidenced by Ma et al. who found that *Salmonella* could grow on sliced tomatoes and jalapenos at 12°C *(63)*. Even after post-harvest washing with water, contaminated produce has the potential to transfer the pathogen to other utensils and preparation surfaces and cause illness. *Salmonella* is resistant to freezing but heat and ultraviolet light can readily destroy it *(89)*. It is susceptible to ethanol-based hand wipes and sanitizers, which can be effective in preventing transmission both on hands and preparation surfaces *(73)*.

Hepatitis A (HAV) is a non-enveloped, single stranded, + sense RNA virus that is part of the Hepatovirus group of the Picornavirus family. Infection leads to liver disease as the virus infects the hepatocytes and liver macrophages *(5)*. The infection is a self-limiting disease and rarely results in chronic infection or chronic liver disease, without underlying liver disease. The infectious dose is estimated to be between 10 and 100 virus particles *(2)*. With an incubation period of approximately 30 days, initial symptoms include fever, nausea, abdominal pain, and diarrhea. Typically, the characteristic symptom of liver disease, jaundice, occurs 7-14 days after initial symptoms *(5)*. Once infected, symptoms may last for two months and in some cases become recurring for up to six months. It is estimated that annually 10 million people are affected worldwide although infected children often may not exhibit any symptoms and mortality is unlikely *(5)*. As of 2000, it is estimated that in the U.S., Hepatitis A cases surpassed 83,000 annually, of which more than 4,000 have been determined to be foodborne *(66)*. HAV infection can be confirmed through HAV specific IgM antibodies collected in the serum of patients *(2)*.

Mammalian cell culture assays can be used for propagation of HAV *(54)*. However, wild type HAV does not initially replicate well in cell culture, but with continued passaging of mammalian cells and further replication of HAV, cell culture HAV variants have arisen *(54)*. It is thought that mutations in the RNA of HAV variants alter the virus phenotype to better utilize host cell factors necessary for translation and replication *(54)*. Tracing the source of an HAV outbreak is difficult due to an incubation period of ~4 weeks. Transmission among people is most common through the fecal-oral route, either directly from one person to another or by consumption of foods contaminated with feces *(5)*. While foodborne outbreaks stemming from produce such as strawberries and green onions are less common compared to norovirus and *Salmonella*, shellfish harvested in polluted waters have often been implicated as a source of HAV illness *(30)*. In addition, in 2002, an outbreak stemming from HAV contaminated green onions sickened more than 640 people in Ohio and Pennsylvania *(28)*. A vaccine currently exists as Hepatitis A has only one serotype, despite the existence of multiple genotypes*(24)*. Hepatitis A vaccination is currently the best measure against HAV infection, while good hygiene including handwashing is recommended as a preventative measure. Inactivation of HAV is difficult as it is resistant to low pH and survives for up to 1 hour at 60°C *(55)*. Complete inactivation has been observed in near boiling water at 98°C for 1 min of exposure time *(51)*.

Human norovirus (NoV) is a single stranded, + sense, non-enveloped RNA virus that is member of the Calicivirus family *(94)*. Causing roughly 67% of the cases of foodborne gastroenteritis and the leading cause of acute gastroenteritis, this virus is estimated to sicken more than 23 million people in the U.S. annually *(94)*. Reasons for the high incidence include a low infectious dose of 1-10 particles, high viral shedding of up to 10^{12} virus/g feces, resistance to ethanol, low pH, and many common surface disinfectants, and the lack of an antiviral or vaccine.

Symptoms include nausea, diarrhea, vomiting, and stomach pain and can last between 6 hours to five days, with symptoms typically lasting less than 24 hours *(94)*. Some individuals may be infected with NoV and exhibit few or none of the typical symptoms but still shed the virus. NoV is generally non-lethal although it can be if severe dehydration results from fluid loss. Additionally, NoV can persist and remain infectious on fomite surfaces for over seven days on dry surfaces *(26)*.

Human NoV cannot be cultured via a mammalian plaque assay so detection relies on real-time reverse transcriptase PCR (rt-PCR) *(26)*. As this is the case, surrogate viruses that are similar to noroviruses in terms of genetic structure and organization are used in infectivity studies, the most common of which are murine norovirus (MNV) and feline calicivirus (FCV). Foods that are often implicated in norovirus outbreaks include green onions, small berries, raw oysters, lettuce, and drinking water *(94)*. With such high viral shedding, NoV is easily transferred from hands to surfaces, putting RTE foods at risk *(11, 26)*. The fecal-oral route is the most common means of transmission among people although aerosolization following vomiting can sicken others by contaminating food or directly from person to person *(91)*. As many as 3 x 10^7 virus particles may be present in a 30 ml bolus of vomit which can aerosolize and disperse during vomiting *(19)*. Outbreaks rapidly spread throughout close quarters such as cruise ships, nursing homes, prisons and hospitals. An issue of concern with human norovirus is its stability in the environment as it has been detected in samples of groundwater stored at 12°C for greater than 2 years using rt-PCR as a detection method *(20)*. As it is non-enveloped, NoV is resistant to ethanol hand sanitizers but inactivation results from heat and chlorine treatments *(27)*. Handwashing after using the restroom has been determined as effective in reducing the risk of transmission *(6)*.

NoV is classified under five genogroups, which can be subdivided again into genetic clusters or genotypes. Most human infections are the result of the genogroup I or II with the genogroup (GII) genotype 4 (GII.4) cluster responsible for a majority of the outbreaks *(94)*. NoV outbreaks are thought to have a general seasonality, with an increase in outbreaks occurring during winter months.

Ready-to-Eat Foods and Fresh Produce

All foods can become contaminated with pathogens; however, foods posing a greater risk of exposure include fresh produce and ready-to-eat (RTE) foods such as lunch meats, bakery items, fresh fruits and vegetables, and prepared salads and sandwiches. RTE foods have seen a significant increase in availability in recent years due to advances in cultivation, cold storage and transport, food preservation, packaging, and consumer demand for convenience products *(43, 69)*. These foods often undergo minimal or no thermal processing prior to human consumption and often are eaten right out of the packaging. Contamination of RTE foods can occur in the supply chain or at the origin. The lack of a thermal processing step is disadvantageous as pathogens can persist long enough to be consumed. Although many RTE foods are transported under cold conditions, this only slows or prevents growth of most bacteria and fungi instead of killing them. Heating RTE foods prior to eating can destroy or inactivate microbes, but heat application may also alter the color, texture, nutritional content, and taste of the final product. Non-thermal processing methods such as chemical sanitizers, gamma irradiation, high hydrostatic pressure, ultraviolet light, and high voltage pulse electric fields (PEF) can inhibit growth or reduce bacterial and fungal numbers prior to final packaging while retaining many of the physical and organoleptic characteristics that are lost with thermal heating *(22, 44, 86)*. However, these processes can be costly, time consuming, and may require skilled laborers for

operation. In addition, such processing steps are not appropriate for all foods and have the potential to alter the sensory properties of the food.

Prepared and fresh fruits and vegetables have been implicated as a source of foodborne illness at increasing rates over the past four decades with tomatoes, lettuce, hot peppers, cantaloupes, berries, and sprouts as products considered to have a high risk of contamination *(62)*. Recent health initiatives by both government and private sectors are pushing for the increased consumption of fresh fruits and vegetables. Initiatives such as "Fruits & Veggies More Matters" emphasize the importance of eating five servings of fresh fruits or vegetables daily *(58)*. This has led to increased production and wider availability of fresh produce year round, facilitated by improved agronomical practices and harvesting methods, cold chain storage and distribution, and globalization of many food industries.

Produce Sanitation

One reason for the increased risk associated with fresh produce is that there is no thermal "kill" step. While non-thermal steps including chemical sanitizers, water rinses, or irradiation are implemented, a standardized method for produce sanitation does not exist. Sanitizing produce can be particularly difficult, especially if pathogens become sheltered in crevices and indentions on the surface of the produce, shielding them from sanitation steps such as rinsing and chemical sanitizers *(48, 49)*. While produce may receive a wash/rinse in water after harvest, not all fruits can be soaked in water or a sanitizing solution as it may alter organoleptic properties or hasten the growth of fungi. A study measuring the efficacy of soaking and rinsing produce with water yielded as high as a 2.89 log reduction of *Listeria monocytogenes* on tomatoes and apples *(48)*. Soak and rinse methods are less effective on produce with a coarse surface such as broccoli. For example, a 1.88 log reduction of *Listeria monoycytogenes* was observed after

rinsing broccoli with water, indicating the morphology of the produce surface may affect bacterial attachment and how well pathogens may be eluted off *(48)*. Shredded lettuce inoculated with 7 logs of murine norovirus as a surrogate model for human NoV yielded 1.14 log reduction after soaking and washing in water for 5 minutes *(7)*. However, after soaking and washing the shredded lettuce in a 200 ppm solution of bleach for 5 minutes, a >2.18 log reduction was observed *(7)*. On cantaloupes and honeydew inoculated with 6 logs of *Salmonella*, soaking the fruit in water for 1 minute yielded a 0.7 log reduction *(79)*. Alternatively, the same procedure was applied to the cantaloupe and honeydew but with a 200 ppm solution of bleach and a 1.8 log reduction was observed *(79)*. Soaking and scrubbing the cantaloupe and honeydew with a vegetable brush in both water and a 200 ppm bleach solution yielded a 0.9 increase in log reduction from the initial inoculation site, but with the water only scrub, *Salmonella* was detected on other portions of the rind and on the brush *(79)*.

Current commercial methods of soaking produce include soaks in chlorinated water with concentrations of 50-200 ppm, however the reduction of bacterial pathogens is often less than 2 logs *(48)*. Numerous other chemical sanitizers have been tested including chlorine dioxide, hydrogen peroxide, organic acids, and calcium solutions, but have shown no significant benefit over chlorinated water in reducing bacteria *(50)*. Both acidic electrolyzed water and ozonated water containing 5 ppm ozone have shown similar log reductions of coliforms and fungi on strawberries and cucumbers when compared to chlorinated water *(50)*. While numerous sanitizers exist, no one sanitizer is appropriate for all produce and further investigation is merited.

Gaseous sanitizers such as chlorine dioxide and ozone have been used for sanitation of produce but have also yielded mixed results with some studies showing less than 2 log

reductions for *Salmonella* after 30 minutes of chlorine dioxide exposure while others have measured a 5 log reduction for *Salmonella* in the same amount of time *(53, 82)*. Ozonation of orange juice inoculated with 8 logs of *E. coli* O157:H7 has yielded greater than 6 log reduction after 1 minute of exposure *(80)*.

Irradiation is considered an effective method of inactivating pathogenic bacteria and parasites on food *(92)*. At 1.5 kilogray (kGY) of gamma radiation, *Listeria monocytogenes* was completely inactivated on tomatoes. Higher doses of 10-50 kGy were required for similar levels of inactivation of foodborne viruses *(25)*. These higher doses have shown measurable loss of water soluble vitamins and in some foods a loss of color and flavor *(25)*. Concerns of public health near irradiation facilities, negative ad campaigns, and a lack of consumer acceptability stemming from false perceptions of health issues related to consumption of irradiated foods have hindered efforts to increase the presence of irradiated food in the United States *(25)*.

While numerous chemical and non-thermal sanitation steps have been used by the food industry, currently there does not exist a standardized method for complete inactivation/removal of pathogens from fresh produce or one method that can be applied to all produce. In the effort to reduce the likelihood of contamination during harvesting and processing of fresh fruits, implementing more or improving upon current preventative measures is essential.

Role of Food Workers in Food Contamination

Another way in which fresh produce and RTE foods can become contaminated is by food workers. By definition, a food worker is anybody who conducts work related to harvesting, processing, preparation, and cooking *(37)*. Improper hand sanitation, handling fresh produce and RTE foods without gloves or with contaminated gloves, unsanitary preparation areas, time/temperature abuse, and undercooking of foods are all means by which workers are

responsible for contaminating or allowing pathogens to persist on already contaminated food. In a review of foodborne outbreaks from 2000-2006, it was discovered that of the 227 foodborne outbreaks involving food workers with a pathogen as the causative agent, 179 were due to viruses, 42 from bacteria, and 6 due to parasites *(37)*. Improved molecular epidemiology combined with more rapid and sensitive detection methods will likely confirm that a much higher percentage of illnesses stem from food workers.

Infected food workers can harbor and shed hundreds of billions of pathogens, which can contaminate food and sicken people by many different routes of transmission. The most common means of transmission of foodborne pathogens among the sick, colonized or convalescent to healthy individuals is via the fecal-oral route *(91)*. However, individuals afflicted with a pathogen-caused foodborne illness can also contaminate food and food contact surfaces and spread the disease directly to other people through aerosolized vomit, and in rare instances through urine and sweat *(91)*. Individuals afflicted with enteric pathogens typically have semi-solid or liquid stools, increasing the likelihood that feces can penetrate the toilet paper and contaminate hands *(91)*. During illness, an individual may shed as high as 10^{12} infectious particles/g of feces as is the case with norovirus while bacterial concentrations of *Salmonella* may be as high as 10^{9} colony forming units/g of feces *(91)*. Another reason by which food workers can transmit their illness is that individuals who appear healthy can still shed infectious pathogens. A study conducted in Japan among 55 norovirus outbreaks discovered that both symptomatic and asymptomatic individuals shed similar viral loads *(78)*. This presents a risk that seemingly healthy workers may contaminate food. Vomitus presents another human source of pathogens that may directly contaminate hands and nearby surfaces through aerosolization *(21, 60)*. The risk of airborne transmission with norovirus is high as it has been determined that

individuals in a hospital walking in close proximity with a vomiting patient have become sickened with norovirus *(84)*. Though a less likely source of contamination, pathogens including Hepatitis A and *Salmonella* have been detected in urine if a systemic infection has occurred *(91)*.

Food workers can contaminate food either through cross-contamination or direct contact. Hands or gloves can become contaminated with pathogens after contact with previously contaminated fomite surfaces and food, following restroom use, after vomiting or through infectious cuts and abrasions *(91)*. Improper hand sanitation in restroom facilities allows for some pathogens to remain on the hands of individuals in high quantities, but also presents the risk for leaving pathogens on contact surfaces such as door handles, which can transfer to healthy individuals upon subsequent contact *(87)*. While handwashing is mandatory for employees in foodservice settings, it is difficult to enforce and even those that wash their hands may do it insufficiently. A study looking at awareness and compliance among food workers in Minnesota found that only 52% of kitchen managers knew the handwashing guidelines from the state's health code and only 48% of kitchen workers could demonstrate a hand wash that complied with the state health code *(1)*. Improper hand sanitation has shown to greatly contribute to the likelihood that handlers will contaminate food. Food worker's hands can become contaminated with transient pathogens after contact with fomite surfaces and contaminated food, or through infectious cuts and abrasions *(91)*. Once on the hands, pathogens can remain in crevices and pores, even after a handwashing application *(23, 29, 72)*. Hand sanitation will decrease the likelihood that food is contaminated but implementation must include the compliance of food workers, employee training, proper documentation and instructions, and available facilities.

Likelihood and Degree of Pathogen Cross-Contamination During Handling

Once on the hands, a pathogen may be readily transferred to foods or food contact surfaces. A study determining the risk of Hepatitis A virus contamination from hands to lettuce during handling found that 9.2% of the virus was transferred *(10)*. Norovirus transfer from hands to food, using feline calicivirus as a surrogate, was found to be as high as 46% when inoculated finger pads came into contact with ham *(11)*. However, when *Salmonella* transfer was measured from food workers to bell peppers, the transfer rate was 0.84% for gloved hands and 0.21% from bare hands *(45)*. Although the transfer rate may vary widely, with 9 or more logs of pathogen potentially contaminating a hand, the likelihood of illness remains high as even a 1% transfer of virus leave enough virus on the food surface to cause infection after consumption. Transfer rate is likely determined by the morphology of a food's surface, the amount of pressure applied to the area during contamination, and the moisture content of the surface, but further research requires collecting quantitative data to build a risk assessment model.

In a study examining the likelihood of norovirus contamination, fingers that touched toilet paper inoculated with 150 μl of fecal suspension containing norovirus showed that after consecutively touching 8 plastic surfaces, which re-created a scenario where 8 household objects were touched, as many as 7 of the surfaces then tested positive for norovirus *(8)*. Secondary transfer was then observed by taking clean hands and touching plastic coated surfaces which were artificially contaminated with norovirus followed by touching clean contact surfaces, and 4/10 door handles, 5/10 telephones, and 3/10 bar taps tested positive for norovirus *(8)*. Another study by D'Souza et al. found that norovirus is readily transferred from stainless steel to both wet and dry lettuce when 10 g of pressure were applied to 9 cm^2 surface area, even when the

inoculum was allowed to dry for 10 min *(26)*. The overall number of lettuce samples testing positive for norovirus was higher when the lettuce was moist, indicating that the presence of moisture increases virus transfer *(26)*.

Cross contamination of food results when foods come into contact with previously contaminated surfaces, equipment, utensils, and other foods. Besides hands, raw ingredients and utensils are also thought to be sources of cross contamination *(23)*. A study measuring the potential for cross-contamination of *Campylobacter* from touching raw chicken found that >3 logs were present on hands, >2 logs on cutting boards and knives, and >1 log of bacteria on cucumber slices cut with a contaminated knife *(61)*. A study identifying the transfer of *Salmonella* from pork to hands found that as high as 38.8% of the pathogen could be detected on gloves *(41)*. Kitchen sponges contaminated with *Salmonella* can transfer as high as 29% of the bacteria to stainless steel kitchen surfaces *(52)*. Even if a pathogen is present on the non-edible outside portion of fruits and vegetables, cutting into the food with a knife can contaminate the edible flesh. A study measuring transfer from the contaminated rind of an orange to the inner tissue by cutting found that when the fruit had 3.6 log CFU/ cm^2 of *E. coli* O157:H7 on the surface, after cutting the orange in half with a knife, the knife had an average of 1.2 log CFU/ cm^2 of *E. coli* and the extracted juice an average of 0.4 logs *(65)*.

While food workers in both the harvest environment and retail foodservice represent a major source of contamination, microorganisms can also be found in and around the fields and farms where produce is grown and harvested. Foods may become contaminated by runoff or contaminated irrigation water containing pathogens *(39, 74)* from untreated or improperly treated sewage, animal manure and compost, runoff from adjacent land, and water used for washing can also carry harmful pathogens if it becomes contaminated *(9)*. Wash and rinse water may contain

pathogens from contaminated produce, contaminating all produce washed afterwards. Additionally, irrigation and flood water drainage may carry pathogens from animal feces to fields of produce *(76)*.

Persistence of bacteria and viruses on fomite surfaces can last for hours to weeks, leading to contamination of food if the contact area is not sanitized prior to use. While plants are not likely to internalize pathogens via their roots, contaminated water may come into contact with edible portions of the plant where pathogens can persist long enough to reach consumers *(32)*. Many pathogens including *Salmonella*, *Listeria monocytogenes, Clostridium botulinum,* and *Bacillus cereus* can also be found in soil where produce may come into direct contact *(9)*. Animal manure is often incorporated as a fertilizer and may carry pathogens if it is raw, not properly composted, or re-contaminated after composting *(75)*. Some pathogens are capable of surviving on produce for periods of time greater than 7 days. *E. coli* O157 H7 has shown to persist in soil for greater than 90 days and survive on lettuce for more than 15 days *(42)*. Animals present another source of pathogens both in fields and processing plants with birds, rodents, reptiles, and insect vectors representing the main source of *Salmonella* and *Campylobacter* in the environment *(68)*. While these animals may harbor bacterial pathogens on their skin or hair and can come into direct contact with produce, their feces may also contact fruits and vegetables prior to harvest, increasing the risk of illness not only from consumption but also that both human and machine harvesters may transfer the pathogen to other contact surfaces.

Methods for Hand Sanitation and Surface Disinfection and Interruption of Pathogen Transfer

Handwashing

Common methods for the disinfection or removal of pathogens from hands and fomites include handwashing and drying, hand sanitizing wipes, and sanitizing solutions. Handwashing is a common method of cleaning that is recommended both before and after working with food, although it does not completely eliminate pathogens *(6, 31, 72)*. Both the American Society for Microbiology and the Centers for Disease Control and Prevention recommend that proper handwashing include lathering of wrists, palms, back of hands, fingers, and under finger nails with soap and warm water for 15-20 seconds followed by rinsing with warm water and drying with a clean towel or air dryer *(71)*. The ability to remove pathogens from hands depends upon external force that is equal to or more than the adhesion of the pathogen *(14)*. Brouwer et al. note that handwashing can be categorized into three steps: "mechanical action, hydrodynamic drag, and wet chemical action" *(14)*. Handwashing is simple and economical, it can be a highly effective mechanism for prevention of foodborne disease. Worker negligence, improper washing methods, or a lack of adequate handwashing facilities can obstruct positive disease outcomes. While glove use is considered a barrier in preventing contamination from hands, incorrect usage may allow for hands to contaminate the outside of clean gloves. An observational study of 321 food workers in retail and home settings found that during only 27% of the activities where food was handled with gloves, a worker had properly washed their hands previously with warm water and soap by vigorously scrubbing for a minimum of 15 seconds and then drying with a cloth or paper towel *(36)*. Additionally, food workers are more likely to wash before initially starting to work with food, but not on a consistent basis afterwards and also more likely to wash appropriately only when work was involved that did not require the use of gloves *(36)*. For all

activities related to food preparation where gloves were not used, handwashing was attempted 51% of the time and handwashing was correctly attempted just 45% of the times *(36)*. Handwashing may reduce the likelihood that foods may become contaminated, but a combination of treatments to increase the reduction of pathogens from hands may be required to further reduce the risk.

Hand Wipes

Hand wipes are another method of removing microbes off of skin with physical manipulation and possibly inactivating microbes by chemical disinfection *(14)*. They are beneficial both in terms of portability and their ease of use. Many of the wipes commercially available are used for cleansing or sanitizing purposes with the difference being that sanitizing wipes can inactivate some pathogens while cleansing wipes will only remove them. Efficacy of hand wipes is based upon the medium being wiped, surface size of the contaminated area; whether or not there is a wetting medium, the type and number of swipes, wipe material, and variations among skin surfaces. Similar to handwashing, the main goal of hand wipes are to provide an external force that is equal to or greater than the adhesion of the pathogen to the skin, resulting in removal *(14)*. In a study comparing handwashing with non-antimicrobial soap versus ethanol based hand wipes, using *Serratia marcescens* and MS2 bacteriophage, a maximum of 0.84 and 0.21 log reductions were recorded for hand wipes containing 40% ethanol while 1.87 and 2.03 logs reductions were observed for *Serratia marcescens* and MS2 bacteriophage, respectively using the soap *(88)*. In a comparison of the efficacy of hand hygiene practices in the removal of pathogens using handwashing or hand wipes, it is likely that handwashing will remove a greater percentage than wipes *(15, 88)*. Although not the most

efficient method of removing pathogens from skin, hand wipes may be effective when handwashing facilities are not available or as an additional step post-washing.

Hand Sanitizers

Liquid, gel or foaming sanitizers present a different method of hand sanitation in that they rely on pathogen inactivation or killing instead of removal with mechanical force. While many sanitizers exist, those that are alcohol based remain the most common. They are used in a variety of situations and have shown to supplement prevention and control strategies for reducing absenteeism in schools, reduce the risk of cross-contamination during handling of food, and decrease the prevalence of infections in health care settings *(33, 40, 85)*. Alcohol sanitizers are ineffective against many non-enveloped viruses, which lack a lipid outer layer. Viral pathogens such as norovirus and hepatitis A virus are likely not inactivated upon exposure *(59)*. Chlorine based sanitizers with >200 ppm chlorine are effective against non-enveloped viruses, but they can irritate skin and are less effective in the presence of organic material *(95)*. In the presence of fecal material, a solution of 5,000 ppm chlorine was used to disinfect 14 household contact surfaces, and it was found that 28% of the surfaces still tested positive for human norovirus using rt-PCR after 5 minutes of exposure with a cloth soaked in the bleach solution *(8)*. Sanitizers can come in the form of liquid, gel, or foam. A comparison on the efficacy of handwashing, hand wipes, and hand sanitizers found that log reductions for *Clostridium difficile* averaged 2.14 logs for warm water and soap, 1.88 logs with cold water and soap, 0.57 logs for antiseptic hand wipes, and 0.06 logs for an ethanol based sanitizer *(77)*. Recent research regarding developing new hand sanitizers has included using ethanol-based sanitizers in conjunction with new chemicals to create a broad range disinfectant as well as the development of novel sanitizers. A recently developed ethanol based sanitizer from GOJO industries

containing 70% ethanol vol/vol combined with a synergistic blend of polyquaternium-37 and citric acid has shown log reductions in viral suspension tests of >4.74 for feline calicivirus (FCV), >3.67 for murine norovirus (MNV), and 1.75 for hepatitis A (HAV) *(64)*.

Levulinic Acid Plus Sodium Dodecyl Sanitizer (SDS)

Researchers at the Center for Food Safety in Griffin, Georgia have recently developed a levulinic acid plus sodium dodecyl sulfate sanitizing solution that is capable of >7 log reduction of *E. coli* O157:H7 and *Salmonella* on chicken carcasses and lettuce within 10 seconds at 21°C *(95)*. Organic acids, such as levulinic acid, have shown to increase their efficacy as antimicrobials in the presence of surfactants *(81)*. While levulinic acid by itself has shown <1 log reduction of *Salmonella* over 30 minutes, in conjugation with SDS the synergistic effect of the two compounds has yielded as high as an 7.2 log reduction of *E. coli (95)*. Levulinic acid is generally recognized as safe by the U.S. Food and Drug Administration *(95)*. While its efficacy as a anti-bacterial has been noted, less is known about the capabilities of levulinic acid/SDS and its efficacy against foodborne viruses both *in vitro* and *in vivo* scenarios.

Microorganism Interaction with Positively Charged Surfaces

Positively charged water filters have been used for decades as a means of concentrating and recovering pathogens from water *(56)*. These wipes have a net positive charge on their surface either from the wipe material or from the addition of a cation. Pathogens with a net negative charge on their surface are attracted to these positively charged wipes by means of electrostatic interactions. As viruses are much smaller than bacteria, with their diameter typically ranging between 10-300 nm, the pore size of the filter would need to be smaller than this to capture any virus *(57)*. A positively charged filter aids in capturing the virus without the slowed filtering rate seen with a smaller pore size filter. Gram-negative bacteria such as

Salmonella contain lipopolysaccharide (LPS) in their cell membrane. While contributing to overall stability, LPS is responsible for the negative charge of the cell membrane. In foodborne viruses, the capsid proteins of NoV and HAV have a net negative charge at neutral pH. This is attributed to the isoelectric point (pI) of the proteins, which is the pH value at which a molecule or surface has a net neutral charge. Norovirus genogroups GI and GII have pI values ranging from 5.2-5.7 and HAV estimated to be between 2 and 6, and it is thought that most viruses have a pI value between 3 and 7 *(35, 70)*. At pH values above the pI, a molecule will have a net negative charge and at pH values below the pI, a net positive charge will exist. Recovery of adenovirus has been measured using positively charged water filters. It was determined that as high as 91% of adenovirus could be recovered in solution while recovery of poliovirus 1 was 96% *(56, 57)*. The United States Environmental Protection Agency recommends that for the collection and concentration of enteric viruses from water, the Virosorb 1MDS (Cuno, Meriden, CT) brand positively charged filter be used *(47)*. This filter contains charge-modified glass in a cellulose medium. These filters are not used for extensive virus monitoring because they have a high cost and do not work for all viruses *(47)*. Cost efficient alternatives have been tested including the NanoCeram (Argonide, Sanford, FL) filter yielding similar or higher percent recoveries when compared to the 1MDS filter. The NanoCeram filter contains nano alumina fibers woven in a microglass fiber matrix *(47)*. While charged water filters are effective in yielding a high percent recovery of viruses and bacteria in solution *(47, 56)*, these filters have yet to be tested under dry conditions as wipes for the removal of pathogens from hands and food contact surfaces. Therefore, the potential for removal of pathogens by electrostatic interactions using positively charged wipes should be assessed.

CHAPTER 3

Materials and Methods

Human Norovirus Transfer

***Virus Stock Preparation and Quantification*.** Stool specimens were obtained from an outbreak of human NoV genogroup II (a gift of Andrea Maloney, South Carolina Department of Health and Environmental Control) and an outbreak of GI norovirus (obtained from an anonymous donor with a child ill with gastroenteritis). Each was prepared in a 20% suspension in phosphate buffered saline (PBS) pH 7.2 and stored at -80°C until used. For MNV, a cell culture lysate was purified by centrifugation at 2,000 x g for 15 minutes at 20°C and filtered using a 0.2 µm filter (Millipore) and concentrated by ultracentrifugation at ~85,000 x g for 1 hour at 20°C and resuspension in 1/10 the volume of the supernatant. A 20% suspension was made by combining this supernatant with a stool specimen that tested negative for GI or GII norovirus. Viral RNA was extracted using a Viral RNA mini-kit (Qiagen, Valencia, CA) and amplified by real time-RT-PCR (Step One, Applied Biosystems, Foster City, CA) using NoV specific primers and probes (8, 10) and a Quantitect one-step RT-PCR kit (Qiagen). Viral RNA was quantified by comparison to a standard curve of NoV RNA transcript of a known concentration. The GII stock was determined to be 11 logs of genomic copies/ml. Once the titer of the GI and MNV stocks were determined, all suspensions were diluted with PBS to a concentration of ~8.5 logs/ml. The 3 virus suspensions were then mixed in equal parts and aliquots were made into sterile Eppendorf tubes for storage at -80°C until use.

Preparation of Food Items and Food Contact Surfaces. All fruits (strawberries, raspberries, red grapes, and blueberries) were soaked in a 10% bleach solution for 10 min, rinsed three times in Milli-Q sterile DI water, dried for 30 min and exposed to germicidal ultraviolet light (UV) for 10 min in a sterile weigh boat before use. Stainless steel coupons (5 cm x 2 cm finish #4) were soaked in 70% ethanol for 1 h, rinsed with Milli-Q sterile water, then autoclaved at 121°C for 30 min at 17 psi and stored in a sterile beaker until use. One-ply toilet paper and latex gloves were exposed to germicidal UV for 10 min on each side prior to use. Six sheets of 1-ply toilet paper were folded over to make a single stack of 6 sheets and a 2.5 cm x 2.5 cm square was cut out using sterilized scissors.

Virus Inoculation and Transfer. The starting item for each transfer was inoculated with 10 one-µl iterations of stool suspension and either allowed to dry for 30 min, representative of a dry inoculation transfer, or immediately used as a wet inoculation transfer. For high titer GII experiments, 9 logs of GII were present in each 10 µl inoculum and for low titer, mixed, "cocktail" inoculum experiments, 6 logs each of GI, GII, and MNV were present in each 10 µl inoculum. For transfer experiments, two scenarios were simulated for contamination of fruit during handling: 1) the hand of a food worker is contaminated in the bathroom which touches the bathroom door handle, then proceeds to handle small fruits, 2) a non-shedding individual touches a contaminated bathroom door handle, then proceeds to handle small fruits. Each scenario was simulated using the right index finger of a gloved hand, pressure was applied during each transfer with 50 g pressure ± 5 g for 5 sec. Pressure was monitored by placing the item on an electronic scale with pressure exerted by the gloved finger tip. Initially, four individual experiments were run (toilet paper to gloved finger tip, gloved finger tip to stainless steel coupon, stainless steel coupon to gloved finger tip, and gloved finger tip to fruit), each

replicating one stage of transfer from the two previously mentioned scenarios. Subsequently, consecutive transfer of virus was investigated to more directly simulate the aforementioned scenarios. Scenario 1 (as outlined in Figure 1) was simulated by artificially contaminating a gloved finger tip, followed by touching a stainless steel surface (representing a door handle), and followed by touching a fruit (strawberry, raspberry, grape or blueberry) with 50 g of pressure ± 5 g for 5 sec for each item touched. Scenario 2 (as outlined in Figure 2) was simulated by artificially contaminating a stainless steel surface (representing a door handle), touching the stainless steel with a gloved finger tip, which was followed by touching a fruit (strawberry, raspberry, grape or blueberry) with 50 g of pressure ± 5 grams for 5 sec for each item touched. After inoculation of toilet paper, the 2.5 cm x 2.5 cm single ply sheets were inverted before applying pressure to determine the amount of virus transferred through the 6 sheets of paper. All experiments involving the high titer GII inoculum were performed in triplicate for all transfer steps. For experiments using a mixed virus inoculum, triplicate experiments were performed for all single stage transfer steps, but for experiments involving two transfer steps, 6 repetitions were made.

Virus Elution

After viral transfer, stainless steel coupons and glove tips were placed in a 50 ml tube containing 10 ml of 0.1M PBS with 1M NaCl pH 7.2 and vortexed for 30 sec. A 200 µl aliquot was transferred to a sterile Eppendorf tube for RNA extraction. Fruit was placed in a sterile 50 ml centrifuge tube containing 10 ml of 0.1M PBS with 0.05% v/v Tween 20 and 1M NaCl pH 7.2. For blueberries and grapes, virus was eluted by vortexing the centrifuge tube for 30 sec. Virus was eluted from strawberries and raspberries by placing the fruit in the same 50 ml centrifuge tube but instead inverting the tube upside down 30 times. A 200 µl aliquot of virus eluate from

all fruits underwent RNA extraction. All samples of extracted RNA either were immediately used for RT-PCR or stored at -80°C for later usage. Due to the dilution factor, the lower limit of detection for the recovery procedure was 2,500 genomic copy numbers (or 3.4 logs of virus) for all experiments involving virus transfer.

Scenario 1

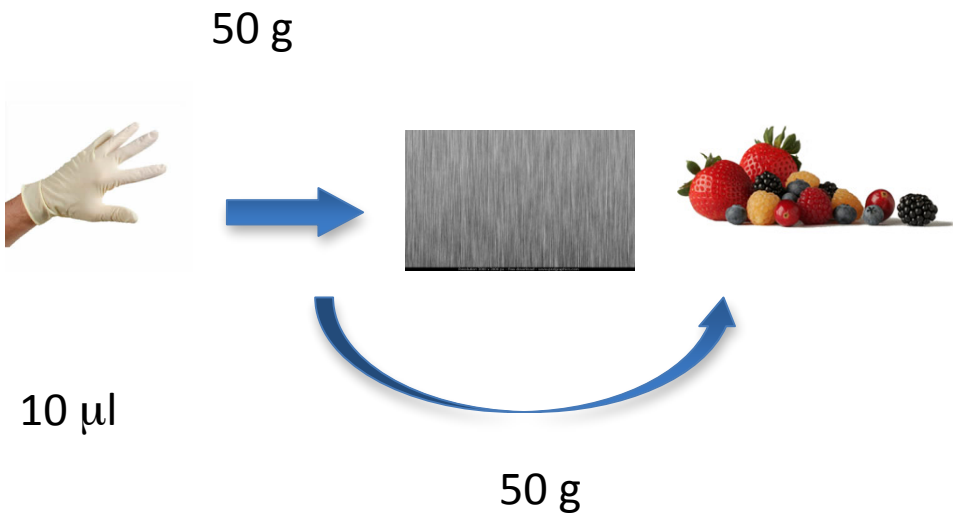

Figure 1: Flow of contamination where a gloved hand is contaminated with NoV, touches a stainless steel coupon (door handle), then subsequently handles small fruits

Scenario 2

Figure 2: Flow of contamination where a clean gloved hand touches a stainless steel coupon (door handle) previously contaminated with fruit, then subsequently handles small fruits

Removal of MNV, HAV, and *Salmonella enterica* with Hand Wipes

MNV and HAV Cultivation and Plaque Assay. RAW 264.7 cells (ATCC# TIB-71) for MNV infection were maintained in complete Dulbecco's modified eagles medium (DMEM) containing 10% low endotoxin fetal bovine serum (FBS) (HyClone, Logan, UT), penicillin (100 U/ml), streptomycin (100 μg/ml), 100 mM HEPES, and 1 mM sodium pyruvate. Fetal Rhesus monkey kidney cells (FRhK) were maintained in complete HAV modified eagles medium (MEM) containing 8% FBS, 1% penicillin, 1% streptomycin, 1% non-essential amino acids, and 1% L-glutamine. MNV and HAV were cultured by infecting 80-90% confluent monolayers of RAW or FRhK cells, respectively. Virus was harvested after complete cytopathic effect (CPE) was apparent (48 h for MNV and 7 d for HAV) by three cycles of freeze-thawing. Cellular debris was removed by centrifugation for 10 min at 1,700 x g and the supernatant was filtered through a 0.2 μm membrane filter (Millipore, Billerica, MA) before storing 1-4 ml aliquots at 4°C for MNV or -80°C for HAV until used.

To determine infectious titer of the virus, standard plaque assay techniques were employed as previously reported *(18)*. Briefly, RAW or FRhK cells were dispensed in 60 mm diameter cell culture plates at a density of $2x10^6$ cells per plate and grown to 80-90% confluence. Immediately preceding infection, the cell culture media was replaced with 0.5 ml of complete MEM without phenol red (Cellgro, Mediatech, Inc, Manassas, VA), supplemented with 5% low endotoxin FBS, penicillin (100 U/ml), streptomycin (100 μg/ml), 100 mM HEPES, and 10 mM sodium pyruvate for MNV and 0.5 ml 2x complete HAV MEM without phenol red (Cellgro), supplemented with 4% FBS, 2% penicillin, 2% streptomycin, 2% non-essential amino acids, 5% sodium bicarbonate, and 2% nystatin for HAV. Ten-fold serial dilutions of virus were prepared in phosphate buffered saline (PBS), pH 7.5 and cell monolayers were infected in duplicate with

0.1 ml of each virus dilution for 1 h at 37°C and 5% CO_2 with gentle rocking every 15 min. Subsequently, infected RAW cells were overlaid with 3 ml complete MEM (without phenol red) (Cellgro) and infected FRhK cells with 5 ml complete HAV MEM supplemented as described above but also containing 0.5% agarose (SeaKem GTG, Lonza, Rockland, ME). RAW cells with MNV were incubated for 48 h and FRhK cells with HAV for 7 d at 37°C and 5% CO_2. Plaques were subsequently counted 5-12 h after a second agarose overlay (complete MEM without phenol red and containing 0.5% agarose) including 1% neutral red solution (Sigma-Aldrich, St. Louis, MO) was added. Plates with 5 to 100 plaques were used to determine the virus titer in plaque forming units (PFU). Log reduction of pathogen was calculated as follows: log value of PFU recovered from positive control - log value of PFU recovered from wiped coupon or glove.

Bacteria Cultivation and Plaque Assay

Five serovars of *Salmonella enterica*, (Typhimurium, Enteritidis, Gaminara, Agona, and Montevideo) (from Dr. Larry Beuchat) were inoculated separately in 10 mL of tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD). Cultures were incubated at 37°C and transferred twice to fresh vials of TSB, 24 h apart. For each strain, 5 ml of inoculated broth was transferred to a conical 50 ml tube and centrifuged at 8,000x g and 4°C for 10 min. The supernatant was removed and replaced with 25 ml of 0.1M PBS pH 7.2 and the tube was vortexed for 30 sec to re-suspend the pellet.

To determine the infectious titer of *Salmonella*, 1:10 dilutions of the stock were made in 0.1% wt/vol peptone to a final concentration of $1x10^{-6}$ of the original stock. A wasp spiral plater (Don Whitley Scientific, West Yorkshire, UK) was used to inoculate 0.1 ml of diluted stock on 100 mm diameter media plates of both tryptic soy agar (Becton Dickinson) plates as a general

media and XLT4 agar (Becton Dickinson) as a selective media. Plates were incubated at 37°C for 24 h. Counting of colonies was performed on a colony plate counter (Acolyte, Don Whitley Scientific, West Yorkshire, UK). Plates with 25-250 colonies were used to determine the bacteria titer in colony forming units (CFU). Percent injury was calculated as follows:

$$\% \text{ Injury} = \frac{\text{CFU on non-selective media} - \text{CFU on selective media}}{\text{CFU on non-selective media}} \times 100\%$$

Protocol for Removal of Pathogens on Stainless Steel and Gloves

Sterile stainless steel coupons (4 cm x 2.5 cm) were inoculated with 50 µl of MNV or HAV partially purified cell culture lysate (~4 x 10^6 PFU/ml stock) spread over the coupon with the pipet tip and 100 µl of *Salmonella* stock (~6 x 10^6 CFU /ml stock) as ten 10 µl iterations before allowing to dry in a BSC-2 for 40 min at 21°C or until visibly dry. Coupons were placed on an electronic scale, which was sanitized by exposure to UV light for 15 min. For each repetition, 1 or 5 swipes was made over the inoculated area using a gloved hand at 50±5 g pressure. All wipes were cut into 1.5 x 1.5 cm squares and autoclaved at 121°C and 16 psi for 30 minutes prior to use. Wipes tested included water filters containing nano-alumina fibers (Nanoceram, Sanford, FL), charge modified glass fibers (Virosorb 1MDS, Cuno, Meriden, CT), negative charge cellulose filters (Millipore, Billerica, MA) and neutral charge cellulose filters (Whatman, Kent, UK). For wet wipes, each wipe was immersed into a 50 ml conical tube containing the desired sanitizer (2% levulinic acid plus 1% SDS, 3% levulinic acid plus 2% SDS, 5% levulinic acid plus 1% SDS or 5% levulinic acid plus 2% SDS) or Milli-Q water, removed with a pipet tip and

gently squeezed by pressing the wipe against the side of the tube with the tip to remove excess moisture. Wipes were discarded of after use.

To mimic hand sanitation procedures, the index fingers of latex gloves sterilized by UV light for 15 min were placed over a sterile 15 ml conical tube. The finger tip was inoculated with 10 µl of MNV stock (~$1x10^6$) and dried in a BSC-2 for 30 min or until visibly dry. The tube was placed on an electronic scale and secured with tape. Wiping was performed as previously described.

Recovery of Pathogen

Post-wipe, coupons inoculated with MNV or HAV were immediately placed in a 50 ml tube containing 10 ml 0.1M PBS with 1M NaCl and vortexed for 30 sec to neutralize the sanitizer. The eluate was plated in 1:10 dilutions on cell culture monolayers using previously described methods. For *Salmonella*, coupons were placed in a stomacher bag (Seward, West Sussex, UK) containing 50 ml 0.1M PBS with 0.02% vol/vol Tween 80. Using a Seward stomacher 400 model (Seward, West Sussex, UK), the bags were stomached at 230 rpm for 1 min. Homogenate was plated in 1:10 dilutions on TSA and XLT4 media as previously described. Duplicate cell culture plates for all pathogens were averaged and used to calculate titer after treatment. Negative controls, stainless steel without inoculation, were performed in duplicate and included with each experimental trial. For a positive control, an inoculated coupon or latex glove finger was dried and pathogen was recovered without any wipe treatment. Percent recovery was calculated as follows:

$$\frac{\text{Total PFU recovered from coupon inoculated with 50 µl virus stock}}{\text{Total PFU in 50 µl of stock}} \times 100$$

The limit of detection for viral plaque assays was 3.3 logs or 2,000 virus particles while for *Salmonella*, the limit of detection was 2.7 logs or 500 CFU.

Statistical Analysis

All differences among experimental variables were determined with Analysis of Variance (SAS/English statistical package version 9.2). This method compared the average log transformed values of the responses or outcomes (either log reduction or log transfer) among the various levels of each treatment, and determined if the average values of the outcomes significantly differed at the $\alpha = 0.05$ level. If an overall difference was found within the model, each outcome was further analyzed using the Generalized Linear Model procedure with the Tukey-Kramer adjustment for determining differences among the mean log transformed responses or outcomes (either log reduction or log transfer) among the various levels of each treatment or interaction within the GLM. The $\alpha = 0.05$ level was again selected for determining significance.

CHAPTER 4

Results

Percent Recovery of Norovirus after Elution

To determine the effectiveness of the elution protocols, percent recovery of virus from stainless steel coupons, gloved finger tip, and fruits was determined (Table 1). This was first examined for the high titer GII norovirus inoculum. When items were inoculated with 9.0 log genomic copy number of human norovirus virus GII per 10 μl inoculum, percent recoveries were 93 ± 11% and 90 ± 3% for stainless steel coupons, and 87 ± 7% and 51 ± 19% for gloved finger tips using wet and dry virus inoculation procedures, respectively. For fruit, percent recoveries were 55 ± 5%, and 8 ± 7% for strawberries, 25 ±8% and 15 ± 4% for raspberries, 108 ± 14% and 65 ± 15% for red grapes and 72 ± 14% and 53 ± 21% for blueberries using wet and dry virus inoculation procedures, respectively.

Table 1: Average percent recovery of high titer norovirus stock (9 logs/10 μl) from items used in transfer experiments (n=3)

Pathogen	Item	Wet or Dry Inoculum	Percent Recovery±SD
GII	Coupon	Wet	93 ± 11%
GII	Coupon	Dry	90 ± 3%
GII	Gloved Finger	Wet	87 ± 7%
GII	Gloved Finger	Dry	51 ± 19%
GII	Strawberry	Wet	55 ± 5%
GII	Strawberry	Dry	8 ± 7%

GII	Raspberry	Wet	25 ±8%
GII	Raspberry	Dry	15 ± 4%
GII	Grape	Wet	108 ± 14%
GII	Grape	Dry	65 ± 15%
GII	Blueberry	Wet	72 ± 14%
GII	Blueberry	Dry	53 ± 21%

Next, 10 μl inoculums containing the virus cocktail of 6.0 logs each of human NoV GII, human NoV GI, and MNV/10 μl were employed and recovery efficiencies were examined for stainless steel coupons, gloved finger tips and fruits for the second set of virus transfer experiments (Table 2). Percent recoveries from gloved finger tips were 42 ± 19%, 33 ± 8%, and 50 ± 22% for GI, GII, and MNV respectively under dry inoculation procedures. Using wet inoculation methods, percent recovery for gloved finger tips was 83 ± 14%, 89 ± 17%, and 104± 22% for GI, GII, and MNV respectively. For stainless steel coupons, percent recovery was 44 ± 12%, 82 ± 10%, and 81 ± 22% under dry inoculation and 104 ± 24%, 111 ± 33%, and 99 ± 11% under wet inoculation for GI, GII, and MNV, respectively. Percent recovery for wet inoculated strawberries was 62.7 ± 22%, 9 ± 12%, and 53 ± 18% while recovery from dry inoculated strawberries was 40 ± 17%, 15 ± 12, and 23 ± 9% for GI, GII, and MNV, respectively. For raspberries, the percent recovery from wet inoculation for GI, GII, and MNV was 104 ± 32%, 42 ± 10%, 54 ± 11% and recovery by dry inoculation was 15 ± 8%, 9 ± 4%, 5 ± 3%, respectively. Recovery from wet inoculated grapes was 32 ± 15%, 95 ± 29%, and 60 ± 8%, and dry inoculated 77 ± 11%, 14 ± 12%, 31 ± 7% while recovery from wet inoculated blueberries was 34 ± 22%,

101 ± 15%, 74 ± 17% and dry inoculation was 57 ± 6%, 44 ± 11%, and 60 ± 18% for GI, GII, and MNV, respectively.

Table 2: Average percent recovery of virus cocktail stock (6 logs of each pathogen/10 μl) from items used in transfer experiments (n=3)

Pathogen	Item	Wet or Dry Inoculum	Percent Recovery±SD
GI	Coupon	Wet	104 ± 24%
GI	Coupon	Dry	44 ± 12%
GI	Gloved Finger	Wet	83 ± 14%
GI	Gloved Finger	Dry	42 ± 19%
GI	Strawberry	Wet	63 ± 22%
GI	Strawberry	Dry	40 ± 17%
GI	Raspberry	Wet	64 ± 32%
GI	Raspberry	Dry	15 ± 8%
GI	Grape	Wet	77 ± 11%
GI	Grape	Dry	32 ± 15%
GI	Blueberry	Wet	57 ± 6%
GI	Blueberry	Dry	34 ± 22%
GII	Coupon	Wet	111 ± 33%
GII	Coupon	Dry	82 ± 10%
GII	Gloved Finger	Wet	89 ± 17%
GII	Gloved Finger	Dry	33 ± 8%
GII	Strawberry	Wet	9 ± 12%
GII	Strawberry	Dry	15 ± 12%
GII	Raspberry	Wet	42 ±10%
GII	Raspberry	Dry	9 ± 4%
GII	Grape	Wet	95 ± 29%
GII	Grape	Dry	14 ± 12%
GII	Blueberry	Wet	101 ± 15%
GII	Blueberry	Dry	44 ± 11%

MNV	Coupon	Wet	99 ± 11%
MNV	Coupon	Dry	81 ± 22%
MNV	Gloved Finger	Wet	104± 22%
MNV	Gloved Finger	Dry	50 ± 22%
MNV	Strawberry	Wet	53 ± 18%
MNV	Strawberry	Dry	23 ± 9%
MNV	Raspberry	Wet	54 ± 11%
MNV	Raspberry	Dry	5 ± 3%
MNV	Grape	Wet	60 ± 8%
MNV	Grape	Dry	31 ± 7%
MNV	Blueberry	Wet	74 ± 17%
MNV	Blueberry	Dry	60 ± 18%

Percent Recovery of Pathogens in Sanitizing Wipe Experiments

Using an inoculum of 6 logs MNV/50 µl, 6 logs HAV/µl, or 6 logs *Salmonella* per 100 µl, percent recoveries from stainless steel coupons were calculated for experiments with the sanitizing wipes. Recovery of MNV from stainless steel coupons was 92 ± 14%. HAV was recovered at 88 ± 9% from stainless steel coupons. *Salmonella enterica* was recovered from stainless steel coupons at 74 ± 15%. All reported percent recoveries were averaged from n=3 trials.

Transfer of Human Norovirus with High Titer GII Virus Stock

The amount of virus ($\log_{10}$) detected on gloved finger tips and stainless steel coupons after transfer of human norovirus from toilet paper to gloved finger tips, gloved finger tips to stainless steel, and stainless steel coupon to gloved finger tips using an initial titer of 9 log genomic copy number of GII norovirus per 10 µl is depicted in Fig 3. An average of 4.65 ± 0.40 logs was detected on gloved finger tips after transfer from inoculated toilet paper under "wet

conditions" of inoculation and transfer. When the virus inoculum was allowed to dry for 30 min prior to contact with the gloved fingertips, an average of 3.59 logs ± 0.35 logs was detected on gloved finger tips. Average NoV transfer from inoculated gloved finger tips to stainless steel coupons was 8.74 ± 0.15 logs and 4.13 ± 0.33 logs. Virus transfer from stainless steel coupons to gloved finger tips was 8.09 ± 0.10 logs and ≤3.39 ± 0.0 logs for wet and dry transfer, respectively.

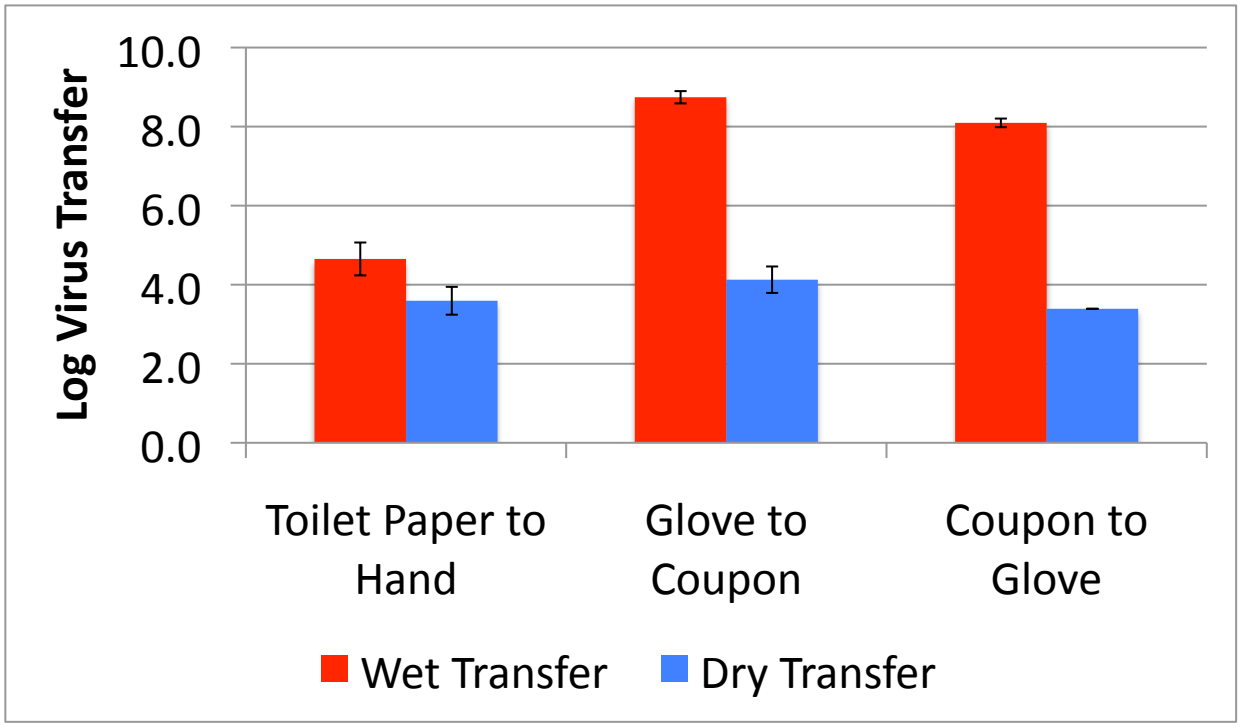

Figure 3: Average amount of high titer GII norovirus (log_{10} genomic copy number) contamination that can be detected on gloves and stainless steel after virus is transferred from contaminated toilet paper, gloves, or stainless steel surfaces using a gloved finger tip for transfer (n=3). Error bars indicate the standard deviations.

Average logs of virus detected on strawberries after contact and transfer from contaminated gloved finger tips measured 6.92 ± 0.10 and 4.53 ± 1.32 logs, while raspberries had 7.23 ± 0.11 and ≤3.39 ± 0.0 logs for their respective wet and dry transfer conditions (Figure 4). For grapes and blueberries, the average logs of virus transferred from contaminated gloved

finger tips to grapes and blueberries was 8.73 ± 0.9 and ≤3.39 ± 0.0 logs for grapes and 8.69 ± 0.42 and 4.61 ± 1.73 logs for wet and dry transfer to blueberries (Figure 4).

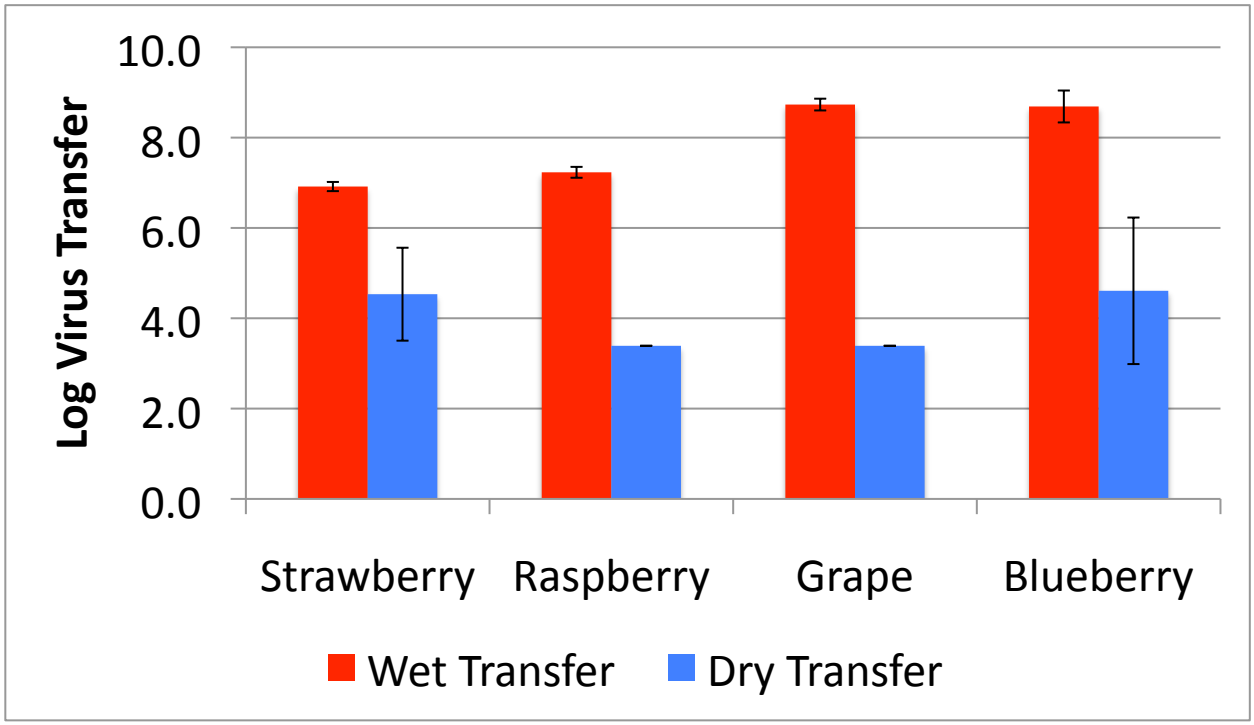

Figure 4: Average amount of high titer GII norovirus (log_{10} genomic copy number) contamination that can be detected on fruit after virus is transferred from gloved finger tips (n=3). Error bars indicate the standard deviations.

Two scenarios depicting the likelihood of food handler contamination of fruits after restroom use in the absence of hand sanitation are depicted in Figures 5 and 6. In the first scenario, an ill field worker contaminates his or her hand while using the restroom, then contaminates the door while exiting, and contaminates fruits after returning to work. This was recreated in the lab by having a gloved finger tip inoculated with human norovirus touch a stainless steel coupon and then proceed to touch various fruits. The likelihood of fruit contamination by this individual is high, with the average logs of virus on strawberries as high as 5.73 logs, raspberries had up to 6.64 logs of virus, grapes had 6.84 logs of virus and blueberries had as high as 7.71 logs detected post-handling for the transfer of virus under wet inoculation

(Figure 5). Dry transfer yielded significantly lower values ($p<0.0001$) but the level of contamination was still high with an average of 4.61 logs of virus detectable on blueberries after transfer of a dry inoculum.

For the second scenario (Fig 6), the likelihood of fruit contamination is examined for an individual who is not shedding virus, but touches a previously contaminated door handle inoculated with 9 logs of virus. This was recreated by having a gloved finger tip touch an inoculated door handle and subsequently handle fruit. Dry contamination of fruit occurs at a much lower degree; however, the limit of detection for this experiment was 3.39 logs of virus, so any positive values below this value were converted to ≤3.39 logs. In contrast, wet transfer is nearly as likely by the healthy worker as contamination by the ill worker with the average contamination of strawberries as high as 6.70 logs, raspberries had up to 6.43 logs, grapes were contaminated with as many as 6.50 logs, and blueberries up to 7.52 logs.

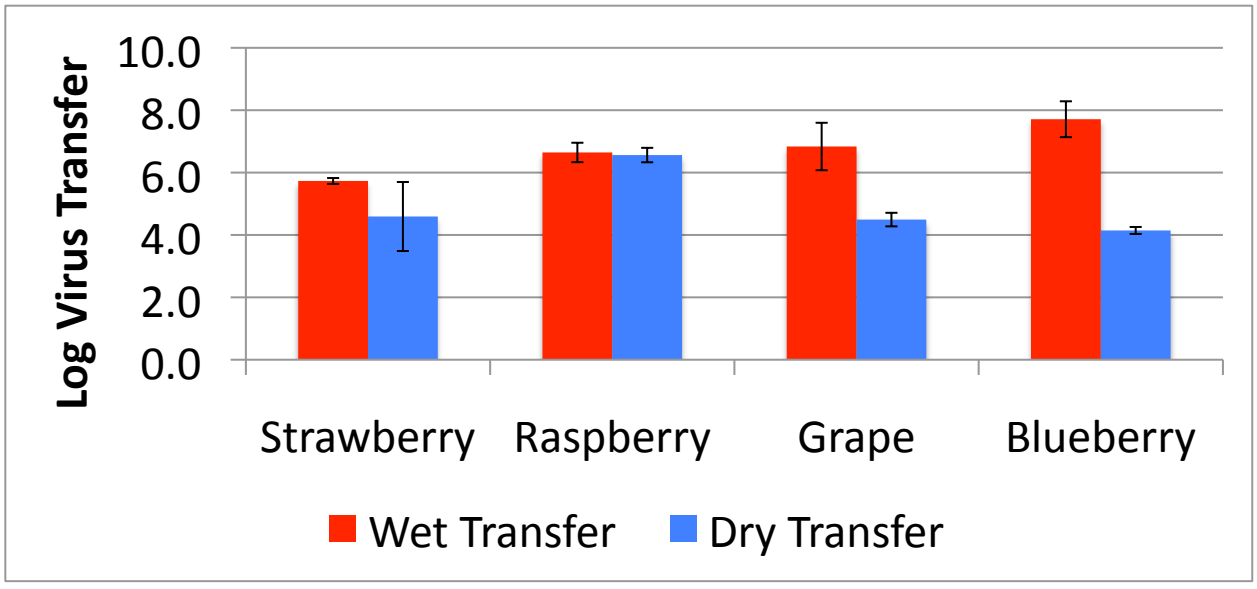

Figure 5: Average amount of high titer GII norovirus (log_{10} genomic copy number) contamination that can be detected on fruit after an inoculated gloved hand touches a stainless steel coupon then subsequently handles fruit (n=3). Error bars indicate standard deviations.

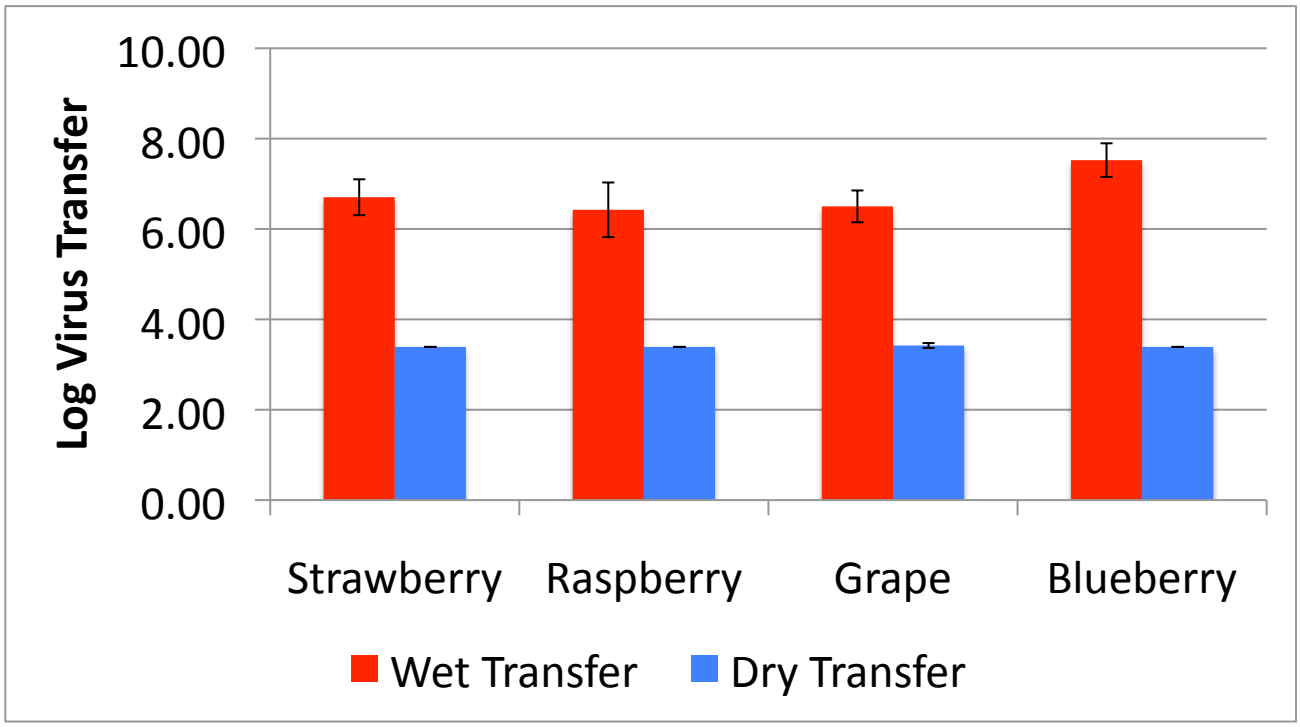

Figure 6: Average amount of high titer GII norovirus (log_{10} genomic copy number) contamination that can be detected on fruit after a gloved finger tip touches an inoculated stainless steel coupon then subsequently handles fruit (n=3). Error bars indicate the standard deviations.

Transfer of Virus to Small Fruits During Handling with Human NoV GI, GII, and Murine Norovirus Cocktail

Transfer experiments were repeated; however, the inoculum consisted of a virus "cocktail" containing 6 logs each of norovirus GI, GII, and MNV per 10 µl inoculum. Compared to the high titer GII stock, the 6 logs seen here are more reasonable representations of the amount of virus that may contaminate a hand after defecating if one is shedding virus during illness. Using the virus "cocktail" allows us to determine if there are genogroup specific differences that may affect virus transfer. Additionally, including Murine Norovirus will help determine if for future transfer studies, MNV is an appropriate surrogate for estimating the behavior of human norovirus. Human norovirus GI, GII, and MNV were inoculated on toilet paper, gloved finger

tips or stainless steel coupons with an initial titer of 6.0 logs of each virus/10 µl. Detection of all 3 viruses was below the detection limit for gloved hands after touching toilet paper (data now shown). Determining the logs of virus transferred from gloved finger tips to stainless steel, an average of 5.41 ± 0.16, 5.71 ± 0.14, and 5.55 ± 0.12 logs were detected under wet inoculation methods while 4.72 ± 0.21, 4.64 ± 0.34, and 4.44 ± 0.26 logs were detected under dry inoculation methods for GI, GII, and MNV, respectively (Fig 7). Average logs transferred from stainless steel to gloved finger tips yielded 5.31 ± 0.16, 5.24 ± 0.14, and 5.27 ± 0.36 logs detected for wet inoculation and transfer and 5.01 ± 0.21, 4.30 ± 0.34, and 4.83 ± 0.26 logs for dry inoculation and transfer (Fig 8).

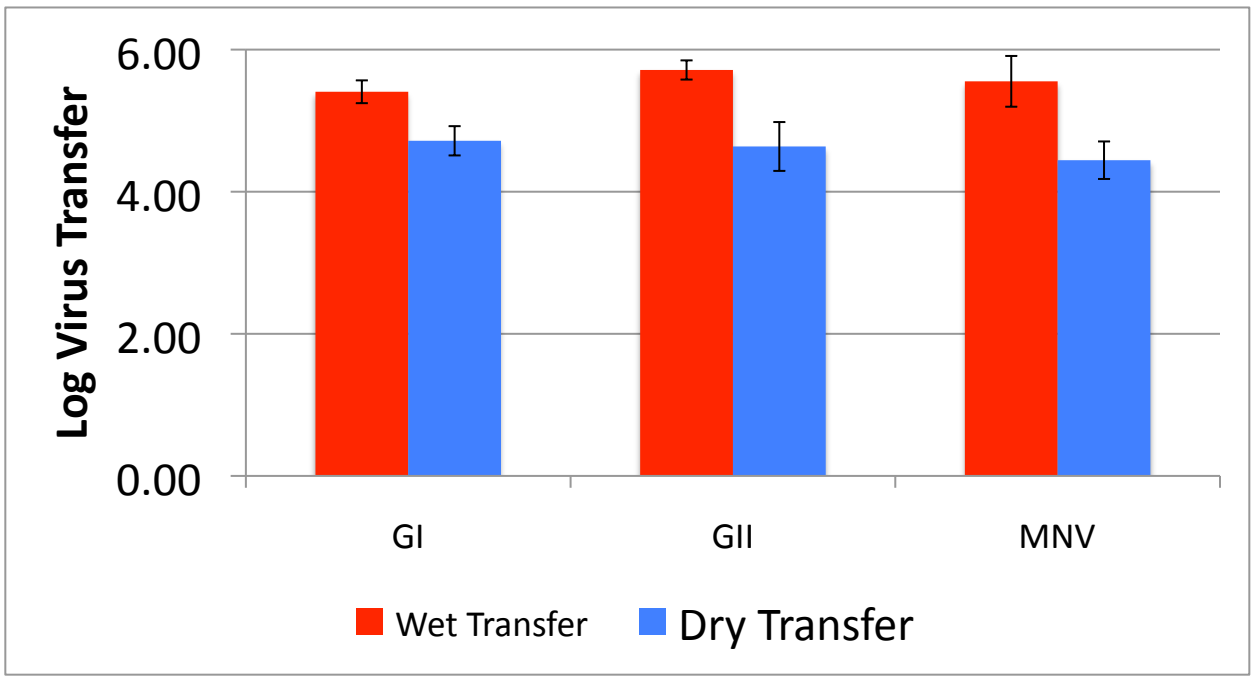

Figure 7: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a stainless steel coupon after an inoculated gloved finger tip touches it (n=3). Error bars indicate the standard deviations.

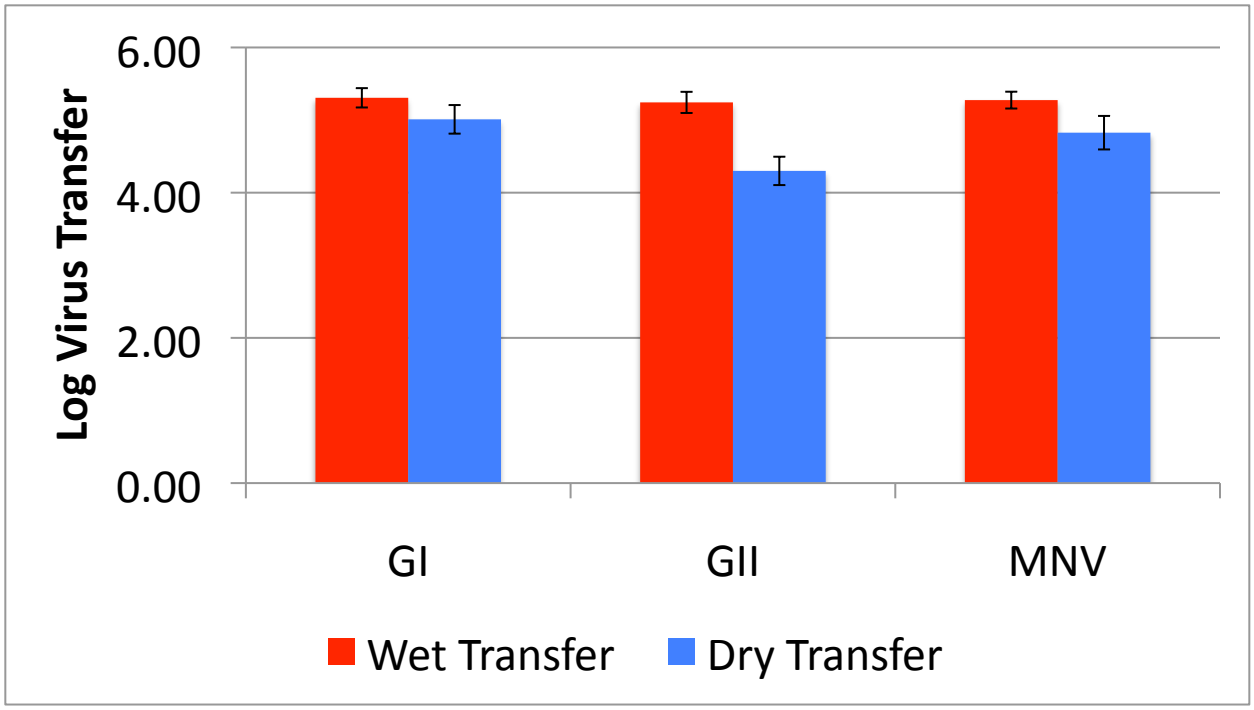

Figure 8: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a gloved finger tip after touching an inoculated stainless steel coupon (n=3). Error bars indicate the standard deviations.

Virus detected on strawberries after transfer from contaminated gloved finger tips measured 4.58 ± 0.19, 4.87 ± 0.14, and 4.72 ± 0.19 logs under dry inoculation transfer while under wet inoculation transfer, 5.11 ± 0.1, 5.3 ± 0.12, and 5.5 ± 0.11 logs were detected for GI, GII, and MNV, respectively (Fig 9). Logs of virus transferred from gloved finger tips to raspberries were measured at ≤3.39 ± 0.0, 3.40 ± 0.24, and ≤3.39 ± 0.0 under dry transfer while for wet transfer, 5.26 ± 0.31, 5.03 ± 0.08, and 5.0 ± 0.22 logs for GI, GII, and MNV (Fig 10). Transfer of virus from gloved finger tips to grapes showed that 4.66 ± 0.2, 3.93 ± 0.22 and 3.68 ± 0.21 logs were present on the contaminated grape after dry transfer and under wet transfer, 5.52 ± 0.21, 5.56 ± 0.17, and 5.13 ± 0.27 logs were transferred for GI, GII, and MNV, respectively (Fig 11). The logs of virus transferred to blueberries was measured at 4.59 ± 0.16,

4.03 ± 0.25, and 3.92 ± 0.28 logs for dry transfer and 5.51 ± 0.16, 5.78 ± 0.30, and 5.71 ± 0.1 logs for wet transfer of GI, GII, and MNV (Fig 12).

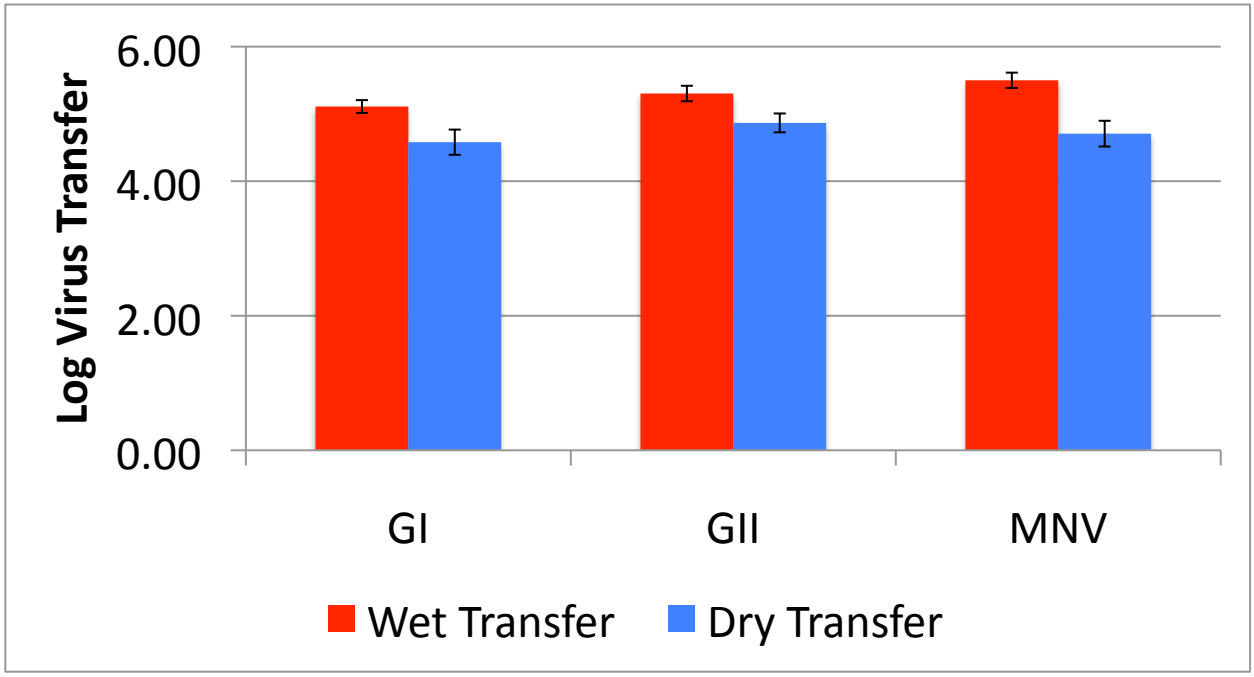

Figure 9: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a strawberry after it is touched by an inoculated gloved finger tip (n=3). Error bars indicate the standard deviations.

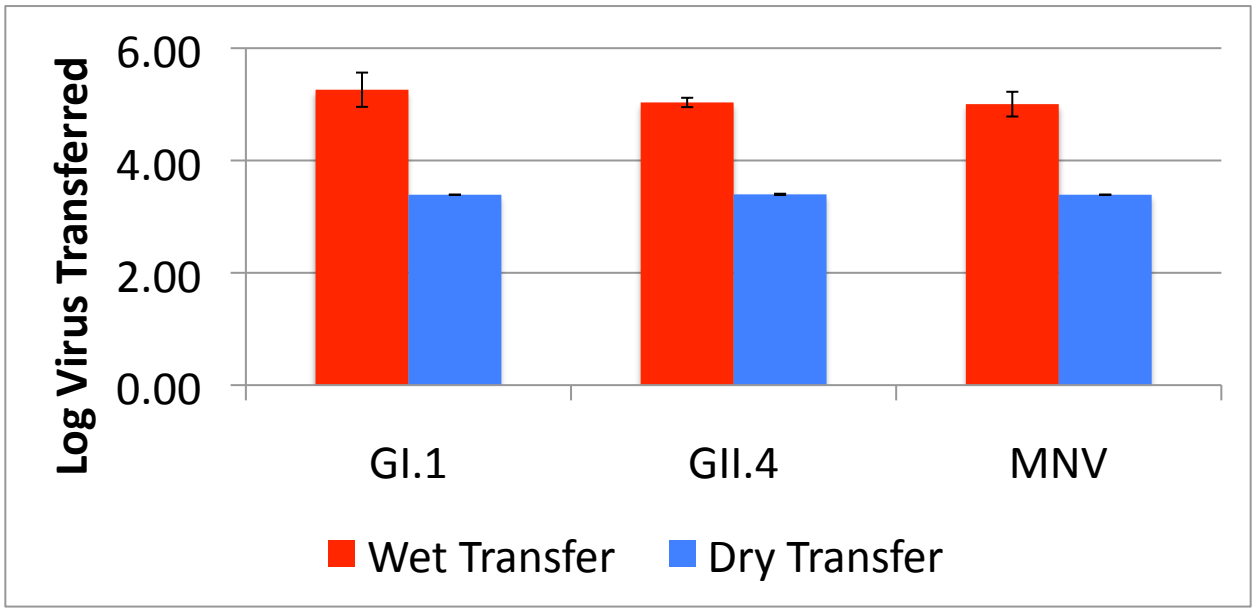

Figure 10: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a raspberry after it is touched by an inoculated gloved finger tip (n=3). Error bars indicate the standard deviations.

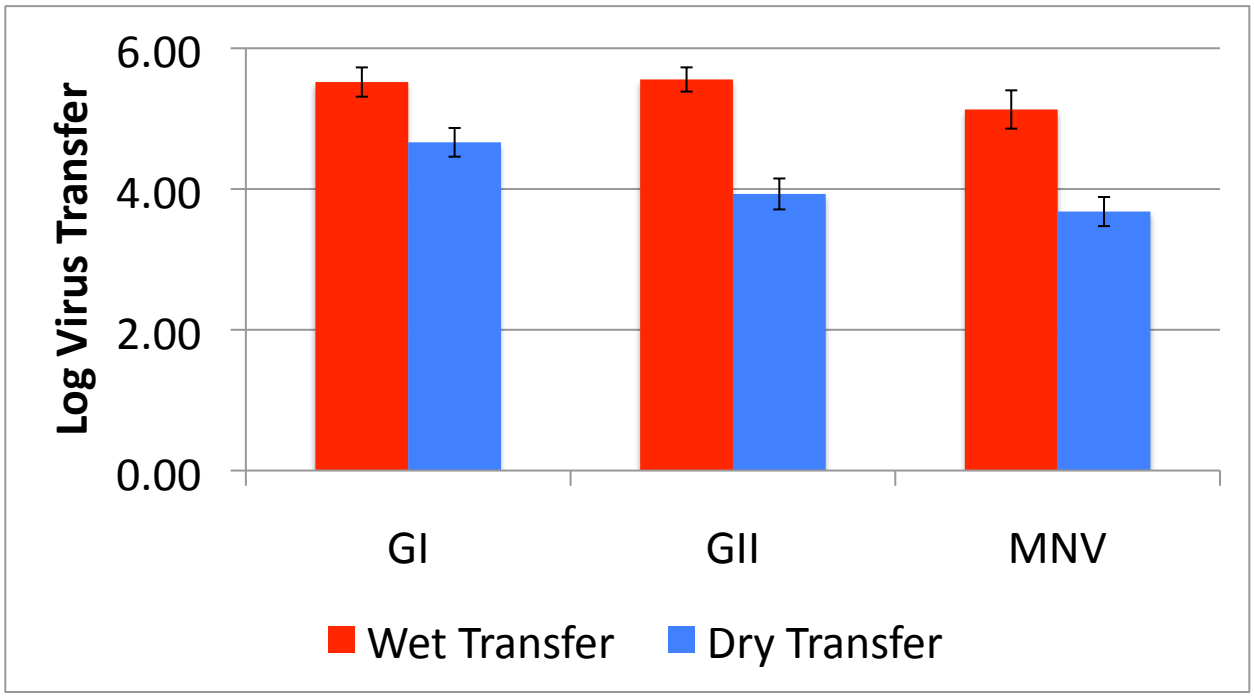

Figure 11: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a grape after it is touched by an inoculated gloved finger tip (n=3). Error bars indicate the standard deviations.

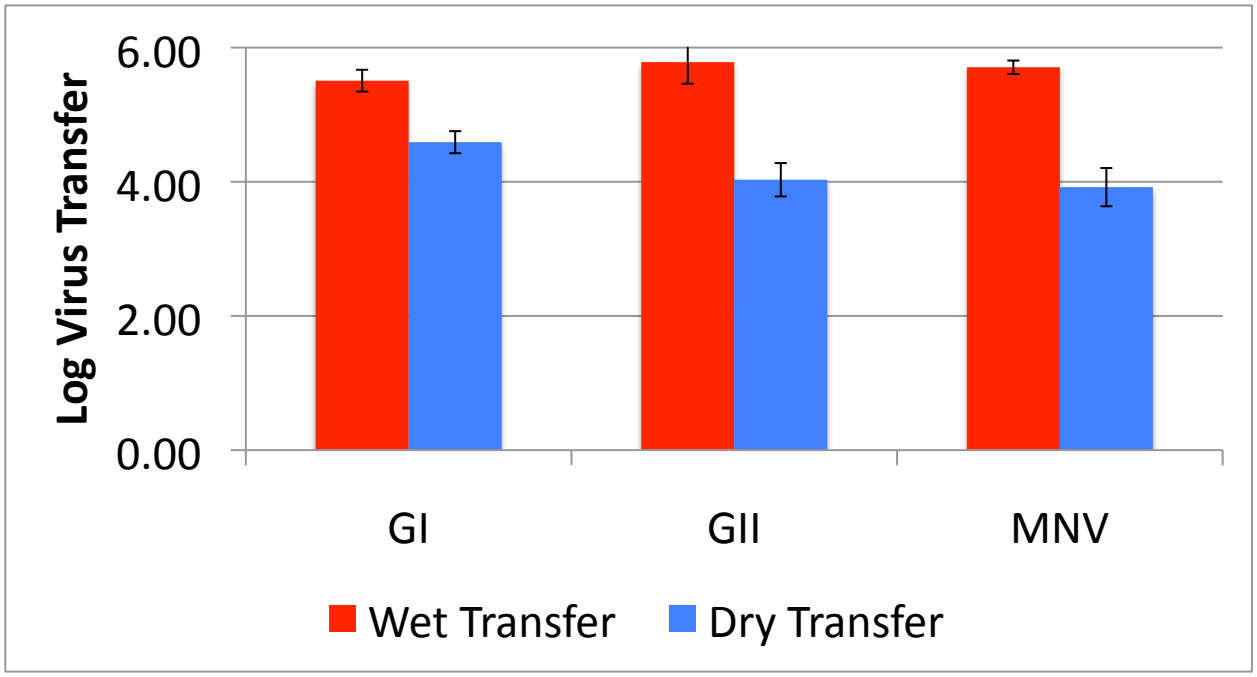

Figure 12: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a blueberry after it is touched by an inoculated gloved finger tip (n=3). Error bars indicate the standard deviations.

The two scenarios depicting the likelihood of food handler contamination of fruits after restroom use in the absence of hand sanitation were again recreated; however, the virus inoculation was 10 μl containing 6.0 logs each of human NoV GI, GII, and MNV (Tables 9 and 10). In the first scenario (Figures 13-16), an ill field worker contaminates their hand (gloved finger tip) while using the restroom, then contaminates the door (stainless steel coupon) while exiting, and then contaminates fruits after handling. The likelihood of fruit contamination by this individual is high, as up to 5.52 logs and 4.50 logs of virus are detected on fruit post-handling for the respective transfer of wet and dry virus suspension. For the second scenario, the likelihood of fruit contamination is examined for an individual who is not shedding virus (Figures 17-20). The healthy individual touches a contaminated door handle and subsequently handles fruit. This was recreated by a gloved finger tip touching an inoculated stainless steel coupon then touching the fruit. Average contamination of fruit under dry inoculation and transfer occurs to a similar degree under both scenarios with ≤3.39-4.50 logs virus transferred for scenario 1 and 3.43-4.12 logs for scenario 2. Using wet inoculation and transfer, similar levels of contamination are likely by both the healthy worker and the ill worker with 4.27-5.52 logs detected on fruit post handling for scenario 1 and 3.98-5.02 logs detected on fruit for scenario 2.

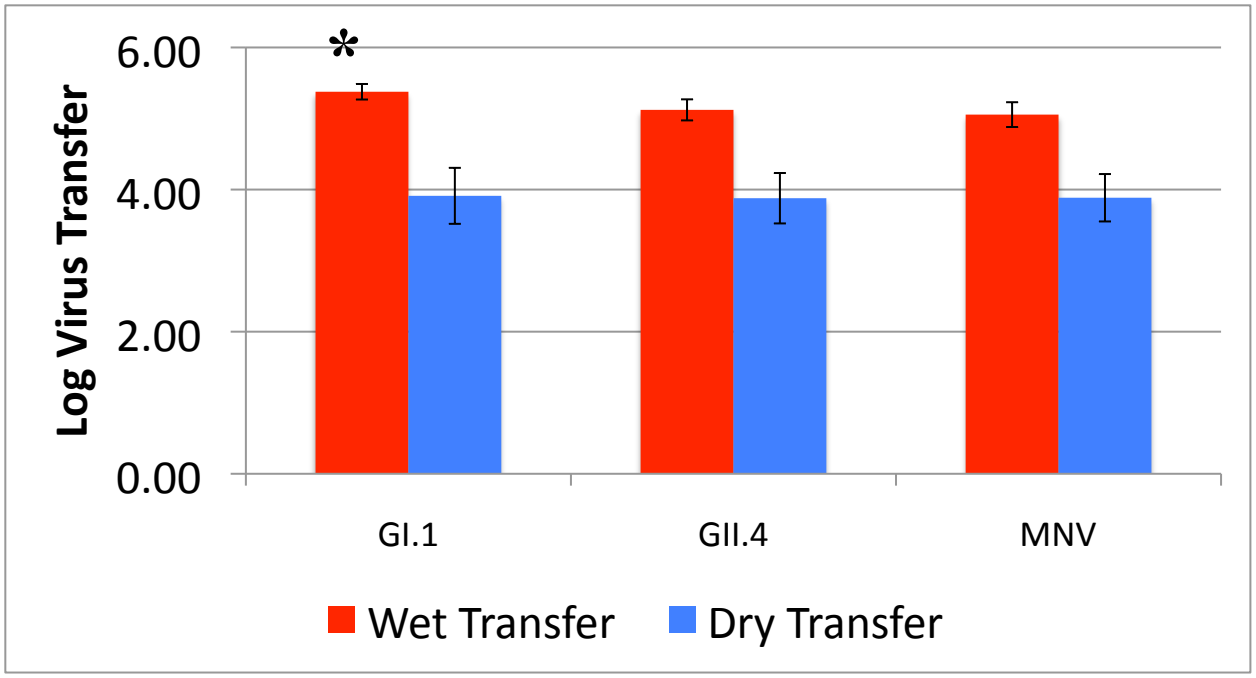

Figure 13: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a strawberry after an inoculated gloved finger tip touches a stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations. * indicates a significant difference in the mean log transfer between the three norovirus genotypes ($p \leq 0.05$).

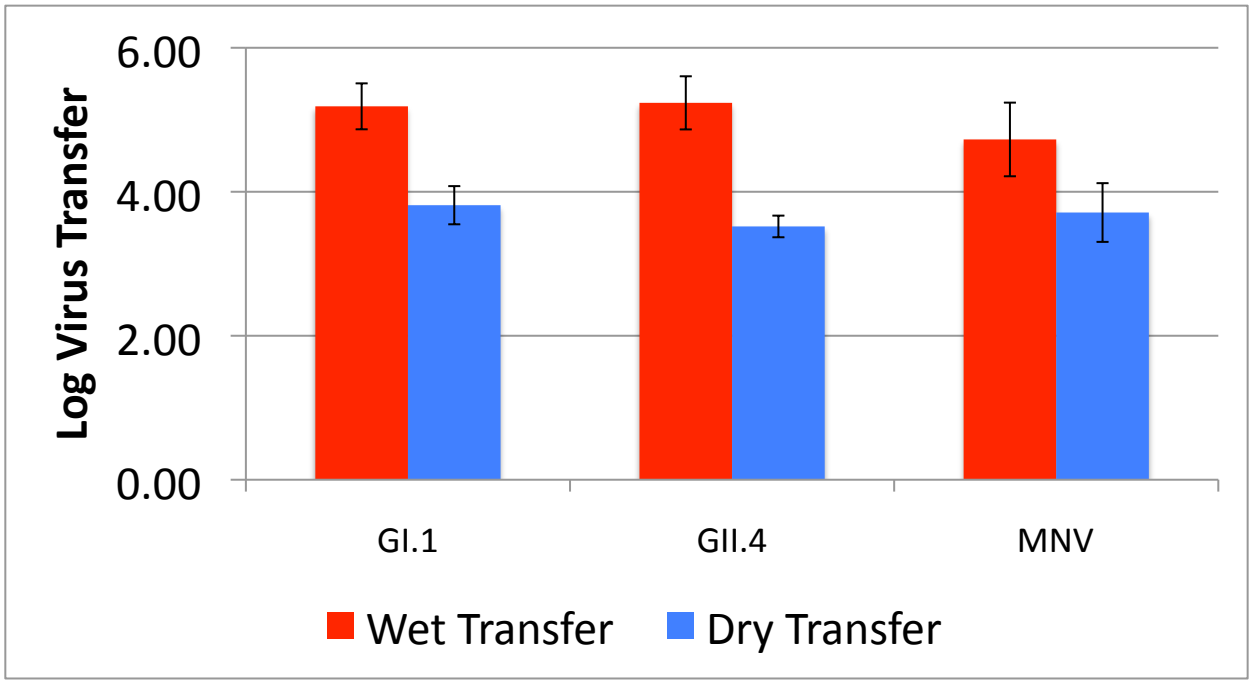

Figure 14: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a raspberry after an inoculated gloved finger tip touches a stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations.

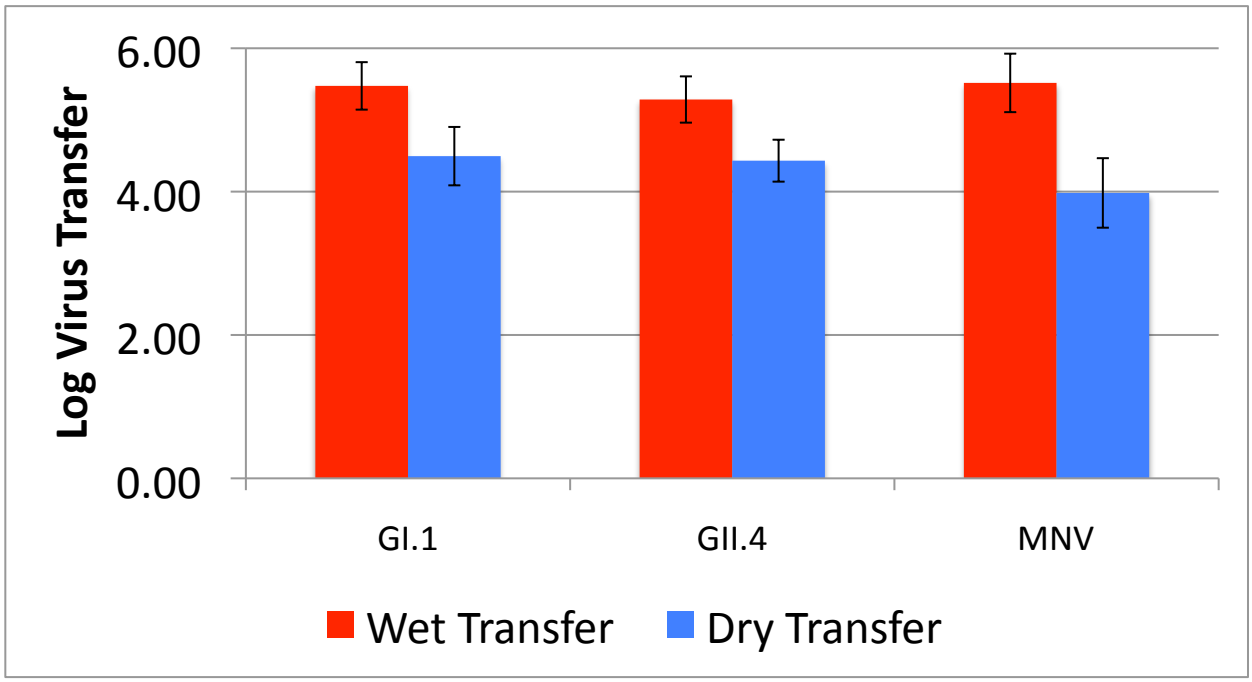

Figure 15: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a grape after an inoculated gloved finger tip touches a stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations.

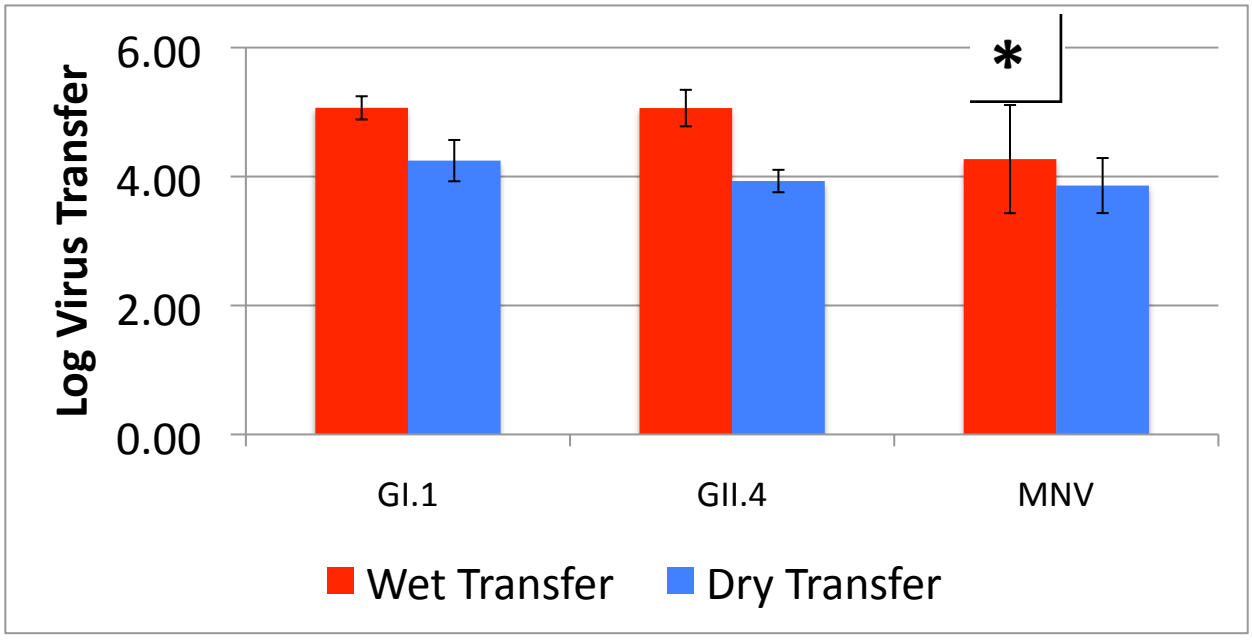

Figure 16: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a blueberry after an inoculated gloved finger tip touches a stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations. * indicates a significant difference in the mean log transfer between the three norovirus genotypes ($p \leq 0.05$).

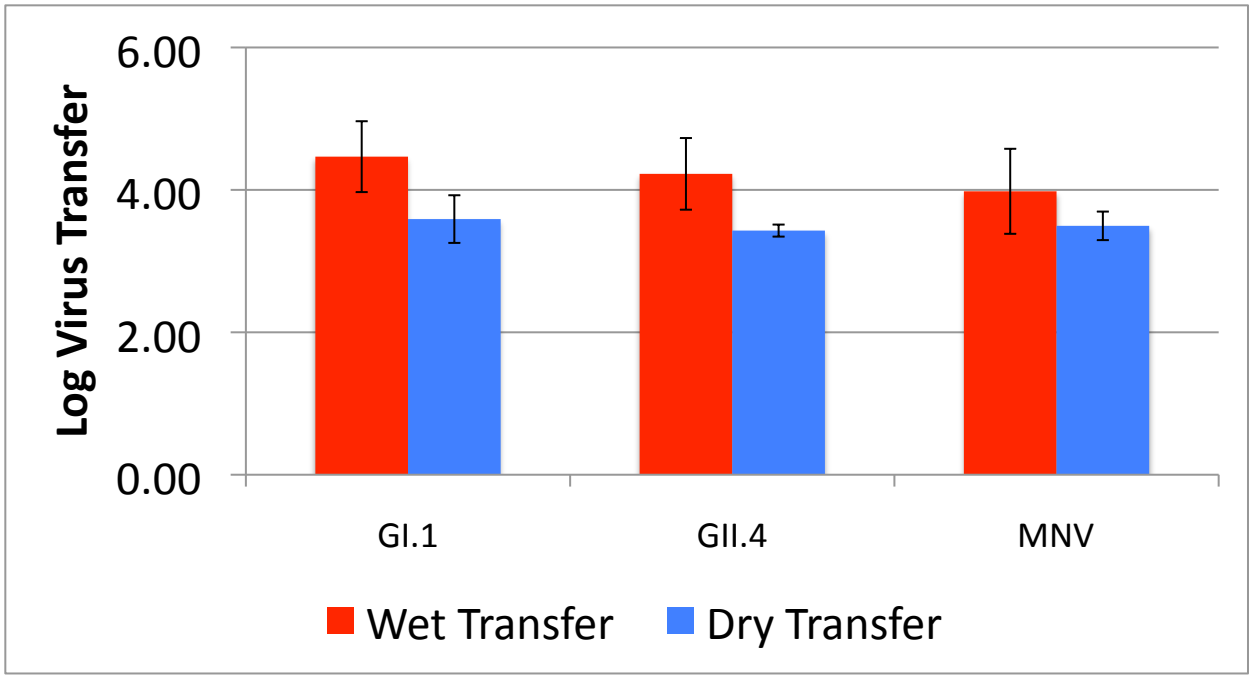

Figure 17: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a strawberry after a gloved finger tip touches an inoculated stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations.

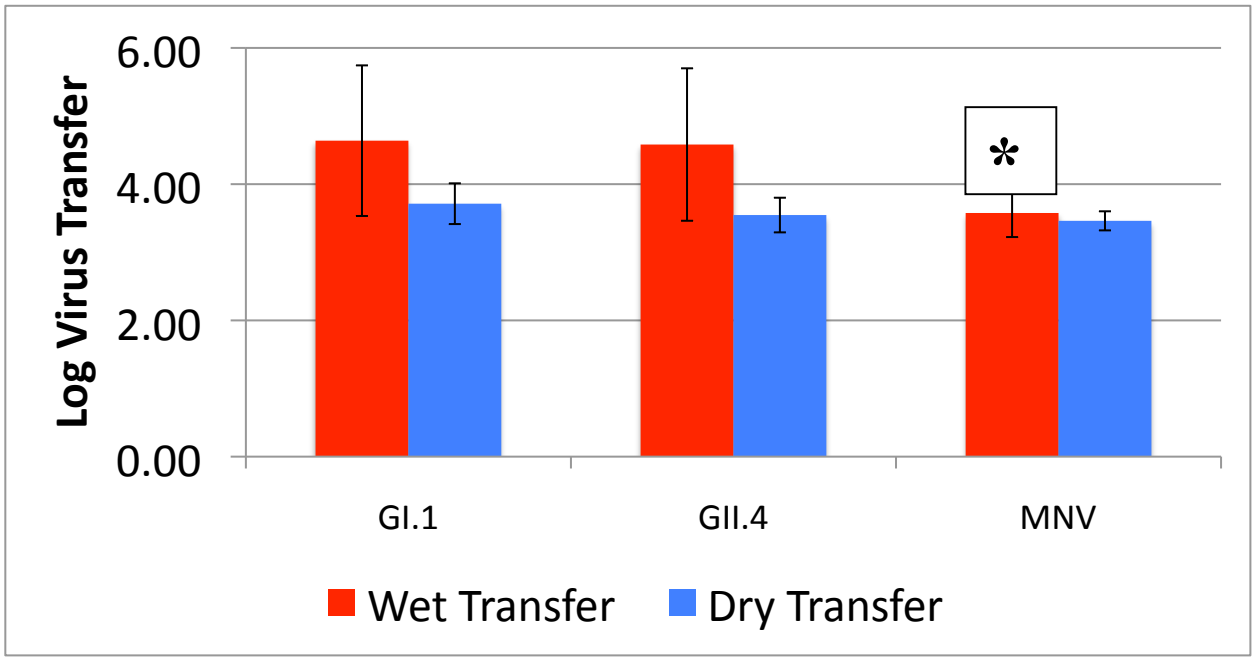

Figure 18: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a raspberry after a gloved finger tip touches an inoculated stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations. * indicates a significant difference in the mean log transfer between the three norovirus genotypes ($p \leq 0.05$).

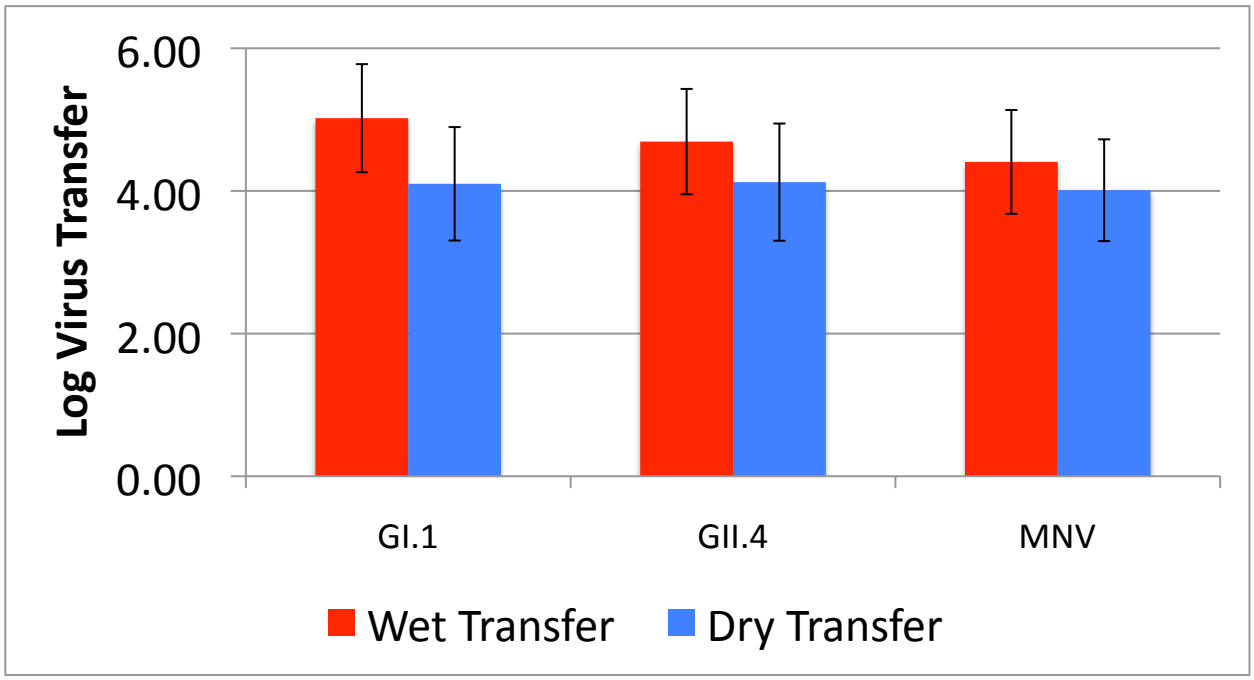

Figure 19: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a grape after a gloved finger tip touches an inoculated stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations.

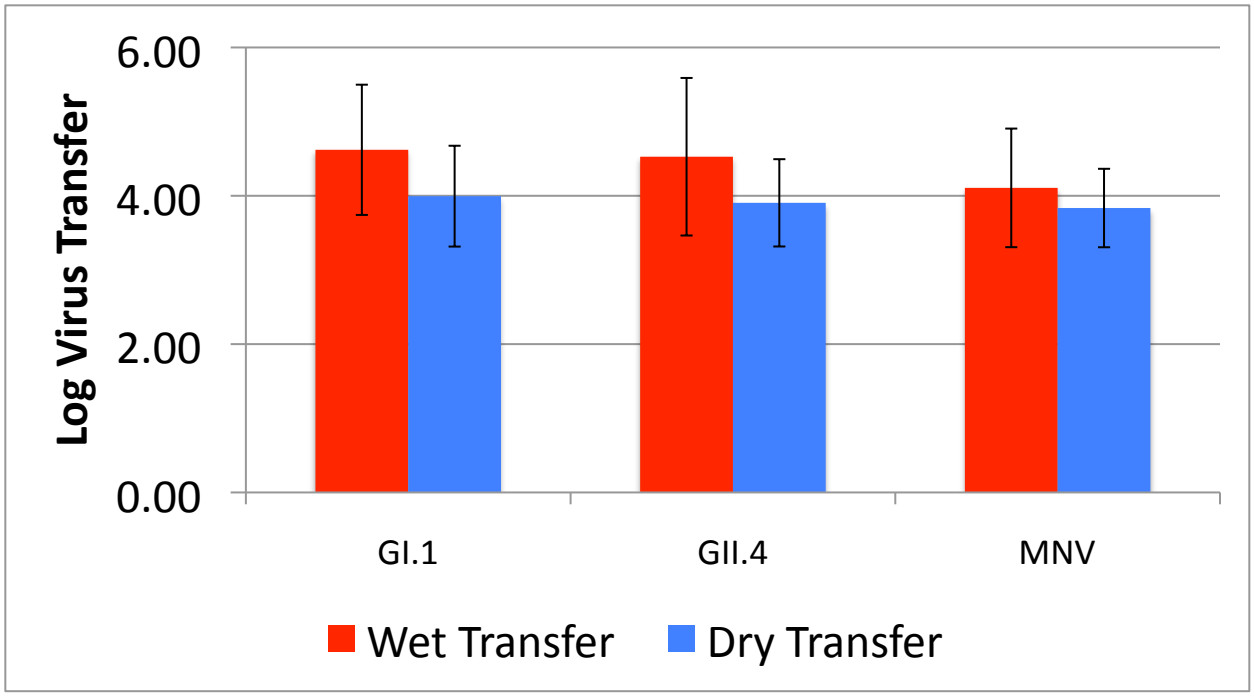

Figure 20: Average amount of virus (log_{10} genomic copy number) contamination that can be detected on a blueberry after a gloved finger tip touches an inoculated stainless steel coupon then touches the fruit (n=6). Error bars indicate the standard deviations.

For all experiments involving virus transfer, average contamination levels were significantly different ($p \leq 0.05$) under wet vs. dry inoculation and transfer. Overall, transfer with a starting contamination of 9 logs transferred significantly more virus than starting with 6 logs (Table 3). Under conditions of wet transfer, average transfer of GII was significantly higher with a 9 log inoculation vs. a 6 log inoculation (Table 5); however, under dry inoculation and transfer, significantly lower transfer was observed when starting with a contamination level of 6 logs vs. 9 logs (Table 4).

Table 3: Average logs transferred of GII norovirus under wet and dry conditions with initial logs contamination values of 6 or 9 logs. Same letter indicates not significantly different.

Amount of Initial Contamination (Logs)	Logs Transferred Least-Square Mean
6	3.95[a]
9	4.60[b]

Table 4: Average logs transferred of GII norovirus under dry conditions with initial logs contamination values of 6 or 9 logs. Same letter indicates not significantly different.

Amount of Initial Contamination (Logs)	Logs Transferred Least-Square Mean
6	3.43[a]
9	2.42[b]

Table 5: Average logs transferred of GII norovirus under wet conditions with initial logs contamination values of 6 or 9 logs. Same letter indicates not significantly different.

Transfer = Wet	Logs Transferred Least-Square Mean
6	4.48[a]
9	6.79[b]

No significant difference between the fruits (strawberry, blueberry, raspberry or grape) was found regarding the transfer of virus from hands to fruit for either of the two scenarios for either inoculum ($p > 0.05$). Ill food workers, represented in scenario 1 for both high titer inoculation and inoculation with the virus cocktail transferred significantly more virus to fruit than healthy individuals represented in scenario 2 ($p \leq 0.05$).

For virus transfer using the high titer GII.4 virus stock, significant differences were seen comparing wet vs. dry transfer (Table 6).

Table 6: Average logs transferred of GII norovirus under wet or dry conditions with initial log contamination value of 9 logs. Same letter indicates not significantly different.

Transfer	Logs Transferred Least-Square Mean
Dry	3.43[a]
Wet	7.15[b]

For virus cocktail transfer, overall, significant differences were found between wet vs. dry transfer and among genotypes, with MNV transfer found to be significantly lower to that of GI and GII (Table 7). Comparison of wet and dry transfer for all three viruses found that wet transfer resulted in significantly higher contamination (Table 8).

Table 7: Average logs transferred of GI, GII, and MNV under wet and dry conditions with initial log contamination value of 6 logs. Same letter indicates not significantly different.

Genotype	Logs Transferred Least-Square Mean
MNV	4.01[a]
GII.4	4.22[b]
GI.1	4.38[b]

Table 8: Average logs transferred of GI, GII, and MNV under wet or dry conditions with initial log contamination value of 6 logs. Same letter indicates not significantly different.

Transfer	Logs Transferred Least-Square Mean
Dry	3.79[a]
Wet	4.61[b]

Table 9. Virus log transfer from a simulated sick individual to fruit after touching a stainless steel coupon, n=6

Pathogen	Wet/Dry Transfer	Fruit	Logs Transferred Average±SD
GI	Dry	Strawberry	3.91 ± 0.39
GI	Wet	Strawberry	5.38 ± 0.11
GI	Dry	Raspberry	3.81 ± 0.27
GI	Wet	Raspberry	5.19 ± 0.32
GI	Dry	Grape	4.50 ± 0.41
GI	Wet	Grape	5.48 ± 0.33
GI	Dry	Blueberry	4.25 ± 0.32
GI	Wet	Blueberry	5.07 ± 0.18
GII	Dry	Strawberry	3.88 ± 0.35
GII	Wet	Strawberry	5.12 ± 0.15
GII	Dry	Raspberry	3.52 ± 0.15
GII	Wet	Raspberry	5.24 ± 0.37
GII	Dry	Grape	4.43 ± 0.29
GII	Wet	Grape	5.29 ± 0.32
GII	Dry	Blueberry	3.93 ± 0.17
GII	Wet	Blueberry	5.06 ± 0.28
MNV	Dry	Strawberry	3.89 ± 0.33

MNV	Wet	Strawberry	5.06 ± 0.17
MNV	Dry	Raspberry	3.71 ± 0.41
MNV	Wet	Raspberry	4.73 ± 0.51
MNV	Dry	Grape	3.98 ± 0.48
MNV	Wet	Grape	5.52 ± 0.41
MNV	Dry	Blueberry	3.86 ± 0.43
MNV	Wet	Blueberry	4.27 ± 0.84

Table 10. Log virus transfer from a simulated healthy individual to fruit after touching a contaminated stainless steel coupon, n=6

Pathogen	Wet/Dry Transfer	Fruit	Logs Transferred Average±SD
GI	Dry	Strawberry	3.59 ± 0.33
GI	Wet	Strawberry	4.47 ± 0.50
GI	Dry	Raspberry	3.71 ± 0.30
GI	Wet	Raspberry	4.64 ± 1.11
GI	Dry	Grape	4.10 ± 0.80
GI	Wet	Grape	5.02 ± 0.76
GI	Dry	Blueberry	4.00 ± 0.68
GI	Wet	Blueberry	4.62 ± 0.88
GII	Dry	Strawberry	3.43 ± 0.08
GII	Wet	Strawberry	4.23 ± 0.50
GII	Dry	Raspberry	3.55 ± 0.25
GII	Wet	Raspberry	4.58 ± 1.12
GII	Dry	Grape	4.12 ± 0.82
GII	Wet	Grape	4.69 ± 0.74
GII	Dry	Blueberry	3.91 ± 0.59
GII	Wet	Blueberry	4.53 ± 1.06
MNV	Dry	Strawberry	3.50 ± 0.20

MNV	Wet	Strawberry	3.98 ± 0.60
MNV	Dry	Raspberry	3.46 ± 0.14
MNV	Wet	Raspberry	3.58 ± 0.35
MNV	Dry	Grape	4.01 ± 0.71
MNV	Wet	Grape	4.41 ± 0.73
MNV	Dry	Blueberry	3.84 ± 0.53
MNV	Wet	Blueberry	4.11 ± 0.80

Removal of Murine Norovirus, Hepatitis A, and *Salmonella* with Positively Charged Wipes Alone and in Conjunction with a Levulinic Acid/Sodium Dodecyl Sulfate Sanitizer

Dry Wipes

Hand wipes were tested for their ability to remove pathogens from stainless steel coupons and gloved hands. For each pathogen, 50 μl of virus stock or 100 μl of bacteria suspension equaling approximately 6 logs was inoculated onto either the coupon or gloved hand and 1 or 5 swipes was made at 50 ± 5 g of pressure using positively, negatively, or neutrally charged wipes. For MNV, dry wipes yielded < 0.5 log reduction for all wipes regardless of surface charge or number of swipes (Fig 21). While positively charged Nanoceram wipes had the highest log reduction value (0.49) after 5 swipes, no significant differences were found between the different types of wipes or the number of swiping motions ($p>0.05$). Dry wipes tested with HAV yielded a maximum of 0.5 logs reduced using the positively charged 1MDS wipe with 5 swipes (Fig 22). While a positive charge did not improve HAV removal from stainless steel surfaces, both positive and neutral charge wipes were more effective than negatively charged (Millipore) wipes using 5 swiping motions ($p\leq0.05$). No significant difference was found when comparing 1 versus 5 swipes ($p>0.05$). Neutral charge, dry Whatman wipes yielded a maximum reduction of

0.59 logs using 5 swipes for *Salmonella enterica*, (Fig 23), however, this value was not significantly greater than the log reduction achieved by the positive or negative charge wipes ($p>0.05$) and no significant difference in removal was found using either 1 or 5 swipes ($p>0.05$).

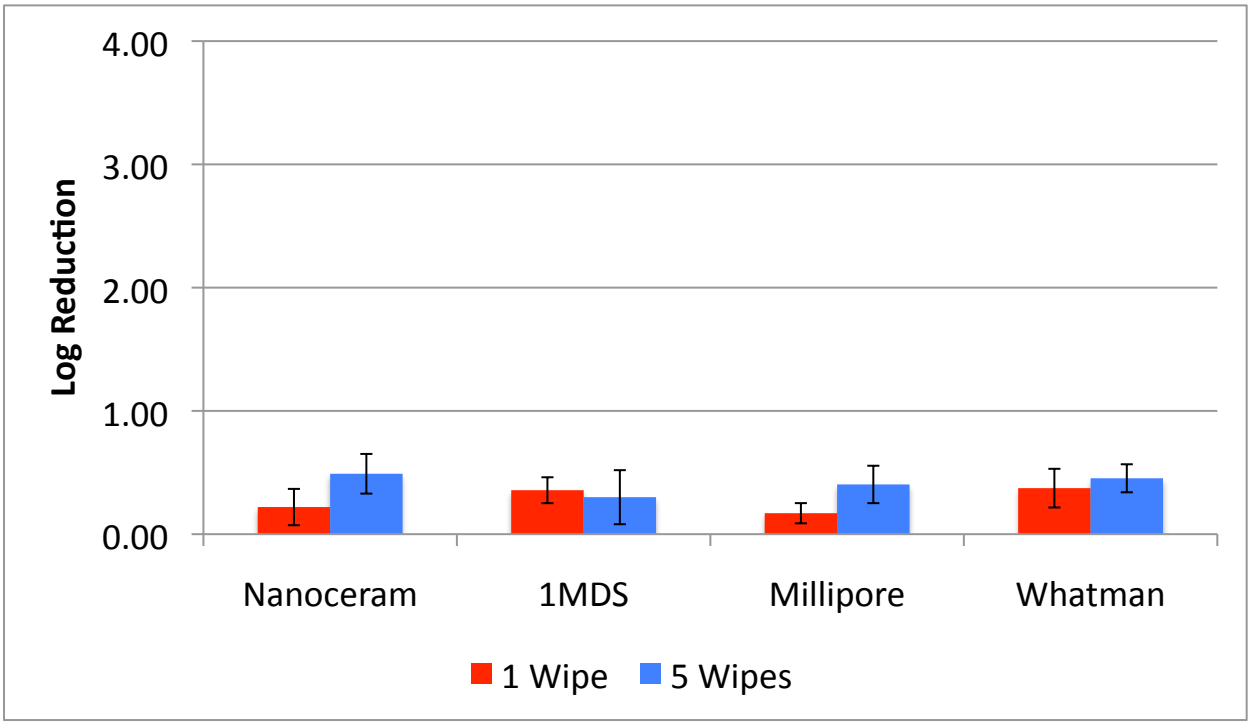

Figure 21: Log reduction of MNV dried on stainless steel coupons after treatment with dry wipes of various surface charges (Nanoceram +charge, 1MDS +charge, Millipore –charge, Whatman 0charge) using 1 or 5 swipes. Error bars indicate standard deviation (n = 3).

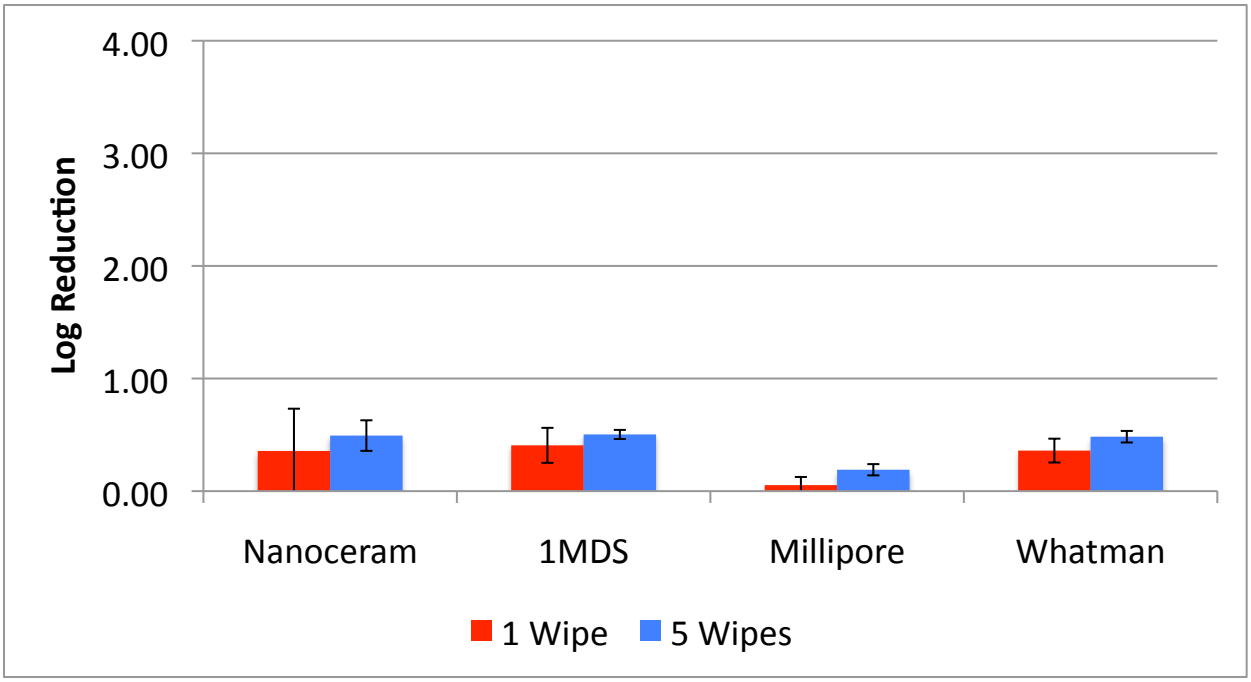

Figure 22: Log reduction of HAV dried on stainless steel coupons after treatment with dry wipes of various surface charges (Nanoceram +charge, 1MDS +charge, Millipore –charge, Whatman 0charge) using 1 or 5 swipes. Error bars indicate standard deviation (n=3).

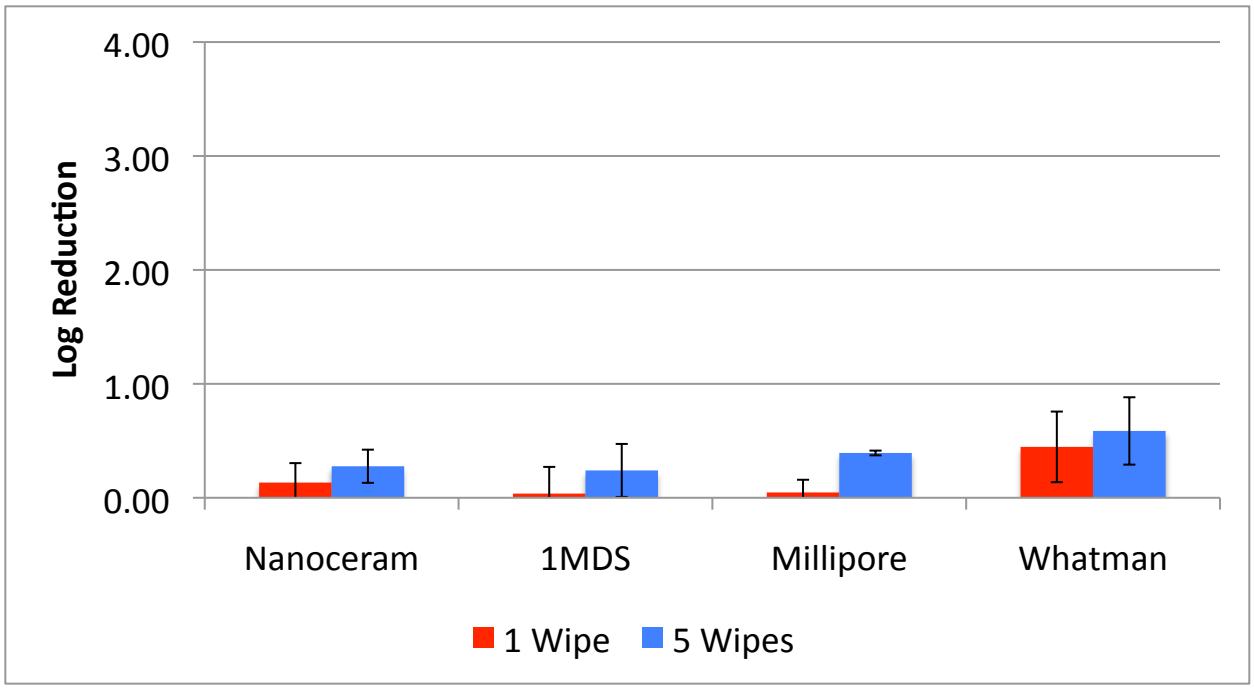

Figure 23: Log reduction of *Salmonella enterica* dried on stainless steel coupons after treatment with dry wipes of various surface charges (Nanoceram +charge, 1MDS +charge, Millipore –charge, Whatman neutral charge) using 1 or 5 swipes. Error bars indicate standard deviation (n=3).

Wet Wipes

Average log reductions for MNV from stainless steel coupons using wet wipes are seen in Fig 24 and Table 21. Overall, significant differences for MNV were observed when comparing wipe charge, number of wipes, sanitizer, and an additional interaction was observed between number of swipes and sanitizer ($p \leq 0.05$). As wiping with 5 swipes yielded higher log reductions for all pathogens, regardless of different combinations of treatments, statistical comparisons between sanitizers and charge were only made for treatments when 5 swipes were used and not for 1 swipe. For wipes soaked in water using 5 swiping motions, both types of positively charged wipes were capable of more MNV removal than dry wipes (1.58-1.63 average log reduction ($p \leq 0.05$) (but this was not significantly different than was observed for neutrally charged wipes (1.47 average log reduction, $p > 0.05$). Wiping with positively charged wipes (5 swipes) soaked in different combinations of levulinic acid plus SDS resulted in greater log reductions of MNV than did wiping with wipes soaked in water ($p \leq 0.05$) for all treatments except 3% Lev/2% SDS. Using 5 swiping motions, average log reductions were 2.29, 2.35, and 1.83 for Nanoceram, 1MDS, and Whatman with the 2% Lev/1% SDS level and 2.27, 1.99, and 2.08 for the same wipes at the 3% Lev/2% SDS level (data not shown). The greatest log reduction for MNV using wet wipes yielded as high as a 2.77 log reduction using the 1MDS wipe in combination with a 5% Lev/2% SDS solution. No differences in MNV log reduction were observed between treatment with 5% Lev/1% SDS or 5% Lev/2% SDS ($p > 0.05$). Using 5 swipes removed significantly more MNV than 1 swipe did (Table 11).

Table 11: Average log reduction for MNV using either 1 or 5 swipes. Same letter indicates not significantly different.

# of Swipes	Log Reduction Least-Square Mean
1	0.982[a]
5	2.267[b]

Examining the effect of wipe charge on MNV, both positively-charged wipes removed significantly more pathogen than the neutral charge wipe when using 5 swipes (Table 12).

Table 12: Average log reduction for MNV for each charged wipe when using 5 swipes. Same letter indicates not significantly different.

Wipe Charge	Log Reduction Least-Square Mean
Neutral	2.01[a]
Positive (Nanoceram)	2.43[b]
Positive (1MDS)	2.36[b]

Comparing different combinations of sanitizer among MNV wipes when using 5 swipes, 5% Lev/1% SDS was found on average to remove significantly more pathogen than 3% Lev/2% SDS and water (Table 13).

Table 13: Average log reduction for MNV based on sanitizer used when making 5 swipes. Same letter indicates not significantly different.

Sanitizer	Log Reduction Least-Square Mean
Water	1.56[a]
3% Lev/2% SDS	2.17[b]
2% Lev/1% SDS	2.34[bc]
5% Lev/2% SDS	2.51[bc]
5% Lev/1% SDS	2.76[c]

For HAV, average log reductions are seen in Fig 25 and Table 22. Using wipes soaked in water with 5 swiping motions, both types of positively charged wipes and the neutral wipe were capable of more HAV removal than dry wipes (1.65-1.84 average log reduction, $p<0.0001$). Comparing the differences among sanitizer and wipe charge, significant differences in HAV removal were found when using 5 swipes. Wet wipes with Lev/SDS tested against HAV yielded a maximum of 2.29 logs of virus reduced when 5% Lev/2% SDS was used in conjugation with the Nanoceram wipe for 5 swipes. Positively charged wipes were not tested with 2% Lev/1% SDS or 3% Lev/2% SDS nor were any concentrations of 5% Lev/1% SDS or 5% Lev/2% SDS for any wipe tested with 1 swipe. In all instances where 1 versus 5 swipes was compared, 5 swipes was significantly greater (Table 14).

Table 14: Average log reduction for HAV based on the number of swipes made. Same letter indicates not significantly different.

# of Swipes	Log Reduction Least-Square Mean
1	0.869[a]
5	1.719[b]

Examining the removal of HAV by wipe charge, it was found that the Nanoceram wipe had a significantly higher log reduction compared to the 1MDS wipe which was significantly higher than the neutral Whatman wipe (Table 15).

Table 15: Average log reduction for HAV for each charged wipe when using 5 swipes. Same letter indicates not significantly different.

Wipe Charge	Log Reduction Least-Square Mean
Neutral	1.42[a]
Positive (1MDS)	1.72[b]
Positive (Nanoceram)	1.98[c]

Comparing sanitizer concentrations for removal of HAV when using 5 swipes, 5% Lev/2% SDS was found to be most effective at removal.

Table 16: Average log reduction for HAV based on sanitizer used when making 5 swipes. Same letter indicates not significantly different.

Sanitizer	Log Reduction Least-Square Mean
Water	1.43^{ab}
2% Lev/1% SDS	1.38^{a}
3% Lev/2% SDS	1.74^{ab}
5% Lev/1% SDS	1.88^{b}
5% Lev/2% SDS	2.10^{b}

Average log reductions of *Salmonella enterica* are seen in Fig 26 and Table 23. For wipes soaked in water and using 5 swiping motions, both types of positively charged wipes and the neutral wipe were capable of more *Salmonella* removal than dry wipes but the positively charged wipes had a 2.34-2.51 average log reduction, $p \leq 0.05$ which was significantly different than was observed for neutrally charged wipes (1.14 average log reduction, $p \leq 0.05$). When wipes were combined with 5% Lev/1% SDS, the 1MDS wipe had a lower log reduction than did the neutral Whatman wipe although not significantly lower ($p > 0.05$). Using 5 swiping motions, average log reductions were 2.57, 2.67, and 0.97 for Nanoceram, 1MDS, and Whatman with the 2% Lev/1% SDS level and 2.65, 2.45, and 1.67 for the same wipes at the 3% Lev/2% SDS level (data not shown). The greatest log reduction for *Salmonella* using wet wipes yielded as high as a 2.64 log reduction using the 1MDS wipe in combination with a 5% Lev/2% SDS solution. No differences in log reduction were observed between treatment with 5% Lev/1% SDS or 5%

Lev/2% SDS ($p>0.05$). One swiping removed significantly less *Salmonella* than 5 swipes for experiments involving wet wipes (Table 17).

Table 17: Average log reduction for *Salmonella* based on the number of swipes made. Same letter indicates not significantly different.

# of Swipes	Log Reduction Least-Square Mean
1	2.05[a]
5	2.31[b]

Comparing wipe charge using 5 swipes, both positively charged wipes removed significantly more pathogen than the neutral charge wipe did (Table 18).

Table 18: Average log reduction for *Salmonella* comparing each charged wipe when making 5 swipes. Same letter indicates not significantly different.

Wipe Charge	Log Reduction Least-Square Mean
Neutral	1.73[a]
Positive (1MDS)	2.54[b]
Positive (Nanoceram)	2.67[b]

Comparing sanitizers when 5 swipes were made, both sanitizer combinations involving 5% levulinic acid removed significantly more *Salmonella* compared to water (Table 19).

Table 19: Average log reduction for *Salmonella* based on sanitizer used when making 5 swipes. Same letter indicates not significantly different.

Sanitizer	Log Reduction Least-Square Mean
Water	1.94[a]
2% Lev/1% SDS	2.17[ab]
3% Lev/2% SDS	2.35[ab]
5% Lev/1% SDS	2.48[b]
5% Lev/2% SDS	2.63[b]

A further interaction was found between wipe charge and sanitizer (Table 20). Comparing each combination of treatments when making 5 swipes, the neutral Whatman wipe was found to have a significant interaction depending on the sanitizer used. No interaction was observed for either of the positive charge wipes in combination with a sanitizer when comparing 5 swipes.

Table 20: Average log reduction for *Salmonella* based on sanitizer when neutral charge wipe and 5 swipes are used. Same letter indicates not significantly different.

Wipe Charge = Neutral	Log Reduction Least-Square Mean
Water	1.14[a]
2% Lev/1% SDS	0.97[a]
3% Lev/2% SDS	1.67[ab]
5% Lev/1% SDS	2.34[b]
5% Lev/2% SDS	2.51[b]

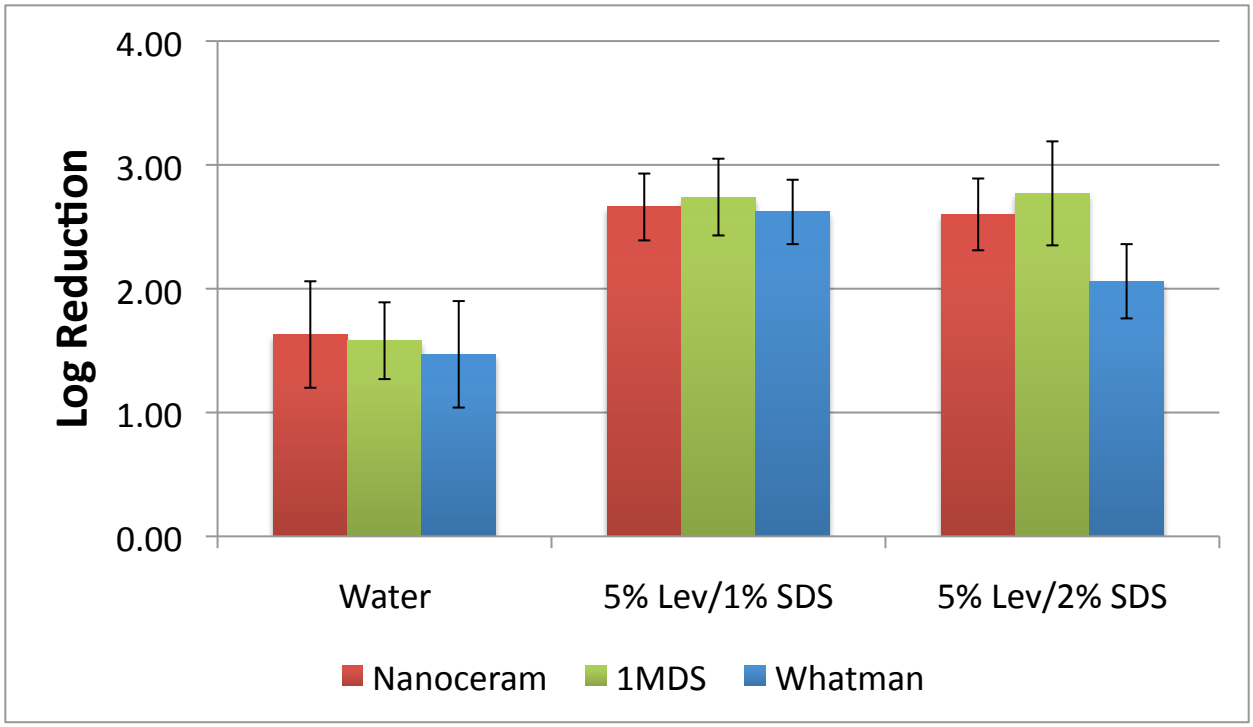

Figure 24
Average log reduction of MNV using 5 swipes for charged and neutral wet wipes (Nanoceram +charge, 1MDS +charge, Whatman neutral charge) n=9.

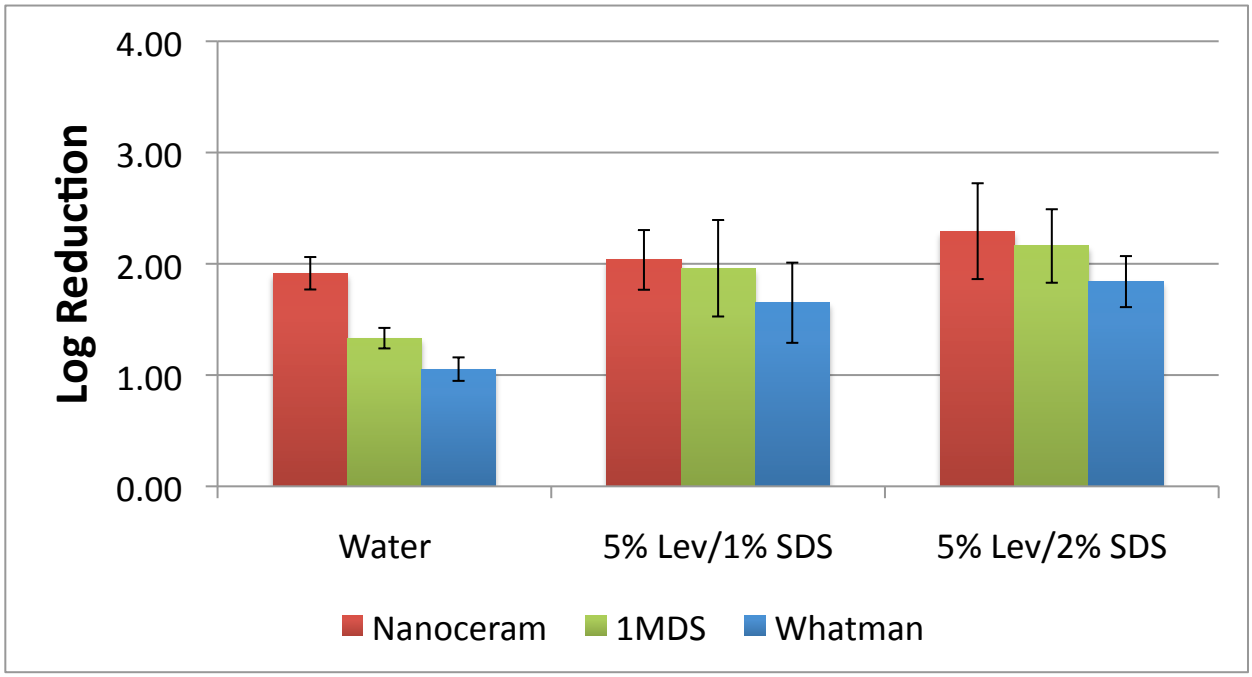

Figure 25
Average log reduction of HAV using 5 swipes for charged and neutral wipes (Nanoceram +charge, 1MDS +charge, Whatman neutral charge) n=6

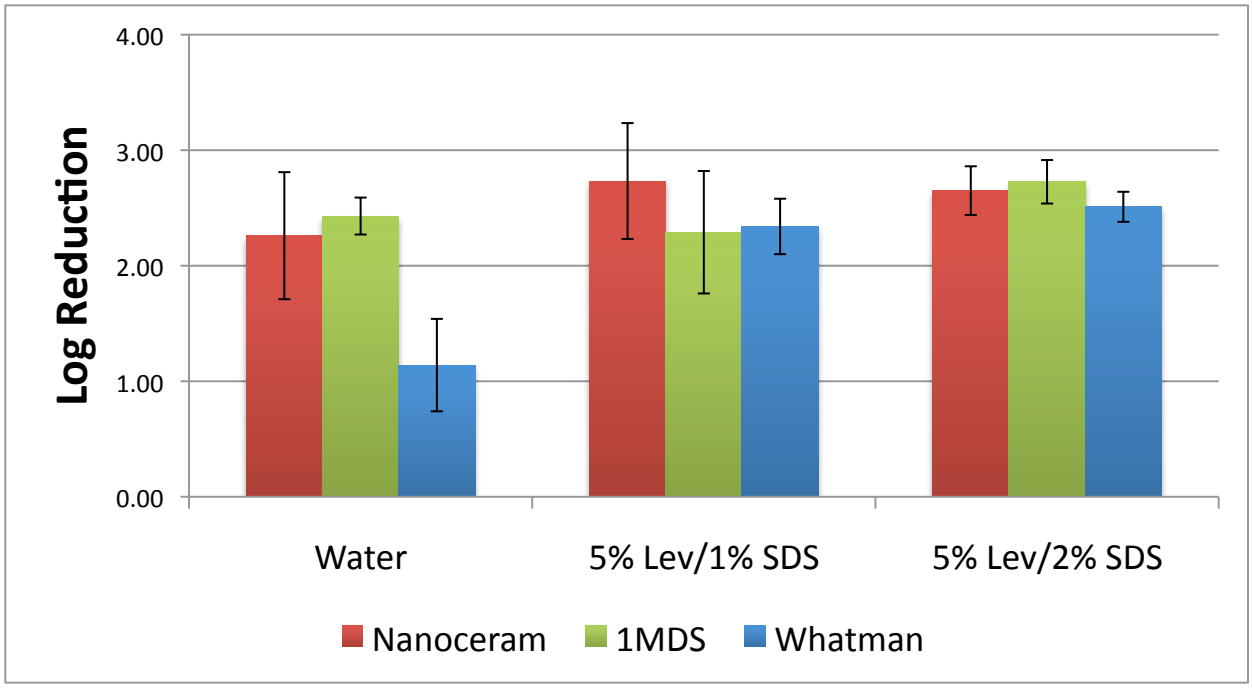

Figure 26
Average log reduction of *Salmonella enterica* using 5 swipes for charged and neutral wipes (Nanoceram +charge, 1MDS +charge, Whatman neutral charge) n=3

Positive charge Nanoceram and 1MDS and neutral charge Whatman wipes all yielded <1 log reduction of MNV on latex gloved hands, regardless of the number of swipes (Fig 27). Data was not collected for concentrations of 2% Lev/1% SDS and 3% Lev/2% SDS using either 1 or 5 swiping motions. No significant differences were observed regardless of the charge of the wipe, sanitizing treatment of the wipe or number of swiping motions. The percent sublethal injury sustained by *Salmonella* during the treatment was calculated and >97% for all treatments.

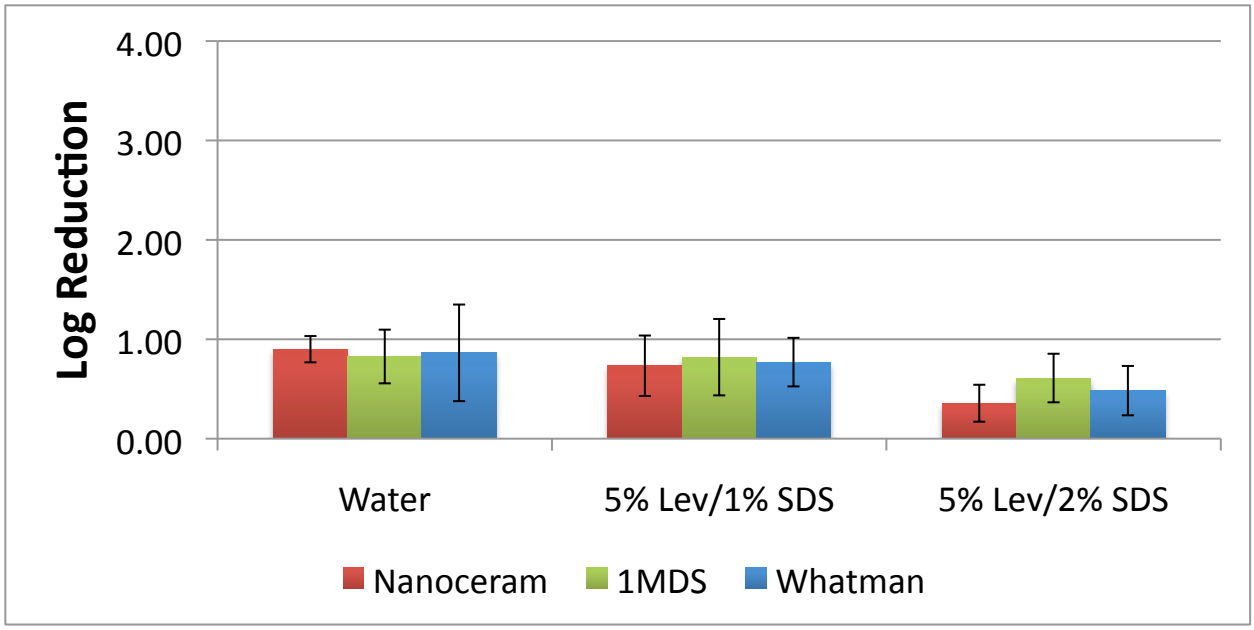

Figure 27
Average log reduction of MNV from gloved finger tips using 5 swipes for charged and neutral wipes (Nanoceram +charge, 1MDS +charge, Whatman neutral charge) N=3

Average log reduction of MNV from gloved fingertips was less than 1 log for all wipes, regardless of charge, treatment, or number of swiping motions. Comparing 5 swipes to 1 swipe resulted in higher log reductions that were statistically different ($p \leq 0.05$). No statistical difference was found comparing charge ($p > 0.05$) and only 5% Lev/1% SDS was statistically greater then water at removal ($p \leq 0.05$). Removal of MNV was significantly less ($p \leq 0.05$) from gloved hands than from stainless steel coupons for all wipe treatments.

Table 21. MNV log reduction from stainless steel coupon using charged and neutral water filters and levulinic acid/SDS

Wipe	# Swipes	Sanitizer (%Lev/%SDS)	Log Reduction Average±SD, n=x
Nanoceram	5	None	0.49 ± 0.16, n=3
Nanoceram	5	Water	1.63 ± 0.43, n=9

Nanoceram	5	5%/1%	2.66 ± 0.27, n=9
Nanoceram	5	5%/2%	2.60 ± 0.29, n=9
1MDS	5	None	0.30 ± 0.22, n=3
1MDS	5	Water	1.58 ± 0.31, n=9
1MDS	5	5%/1%	2.74 ± 0.31, n=9
1MDS	5	5%/2%	2.77 ± 0.42, n=9
Whatman	5	None	0.45 ± 0.11, n=3
Whatman	5	Water	1.47 ± 0.43, n=9
Whatman	5	5%/1%	2.62 ± 0.26, n=9
Whatman	5	5%/2%	2.06 ± 0.30, n=9

Table 22. HAV log reduction from stainless steel coupon using charged and neutral water filters and levulinic acid/SDS

Wipe	# Swipes	Sanitizer (% Lev/%SDS)	Log Reduction Average±SD, n=x
Nanoceram	5	None	0.49 ± 0.14, n=3
Nanoceram	5	Water	1.92 ± 0.15, n=6
Nanoceram	5	5%/1%	2.04 ± 0.27, n=6
Nanoceram	5	5%/2%	2.29 ± 0.43, n=6
1MDS	5	None	0.50 ± 0.04, n=3
1MDS	5	Water	1.33 ± 0.09, n=6
1MDS	5	5%/1%	1.96 ± 0.43, n=6

1MDS	5	5%/2%	2.16 ± 0.33, n=6
Whatman	5	None	0.48 ± 0.05, n=3
Whatman	5	Water	1.05 ± 0.11, n=6
Whatman	5	5%/1%	1.65 ± 0.36, n=6
Whatman	5	5%/2%	1.84 ± 0.23, n=6

Table 23. *Salmonella enterica* log reduction from stainless steel coupon using charged and neutral water filters and levulinic acid/SDS, n=3

Wipe	# Wipes	Sanitizer (% Lev/%SDS)	Log Reduction Average±SD
Nanoceram	5	None	0.28 ± 0.15
Nanoceram	5	Water	2.26 ± 0.55
Nanoceram	5	5%/1%	2.53 ± 0.16
Nanoceram	5	5%/2%	2.60 ± 0.15
1MDS	5	None	0.24 ± 0.23
1MDS	5	Water	2.43 ± 0.16
1MDS	5	5%/1%	2.29 ± 0.53
1MDS	5	5%/2%	2.64 ± 0.12
Whatman	5	None	0.59 ± 0.3
Whatman	5	Water	1.14 ± 0.4
Whatman	5	5%/1%	2.34 ± 0.24
Whatman	5	5%/2%	2.51 ± 0.13

CHAPTER 5

Discussion

There is little published quantitative data regarding the likelihood and degree of food contamination with norovirus that occurs during food handling. While studies have shown the rate of transfer from hands to food and contact surfaces to food using semi-quantitative methods, few studies have examined virus transfer using sensitive realtime RT-PCR methods. Such studies are important for developing quantitative risk assessment models, which will allow the potential of both sick and healthy individuals to contaminate foods during handling to be estimated. Transfer rates have been quantitatively determined to be up to 46% from fingerpads to RTE foods for human norovirus surrogates, such as feline calicivirus *(11)*, yet very few studies have quantitatively examined the likelihood of transferring human norovirus from hands to food and compared that to the transfer rates of surrogate viruses. D'Souza et al. found that using a semi-quantitative model, human norovirus GI could be transferred after drying on a stainless steel coupon for 10 min to wet lettuce for 3/3 replicates after 100 g/9 cm^2 of pressure was applied but 0/3 replicates after the inoculum had dried on the coupon for 60 min *(26)*. Repeating the same procedure using feline calicivirus to determine quantitative transfer, transfer of norovirus from stainless steel to dry lettuce immediately after inoculation, an average transfer rate of 4.93% was revealed for a wet inoculum, while drying the inoculum for 60 min yielded a mean 0.24% transfer rate *(26)*. While the transfer rate of feline calicivirus was quantitatively determined, no comparison was made to human norovirus to determine if human norovirus surrogates would be an acceptable alternative to use during a transfer study.

The removal of pathogens from hands has been widely researched and while a great deal of literature exists regarding the use of sanitizers and handwashing *(13, 14, 72)*, to date there does not exist a method or product that is appropriate for all situations and is effective against a broad range of pathogens. There is a current lack of understanding regarding the potential for RTE produce becoming contaminated with norovirus during handling which is coupled with the fact that few hand sanitizers or sanitizing wipes exist that are effective against human norovirus and other enteric pathogens. A recently developed sanitizer demonstrated efficacy against norovirus, using murine norovirus a surrogate. The chemical blend of 70% ethanol with polyquaternium-37 and citric acid yielded a ≥3.68 logs reduction of MNV in solution and a 2.48 log reduction from fingerpads *(64)*. While this formulation shows promise as an effective sanitizer against norovirus, it may not be permitted for application on edible items such as produce.

The purpose of this research was to quantify the degree of contamination by human norovirus that occurs during handling of RTE fruits including strawberries, raspberries, grapes, and blueberries under wet and dry transfer conditions and different initial contamination loads. A human norovirus surrogate was included with the human norovirus to determine its acceptability for use in future transfer studies. Attempting to interrupt the transfer of norovirus as well as hepatitis A and *Salmonella enterica*, novel sanitizing hands wipes were tested for their ability to remove pathogens from stainless steel surfaces and gloved hands. Wipes, constructed of positive, negative or neutral charge materials, were tested dry, wetted with water or wetted with a sanitizer consisting of combinations of levulinic acid plus sodium dodecyl sulfate at various concentrations to determine which, if any, were capable of removing or inactivating a variety of foodborne pathogens.

Transfer of virus was higher under wet versus dry conditions for all scenarios and all norovirus genogroups. While it has been observed in previous literature that transfer of a pathogen to food is higher when the inoculum has not been allowed to dry *(10, 11, 26)*, increasing the adherence of the liquid medium to the surface, it must be noted that wet transfer is normally the transfer that will be occurring once hands are initially contaminated with fecal material as there typically will not be enough time to dry. However, once the individual leaves the restroom, the virus remaining on the contaminated door handle may have time to dry before another individual makes contact. Virus transfer from toilet paper to hands was detectable after inoculation under wet and dry conditions with the higher titer stock of norovirus GII of 9 logs but not with the virus mixture containing 6 logs each of GI, GII, and MNV. The theoretical limit of detection for the PCR assay and elution procedure of the virus cocktail was <2,500 or 3.4 logs of virus genomic copy numbers indicating that contamination of hands from toilet paper may occur at lower levels. Transfer from hands to stainless steel coupons, representing contamination of a door handle, indicated that transfer readily occurred (up to 8.7 logs for high titer virus stock and 5.7 logs for the lower titer virus cocktail) but that a tremendous amount of norovirus likely remained on the gloved finger tips (5.7 logs for lower titer virus cocktail to 8.7 logs for the high titer stock). This example demonstrates that while ≤50% of virus was transferred, even a low percentage of transfer can lead to very high levels on contamination. Virus transfer from a contaminated door handle to hands (up to 5.3 logs for virus cocktail and 8.1 logs for the high titer stock), representing contamination of a hand after touching the door handle, showed that a majority of virus remained on the door, increasing the likelihood that subsequent individuals who use the restroom would contaminate their hands upon exit.

Transfer of virus from hands to fruit showed that the potential for contamination is high, as between 5.3 and 8.7 logs of virus may be present on the hands after leaving the restroom. Virus detection on the fruits used in this study indicates a very high likelihood that both individuals shedding norovirus and healthy individuals using the same restroom facilities can contaminate fruit during harvest. With as few as 1 norovirus needed to cause illness *(87)*, the potential for causing an outbreak from handling multiple fruits is high, as the fruit may become mixed into a food dish where many people will consume it. Transfer of virus to fruit may be less than 1% as seen with the transfer of the high titer GII stock, but with viral shedding as high as 10^{12} viruses/g of feces *(91)*, while typical viral loads for GII have been measured at $3.8x10^{8}$ and for GI $2.8x10^{7}$ virus genomic copies/g of feces *(78)*. The risk for contamination may remain high, even after handwashing. There was no statistical difference in the level of contamination transferred to each fruit for any of the norovirus genotypes. It must be noted, however, that the percent recoveries overall were lower for strawberries and raspberries indicating that perhaps more virus is actually transferred to those fruits but cannot be detected due to the lower recovery during elution.

Based on the contamination levels from the single transfer studies, the potential for contamination of fruits by an individual rests in the likelihood of the person initially contaminating their hands. Up to 4.7 logs of virus from the high titer stock and ≤3.4 logs using the virus cocktail may contaminate the hands through the transfer of norovirus from toilet paper to hands. However, this only represents the average amount of virus contaminating the hands through 6 sheets of toilet paper when an individual is shedding norovirus and has liquid feces. The 10 μl inoculated on hands during the transfer scenarios is representative of a worst-case scenario where a higher contamination load occurs and handwashing is neglected. As seen in the

transfer from hands to door handle, door handle to hands, and hands to fruit, the likelihood of contamination during a worst case scenario is very high. In the scenario where a healthy individual contaminates his or her hands and then picks fruit, detectable virus on fruit was actually higher with the lower titer inoculation (~10% transfer of original inoculum) compared to the high titer GII stock (≤1% transfer of original inoculum). While this may seem inconsistent, the virus stock for the high titer GII sample was a fecal suspension while for the virus cocktail, 3 different viruses were mixed in a media containing both fecal suspension from two individuals and residual cell culture lysate remaining after ultracentrifugation and resuspension of MNV in PBS. Past studies examining norovirus transfer have used clarified cell culture lysate ,which contains a high percentage of FBS and other nutrients for cell culture, when working with norovirus surrogates and fecal suspensions for the human norovirus stock, but a direct comparison of the transfer rates of viruses using a virus cocktail has not previously been described *(11, 26)*. This may help to explain why more virus was detected on fruits after dry transfer with the lower titer virus cocktail than with dry transfer of the high titer GII stock, as the percentage solids and viscosity of the different medias may play a role in the level of virus transferred.

Overall, there was no statistical difference among transfer to the different types of fruits for both single and double transfer studies. While in some instances strawberries and raspberries had less detectable virus, this can be attributed to the lower recovery of virus from the fruits (8-60% for strawberries and raspberries compared to ~50-100% for grapes and blueberries). These fruits have many more crevices and indents, which unlike grapes and blueberries, may have allowed for the virus to better adhere to the fruit and become sheltered, avoiding elution. Additionally, the method of elution for strawberries and raspberries differed than from grapes

and blueberries. Strawberries and raspberries were not vortexed but instead inverted repeatedly in elution buffer as they would otherwise break apart, potentially releasing compounds that would be inhibitory towards PCR.

Murine norovirus was included as part of the lower titer virus cocktail. While MNV is used as a surrogate for determining the infectivity of human norovirus, previous studies examining the transfer of norovirus from hands to skin using surrogates such as MNV or Feline Calicivirus have not compared the results with the transfer to human norovirus. Seen in this study, a comparison of virus transfer among GI, GII, and MNV found that MNV transfer was significantly lower when compared to human norovirus viruses for all scenarios. This indicates that MNV may not be a good model to use when estimating the transfer of human norovirus.

Removal of MNV, HAV, and *Salmonella* using dry wipes showed that regardless of charge and number of wipes, dry wipes are ineffective (<0.5 log reduction) at removing the pathogens from stainless steel. Results show that positive and neutral charge dry wipes yielded similar log reductions with both of the positive and the negative wipes showing increased log reductions compared to the negative charge wipe for MNV, HAV, and *Salmonella enterica*. These results cannot be explained solely by the fact that these pathogens exhibit a negative charge at neutral pH. The positive and neutral charge wipes were very thick with a coarse surface texture compared to the less textured surface of the negatively charged wipe. Comparing thick vs. thin dry wipes, one may expect to see an increased removal for both dry and wet wipes. When used as dry wipes, the thick, textured wipes have an increased surface area, which may provide a larger area for the pathogen to adhere to and a greater ability to overcome the friction associated with the swiping motion. As a wet wipe, the thicker wipe has the potential to absorb a greater amount of sanitizer. The ability of dry wipes to remove pathogens may depend more on

the texture and thickness of the wipe and its ability to overcome the friction between the wipe and the dried inoculum rather than the interaction between the charge of the pathogen and the wipe. As a whole, dry wipes were ineffective in removal of MNV, HAV, and *Salmonella* from stainless steel coupons, yielding less than 1 log reductions for all combinations of charge and number of wipes.

Removal of pathogens from stainless steel coupons with positively charged wet wipes yielded higher log reductions for MNV and *Salmonella enterica* compared to HAV. Both positive and neutral charge wipes soaked in levulinic acid/SDS yielded higher log reductions compared to wipes soaked in water. In general, wet wipes yield higher log reductions than dry wipes as the moisture helps overcome the adhesion of the contaminant to the surface. Testing different concentrations found that all wipes containing 5%/1% and 5%/2% Lev/SDS yielded higher log reductions compared to 2%/1%, 3%/2%, and water. At lower concentrations of Lev/SDS (0.5%/0.5%), both MNV and FCV are rapidly inactivated in solution, resulting in a ≥ 4.0 log reduction of MNV or 3.6 log reduction of FCV within 1 min of contact time *(17)*. However at 0.5% Lev/0.05% SDS only FCV is inactivated. Additionally, unpublished preliminary data has shown the use of Lev/SDS at 5%/2% as a liquid or a foam will render MNV noninfectious within 5 min of exposure when the virus is dried on stainless steel *(17)*.

Once the coupon was wiped, the time between wiping and placing the coupon in the neutralization buffer was about 10 s. After wiping, a residue of Lev/SDS was present on the stainless steel coupon that may have further inactivated any remaining pathogens during the 10 s stay before being placed in the neutralizing buffer, yielding an increase in log reduction compared to water. It is possible that inactivation of the pathogen occurred both during the

swiping and immediately afterwards, when the Lev/SDS remained on the coupon prior to neutralization.

While the mechanism of Lev/SDS for inactivating pathogens is unknown, unpublished preliminary data has shown that when tested against murine norovirus and human NoV, there is evidence of damage to the genome as shown by decreased detection of virus using rt-PCR *(17)*. This would indicate that the synergistic effect of Lev/SDS may penetrate the capsid protein of the virus and render the virus noninfectious due to capsid and/or damage to the virus genome.

Reduction of MNV levels from gloved hands was significantly lower than that of stainless steel for wet wipes with 5%/1% and 5%/2% Lev/SDS. One reason for this may be that the virus may adhere to the latex surface differently than it does to the stainless steel due to differences in triboelectric potentials (stainless steel is relatively inert as far as its surface charge, while latex is negatively charged) which could disrupt the electrostatic interactions between the wipe and MNV or alter the efficacy of Lev/SDS. Also, the physical nature of the glove was coarse compared to the smooth polished surface of the coupon, potentially allowing virus to become embedded in crevices, protecting it from interaction with the water and sanitizer, and decreasing its ability to be removed by the wipes.

One of the limitations for both the transfer and wipe study is that the interaction between pathogens and gloves is not the same as between those pathogens and human skin. Differences between latex gloves and human skin include the surface charge, surface texture, and presence of oils on the skin all of which could affect the amount of virus reduced after treatment or the amount of virus transferred between surfaces. Gloves were used in these studies because of the time needed for approval when using human volunteers for bare hand experiments. While any comment on the efficacy of the hand wipes tested with Lev/SDS on human hands is speculative,

if these wipes were to be developed and sold for use in real life situations, the log reductions may be greater than what was observed for the experiments involving sanitizing wipes and gloved hands. However, in a real life situation, more than 50 g of pressure would likely be applied, a larger ratio of wipe surface area to contaminated area would exist and more than 5 swipes may be applied, all potentially increasing the log reduction of pathogens. Past research investigating removal of pesticides with hand wipes found that the pressure applied, the number of swipes, and the ratio of surface area to contamination area all factor into the ability of a wipe to remove a pesticide from hands *(14)*. Another limitation of the wipe study was that the limit of detection for elution (3.3 logs in this case) after wiping decreased the maximum possible log reduction. Increasing the amount of virus inoculated on the coupon would allow for a greater amount of virus reduction to be claimed. Also, it is possible that during wipe tests where 5 swipes were made, an increased contact time between the pathogen and sanitizer would yield an increased amount of virus reduction. This increased virus reduction would be due to an extended interaction between the pathogen and sanitizer resulting in inactivation and not from the actual removal by the wipe. For the transfer studies, one limitation was that the inoculation of hands for each scenario was 10 μl of virus stock containing either a 9 or 6 $\log_{10}$ level of virus. While this represented a worst-case scenario, it would be worthwhile to inoculate the hands with the average logs of virus transferred from toilet paper to hands. This would not represent a worst case scenario but rather reenact a typical amount of virus an individual may have on their hands every time they use the restroom and then neglect to wash their hands. Lastly, while a high level of contamination was transferred to fruits, no method was employed to determine how much of the virus is infective.

CHAPTER 6

Conclusion

The results of the virus transfer study are important because they represent the first quantitative study for human and surrogate norovirus transfer studies, which can be used in a quantitative risk assessment model. Overall, there is a significant difference between GI, GII and surrogate MNV during transfer, indicating MNV may not be an acceptable surrogate for transfer studies. From the wipe study, dry wipes are ineffective (<1 log reduction) for removal of MNV, HAV, or *Salmonella* from stainless steel surfaces. Sanitizing wet wipes were more effective for MNV and *Salmonella* but not for HAV. Wet sanitizing wipes were not effective on gloves; however, this does not necessarily correlate to their efficacy on bare hands. In conclusion, the norovirus transfer data supports that there is risk associated with an individual shedding norovirus who uses the restroom and contaminates his or her hands, without washing, will further contaminate both restroom door handles and hand-picked RTE fruits. Additionally, a healthy person who uses the restroom may wash their hands but contaminate them upon exit and proceed to harvest the fruits, contaminating them. While contamination of hands with 10 μl of fecal material represents a worst-case scenario, the data presented here shows one method of how people may potentially cause a norovirus outbreak from contamination during harvest. Subsequently, the interruption of norovirus and other enteric pathogen transfer with hand wipes combined with Lev/SDS shows promise as a surface disinfectant to be used on contaminated stainless steel surfaces in the bathroom (door handles) and/or food contact surfaces. While a 3 log reduction of MNV may not be enough to prevent a norovirus outbreak if handwashing is

neglected, depending on the viral load, it may be enough to cause a reduction in the number of fruits that become contaminated by an ill foodhandler.

Additional research should evaluate the wipes for use on contact surfaces other than stainless steel to determine their efficacy not only as hand wipes but also as general cleansing wipes. While the wipes tested in these models are not effective in removing MNV from gloved hands (<1 log reduction), further optimization of the wipes could include testing different pressures applied, increasing the ratio of the area of the wipe to the area of contamination, wiping on different food contact surfaces, and using bare finger pads instead of gloves as a model for hand wipes. Future wipe studies should also test for removal and inactivation of other enteric pathogens as well as determine if multiple swipes would actually contaminate other areas of the hand. The chemical composition of the Lev/SDS needs further optimization (possibly adding a third or fourth chemical ingredient) to improve its efficacy against HAV. Finally, levulinic acid/SDS has potential to be effective as a hand or surface sanitizer both in foam or gel formulations.

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