

Examining cross contamination pathways for foodborne pathogens in a retail deli environment
using an abiotic surrogate

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Abstract:

Understanding potential cross contamination pathways is essential to reducing the risk of food product contamination. The use of a fluorescing abiotic surrogate (GloGerm™) to visualize the potential spread of bacteria may be beneficial to researchers. To quantify cross contamination during experimental trials in a mock retail deli, a rating method for visualization of fluorescence levels using a trained sensory panel was developed. Panelists' feedback led to a pre-defined strategy allowing for characterization of contamination seen in photographs and reduced variability within responses.

Following validation, GloGerm™ was used to visually represent how bacteria may spread through a deli environment. Six origination sites (slicer blade, meat chub, floor drain, preparation table, employee's glove, employee's hands) were evaluated separately and spread was photographed throughout the mock deli. The trained sensory panel then analyzed the photographs. Five of the six contamination origination sites transferred GloGerm™ to surfaces throughout the mock deli. Contamination from the floor drain did not spread to any food contact surfaces.

To determine the potential of using a GloGerm™/ bacteria mixture to simultaneously track and sample contamination spread; surfaces were co-inoculated with GloGerm™ and bacteria to determine if co-inoculation would affect the recoverability of microorganisms from these surfaces. Three common foodborne bacteria (*E. coli* O157:H7, *Salmonella enterica* ser. Enteritidis, *Listeria monocytogenes*, *Listeria innocua*) were inoculated on 2" by 2" stainless steel

coupons alone and with GloGerm™ . There was no significant difference found ($p > 0.05$) between the recovery of bacteria alone and the mixture for all bacteria.

Finally, the use of co-inoculation was further explored by inoculating two contamination origination sites with either bacteria alone (*L. monocytogenes* and *L. innocua*) or a GloGerm™/bacteria cocktail. Nine recipient sites were sampled after a series of deli procedures were performed. Generally, no significant differences ($p > 0.05$) were seen between the transfer of bacteria inoculated alone and the transfer of bacteria inoculated with GloGerm™ to the selected recipient sites, regardless of contamination source or bacteria. These results suggest there may be potential in using *L. innocua* in combination with GloGerm™ to visually track and sample contamination from a known source throughout a retail deli environment.

Dedication:

I would like to dedicate this to my guardian angels,

David & Mildred Maitland and Jack & Virginia Smith.

Attribution:

Several colleagues and coworkers aided in the writing and research behind the chapters of this dissertation. A brief description of their background and their contributions are included here.

Renee R. Boyer - Ph.D. (Department of Food Science and Technology, Virginia Tech) is the primary advisor and head committee chair. Along with personal mentorship, Dr. Boyer provided constant assistance and guidance as well as resources for funding throughout this research work.

Susan E. Duncan - Ph.D. (Department of Food Science and Technology, Virginia Tech) was an active member of the author's committee. Dr. Duncan's specialized knowledge of sensory methodologies contributed to the development of this work's sensory methods, training, and validation study.

Joseph D. Eifert - Ph.D. (Department of Food Science and Technology, Virginia Tech) was an active member of the author's committee. Dr. Eifert's provided expertise in food safety and food microbiology and contributed to the experimental design and analysis of this work.

Daniel L. Gallagher - Ph.D. (Department of Environmental Engineering, Virginia Tech) was an active member of the author's committee. Dr. Gallagher presented the original proposal for this work as an input to an ongoing FDA/FSIS risk assessment. Dr. Gallagher's expertise in risk analysis and experimental design contributed to the development of this work.

Walter Hartman - (Department of Food Science and Technology, Virginia Tech) provided technical support in designing and constructing the retail mock deli. He built supports to house the hanging UV light boxes and helped to physically move elements into the mock deli space.

Nathan Bauer - D.V.M., MS (Scientific Liaison, United States Department of Agriculture, Food Safety and Inspection Service) provided crucial advice and assistance throughout the development of this work through bi-weekly telephone conferences. He was also in part responsible for the funding of this work.

Janell Kause - (Scientific Advisor for Risk Assessment, Office of Public Health Science, Food Safety and Inspection Service) provided crucial advice and assistance throughout the development of this work through bi-weekly telephone conferences. She was also in part responsible for the funding of this work.

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Chapter 1.

Introduction.

Ready to eat (RTE) deli meats are considered high risk for causing foodborne illness. While there are many different species of pathogens capable of contaminating food products, the pathogen of particular concern in RTE products and retail food establishments is *Listeria monocytogenes*. *L. monocytogenes* has been a serious threat to food safety over the last few decades not only because of its increased prevalence in particular foods but also because of unique characteristics that make it difficult to control. It has been estimated that *L. monocytogenes* causes 1,591 illnesses, 1,455 hospitalizations, and 225 deaths annually in the United States (Scallen et al., 2011).

L. monocytogenes is naturally found in the environment in soil, water, sewage, and decaying plant matter. It can be transferred easily between humans, animals, raw food products, and throughout food processing and retail environments (USDA, 2008). The pathogen is particularly dangerous for individuals who are immuno-compromised, including children, pregnant women, and the elderly. Among those hospitalized for *Listeria* related illness, those most significantly affected are the elderly (> 50 years) at 86.2%. (CDC, 2009) The pathogen is difficult to control because of its ability to grow slowly at refrigerated temperatures (41°F or lower). Without a final intervention step, RTE products that are prepared in a retail deli and kept at refrigeration temperatures are ideal vehicles for infecting consumers with *L. monocytogenes*. A recent FSIS risk assessment found that deli meat sliced at retail was significantly more likely to be contaminated than deli meat pre packaged at the plant (USDA FSIS, 2010). Data from grocery store sampling in 4 different states at a variety of store types revealed that 49 out of 3,518 samples of retail sliced deli meat was contaminated vs. 6 out of 3,522 samples of pre-

packaged deli meat (Draughon, 2006). The risk assessment concluded that approximately 85% of illness and deaths from *Lm* are attributed to deli meat sliced and packaged specifically (Endrikat et al., 2010).

Cross contamination is likely to play a significant role in contamination of RTE foods prepared at retail. In 1998, 2003, and 2008, the FDA surveyed 98 retail deli departments to identify common practices that were out of compliance with FDA standards (FDA, 2009). Two cross contamination related practices that were commonly found to be out of compliance were “hands were not properly cleaned when and as required” (52%) and “visibly contaminated equipment due to lack of proper sanitation” (46%) (FDA, 2009). Over the 10-year period of the study, the percent out of compliance for these two practices improved, but never reached levels considered acceptable by the FDA (80% in compliance) (FDA, 2009).

There are many practices in a retail deli that may contribute to the spread of contamination throughout the environment. Some common sources of contamination include: employees coming to work either dirty or sick, improper hand washing and glove wearing, contact between RTE foods and contaminated equipment, utensils, and pests, purchasing food from unsafe sources, and improper cleaning and sanitizing practices (Penn State University, 2006). Once a location is contaminated, common deli activities can directly transfer contamination between food contact surfaces. For example, industrial slicers and food scales are well-documented sites of *L. monocytogenes* contamination in retail deli environments and contamination can be spread back and forth between meat and slicer (Lin et al., 2006, Sheen et al., 2010, Vorst et al., 2006a, Vorst et al., 2006b).

Food handlers also play a critical role in the transfer of pathogens throughout a retail food service environment. Observational studies at retail food service operations have indicated

several behaviors that may contribute to cross contamination including bare hand contact with raw product and inadequate hand washing. Gloves and hands have been indicated as an important transfer vehicle throughout retail environment. (Montville et al, 2001; Hoelzer et al., 2012).

The high risk of contamination in RTE deli meat sliced at retail and severity of listeriosis for certain populations has generated the need for a full understanding of potential contamination routes. There are currently no studies that track contamination spread between several food contact surfaces and within the retail deli environment. This data gap exists partially because is difficult to track previously unknown pathogen spread without a way to visualize where the contamination may be present. Also the use of pathogens in a functioning retail deli may pose a threat to researchers and the general public, which makes finding a realistic space to run experiments difficult.

This study aimed at using a fluorescing abiotic surrogate (GloGerm™) to visually represent how pathogens, such as *L. monocytogenes*, may potentially spread throughout a mock retail deli facility once introduced by common sources. Once the method was validated and pathways were determined, the potential of using a pathogenic or non-pathogenic biotic surrogate (*L. innocua*) mixed with the fluorescing abiotic surrogate (GloGerm™) to simultaneously track and sample contamination throughout a retail deli environment was explored.

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Objectives.

1. To develop and validate a method to use a sensory panel to evaluate GloGerm™ concentrations on common retail deli surfaces.
2. To track cross contamination transfer dynamics at retail deli markets using the abiotic surrogate, GloGerm™
3. To determine if GloGerm™ and foodborne pathogens could be co-inoculated on to stainless steel and result in consistently recoverable populations similar to when a pathogen is inoculated alone.
4. To compare the recovery rates of a GloGerm™/ bacteria mixture to recovery rates of *Listeria* spp. through environmental sampling of food contact surfaces and meat slices.

Chapter 2.

Literature Review

Listeria monocytogenes

Listeriosis was first observed by Murray et al. in 1926 when researchers noticed the sudden death of young rabbits. Since a large increase in white blood cell production was the most notable characteristic of their death, they named the responsible, yet unknown, microorganism “*Bacterium monocytogenes*” (Murray et al., 1926). A year later, a researcher in Johannesburg, South Africa discovered a microorganism responsible for the deaths of several gerbils with severe damage to the liver. After combining its pathogenic effect with a dedication to Sir Lister (a pioneer of sterile surgery) the researchers named their agent “*Listerella hepatolytica*” (Pirie, 1927). When both groups sent their strains in to an institute in London, the director noticed the similarities and put the two researchers in contact with one another. They eventually agreed to evolve the name into “*Listerella monocytogenes*” (Pirie, 1940). The currently used “*Listeria monocytogenes*” came from an edit by Pirie after the International Committee on Systematic Bacteriology rejected the previous name (Pirie, 1940).

Listeria monocytogenes continues to be a serious threat to food safety not only because of its increased presence in particular foods but also because of the unique characteristics that make it a difficult pathogen to control. *L.monocytogenes* is naturally found in the environment in soil, water, sewage, and decaying plant matter. It is also often transferred throughout humans, animals, raw food products, and food processing and retail environments. The pathogen is particularly dangerous for individuals with weakened immune systems including the immunocompromised, children, pregnant women and the elderly. Among those hospitalized for *L.monocytogenes* related illness, the highest percentage (86.2%) is the elderly (> 50 years)

(CDC, 2009). The pathogen is difficult to control because of its ability to grow slowly at refrigerated temperatures. Ready to eat (RTE) foods without a final intervention step prior to consumption (such as heat or pressure) are at high risk for contamination. It has been estimated that *L. monocytogenes* causes approximately 1,600 illnesses, 1,500 hospitalizations, and 255 deaths annually in the United States (Scallan et al., 2011).

Taxonomy.

The genus contains six species – *monocytogenes*, *ivanovii*, *innocua*, *welshimeri*, *seeligeri* and *grayi* as well as four new species that have been reported over the last 4 years [*racourtaiae*, *marthii*, *weihenstephanensis*, and *fleischmannii*] (LeClerq et al., 2009; Graves et al., 2010; Halter et al., 2012; Bertsch et al., 2012). Out of the two pathogenic *Listeria* species, *L. monocytogenes* is the species commonly associated with infection in humans and *ivanovii* is commonly associated with infections in mammals, particularly ruminants (Guillet et al., 2010). Rare cases of human infection caused by *L. ivanovii* have been documented (Guillet et al., 2010). Although completely nonpathogenic, *L. innocua* is the most closely related species to *L. monocytogenes* and the two often coexist in various environments. Using internalin (*Listeria* surface proteins responsible for cell entry) profiling and multi locus sequence typing, researchers discovered that *L. innocua* is a young species descendent of *L. monocytogenes* (Chen and Wiedmann, 2009). These results are interesting in an evolutionary sense because it is a rare example of bacterial adaptation towards reduced virulence (Chen and Wiedmann, 2009). Because of their ecological, biochemical, and genetic similarities, researchers often use *L. innocua* as a nonpathogenic surrogate to safely study *L. monocytogenes* in real world situations (Chen et al., 2010). *L. grayi*, on the other hand, is so phylogenetically different from the other *Listeria* species that some suggest this species should be put into the genus *Murraya* (Stuart et al., 1974).

Many subtyping studies have shown that *L. monocytogenes* isolates form a structured population, containing different lineages (den Bakker et al., 2008; Orsi et al., 2008; Ragon et al., 2008). The first two lineages (I and II) were identified in 1989 and contain the majority of the serotypes (Piffaretti et al., 1989). Lineage I contains the serotypes 1/2b, 3b, 3c, and 4b, while lineage II contains 1/2a, 1/2c, and 3a. Two additional lineages (III and IV) were discovered in 1995 and 2006 respectively (Rasmussen et al., 1995; Roberts et al., 2006) and currently do not contain any serotypes responsible for human illness. The majority of human listeriosis outbreaks have been linked to lineage I 4b isolates, although some cases have been caused by lineage I 1/2b and lineage II 1/2a isolates (Jeffers et al., 2001). Ward et al. (2010) evaluated a total of 501 *L. monocytogenes* isolates from USDA-FSIS that had been collected from RTE foods and food processing environments between the years of 1998 and 2008. Through multilocus genotyping, the data showed that the majority of RTE isolates belonged to serotypes 1/2a (41.1%), 1/2b (39.5%), 1/2c (12.4%), and 4b (6.4%) (Ward et al., 2010). The higher prevalence of these isolates in foods is due to the inherent general characteristics of the *Listeria* species.

Characteristics.

Listeria is a small (0.5 mm in diameter, 1-2 mm in length) Gram-positive rod shaped bacterium found in single unit or in short chains. The genus is characterized by its ability to grow at low temperatures, from 4°C up to 45°C, and can even grow very slowly in temperatures as low as -1.7 in some experimental conditions (Membre et al., 2005). *Listeria* grow between pH 4.3 and pH 9.2, optimally growing around pH 7 (Tienungoon et al., 2000). The bacterium can also grow in up to 10% (w/v) salt concentration and survive at even higher concentrations (Sleator and Hill, 2010) allowing *Listeria* to qualify as one of the few foodborne bacteria that will grow at an a_w less than 0.93 (Ryser et al., 2007). *L. monocytogenes*' ability to respond and

adapt to a variety of environmental stresses make the pathogen particularly hard to control in both the food products and food contact surfaces.

Survival at low temperatures. *Listeria* is considered a psychrotolerant organism with an optimal growth range between 30°C and 37°C, but may grow slowly at temperatures as low as -0.4°C and up to 45°C. The earliest study identifying that *L. monocytogenes* could grow at refrigeration temperature was done by Gray et al. in 1948. Gray et al. (1946) found that *L. monocytogenes* from bovine brain suspensions could grow at 4°C after being stored for 3 days up to 3 months (Gray et al., 1948). Growth below 4°C is very slow with doubling times of 12 to >50hrs (ICMSF, 1996) and with extended lag phases (Farber et al., 1989) however as the temperature rises above 4°C growth rate increases and lag phase decreases significantly (ILSI, 2005). This means that even the slightest refrigeration temperature abuse for RTE foods could drastically increase the risk for *L. monocytogenes* growth. Although research is ongoing on *L. monocytogenes*' response to low temperatures, researchers have found the bacteria adapts by inducing the production of cold shock proteins (CSPs) and cold acclimation proteins (CAPs). This wide range of proteins are responsible for actions necessary for facilitating growth; such as changing its membrane fluidity and accumulating compatible solutes.

CSPs are a common response in a variety of pathogens to a sudden change in temperature away from that organism's optimal temperature. One study found that 12 proteins were expressed in *L. monocytogenes* after \leq 120min and a temperature drop from 37°C to 5°C (Bayles et al., 1996). Another 2D gel electrophoresis study reported that 38 CSPs were induced in *L. monocytogenes* after a temperature drop from 25°C to 4°C (Phan-Thanh and Gormon, 1995).

One important function of a group of these proteins is to act as an enzyme modifying the lipid membrane's fluidity. Lowering the temperature causes lipid membranes to convert from a

liquid crystalline state to a gel-like state. Lipids in the membrane who have a lower melting point (anteiso-branched fatty acids) will maintain their fluidity, while those who have a high melting point (iso-branched fatty acids) tend to turn crystalline in the gel-like membrane state (Edgcomb et al., 2000). At 35°C, 95% of *L. monocytogenes* membrane is composed of various anteiso- and iso- branched chain fatty acids (Annous et al., 1997). When exposed to cold temperatures (5°C), *L. monocytogenes* shows a switch in its membranes branching from iso-C_{15:0} to anteiso-C_{15:0} (Annous et al., 1997). There is also an inhibition of fatty acid synthetase, which is responsible for adding 2 carbon units to the C_{15:0} fatty acids. Shorter fatty acid length also contributes to membrane fluidity at lower temperatures (Annous et al., 1997).

Ferritin is another cold shock protein expressed by *L. monocytogenes* during exposure to cold temperatures, as well as high temperatures. Protein 2D electrophoresis has shown that ferritin is induced in *L. monocytogenes* at 5°C for ≥ 4 hrs as compared to 30°C (Hébraud and Guzzo, 2000). Ferritin's ability to bind iron has researchers hypothesizing that ferritin is necessary to transport in iron to facilitate growth during cold exposure (Chan and Wiedmann, 2009). Iron is an essential mineral for *Listeria* growth and potentially for virulence as well, as discussed later in this review (Lungu et al., 2009).

Survival under high osmotic pressure. The use of salt to lower the water activity of a food is one of the oldest methods of food preservation. In order to survive this hurdle, *L. monocytogenes* has developed the ability to adapt to changes in osmolarity of its environment (osmoadaptation) (Hill et al., 2002). Similar to the CSPs mentioned before, studies have shown that when subjected to salt stress, *L. monocytogenes* rapidly activated 12 different proteins falling into one of two groups salt shock proteins (SSPs) or salt acclimation proteins (SAPs) (Duche et al., 2002). Many of these proteins are enzymes and transport proteins that aid in the

accumulation (uptake or synthesis) of compatible solutes (osmoprotectants). These small neutral molecules collect and dissolve in cellular fluid to balance out any osmotic differences (Sleator and Hill, 2010). Bayles and Wilkinson(2000) found that (in order of importance) glycine betaine, proline betaine, acetyl carnitine, carnitine, g-butyrobetaine, and 3-dimethylsilylphoniopropionate act as osmoprotectants in *L. monocytogenes*, The pathogen has access to these solutes in food systems; as betaine is commonly found in foods of plant origin and carnitine is found in animal tissues (Sleator and Hill, 2010).

Studies have shown that, when in the presence of these compounds, salt stressed *L. monocytogenes* cells were found to have up to a 2.6fold increase in growth rate as compared to stressed cells without these protectants (Bayles and Wilkinson, 2000). These solutes have been found to act as a cryoprotectant as well (accumulating in the presence of low temperatures). However, when salt stress was removed and temperature kept between 30°C to 37°C, these compounds were found to have little to no growth promoting effect (Smith, 1996).

Survival under high acidity. *L. monocytogenes* will encounter low pH environments in a variety of environments throughout its journey to infection. These environments include acidic foods (both naturally occurring and added as an intervention), the gastric system, and inside the phagosome after ingestion by the macrophage (Cotter and Hill, 2003). The mean pH of a healthy adult's stomach is approximately 2, a level low enough to effectively kill many un-adapted bacteria, including *L. monocytogenes* (Smith et al., 2003). The mechanisms involved in maintaining intracellular pH homeostasis include F_0F_1 -ATPase, the arginine deiminase (ADI) system, and the glutamate decarboxylase (GAD system) (Ryan et al., 2008). *L. monocytogenes* uses these mechanisms to prompt the acid tolerance response (ATR), a condition where the

bacteria becomes resistant to acidic conditions after exposure to mild acidity (O'Driscoll et al., 1996).

These enzyme systems generally work to promote ATR by actively binding and removing excess intracellular protons (H^+) to increase intracellular pH. Aerobically, the coupling of the electron transport chain and respiratory systems carries out this process. Under anaerobic conditions, such as the intestines, the transportation of H^+ molecules is dependent on enzymes using energy from the breakdown of ATP or transport of proton binding molecules. F_0F_1 -ATPase is a multi subunit enzyme crucial for life in many bacterial species (Koebmann et al., 2000). It works to synthesize ATP under aerobic conditions as a result of protons passing into the cell, and also establishes a proton motive force (PMF) under anaerobic conditions using the expulsion of protons (Koebmann et al., 2000). The arginine deiminase (ADI) system uses a 3-enzyme pathway to catabolize the amino acid arginine into ornithine, ammonia, and carbon dioxide (Ryan et al., 2008). During the reaction, 2 moles of ammonia (NH_3) are produced, which then combine with protons to make ammonium (NH_4^+) ions, increasing intracellular pH. The ornithine created is exchanged through an antiporter protein for another arginine, making this an energy independent process (Ryan et al., 2008).

Perhaps the most understood of the mechanisms in *L. monocytogenes* is the glutamate decarboxylase (GAD) system. The system includes an enzyme that decarboxylates the acidic substrate glutamate to produce a neutral compound. This neutral compound is then exchanged over the cell membrane for another molecule of glutamate by an antiporter transmembrane protein. An intracellular proton is required for this reaction, increasing cytoplasmic pH (Cotter et al., 2001a). Cotter et al (2001b) proved that loss of the gene encoding a key GAD enzyme and a glutamate transporter had a significant effect on *L. monocytogenes*' ability to survive in gastric

fluid (> 5 log reduction relative to the wild-type at pH 3.5). The GAD system has also shown to be important in certain food environments. The system is significantly involved in pathogen survival in low pH foods such as fruit juice (tomato, apple, orange), yogurt, mayonnaise, and salad dressing because these foods all have an abundance of glutamate. Even in diluted skim milk, the addition of free glutamate significantly improved survival of the wild type but not the mutant strains (Cotter et al., 2001b). However, Cotter et al (2001a) still observed some acid tolerance in these mutant strains suggesting this system does not work alone and other mechanisms also contribute to the ATR.

Cross protection. While the responses to different environmental stresses may be attributed to some individual mechanisms, there is definite proof that response from one environmental stress may cross protect for another type of stress. Shahamat et al. (1980) found that *L. monocytogenes* survived in tryptic soy broth containing 25.5% NaCl for 4 days at 37°C. Survival increased to 24 days at 22°C and 132 days at 4°C. *L. monocytogenes* that had been acid adapted at pH 5.2 for 2h has shown to have increased resistance to heat shock (52°C), osmotic stress (25-30%NaCl), and alcohol stress (Phan-Thanh et al., 2000). During cheese making, the product is exposed to an acidic environment and then salted. Faleiro et al (2003) showed that acid adapted (pH 5.5) cheese isolates had a significantly greater survival rate in 20% (w/v) NaCl than nonadapted isolates. They also found that certain osmo-adapted (3.5% w/v NaCl) strains induced the ATR response and were able to survive at pH 3.5 for 1 hr (Faleiro et al., 2003). These findings show that it is important for food manufacturers to not assume that the introduction of more interventions will reduce foodborne pathogens simply by addition.

These mechanisms, working together, help *L.monocytogenes* to survive and grow in hostile environments, such as sanitized food contact surfaces or our digestive system, in order to reach a level necessary to cause infection.

Listeriosis

Listeria are primarily environmental organisms (soil) that have evolved to invade eukaryotic cells, it is not host adapted to humans, causing the estimated infectious dose to be larger and more variable than other common foodborne pathogens (McLauchlin et al., 2004). The infectious dose of *L. monocytogenes* is generally considered to be between 10^6 and 10^9 CFU but is very much dependent upon the nature of the food and host susceptibility (FAO/WHO, 2004; FDA/FSIS, 2003). *Listeria monocytogenes* is an opportunistic pathogen because it primarily produces disease in individuals with weakened immune systems. For the immunocompromised, *L. monocytogenes* is one of the more deadly foodborne pathogens, responsible for an estimated 94% hospitalization rate and 15.9% mortality rate (Scallen et al., 2011). Between 2009 and 2011, a majority (58%) of all listeriosis cases were seen in adults aged ≥ 65 years old with a media age of 72 (CDC, 2013). Listeriosis presents itself in one of three clinical manifestations; febrile gastroenteritis, maternal-fetal listeriosis, and bacteremia with or without cerebral infections (Vasquez-Boland et al, 2001). Although the more fatal symptoms of listeriosis have been diagnosable for decades, it was only recently that self-limiting febrile gastroenteritis was considered a symptom in healthy patients (Ooi and Lorber, 2005). The CDC indicates that listeriosis was not added to the list of nationally notifiable diseases until 2001 (CDC, 2012). *L. monocytogenes* has even been isolated from stool samples of volunteers showing no signs of illness. Researchers detected the organism in 3.57% of stool samples from three individuals over a 1-year period using PCR (Grif et al, 2003). As mentioned earlier, not all

strains of *L. monocytogenes* are equally pathogenic. Isolates from only 4 of the 13 known serotypes (1/2a, 1/2b, 1/2c, and 4b) are responsible an overwhelming majority of reported human listeriosis cases (Buchrieser, 2007).

The general pathway of human foodborne listeriosis begins with the cells surviving the hostile environment of the digestive system. As previously reviewed, *L. monocytogenes* has developed resistance mechanisms to adapt to high salt, low pH, and low oxygen environments found in the stomach and intestines. The bacteria that survive the gastric passage will use a variety of proteins to invade the cells lining the intestinal wall (Schluppler and Loessner, 2010).

Internalins are the proteins on the surface of the bacteria that are involved in the adhesion and invasion of bacteria into host cells. The main internalin used by *Listeria* to invade gastrointestinal cells is internalin A (InlA), which acts by binding to E-cadherin receptors on the host cell membrane (Mengaud et al., 1996). Another internalin, (InlB), is not involved in gastrointestinal cell entry but plays a large role in *Listeria*'s entry into hepatocytes and is required, in combination with InlA, for crossing the materno-fetal barrier (Disson et al., 2008). While internalins help *Listeria* to attach and begin internalization into the cell, it has recently been discovered that clathrin, a molecule commonly associated with internalization of other macromolecules, is also essential in *Listeria* entry (Veiga et al., 2007). The molecule is necessary for recruiting other molecules to induce early actin polymerization, the mechanism that will eventually allow *Listeria* cells to move from cell to cell.

After entry into the cell, the bacteria are entrapped in a vacuole for 30 minutes before escaping. *Listeria* uses a toxin called listeriolysin O (LLO) to create pores in the phagosomal membrane when activated by acidic conditions (pH5.5) (Seveau et al., 2007). Eventually, LLO will cause a lysis of the phagosome, releasing the *Listeria* cell into the cytosol to start replicating.

At this point, the surface protein ActA promotes full actin polymerization and the cells begin to form actin filaments (Robbins et al., 1999). These filaments act as tails and the energy produced from polymerization propel the bacteria through the cytosol at a speed of about 10 $\mu\text{m}/\text{min}$ (Cossart and Toledo-Arana, 2008). Once the bacteria reach the plasma membrane of the host cell they form protrusions that begin to invade neighboring cells. These protrusions will eventually produce a double vacuole in the neighboring cell, which *Listeria* will escape, and start the replication cycle again in the second host cell (Cossart and Toledo-Arana, 2008). The ability of *Listeria* to directly spread from cell to cell within the host allows for the bacteria to avoid many host immune responses (Robbins et al., 1999).

After crossing the intestinal barrier, *Listeria* cells can travel through lymph nodes and blood to the liver, where they replicate in the hepatocytes and the spleen. In individuals with weakened immune systems, the bacteria may then use their invasion mechanism to directly invade the blood-brain barrier and materno-fetal barrier (Cossart and Toledo-Arana, 2008) or to enter white blood cells and travel, unnoticed, to sites within the central nervous system (Clauss and Lorber, 2008). *L. monocytogenes* has shown such an affinity for the central nervous system that physicians are advised to prescribe ampicillin doses appropriate to meningitis treatment in all patients showing presence of *Listeria* in the blood until cerebrospinal fluid can be tested (Clauss and Lorber, 2008). While *L. monocytogenes* infections may cause mild illness in pregnant women, once it crosses the placental membrane it may have severe outcomes for the fetus, including preterm labor, neonatal sepsis, meningitis, or death (CDC, 2013). Between 2009-2011, preterm labor was reported in 64% of pregnancy-associated cases, and 46 fetal losses and neonatal deaths were caused by listeriosis infections nationwide (CDC, 2013).

Listeria in Food

While a description of *L. monocytogenes* was first published in 1926 (Murray et al., 1926), until the early 1980s there were relatively few articles published in journals about the incidence of *Listeria* in foods. Sporadic cases were reported associating the bacteria with handling infected animals. For decades, listeriosis was believed to be a “zoonosis”, an illness transmitted directly from animal to humans (Seeliger and hoehne, 1979).

A heavily referenced article published in the New England Journal of Medicine in 1983 titled “Epidemic listeriosis – evidence for transmission by food” suggests that little was previously known connecting the two (Schlech et al., 1983). Schlech et al (1983) investigated a 1981 outbreak of listeriosis that resulted in 41 infections and significant morbidity and mortality rates (9 still births, a 27% mortality rate among neonates, and a 28.6% mortality rate among adults). Contaminated coleslaw was identified as the vehicle after *L. monocytogenes* serotype 4b was isolated from patients’ blood samples as well as from leftover coleslaw in one patient’s refrigerator. After investigating the source of the vegetables, researchers found a cabbage farm adjacent to a flock of sheep with history of deaths from listeriosis. The cabbage fields were fertilized with compost from the infected sheep flocks and then stored in a cold storage shed that essentially served as cold enrichment for the bacteria (Schlech et al., 1983).

Several studies were published following Schlech et al (1983) linking listeriosis with other food commodities. In 1988, a paper was published describing a large US foodborne outbreak of *Listeria* that had occurred three years earlier (Linnan et al., 1988). Between January 1 and August 15, 1985, 142 cases of listeriosis were reported in Los Angeles, CA. Ninety-three (65.5%) of the cases occurred in pregnant women and there were 48 deaths (20 fetuses, 10 neonates, and 18 non-pregnant adults) (Linnan et al., 1988). A case-control study implicated a Mexican style soft cheese had been contaminated with unpasteurized milk. Four years later, in

June of 1989, 10 out of 36 attendees of a club party in New York City reported symptoms of febrile gastroenteritis and muscle aches (Riedo et al., 1994). *L. monocytogenes* was eventually isolated from all patients' blood or stool. Investigation led the epidemiologists to believe the vehicle of *Listeria* to be shrimp but the real significance of this case study was the suggestion that consumption of *Listeria* could result in a milder self limiting illness in healthy individuals (Riedo et al., 1994). The term "*Listeria* hysteria" was coined towards the end of 1980s following this series of listeriosis outbreaks. Recently, this term reemerged after a large outbreak in Canada linked to deli meats (Warriner and Namvar, 2009).

Further evidence towards the varying degree of foodborne listeriosis was seen in an outbreak involving individuals who attended a picnic at a Holstein cow show in Illinois on July 9, 1994 (Dalton et al., 1997). Forty-five illnesses were reported in picnic attendees (aged 3 to 79) with 4 hospitalizations and no fatalities. Traceback investigation discovered illness was associated with chocolate milk and samples from the dairy revealed contamination of *L. monocytogenes* serotype 1/2b ($\sim 10^9$ CFU/mL) from a floor drain and valve connected to the pasteurizer (Dalton et al., 1997).

Since the publication of these principal studies, *L. monocytogenes* has been isolated from a wide variety of foods, from meat and poultry products, to seafood, to fresh produce and dairy products (Ryser et al., 2007). It has also commonly been isolated from food processing environments (Carpentier and Cerf, 2011), retail environments (Hoelzer et al., 2011), and consumer's homes (Kilonzo-Nthenge et al., 2008). In order to control *L. monocytogenes* in the food industry it is important to focus on quality assurance of the food products themselves, as well as on the development and implementation of standards of quality for the environments surrounding the food.

***L. monocytogenes* in RTE Food**

Ready to eat (RTE) food products are defined as “food that is in a form that is edible without additional preparation to achieve food safety” (FDA, 2009a). A variety of commodities may be served in RTE form including raw fruits and vegetables, dairy products; such as milk and cheeses, meat and poultry products; such as hot dogs and delicatessen meat, and combination products; such as deli salads. RTE foods: may still contain indigenous bacteria, are often heavily manipulated at retail sites, and are consumed without a final intervention step. All of these factors contribute to a high risk of contamination with a species like *Listeria*, one that is ubiquitous through the environment and can continue to grow at refrigeration temperatures in high salt, high acid, and low oxygen food products.

Outbreaks.

The first landmark outbreak to link a RTE product as the source of clinical listeriosis occurred in 1988 (CDC, 1989). A woman with cancer was hospitalized in Oklahoma with a fatal case of sepsis caused by listeriosis. *L. monocytogenes* was eventually isolated from a package of opened turkey franks in the patient’s refrigerator. Since this case, several major outbreaks and sporadic cases of listeriosis have been linked to RTE commodities (Table 2.1).

In November of 1998, an epidemiological investigation was initiated after clusters of listeriosis cases were reported in New York, Connecticut, Ohio, and Tennessee (Mead et al., 2006). Telephone surveys, interviews, and samples from patients’ refrigerators lead investigators to determine frankfurters and sliced deli meats were the vehicles of contamination. After sampling the production facility in Michigan where the meat originated from, investigators found a ceiling refrigerator unit had been disassembled with a chain saw during facility upgrades. Environmental samples taken after this demolition increased to 92% positive for psychrophilic

organisms (a marker for potential *L. monocytogenes* contamination) as compared to 25% positive before the demolition (Mead et al., 2006). Investigators concluded that the refrigeration unit may have been previously colonized with *L. monocytogenes* and when disassembled lead to contamination spread to food contact surfaces. In total, this outbreak caused a total of 104 reported cases within 24 states and resulted in 14 deaths and 4 stillbirths.

A large multistate outbreak of listeriosis occurred in 9 states between July and October of 2002 (Gottlieb et al., 2006). In total, 54 case patients were identified resulting in 8 deaths and 3 fetal losses. Using a standard questionnaire, investigators determined that turkey deli meat was the common link between each of the patient's food histories. None of the patients interviewed could remember the brand of the turkey deli meat purchased but many could supply information on where it was purchased. The investigators were able to sample 84% of the purchase sites and could track the deli meat back to over 50 processing plants (Gottlieb et al., 2006). After taking into consideration past food safety infractions, history of recent construction, and on-site sampling results, four plants were chosen for on site investigation. The outbreak strain was found in two of the four plants on the floor drains. This outbreak lead to one of the largest meat recalls in US history and new more stringent government regulations on *Listeria* contamination in RTE meat and poultry products (USDA, 2003b).

Another large multistate listeriosis outbreak concerning RTE food products occurred as recently as 2011. A total of 147 patients across 28 states were diagnosed with listeriosis between August and October 2011 (CDC, 2011a). After interviewing 144 of the patients 93% reported eating cantaloupe in the month before onset of illness. Many of the patients could recall that they were Rocky Ford cantaloupes, which are only grown in a region of southeastern Colorado. Traceback led investigators to Jensen Farms, who had a record of shipping these cantaloupes

between July 29 and September 10, 2011 to at least 24 states. FDA officials eventually isolated outbreak strains of *L. monocytogenes* from equipment and cantaloupes within a packing facility on the farm (CDC, 2011a). This outbreak was significant because fruits had previously not been commonly considered as a RTE vehicle for *Listeria* contamination.

Contamination of RTE foods.

RTE foods have been a consistent source of *Listeria* related outbreaks in the US over the last few decades (Table 2.2.1) as well as internationally. According to a survey done of food sampled in food processing plants, supermarkets, and small food markets between 1990 and 1999 throughout Italy, half of the *L. monocytogenes* positive samples were RTE foods; particularly vegetables, dairy products, fish products, and seasoned meats (Gianfranceschi et al., 2003). Because of the apparent connection between the two, the potential for *L. monocytogenes* growth in a variety of RTE foods has been explored in many studies (Burnett et al, 2005; Byelashov et al., 2008; Erickson, 2010; Francis and O’Beirne, 2005; Gombas et al, 2003; Hwang and Marmer, 2007; Linton and Harper, 2008; Skalina and Nikolajeva, 2010).

Produce. RTE produce may be contaminated with *L. monocytogenes* during growth and processing, as well as during preparation at retail or in the home due to cross contamination. The increased consumption of raw produce purchased from farmer’s markets or as prewashed RTE bagged salads over the last few decades has increased the risk of produce contamination (NACMCE, 1999; Thunberg et al., 2002). High levels of *Listeria* on produce are not associated with poor product quality and even highly contaminated produce may not appear dangerous (Steinbruegge et al., 1988). While finding one particular source of contamination throughout the production chain for produce is difficult, soil, nearby wildlife, and humans seem to be the primary reservoirs for *Listeria* contamination (Beuchat, 1996). Thunberg et al (2002) found

Listeria spp. on a variety of produce items purchased from retail supermarkets and farmers markets around the metropolitan Washington D.C. area. For samples obtained from farmer's markets, three produce items were positive for *Listeria* spp.: celery (2/6 samples), field cress (4/11 samples), and potatoes (4/8 samples). Six produce items were found to be positive from supermarket samples: celery (1/6 samples), lettuce (2/4 samples), mung bean sprouts (3/12 samples), soybean sprouts (6/10 samples), watercress (2/11 samples), and yams (1/4 samples) (Thunberg et al., 2002).

Although many studies have often failed to detect *L. monocytogenes* in raw and RTE produce at retail establishments (Farber et al., 1989; Heisick et al., 1989; Sagoo et al., 2001; Samadpour et al., 2006), there have been documented outbreaks due to contaminated produce (Table 2.1) and the potential for growth of *L. monocytogenes* on produce during refrigerated storage has been demonstrated on a variety of products including asparagus, broccoli, cabbage, cauliflower, chicory endive, and lettuce (Beuchat, 1996). The fate of the pathogen varies and is dependent on the type of produce as well as environmental conditions. Greater survival of *L. monocytogenes* has been observed on cabbage than on raw carrots or onions (Erickson, 2010). Ells and Truelstrup Hansen (2006) found that damage to cabbage tissue occurring during processing could increase *L. monocytogenes* populations by 1.5 log CFU/cm² during storage under refrigeration temperatures. However, between certain types of cabbage, the growth potential of the pathogen varies. *L. monocytogenes* increased 7-fold on white and China cabbage, while populations decreased in red and Savoy cabbage at 7°C (Breer and Baumgartner, 1992). Berrang et al. (1989) discovered that *L. monocytogenes* could proliferate on broccoli, cauliflower, and asparagus packaged under modified atmosphere conditions and held at refrigeration temperatures. Following an outbreak implicating fresh cut celery (Table 2.1),

Vandamm et al. (2013) found that, when inoculated with 3 log CFU/g of *L. monocytogenes*, fresh cut celery populations increased by over 0.5 log CFU/g over 7 days while other common foodborne pathogens (*Salmonella* and *E. coli*) stayed consistent.

Dairy. RTE dairy products such as milk and cheese have been implicated in several listeriosis outbreaks over the last few decades (Table 2.1). Raw milk is one significant source of contamination to dairy products, which may be introduced during the milking process (from animals or the environment) or during dairy product manufacturing (Kozak et al., 1996). *L. monocytogenes* is sensitive to heat and is sufficiently destroyed during the pasteurization process. A survey done by the International Dairy Foods Association found a very low incidence rate (0.018%) of *L. monocytogenes* in fluid pasteurized milk in US retail stores in 2000. Only 1 of 5,519 samples tested positive (Frye and Donnelly, 2005). Therefore, contamination of properly pasteurized RTE products is most likely due to post processing contamination or products containing raw milk.

Mexican style cheese (usually a soft unpasteurized milk cheese) has been implicated in many foodborne listeriosis outbreaks (Table 2.1; Linnan et al, 1988). These soft cheeses are commonly made from raw milk and because of their intrinsic properties (higher pH, high water activity, and lower salt concentration) *L. monocytogenes* may survive during manufacturing (De Reu et al., 2002). After a 2012 multistate outbreak that resulted in 4 deaths was traced back to a ricotta salata cheese (an Italian soft cheese) (CDC, 2012), a study found that *L. monocytogenes* applied to the outside of a ricotta salata wheel at a level of 1 log CFU/g, could proliferate into 7 log CFU/g after 4 months of storage at 4°C (Spanu et al., 2012). Although a high number of outbreaks have been linked to these raw cheeses, *L. monocytogenes* prevalence studies show varying results in this commodity. Samples taken from retail markets in Maryland and California

produced only 5 positive samples out of 2,931 fresh soft “Hispanic-style” cheese (Gombas et al., 2003). In 41 raw milk cheeses obtained from retail specialty shops, farmer’s markets and online sources throughout the US, no positive *L. monocytogenes* samples were detected (Brooks et al., 2012). Internationally, these studies see much higher prevalence. A study done on soft cheese samples from one retail market in Belgium found a high prevalence in both raw (49.7%) and pasteurized (60.0%) milk cheeses (Van Coillie et al., 2004). Little et al. (2008), found 17 positive samples in a survey of 1,819 raw or thermized (a short heating process reaching temperatures lower than pasteurization) milk cheeses manufactured in Europe.

A higher prevalence of *L. monocytogenes* has also been found in those RTE dairy products handled at retail (De Reu et al., 2002) than those left in packaging. Warke et al. (2000), found a higher incidence of *Listeria* spp. in open ice cream containers than in packaged ice cream sold at an Indian market. Loncarevic et al. (1995) found an even distribution of positive *L. monocytogenes* samples in whole and precut wedges in a Swedish retail survey of soft and semisoft cheeses. After multiple outbreaks of listeriosis associated with butter in the United Kingdom, Lewis et al. (2006) isolated *Listeria* spp, even *L. monocytogenes*, from butter samples at catering, retail, and production premises throughout the UK.

Deli salads. In a review of 12 studies examining the prevalence of *L. monocytogenes* in RTE produce and produce containing products, *L. monocytogenes* was an infrequent contaminant of these products with one exception (Erickson, 2010). Throughout these 12 studies, the mean percent prevalence of *L. monocytogenes* in deli mayonnaise-based salads was 10.4% (Erickson et al., 2010). Gombas et al. (2003) reported that *L. monocytogenes* was found in 2.36% (202/8549) of prepared deli salads (potato, pasta, coleslaw) and 4.70% (115/2446) of seafood salads sampled from retail establishments throughout Maryland and California. A study done for the joint FDA

FSIS risk assessment, found a 1 log increase of *L. monocytogenes* in store prepared crab salad over 10 days and a 2 log increase in store prepared shrimp salad at 5°C. (FDA FSIS, 2003).

When sampling RTE mixed deli salads in UK retail facilities, Little et al (2007) found a significantly higher proportion of *Listeria* spp. and *L. monocytogenes* in mixed raw vegetable salads with meat (14.7% and 6.0% respectively) compared to salads with seafood (7.4% and 3.8% respectively). The USDA recommends that anyone at risk for listeriosis not eat store made deli salads such as ham salad, tuna salad, chicken salad, egg salad, and seafood salad (USDA, 2005).

Mayonnaise itself is inhospitable for pathogen growth because manufacturers target a pH of ≤ 4.4 , which is below the reported inhibitory pH of 4.5 for foodborne pathogens (Smittle, 2000). However, in RTE deli salads a variety of components are introduced, each with their own microbiological profile and propensity towards contamination causing the survival and growth of *L. monocytogenes* to widely vary in these types of salads. *L. monocytogenes* populations tend to decrease during storage for items such as coleslaw and potato salad but increase in ham and seafood salads. This variety is most likely due to differences in pH once the components are added. Although acetic acid concentration decreases in coleslaw and potato salad because of adsorption into the vegetable tissues (Wu et al., 2002; Hwang, 2005a), the protein-based salads have a much greater capacity for buffering *L. monocytogenes* from acidic conditions (Hwang and Tamplin, 2005b). Storage temperature abuse of these deli salads is common in retail or home environments and also contributes significantly to *Listeria* contamination (Hwang, 2005a, Hwang and Tamplin, 2005b)

Meat and poultry. Raw meats and particularly raw poultry are sources of *L. monocytogenes* (Jay, 1996). However, they are generally not considered high-risk commodities

provided they are adequately cooked before consumption and cross contamination is avoided (Lianou and Sofos, 2007). Major outbreaks have been associated with RTE meat products such as hot dogs and delicatessen meat and poultry products (CDC, 2000) that may be consumed without a final heat intervention and are susceptible to post processing cross contamination within the retail or home environment (Table 2.1).

Higher *L. monocytogenes* levels have been documented in delicatessen meats with a high pH, high water activity, and low nitrite concentrations (Grau and Vanderlinde, 1992). The potential for the growth of three different strains of *L. monocytogenes* (1/2b, 1/2a, and 4b) were analyzed in various RTE meat products at three different temperatures (5°C, 7°C, 10°C) for up to 14 days (Burnett et al., 2005). Cold smoked salmon, ham, and turkey were found to support the growth of *L. monocytogenes*. While growth rates increased with increasing temperature, *L. monocytogenes* still grew at an average rate of 0.39 log CFU/g per day in these products at common refrigeration temperatures (5°C; 41°F).

RTE deli meat as a high-risk commodity. A 2003 collaborative risk assessment done by the FDA and USDA-FSIS ranked 23 RTE foods based on relative risk (FDA FSIS, 2003). The risk assessment took into consideration how much and how often the food was consumed, the frequency and amount of *L. monocytogenes* already found in these foods, the potential for *L. monocytogenes* to grow within the product, the holding temperature during storage, and length of storage before consumption. Of these 23 products, the risk assessment identified deli meats, soft cheeses, pate, and smoked seafood as having the highest risk per serving. RTE deli meats were ultimately found to be responsible for approximately 67% of all listeriosis cases per year in the US (FDA FSIS 2003). The findings of this risk assessment and others (FSIS, 2003a) along with a recent significant outbreak involving deli meat (Gottlieb et al., 2006) were inspiration for the

adoption of new policies and programs devoted to improved training of the inspection workforce and stricter controls of *L. monocytogenes* within the RTE manufacturing process. As a result, FSIS has seen a steady decline in the number of positive sample from in-plant inspections of RTE manufacturing facilities over the last decade (FSIS, 2012).

However, the decline in positive samples at the plants has not translated into a decrease in reported listeriosis outbreaks (Table 2.1). Data from the CDC shows that over the last decade the incidence of listeriosis has stayed steady around ~0.30 cases per 100,000 populations (CDC, 2009). This incidence level missed the Healthy People of 2010 target of 0.24 cases per 100,000 populations (CDC, 2011b). Because listeriosis rates were not dropping alongside the decrease in positive samples from manufacturing facilities, industry, government, and academia all began to look at the retail environment as a potential contamination source.

A more recent FSIS risk assessment found that deli meat sliced at retail was significantly more likely to be contaminated than deli meat pre-packaged at the plant (FSIS, 2010). Data from grocery store sampling in 4 different states at a variety of store types revealed that 49 out of 3,518 samples of retail sliced deli meat was contaminated vs. 6 out of 3,522 samples of pre-packaged deli meat (Draughon, 2006). The risk assessment concluded that approximately 85% of illness and deaths from *L. monocytogenes* are attributed to deli meat sliced and packaged at retail facilities (Endrikat et al., 2010).

***Listeria* Contamination in RTE Deli Environments.**

Although regulatory agencies and the food industry have spent the last few decades on developing regulations and guidelines and sampling within RTE food production plants, the same scrutiny is just beginning to be applied to retail establishments. A cross sectional study of contamination patterns in 121 retail deli facilities throughout New York was carried out in 2009

(Sauders et al., 2009). After environmental samples in each of the 121 retail establishments, 73 (60.3%) of them had a least one positive *L. monocytogenes* environmental sample. Among 412 samples taken from food contact surfaces, 26 (6.3%) were positive for *L. monocytogenes*. Within the food contact surfaces, *L. monocytogenes* was more commonly isolated from the deli sink (12.2% of samples) and the produce preparation area (12.5% of samples) than the slicer and utensils (3.3%), and deli case (3.3%). Among 749 samples from non-food contact surfaces, 125 (16.7%) were positive for *L. monocytogenes*. Within non-food contact surfaces, the pathogen was more commonly isolated from floor drains in the raw meat area and deli area (34.9% and 19.7% respectively), the inside of dairy cases (10.9%), and grocery cart wheels and floors in the dry aisle (7.9% and 7.3%, respectively) The study also found sporadic contamination present on coolers, display cases, walls, scales, and ice bins (Sauders et al., 2009).

This prevalence of *Listeria* in RTE retail environments is independent of any socioeconomic, racial, or ethnic status of the stores. Research has shown that consumers living in low income areas often depend more on local corner stores for purchasing food items (Moore and Diaz Roux, 2006). These small independently owned stores in lower socioeconomic status areas might face more food safety barriers due to limitations in resources and staff (Yapp and Fairman, 2006). A study done in Philadelphia, PA took samples of a variety of food products, including RTE sandwiches and lunchmeat, from 60 retail stores with differing demographics (Signs et al., 2011). Although, the data showed a low incidence of *Listeria* (1/316 lunchmeat samples, 6/595 prepared sandwich samples), the study concluded that there was no significant difference in the safety of RTE lunchmeat and deli sandwiches available to different demographics (Signs et al., 2011). These findings show that any RTE retail facility, no matter how large or small, could be affected by *Listeria* contamination.

Persistence. Certain strains of *L. monocytogenes* have demonstrated the ability to become part of a food-processing environment and remain a consistent member of the facilities microflora for months or years (Nesbakken et al., 1996; Wenger et al., 1990;). Very few studies have examined the persistence of *L. monocytogenes* within the retail deli specifically. During the longitudinal cross sectional study carried out in 121 retail establishments throughout New York (Sauders et al., 2009), 7 of the establishments were selected for follow up testing 8 to 19 months later. Of the 69 environmental samples taken throughout these 7 establishments, 19 (28%) samples were still positive. Five of these establishments saw persistence of *L. monocytogenes* strains matching the original isolates in the floor drains throughout the deli, floor in the dry good aisle, and the deli sink.

Potential Cross Contamination Sources. In 1998, 2003, and 2008, the FDA surveyed 98 retail deli departments to identify common practices that were out of compliance with FDA standards (FDA, 2009b). Two cross contamination related practices that were commonly found to be out of compliance were improper handwashing (52%) and contaminated equipment due to lack of proper cleaning (46%) (FDA, 2009b). Over the 10-year period of the study, the percent out of compliance for these two practices improved, but never reached levels considered acceptable by the FDA (80% in compliance) (FDA, 2009b). Several sources of *L. monocytogenes* in retail environments have been determined including food products, equipment, employees, and the surrounding environment (Penn State, 2006).

Meat chub. The ubiquitous nature of *Listeria* means it is widely present in plant, soil, silage, sewage, human and animal feces, slaughterhouse waste, processing environments, and catering facilities (Beresford et al., 2001). Therefore, there exists the potential that any raw food product entering a RTE facility may be entering with contamination and could lead to

contamination of other food contact surfaces. For products that have been cooked, *L. monocytogenes* is sensitive to heat treatment so post-processing recontamination during packaging or preparation is the main concern.

Deli Slicers. Industrial slicers are well-documented sites of *L. monocytogenes* contamination in retail deli environments and contamination may spread back and forth between meat and slicer (Lin et al., 2006; Sheen et al., 2010; Vorst et al., 2006a; Vorst et al., 2006b). Lin et al. (2006) studied the effects of different levels on *Listeria*-contaminated slicer blades on three different types of meat (uncured oven roasted turkey, salami, and bologna). In this study, higher levels of inoculation correlated with a higher degree of transfer, resulting in more slices contaminated. This trend is also seen in several other studies (Sheen et al, 2008; Vorst et al., 2006a; Vorst et al., 2006b).

In the Lin et al. (2006) study, transfer back to the three meat chubs from the inoculated slicer blade was also studied by sampling the remaining chubs during 90 days of refrigerated storage. While *L. monocytogenes* levels decreased and eventually became non-detectable on the bologna and salami chubs, the bacteria continued to grow on the turkey chub. The decrease in *L. monocytogenes* populations is most likely due to the low moisture content and low pH value of the fermented salami (Beumer et al., 1996) and the presence of sodium lactate and sodium diacetate in the bologna chub (Mbandi et al., 2001).

Lin et al. (2006) also found that after an initial inoculum levels of 3 log CFU/blade was applied to a slicer blade, positive samples were found on the blade housing, blade surface, conveyor belt, and the left inside corner of the area underneath the blade housing. Positive samples were found on three, eight, and one environmental surface after slicing turkey, salami, and bologna respectively. Researchers noticed a layer of fat on the slicer blade and conveyor belt

after slicing the salami and a layer of clear liquid present after slicing the turkey, concluding that the smearing of fat after salami slicing contributed to the greater contamination spread.

Vorst et al (2006a) used a fluorescing powder to identify areas of contamination spread on the slicer to sample during experimental trials. A turkey chub was bathed in GloGerm™ powder and 5 slices were immediately sliced. When observed under UV light, the researchers identified cross contamination spread throughout the following areas of the slicer; table, back plate, guard, blade, and collection area (Vorst et al., 2006a). Gibson et al (2013) also used an uninoculated fluorescing compound powder to track cross contamination originating from a meat chub throughout a deli slicer. Twenty-one participants came in and sliced 5 slices from 3 separate bologna chubs. They found the surfaces on the deli slicer with the highest concentrations after the slicing events to be the carriage tray, collection area, and meat grip (Gibson et al., 2013).

The transfer of *L. monocytogenes* from an inoculated slicer blade to slices of salmon, as well as the transfer from an inoculated salmon filet to the slicer blade and subsequent slices of uninoculated salmon, was studied by Aarnisalo et al. (2007). The study found that a lower transfer to salmon slices was consistently seen when starting inoculum levels were lower (3.5 vs. 8 log CFU/blade), the temperature was colder (0°C vs. 10°C), or the amount of time the inoculum was left to attach to the blade was longer (2.5 hr vs. 10 min).

Employee's hands and gloves. The majority of foodborne illness outbreaks associated with food service employees, involved transmission of the pathogen to the food product via the employees' hands (Guzewich and Ross, 1999). In a study done soliciting expert opinions on potential cross contamination pathways in retail deli operations, most experts agreed that hands and gloves represented a likely transmission vehicle to a wide variety of environmental sites

(Hoelzer et al., 2012). Gloves and hands may become contaminated by touching a variety of surfaces that are frequently contaminated with *L. monocytogenes* throughout a retail deli. A recent observational study carried out in 9 retail delis throughout Maryland and Virginia reported a high frequency of hand to non-food surface contact, followed by hand to food surface contacts (Lubran et al., 2010). Observers also documented that hand washing was not carried out after touching surfaces such as the food scale face, deli case door handles, and trash cans (Lubran et al., 2010).

Proper hand hygiene includes; adequate hand washing with water and soap, prompt drying of hands with disposable towels or air dryers, trimmed nails, frequent changing of gloves, and avoidance of potentially contaminated food surfaces (Todd et al., 2009). Studies have found, however, that even if hand washing is completed, it is usually not done in a way that sufficiently reduces bacterial amount (Todd et al., 2009). Food workers have been known to wash their hands then quickly wipe their clean hands dry on dirty pants or aprons (Martin et al, 1985). Also bacteria may proliferate on surfaces near the hand washing areas, such as paper towel dispenser or sink handles, and lead to quick recontamination of clean hands (Todd et al., 2009). Nel et al (2004) determined that food handlers working with raw meat in moist environments can expect to encounter pathogenic contamination from raw products as well as equipment, such as knives and conveyor belts. After sampling the hands of ninety-nine food production and retail employees, Kerr et al. (1993) found that 12% of workers had low level *L. monocytogenes* contamination on their hands both before and after washing. Almost all of the workers did not wash their hands well enough to sufficiently remove all the *Listeria* because they either did not use soap, washed for too short an amount of time, or used a dirty towel (Kerr et al., 1993). In two cases washing and drying actually contaminated the workers hands with *L. monocytogenes*, as no

growth was seen in samples pre-handwashing (Kerr et al., 1993). *L. monocytogenes* has even been found to survive on contaminated fingertips. Snelling et al. (1991) found that when suspended in milk, 23% of *L. monocytogenes* could be recovered from fingertips after 30 minutes and 9% could be recovered after 120 minutes.

Although currently there exists a data gap on realistic quantitative transfer of bacteria by gloved and bare hands, Perez-Rodriguez et al. (2006) developed models to predict cross contamination transfer from *L. monocytogenes* contaminated chicken to ham slices via bare and gloved hands as well as washed and unwashed hands. The highest risk was found using the same gloves to handle the contaminated chicken and then the ham slices. Commonly worn latex gloves have even shown permeability to bacteria, so some *L. monocytogenes* contamination could reach the hands and spread throughout a retail establishment even with frequent glove changing (Montville et al., 2001). A combination of proper glove usage and hand washing led to the lowest risk, with only 250 of the 100,000 slices showing simulating contamination (Perez-Rodriguez et al., 2006).

Floor Drain. With the amount of raw material being washed into floor drains during cleaning procedures in food processing environments it is not surprising that a ubiquitous microorganism such as *L. monocytogenes* is often detected in these drains (Berrang et al., 2010). Even after extensive plant sanitation and wash down, certain serotypes of *L. monocytogenes* have persisted over months in the same floor drain (Berrang et al., 2010). Subtypes found in floor drains have been isolated on products and have even been determined to be the only environmental isolate matching the subtype of a previously described listeriosis outbreak in deli turkey meat (Ojeniyi et al., 2000; Gottlieb et al., 2006).

Berreng et al. (2010) found that spraying for 2 second into a wet drain resulted in more airborne *Listeria* than the same spray into an empty drainpipe. *L. innocua* spread from the floor drain as far as 4 meters from the drain and as high as 2.4 m above the floor on the walls within 4 m of the drain. Generally < 2 log CFU/plate was found after spraying a drain with an initial inoculum of 8 log CFU/ml, but even a small amount of *L. monocytogenes* spread to these cool wet surfaces could become a significant source of contamination (Berreng et al, 2010).

Other food contact surfaces. Many outbreaks have been linked to cross contamination originating from other food contact surfaces as well. A large listeriosis outbreak affecting staff and students at two different northern Italy primary schools was traced back to dirty food contact surfaces (Aureli et al., 2000). During investigation, 3 locations within the catering plant (sink drain where utensils were washed, sink drain where vegetables were handled, and the work surface where meals were prepared) were found to be positive for *L. monocytogenes* (Aureli et al., 2000). Samples from food items prepared at the plant and interviews from patients determined that corn kernels, used in a corn and tuna salad served at lunch and corn salad served at dinner, was likely the vehicle of contamination. Investigators determined that cross contamination may have occurred from utensils or food contact surfaces and the salads were then left out for several hours at room temperature before being served, giving *L. monocytogenes* time to grow to high numbers (Aureli et al., 2000). Another study found meat containers, knives, and carts to still produce positive *L. monocytogenes* samples after cleaning and disinfection practices were carried out in a processing plants responsible for a widespread listeriosis outbreak in France that lead to 64 deaths (Salvat et al., 1995).

A niche or a harborage site is any hard to reach area where bacteria may successfully escape cleaning and sanitation and grow to large numbers, eventually large enough numbers to

transfer to other sites. In a retail deli some niches may include, hollow rollers on conveyors, cracked tubular support rods on equipment, the space between close fitting metal-to-metal or metal-to-plastic parts, worn or cracked rubber seals around doors, on off valves and switches on equipment, and saturated insulation (Tompkin, 2002). Harborage sites are also of concern during times of construction, when the disruption may break up these sites and introduce a strong virulent strain of *L. monocytogenes* into the environment (i.e. when replacing floor drains, walls, or cooling units) (Tompkin, 2002). For example, a previously described deadly outbreak (Mead et al., 2006) was traced back to a refrigeration unit that may have been colonized with *L. monocytogenes* and when disassembled lead to contamination of food contact surfaces.

Control of *Listeria* in RTE Deli Products and Environment.

Regulations. Following the landmark outbreaks of listeriosis in the 1980s, USDA-FSIS established a “zero tolerance” (no detectable level of viable pathogens permitted in a 25g food sample) in RTE meats and poultry products (FSIS, 2003a). Those products that tested positive for *L. monocytogenes* or came in contact with surfaces contaminated with *L. monocytogenes* are considered “adulterated” under the Federal Meat Inspection Act or the Poultry Products Inspection Act (FSIS, 2003b). Some oppose these regulations claiming that a blanket rule strategy doesn’t effectively control contamination (Chen et al., 2003). Instead they suggest a strategy similar to the Canadian and European policies on *L. monocytogenes* contamination in those products that don’t support growth, where a maximum of 100 CFU/g at point of consumption is allowed (Chen et al., 2003).

In order to allow for some variation, The *Listeria* Rule (or 9 CFR part 430) establishes three alternatives that establishments can adopt to control post processing *L. monocytogenes* contamination in RTE products (FSIS, 2003b). Under Alternative 1, an establishment agrees to

use a post-lethality treatment (PLT) to reduce or eliminate existing *L. monocytogenes* along with an antimicrobial agent or process (AMA or AMP) to suppress growth of *L. monocytogenes*. These interventions must be validated and included in the establishments Hazard Analysis Critical Control Points (HACCP) plan or SSOPs (Sanitation Standard Operating Procedures) (FSIS, 2003b). Alternative 2 establishments can either use a PLT or an AMA, AMP and Alternative 3 establishments are ones that choose to not use either intervention but must still have a detailed sanitation program on record. Verification testing frequency by FSIS is based on which alternative an establishment falls under. An establishment using Alternative 3 will be tested more frequently because of the higher risk of post-lethality *L. monocytogenes* contamination than with products produced from facilities following Alternative 1 or 2. In the same respect, facilities following Alternative 2 will be sampled more frequently than those under Alternative 1.

Antimicrobials. An antimicrobial agent is a substance that either occurs naturally or is added to a RTE meat or poultry product in order to reduce or eliminate pathogens or suppress or limit growth of a pathogen throughout the food product's shelf life (FSIS, 2011a).

Antimicrobials that have shown a reduction or elimination of *L. monocytogenes* in RTE meat and poultry products include; combination of sodium lactate and sodium diacetate, potassium lactate alone, and the combination of potassium lactate and sodium diacetate (Semen et al., 2008; Pal et al., 2008). A full list of approved antimicrobials for processed meat and poultry products can be found in 9 CFR 424.21 CFR (2011f) and in Directive 7120.1 (FSIS, 2011a; FSIS, 2011b).

Common antimicrobial processes for the reduction of *L. monocytogenes* in RTE meat and poultry products is the addition of salt, nitrates, and other additives to achieve a lower water activity, pH, or moisture:protein ratio to reduce the level of bacteria present and continue to

inhibit *Listeria* growth throughout the shelf life of the product (FSIS, 2006). Products using these antimicrobial processes are not shelf stable and still need to be refrigerated, but because of their pH and water activity *L. monocytogenes* should be inhibited and not grow at these refrigerated temperatures (FSIS, 2006).

A recent interagency risk assessment run by FDA and FSIA determined that adding growth inhibitors to RTE foods to act on products both at retail and at home lead to a dramatically significant reduction in the predictive risk of listeriosis (ca. 95%) (FDA, FSIS, 2013).

Post- lethality treatments. Post lethality treatments, such as hot water pasteurization, high pressure processing, radiant heating, and steam pasteurization, are developed to prevent or eliminate any potential post processing *L. monocytogenes* contamination. These treatments may either be applied pre-packaging (i.e. radiant heating) or post-packaging (i.e. high pressure processing). Ultra violet treatments may be classified as a post lethality treatment or antimicrobial process depending on if it is used to eliminate or reduce and suppress *L. monocytogenes* growth. Studies on approved post lethality treatments have shown a 1 to 7 log reduction in *L. monocytogenes* based on product type, duration of treatment, and temperature (FSIS, 2006). Even higher log reductions were obtained when combining both a pre and post packaging treatment to a single product (FSIS, 2006).

Education. Perhaps the biggest challenge for retail deli food managers is keeping a staff trained amidst incredibly high turnover rates. In 1990, the average turnover rate for a retail deli was 67% (German and Hawkes., 1990). Although this data is old it most likely follows the same trend as turnover rates in the full service restaurant industry over the last two decades, which has increased from 78% to 107% (Berta, 2006). While many hours and dollars have gone into

developing controls to limit *L. monocytogenes* contamination in RTE deli products, this literature review has highlighted just how important the deli worker's role is in cross contamination transmission. This high turnover rate makes it challenging to keep deli workers up to date with training crucial to reducing cross contamination risk.

After consulting with 15 experts currently working in or managing a retail deli, Kim et al (2013), translated all 285 food safety objectives from the Food Code into 3 baseline objectives for retail deli training. They include; 1) knowledge of food safety standards identified by either the FDA Food Code, state, county or local ordinances, 2) identification of specific risks within the deli that contribute to foodborne illness, and 3) key practices to ensure food safety within the deli were the main priorities for deli food safety training programs (Kim et al., 2013).

Currently online food safety training programs do not focus specifically on retail deli specific training and these baseline objectives. More research is needed to develop deli specific programs around these 3 baseline needs. One way to develop an easily understood visual representation of cross contamination throughout a retail deli may be through the use of fluorescing surrogates.

Surrogates

A surrogate is defined as something that takes the place of something else, for example, a person or an animal that function as a substitute for another. In microbiology research, a surrogate is referred to as a microorganism that responds to a variety of stresses in a similar manner as that of another particular target organism (Busta et al., 2003). Researchers have used surrogate microorganisms to mimic other microorganisms in a wide range of biological systems for several decades. One of the primary reason surrogates are used in place of another organism being studied is to keep from introducing potentially pathogenic organisms into a working

production system (Busta et al., 2003). From a legal and safety standpoint, researchers working with harmful organisms near other workers, equipment or even the final product leads to a high risk of contamination. Another advantage for the use of surrogates is that target organisms may only be present in the system in very low numbers, surrogate organisms can instead be inoculated into the target system in larger concentrations and its presence can be easily measured to determine the efficacy of the process (Busta et al., 2003).

Selection and Use of Surrogates in Food Safety.

The most commonly used surrogate in recent published food microbiology research is the use of a nonpathogenic strain of the target organism being studied (Busta et al., 2003; Cabrerra-Diaz et al., 2008; Eblen et al., 2004; Gurtler et al., 2010; Kim and Harrison, 2009). The selection of an appropriate surrogate strain is critical to the value of the research. The FDA has listed criteria as characteristics that make a surrogate desirable (Table 2.2; Busta et al., 2003).

Eblen et al (2004) took several of these characteristics into consideration when selecting a surrogate for *E. coli* O157:H7 and *Salmonella enterica* for use in pilot plant studies. Fifteen non-pathogenic *E. coli* strains and one non-pathogenic *E. coli* O157:H43 were compared against two *E. coli* O157:H7 cider outbreak strains and two *Salmonella* produce outbreak strains (Eblen et al., 2004). All strains were tested for the presence of known O157:H7 virulence genes and all but one of the fifteen strains tested negative. Then changes in pH and growth characteristics (generation time, lag phase duration, and maximum growth) of all strains were measured in five different broths: (1. standard tryptic soy broth, TSB, 0.25% glucose; 2. TSB+ 1% Glucose; 3. TSB – G, no glucose; 4. TSB – G + HCl, adjusted to pH 5; and 5. brain heart infusion, BHI) (Eblen et al., 2004). There were few differences identified between the pathogenic and non-pathogenic strains, indicating that the non-pathogenic strains mimicked the pathogenic strains

under the specific conditions tested. When the stains were subjected to greater stressors (attachment and survival on apple surfaces, resistance to H₂O₂ wash treatments, and thermal inactivation) only five non-pathogenic strains remained comparable to the pathogenic strains (Eblen et al., 2004). After considering that strains with higher thermal resistance may result in unnecessary use of energy or over processing of the food product, only one of the fifteen potential surrogates was considered an optimal surrogate for the pathogens (Eblen et al., 2004).

Listeria innocua.

The ability to use non-pathogenic *Listeria innocua* in place of the pathogenic *L. monocytogenes* has been extensively evaluated. Foegeding and Stanley (1991) were the first to suggest the use of *L. innocua* as a biological indicator for *L. monocytogenes*. The researchers used a plasmid to transform *L. innocua* for resistance to erythromycin and chloroamphenicol and to promote enumeration (Foegeding and Stanley, 1991). In order for the surrogate to successfully mimic the pathogen, the researchers determined that it must have equal or greater resistance to heat and to be efficiently used in processing environments in order to assume that reduction of the surrogate correlates with reduction of the pathogen (Foegeding and Stanley, 1991). They found that the transformed *L. innocua* was 1.5 to 3.0 times more heat resistant than *L. monocytogenes* at 56 and 66 °C (Foegeding and Stanley, 1991). The researchers concluded that this modified *L. innocua* would be an effective surrogate for *L. monocytogenes* but warned that the plasmid-encoded resistance may not be stable in food processing environments (Foegeding and Stanley, 1991).

Fairchild and Foegeding (1993) continued the research by developing a *L. innocua* strain that was resistant to antibiotics through selective enrichment instead of encoding with a plasmid. The strain was made resistant to both streptomycin and rifampin by subsequent plating on

Tryptose Soy Agar (TSA) containing increasing concentrations of the antibiotics until the *L. innocua* M1 was resistant at levels of 50mg/L of rifampin and 250 mg/L of streptomycin (Fairchild and Foegeding, 1993). The researchers determined that this method of transforming the strain allowed for an easily enumerated and recoverable surrogate. The thermal resistance of the modified *L. innocua* was compared to *L. monocytogenes* through studying D-values of inoculated milk samples. The study found that the D-values were not significantly different in both sterile and raw milk. The researchers concluded that *L. innocua* M1 was an effective surrogate for *L. monocytogenes* as well as being stable in naturally occurring environmental conditions (Fairchild and Foegeding, 1993).

Since the publication of these initial studies, *L. innocua* has been used as an acceptable surrogate in a wide range of food products and experimental interventions. (Aguado et al., 2003; Boyer et al., 2009; Buchrieser et al., 2003; Buchrieser et al., 2007; Carvalheira et al., 2010; Cornu et al., 2002; Enns et al., 2007; Friedly et al., 2008; Girardin et al., 2005; Joyce and Gahan., 2010; Kamat and Nair, 1996; Kim and Linton, 2008; Ma et al., 2007; Niemira et al., 2003; Nolan et al., 1992; Nufer et al., 2007; Orsi et al., 2011; Rodriguez et al., 2005; Schmid et al., 2005; Vaz-Velho et al., 2000; Williams et al., 2011; Zhang et al., 2007).

The Use of Fluorescent Surrogates.

Once a surrogate strain has been selected for a particular application, enumeration and detection of the surrogate among other naturally occurring microorganisms in the environment or on the food product being tested can be challenging. Inserting fluorescent marking proteins into the surrogate strain is one method to easily isolate the desired organism. Although the addition of fluorescent proteins into a surrogate's genome may alter comparability to the target organism

(Oscar et al., 2002), some researchers have successfully incorporated fluorescence into a surrogate without altering comparability (Cabrerria-Diaz et al., 2008; Noah et al., 2005).

Cabrerria-Diaz et al. (2008) compared the growth, resistance and attachment of non-pathogenic *E. coli* strains (with fluorescent markers) to pathogenic *E. coli* O157:H7. Researchers wanted to use non-pathogenic *E. coli* as a surrogate to study the efficacy of beef carcass cleaning strategies and needed to determine which strains could be used as successful surrogates (Cabrerria-Diaz et al., 2008). Four non-pathogenic *E. coli* strains isolated from cattle hides were selected and genetically modified to express fluorescent proteins (Cabrerria-Diaz et al., 2008). No differences were seen in the growth rate or doubling time between the fluorescent surrogates and parent strains (Cabrerria-Diaz et al., 2008). When exposed to lactic acid interventions (a common practice in reducing contamination in beef), log reductions of the marked surrogates were either the same or lower than the parent strains (Cabrerria-Diaz et al., 2008). Overall, the thermal resistance and cell surface hydrophobicity of all the surrogates was also found to be the same or higher than the parent strains (Cabrerria-Diaz et al., 2008). After being exposed to antimicrobial interventions (high pressure water wash, hot water wash, lactic acid spray, and combinations of all three) the four surrogates enumerated as a cocktail had similar or slightly lower reductions than the parent strains (Cabrerria-Diaz et al., 2008). The study concluded that all four of the nonpathogenic fluorescent strains could be effectively used as surrogates for *E. coli* O157:H7 and *Salmonella* as a validation of hot water and acid wash interventions.

Abiotic Surrogates.

The use of abiotic (non living) surrogates is not as common as the use of non-pathogenic strains in food microbiology. However, the use of abiotic surrogates has the potential to aid

researchers in visualizing contamination routes and validating decontamination strategies. While these types of surrogates would not be as comparable as another microorganism to track a pathogen, their use can inform future research using actual bacteria and help to create targeted, well planned studies. Non-living fluorescent surrogates have made a large impact in researching the risk of contamination in groundwater quality (Harvey et al., 1989; Harvey and Harris, 2002; Auckenthaler et al., 2002; Sinreich et al., 2008) because they are easy to trace through the system and injecting pathogenic bacteria into these natural environments would be incredibly dangerous. Sinreich et al (2008) injected 1- μ m fluorescent polystyrene microspheres as well as a non-pathogenic microorganism underground to trace how similarly sized bacteria may travel through aquifers and contaminate drinking water. The authors found that the non-pathogenic bacteria more closely mimicked the transport of a target microorganisms than the artificial microspheres in both field and laboratory situations but the researchers emphasized the benefit of using microspheres for this work due to their simplicity and availability and suggested more research should be done (Sinreich et al., 2008).

Renken et al. (2005) performed a study to determine if limestone rock mining would cause contamination of a nearby well field of drinking water in Miami, Florida. Using fluorescent microspheres the researchers successfully found that the speed and recovery of the surrogates into the drinking water well field was higher than their original assumption (Renken et al., 2005). These findings helped researchers to realize that they may need go back and reassess the distance between the mine and well field in order to reduce potential for microbiological contamination (Renken et al., 2005).

Fluorescent microspheres have not just been used out in the natural environment but have also been used to monitor the contamination levels in water treatment plants. Baeza and Ducoste

(2004) tested the ability of fluorescent 1- μ m microspheres to mimic *Cryptosporidium parvum* oocysts in water treatment systems that use a variety of disinfectant combinations. To measure survival rates of the non-biological surrogate a fluorescence intensity threshold was developed using histograms to duplicate the inactivation of different strains of *C. parvum* oocysts (Baeza and Ducoste, 2004). The mechanism for detection was that as the disinfectants react with and damage the surface and interior of the microspheres (as they would the oocysts), the level of fluorescence decreases (Baeza and Ducoste, 2004).

GloGerm™.

The primary objective of this project is to use an abiotic fluorescent surrogate to visually mimic cross contamination through a retail deli environment. The abiotic surrogate selected is a commercially available product called GloGerm™ Gel (GloGerm, Moab, UT). The product is well known for use to teaching proper hand washing techniques and aseptic procedures in schools, labs, and hospitals. GloGerm™ is invisible under regular light sources but will fluoresce under UVA or black light (400 – 360nm). Although GloGerm™ is also available in powder and liquid forms, the gel form was chosen for this study because of its uniform coverage of surfaces. It glows a bright blue under the UV light and was the easiest to distinguish on all meat, handler, and equipment surfaces in the mock deli environment.

GloGerm™ uses in research.

GloGerm™ and similar luminol based products have been used in previous research (Oberyszyn and Robertson, 2001; Perfetto et al., 2003; Bergervoet et al., 2008; Snyder et al., 1999). Luminol ($C_8H_7N_3O_2$) is a chemical that fluoresces when reacting with an oxidizing agent (usually hydrogen peroxide). Bergervoet et al (2008) uses a luminol-based spray to detect traces of blood in the typical hospital environment. When the spray comes in contact with blood a

chemical reaction with the blood's hemoglobin (an oxidizing agent) emits a blue fluorescence under UV light. Typical hospital environments, cleaned according to industry standard, were sprayed down with the luminol-spray and photographed to identify left over blood residue (Bergervoet et al., 2008). This process helps inform hospital workers of the presence of blood residue that could contribute to transferring blood-borne infections (Bergervoet et al., 2008). A follow up letter to the editor citing the previous article expressed interested in combining these techniques with the use of GloGerm™ as a surrogate to determine both actual and potential blood contamination hazards (Blenkharn, 2008).

GloGerm™ products have also been used to track the potential for spread of contamination as a result high-speed cell sorting during cytometry analysis. These high-speed sorters help separate and examine small particles in a thin stream of fluid that runs by a detector in the machine. The contamination from these sorters may occur from aerosols formed by micro-droplets (3-7 mm). These micro-droplets may be inhaled by operators or contaminate other nearby surfaces (Oberyszyn et al., 2001). GloGerm™ oil was used as a surrogate for these micro-droplets because of its comparable droplet size (5 mm) (Oberyszyn and Robertson, 2001). The GloGerm™ oil suspension was run through the high-speed sorter under normal and failure modes, and photographs were taken to visualize the spread of contamination of the aerosols. The authors found no florescence on the outside of chamber under all normal modes and failure modes when the chamber door was closed. If the chamber door was left open aerosol formation was seen on the lab bench (Oberyszyn and Robertson, 2001). The research concluded that GloGerm™ is an easy and accurate method of assessing the potential of aerosol contamination from high-speed cell sorters (Oberyszyn and Robertson, 2001).

A similar study done a few years later by Perfetto et al (2003) introduced a potential

Aerosol Management System (AMS) to ensure aerosol containment during high speed cell sorting. GloGerm™ oil was again used to visualize aerosol contamination after the AMS subjects escaped aerosols to six vacuum ports, a povidone-iodine trap and finally a HEPA filter. All operators are also required to wear a full body suit made out of HEPA filter material and connected to a vacuum system. Overall the AMS system achieved a 4-log reduction in potential aerosol contamination compared to not using the system.

GloGerm™ uses in food safety.

Although there have been no published research articles released on the uses of GloGerm™ in validated the efficacy of food safety procedures, it has been used in a variety of training and experimental ways. Dr. Peter Snyder (1999) wrote a short experimental paper on the use of GloGerm™ to determine if single-use gloves are effective in food service if food handlers do not wash their hands according to suggested guidelines. Subjects applied GloGerm™ to their fingertips then applied and removed gloves. Photographs show a high concentration of GloGerm™ spread throughout the inside of the glove as well as on the glove box. This study determined that if hands, even just fingertips, are not properly washed before putting on gloves the chance of spreading contamination is still high (Snyder, 1999).

The state of Alaska developed a series of exercises using GloGerm™ to teach food workers proper food safety techniques (ASFW, 2007). The proper foods safety techniques for which exercises were created include correct hand washing, glove use, and cross contamination. GloGerm™ lotion was used to show that even perceived adequate hand washing is not completely effective and does not always remove germs around cuticles, rings or bandages. GloGerm™ powder was used in one exercise in the series to stress the importance of washing hands before putting on gloves, and to show how using the incorrect instrument to do a task (i.e.

a paper cup to use as an ice scoop) can end up contaminating large areas (ASWF, 2007). The powder is commonly used to show food workers the importance of thoroughly cleaning cutting boards to reduce cross contamination, especially when processing raw material and ready to eat food products.

GloGerm™ lotion and powder are very helpful at helping food industry researchers determine the efficacy of sanitation plans and helps food handlers visualize the importance of proper cleaning. There are several advantages to using GloGerm™ as a surrogate for testing the efficacy of a cross contamination strategy. GloGerm™ has no pathogenic or hazardous ingredients so there is no danger in contaminating product, surfaces or handlers (Oberyszyn and Robertson, 2001). Also using GloGerm™ in research requires little previous knowledge of microbiology and the results are easy to explain to individuals from a range of educational backgrounds (Oberyszyn and Robertson, 2001). Unlike regular microbiological research there is very little preparation time and clean up time since no cultures or growth agars needs to be prepared, grown or disposed of and the results are immediate as opposed to waiting for plates to incubate overnight or longer (Oberyszyn and Robertson, 2001).

In terms of this project, GloGerm™ lotion has been selected as an abiotic surrogate for *L. monocytogenes* based on its ability to be tracked throughout the mock deli environment, ease of purchasing, and because it poses no pathogenic threat to any of the researchers.

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Table 2.1. Reported listeriosis outbreaks by year – Foodborne Disease Outbreak Surveillance System, United States, 1998-2013.

Year	Multistate	Total # of cases (deaths)	Implicated food vehicle
1998	Yes	108 (14)	Frankfurters
	No	4	Frankfurters
1999	No	6	Unknown
	No	4	Unknown
	No	5 (1)	Deli meat
	Yes	11	Pâté
	No	2 (1)	Deli meat
2000	No	13	Mexican-style cheese
	Yes	30 (4)	Deli meat
2001	No	28	Deli meat
2002	Yes	54 (8)	Deli meat
2003	No	3	Unknown
	No	12 (1)	Mexican-style cheese
2005	No	6	Unknown
	No	3	Grilled chicken
	Yes	13 (1)	Deli Meat
	No	12	Mexican-style cheese
2006	No	2 (1)	Unknown
	No	2	Taco or nacho salad
	No	3 (1)	Cheese
2007	No	5 (3)	Milk
2008	No	5 (3)	Tuna Salad
	Yes	20	Sprouts
2009	Yes	18	Mexican-style cheese
	Yes	8	Mexican-style cheese
2010	No	8	Hog head cheese
	No	2	Sushi rolls
	No	4	Unknown
	No	10	Pre-cut celery
	Yes	6	Mexican-style cheese
2011	No	2	Unknown
	No	2	Chive and ackawi cheese
	Yes	147 (33)	Whole cantaloupe
	No	2	Mexican-style cheese
	Yes	15	Aged, blue-veined cheese
2012	Yes	22 (4)	Ricotta salata cheese
2013	Yes	5 (1)	Washed rind, soft-ripened cheese

*Source: modified from Cartwright et al, 2013 and CDC MMWR Vital Signs, June 7, 2013.

Table 2.2. Desirable characteristics for the selection of an appropriate surrogate (Busta, 2003)

1) Nonpathogenic
2) inactivation characteristics and kinetics that can be used to predict those of the target organism
3) behavior similar to target microorganisms when exposed to processing parameters (i.e. pH stability, temperature sensitivity, and oxygen tolerance)
4) stable and consistent growth characteristics
5) easily prepared to yield high-density populations
6) constant population until utilized
7) easily enumerated and differentiated
8) attachment characteristics that mimic those of target
9) genetically stable so results can be reproduced
10) will not establish itself as a spoilage organism
11) susceptible to injury similar to that of a target organism

Chapter 3.

Tracking Cross Contamination Transfer Dynamics at Retail Deli Markets using GloGerm™.

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Abstract.

Ready-to-eat (RTE) deli meats are considered a food at high risk for causing foodborne illness. Deli meats are specifically listed as the highest risk RTE food vehicle for *Listeria monocytogenes*. Cross contamination in the retail deli may contribute to spread of pathogens to these products. Understanding potential cross contamination pathways is essential to reducing the risk of contaminating these products. The objective of this study was to track cross contamination pathways through a retail deli using an abiotic surrogate, GloGerm™, to visually represent how pathogens may spread through the deli environment via direct contact on food contact surfaces. Six contamination origination sites (slicer blade, meat chub, floor drain, preparation table, employee's glove, employee's hands) were evaluated separately. Each site was inoculated with 20 ml of GloGerm™ and a series of standard deli operations were completed (approximately 10 minutes of work). Photographs were then taken under UV illumination to visualize spread of GloGerm™ throughout the deli. A sensory panel evaluated the levels of contamination coverage and intensity on the resulting contaminated surfaces. Five of the six contamination origination sites transferred GloGerm™ in varying degrees to the deli case door handle, slicer blade, meat chub, preparation table and the employee's gloves. Additional locations became contaminated (i.e. deli case shelf, prep table sink, glove box), but not consistently across all trials. Contamination did not spread from the floor drain to any food contact surfaces. The findings of this study reinforce the need for consistent equipment cleaning and food safety practices among deli workers in order to minimize cross contamination.

Introduction.

Ready-to-eat (RTE) deli meats are considered a food at high risk for causing foodborne illness. The 2003 U.S. Food and Drug Administration and U.S. Department of Agriculture, Food Safety and Inspection Service risk assessment ranked deli meat as the highest risk RTE food vehicle for *Listeria monocytogenes* (USDA FDA, 2003). Studies have shown that the likelihood of contamination with *L. monocytogenes* is highest in deli meats sliced at the retail market versus those products sliced and prepackaged at a processing plant (Draughon, 2006; Endrikat et al., 2010; Pradhan et al., 2010). This pathogen is responsible for an estimated 1,591 illnesses and 255 deaths annually in the United States (Scallen et al., 2011). Approximately 83% of cases and deaths associated with deli meats result from product sliced and packaged at retail facilities (FDA, 2009). This high risk of contamination is particularly dangerous to consumers, considering there is usually no final intervention step between production and consumption of RTE foods.

Cross contamination is likely to play the biggest role in contamination of product in the retail deli. In 1998, 2003, and 2008, the FDA surveyed 98 retail deli departments to identify common practices that were out of compliance with FDA standards (FDA, 2003; FDA, 2004; FDA, 2009). Two cross contamination related practices that were commonly found to be out of compliance were improper hand washing (52% in 2009) and contaminated equipment due to lack of proper cleaning (46% in 2009) (FDA, 2009). Over the 10-year period of the study, the percent in compliance for these two practices improved (from 45.8% to 48% for hand washing, from 47.4% to 54.1% for cleaning and sanitizing). The values obtained never reached levels considered acceptable by the FDA (80% in compliance) (FDA, 2009).

There are many practices in a retail deli that can contribute to cross contamination through the environment. Some common sources where contamination may be introduced into the deli include improper hand washing and glove wearing, contact between RTE foods and contaminated equipment, utensils, pests, and improper cleaning and sanitizing practices (Penn State University, 2006). Identifying the most common routes that contribute to bacterial contamination of retail deli meats is an important topic of research, and tracking these potential paths throughout the deli environment is essential (Lin et al., 2006; Lunden et al., 2002; Perez-Rodriguez et al., 2007; Sheen and Hwang, 2011; Snelling et al., 1991; Snyder, 1999; Thompkin, 2002). Once a site is contaminated, common deli operations and movement can directly transfer contamination between food contact surfaces. For example, industrial slicers and food scales are well-documented sites of contamination in retail deli environments because contamination may spread back and forth between meat and slicer (Lin et al., 2006; Sheen and Hwang, 2008; Vorst et al., 2006). Higher levels of initial contamination are correlated with a higher degree of transfer between meat and slicer (Lin et al., 2006).

It is difficult to track pathogen spread due to the labor involved in environmental sampling and inability to identify possible niches where spread may occur without a researcher's knowledge. To compensate, the project used an abiotic fluorescing surrogate. The objective of this study was to track cross contamination through a retail deli from different introduction sites (contamination origination sites) using an abiotic surrogate to represent how pathogens may spread through the environment visually via direct contact on food contact surfaces.

Materials and Methods.

Creation of mock deli. A mock deli was created in the Food Science & Technology department at Virginia Polytechnic Institute and State University. The general layout was shaped

following observations at local retail delis, as well as conversations with representatives from established retail deli operations. The deli layout and dimensions are outlined in Figure 3.1. A traditional style deli case (48 in x 33 ½ in x 52 in high; Bush refrigeration, Camden, N.J.) served as the retail front of the deli. The case contains two shelves for display as well as a bottom drawer for extra storage. A food scale (ADAM equipment, Danbury, CT), selected for ease in cleaning and sanitation, was placed on top of the deli case. An electric slicer (model #2612C; Hobart Corporation, Troy, OH) was used to slice meats and cheeses and is considered an adequate representation of slicers typically found in delis. The slicer was located on a table with a backsplash behind the deli case. Two stainless steel tables (one with a backsplash to be used as a preparation table, and one containing a separate hand-washing sink) and a three-compartment sink completed the space.

To illuminate the space properly for fluorescence, six black light cases (containing two GE brand 48 in 40-watt bulbs each; Lowes, Christiansburg, VA) were evenly spaced around the exterior of the setup (Figure 3.1). The light cases were placed to allow complete illumination over the tables, drains, and into the deli case to achieve adequate fluorescence for photography.

Abiotic Surrogate Selection. The abiotic surrogate selected for the study is a commercially available product called GloGerm™ Gel (GloGerm, Moab, UT). GloGerm is invisible under regular light sources but will fluoresce under UV A (315–400 nm). The product is well known for use in teaching proper hand washing techniques and aseptic procedures in schools, labs, and hospitals and has been used to determine the efficacy of sanitation plans. This product also helps food handlers visualize the importance of proper cleaning and has been used to track food contact surface contamination on a deli slicer to identify sites commonly contaminated during slicing (Bergervoet et al., 2008; Oberyszyn and Robertson, 2001; Perfetto et

al., 2003; Snyder, 1999; FDA, 2009). GloGerm™ is available in powder, gel, and liquid forms. The gel lotion form was chosen for this study because it was the easiest to distinguish on all meat, food handler, and equipment surfaces evaluated in the mock deli environment (results not shown).

Measuring cross contamination throughout the mock deli. To measure both presence and level of contamination throughout the mock deli, a trained sensory panel was used. Preliminary work was done to validate that a trained panel was an effective way to quantify contamination throughout the trials.

Training and validation of sensory panel. Eighteen participants (students, faculty, and staff from the Virginia Tech Food Science and Technology department) were recruited and trained (Institutional Review Board, IRB# 10-654) at Virginia Polytechnic Institute and State University. The panel went through three training sessions before completing the preliminary sensory validation experiments. Preliminary tests documented that the panel (n=18) could determine the differences between five concentrations of GloGerm™ gel on different surfaces, based on how brightly the gel appeared in photographs (data not shown). The subjects could also identify the difference in dilutions when the gel spread from an inoculated blade to meat slices and to a glove used by the operator. The training sessions were validated by achieving a low (less than ± 0.35) standard deviation between participant's responses. These initial samples were used as a control to aid panelists in rating future photographs in this study based on level of brightness.

Contamination and spread through the mock deli. In each experimental trial, one location in the deli was contaminated and a series of standard deli operations were completed (series of actions outlined in Figure 3.2). Six different contamination origination sites were

chosen (deli slice blade, rim of floor drain, surface of deli meat chub, employees bare hands, gloves, and the preparation table surface) based on previously described contamination sites (Berrang et al, 2008; Kovacevic et al., 2009; Kushwaha and Muriana, 2009; Lianou and Sofos, 2007; Lin et al., 2006; Penn State University, 2006; Strohbehn et al., 2008, Thompkin, 2002). For each trial, 20 ml of GloGerm™ lotion was applied to one of the initiation point surfaces in order to simulate that area as the point of contamination. Following contamination, a series of standard deli operations were performed as described previously by Lubran et al. (2010) (Figure 3.2).

Upon completion of the series of deli operations (without disturbing the spread of GloGerm™ through the mock deli), photographs of all surfaces, meat slices, used gloves, and the floor were taken under 350 nm, a mid-range value in the wavelength range of UVA light illumination. Photographs were taken using a Nikon D90 camera mounted on a tripod. The camera was set at ISO 400 with an exposure time of 1.3s at f/6.3. These settings were adapted from Blenkham (2008). In order to ensure consistency, tape was placed at edges of each contaminated surface, and the camera was placed in the same location at the same angle for each photo. The floor was sectioned off into 3ft by 3ft blocks using tape. Following initial evaluation of the slicer, the slicer was broken down and images of each specific component (blade, bed, shelf, handle and carriage; Figure 3.3) were taken and evaluated for contamination. Additional photos were taken of any other locations in the deli where GloGerm™ was observed.

Each contamination start point was assessed during three separate replications of contamination and deli operations series of events. In between each replication the mock deli was sanitized with 70% ethanol. All surfaces were allowed 15 minutes to air dry and then researchers took additional photographs under UVA illumination to ensure that all surfaces were

cleaned and there was no remaining GloGerm™ . The resulting photographs were compiled into photo sets and grouped according to surface.

Sensory panel evaluation. Eight participants from the original validation studies were selected to serve on the sensory panel for this research based on availability and consistency of responses from the preliminary training. This panel of eight members was additionally trained (IRB# 11-014) to rate a single photograph instead of ranking a series of photographs as completed for the validation process. The sensory panel analyzed photographs to determine contamination level of twelve contaminated surfaces (slicer bed, slicer carriage, slicer shelf, slicer blade, slicer handle, preparation table, first meat slice, third meat slice, refrigerated case handle, employee's gloves, meat chub, and floor). Each photo set contained two photos from each starting contaminant point (selected randomly from photos from all three trials). As a control, two photos of the same surface without any GloGerm™ and two duplicate photos were included, for a total of 16 photos. Three of the surfaces (meat slice 1, meat slice 3, and floor) were viewed in sets of 20 photos in order to accommodate different sample numbers. All photo sets were viewed on 17 in. computer monitors in total darkness in the sensory booths located in the Food Science and Technology Building at Virginia Polytechnic Institute and State University (Blacksburg, VA). Every photograph was labeled with a random three-digit code and presented in a different random order to the eight panelists. An example of typical photos that each panelist would see is shown in Figure 3.4. When viewing the photographs, the panelists were asked to rate the presence of GloGerm™ based on two factors, coverage and intensity. Coverage was considered the area in the photograph that contained the GloGerm™ lotion, and intensity was considered the brightness of the GloGerm™ in the photograph. Both values were rated as either

none, slight, moderate, or heavy. After evaluating intensity and coverage separately, the panelists determined one overall contamination rating from a chart that was provided to them (Table 3.1).

Data Analysis. For statistical purposes, the one word overall ratings (given by each panel member) were converted into numerical form: 0 = none; 1= slight; 2 = moderate; and 3= heavy; an average rating for each surface was calculated. Following this conversion, a descriptive value (none, slight, moderate, heavy) was applied to the average. To determine the descriptive value, averages of 0.5 or above were rounded up to the next integer, 0.49 and below rounded down to the next integer. The eight panelists were given photographs from the meat chub, preparation table, slicer blade, deli case door handle, gloves, a floor surfaces, and two replicates of each of the six starting surfaces (n=16). The floor drain set contained photos from two reps of each of the eight floor blocks (n=16). Multiple comparisons are based on the nonparametric-Behrens-Fisher test out of the NPMC library from R version 2.13.2 using $\alpha=0.05$.

There were three types of control samples included in the data: initial GloGerm™ inoculation concentration, surface of site after cleaning, and duplicate photographs of GloGerm™ concentration after running the mock deli. For each, the same eight panelists evaluated the photos twice. The data were analyzed with a paired Wilcoxon test (i.e. a nonparametric version of a paired t-test) using $\alpha=0.05$. Each comparison was conducted separately for coverage, intensity, and overall score. Analysts evaluated five sites for initial concentration, 11 sites following cleaning, and 20 start-end site combinations for the comparisons between deli runs. A one-sample Wilcoxon test was also conducted on the first panel evaluation of the initial concentration and the control concentration. The initial concentration was tested against a mean of 3 for each site, and the cleaned concentration against a mean of 0 for each site.

Results.

Statistical Analysis of Controls. The evaluation of controls is used to validate that the panel is well trained and validate the methods chosen for the experimental design. There were no statistically significant differences for the paired data based on the initial contamination for any of the sites. For the initial GloGerm™ inoculation concentrations, a significant difference from a mean of 3 was only found for floor drain coverage ($p=0.026$). Additionally, there were no statistically significant differences for the paired data based on the cleaned surfaces for any of the sites. None of the sites rated a mean statistically different from 0.

The following sites for the replicated photos within each photo set had statistically significant differences: intensity for blade to gloves ($p=0.037$), intensity for glove to meat slice #7 ($p=0.019$), overall for glove to meat slice #7 ($p=0.048$), intensity for hands to slicer bed ($p=0.020$), and overall for hands to slicer bed ($p=0.020$). The differences may be attributed to slight differences in hand movements by the researcher during each replicate.

Comparison of six commonly contaminated areas. Spread of contamination throughout the mock deli was evaluated from six different contamination origination sites. Contamination introduced at these six sites (rim of floor drain, employee's gloves, deli slice blade, surface of deli meat chub, preparation table surface, and employee's bare hands) most commonly spread to gloves, deli slicer blade, meat chub slices, prep table and deli case door handle (Table 3.2). Additional surfaces infrequently found to be contaminated were hand washing sink faucet, glove box, cart handles, scale face, deli case shelf, prep table sink, slicer table, employee, and cart wheels (Table 3.3).

Contamination originating from the floor drain spread the least to food contact surfaces. A few panelists rated slight values of contamination spread from the floor to the meat chub and

door handle, but the values were not statistically different from 0.00. In the case of the meat chub, the writing on the chub packaging fluoresced and may have contributed to a higher rating (Table 3.2). Contamination at the floor drain, however, did spread to other locations on the floor. Photographs of contamination on the floor were taken (3 ft. by 3 ft. sections of the floor) and analyzed by the panel. Figure 3.5 provides a visual example of where contamination was seen on the floor. The panel analyzed only those areas that showed fluorescence. Floor contamination was highest immediately around the drain at the inoculation site and contamination spread to areas farther away from the contaminated drain (Figure 3.5, Table 3.4). Areas that were noted as having trace presence (not evaluated by sensory panel) of GloGerm™ also included bottom of employee's shoes and the cart's wheels, which spread GloGerm™ from the initial floor drain contamination (Table 3.3).

Contamination spread most from the food handler's gloves to other surfaces. When gloves were contaminated, the heaviest spread was to the door handle and the meat chub. When bare hands were initially contaminated and not washed before putting on gloves, the heaviest spread was to the gloves, meat chub, and prep table. Spread from bare hands was minimized because they were covered with gloves following contamination indicating that gloves provide a good barrier protecting contaminated hands from other surfaces.

Slicer breakdown. Specific sites within the slicer were evaluated separately. Following photography of the most commonly seen contaminated sites, the slicer components were assessed. Contamination was transferred to sections of the slicer (handle, bed, carriage, and shelf; Figure 3.3) at various levels throughout the trials (Table 3.5). Contamination spread most heavily through the slicer from the blade itself and the meat chub. Employee's gloves were strongly correlated with contamination spread to the handle of the slicer.

Comparison of meat slice contamination. The sensory panelists also analyzed several meat slices sliced during the deli trials to determine whether there was a change in level of contamination on slices during slicing. Generally, for all starting points of contamination that transferred contamination to the chub, there was a decrease in contamination from slice 3 to slice 9 (Figure 3.6, Table 3.7). Since the meat was sliced in multiples of three, these slices represent the end of the first and third slicing operation. The contaminated blade spread the heaviest level of contamination to the slices of meat. Contamination on the meat chub and gloves decreased from the third to ninth slice. When the initial contamination site was the prep table and employee's hands, resulting meat slice contamination did not follow this pattern; meat slice 9 received a slightly higher rating than meat slice 3 (Table 3.6).

Discussion.

The prevalence of contaminants in RTE retail facilities has been previously studied, but only recently has research begun to focus on the possible routes and spread of contamination. Although the potential for transfer applies to all bacteria and viruses, researchers in the deli environment focus on *L. monocytogenes* in particular, due to its ubiquitous nature and the severe threat it poses to susceptible consumers. The highest predictive risk of causing listeriosis on a per serving and per annum basis is from deli meat (FDA, 2003). One study found that out of 121 New York retail delicatessens tested, 60% were positive for *L. monocytogenes* on surfaces such as the deli case, sink, and slicer (Saunders et al, 2009). Food contact surfaces are a major source of cross contamination in RTE facilities (Guzewich and Ross, 1999). It is critical to understand where contamination originates and how it is spread throughout these environments. Aside from proper sanitation, there are no intervention steps between deli preparation and consumer consumption. The data from this work provides insight into the potential locations throughout a

retail deli that may become contaminated when contamination enters the deli from six different “origination” sites. Additionally, the work provides insight into spread throughout the slicer and to individual meat slices. A study done by Hoelzer et al (2011) surveyed 41 experts from industry and state regulatory agencies to identify sites within a retail deli that pose the greatest cross contamination risk. The surveys contained questions asking the maximum, minimum, and percent probability that contamination from certain source sites (hands, product, slicer blade, food preparation table, and central floor drain) would transfer to the product, hands, or food contact surfaces. The expert’s agreed that employee’s gloves were an important site that could contribute significantly to cross contamination. The data collected in our study supports this, with gloves contributing to the spread of contamination to the meat chub, slicer, prep table and door handle (Hoelzer et al., 2011).

This study found that the six originating contaminated sites were also generally the six most commonly contaminated from other surfaces, with the exception of the floor drain. The deli case door handle also saw a significant amount of contamination spread.

The highest degree of spread came from food handlers gloves, which has been observed as a primary source of cross contamination, especially when workers wear the same pair for extended times without washing hands or changing regularly (Green et al, 2006; Lynch et al., 2005). *L. monocytogenes* can survive on fingertips for up to one hour and similar Gram-positive bacteria can survive on glove and cloth surfaces for up to four hours, particularly in the presence of food particles (Kerr et al, 1993; Scott and Bloomfield, 1990; Snelling et al, 1991). Viruses also spread this way, with a transfer rate of nearly 46% between a norovirus surrogate on fingertips and ham slices (Bidawid et al., 2003). When employee’s bare hands were the initial source of contamination and gloves were regularly changed, the level and spread of contamination was

less, confirming that following proper hand washing and glove procedures can reduce spread. However, proper hand washing and glove use is often lacking. Observational studies show that deli food handlers wash hands 73 (17%) of the 439 recommended times (according to actions) and may only follow the correct procedure less than 48% of the time (Lubran et al, 2010, Robertson et al., 2010). Additionally, food handlers only washed their hands 48% of the time before putting on new gloves (Lubran et al, 2010). Taking steps to reduce the amount of human contact with foods as much as possible will also help reduce the spread. For example, slicing meat onto a clean piece of wax paper instead of directly onto gloves or mixing deli salads with a sanitized spoon or fork instead of by hand may reduce contamination (Richards, 2001).

Inadequately cleaned food contact surfaces are also common sources of cross contamination throughout food service facilities. Two surfaces with high food contact rates are the preparation table and deli slicer. The ability for pathogens such as *L. monocytogenes* to attach to and develop protective biofilms on stainless steel surfaces is well documented (Beresford et al., 2001; Lunden et al, 2002; Penn State University, 2006; Tompkin, 2002). Optimal sanitation procedures vary depending on the different factors of each specific deli facility. It is suggested that retail establishments work with sanitation companies to determine the best cleaner and sanitizer regimens for their particular equipment and type of food product residue (Penn State University, 2006).

The deli slicer is one of the primary sources for contamination. Several studies have explored the mechanics of contamination transfer between the slicer blade and meat slices (Keskinen et al., 2007; Lin et al., 2006; Rodriguez and McLandsborough, 2006; Rodriguez et al., 2007; Sheen and Hwang, 2008; Vorst et al., 2006). The level and spread of contamination transfer depend on both the initial level of pathogen on the blade and the type of product being

sliced (turkey breast, salami, or bologna) (Lin et al., 2006). The current study evaluated particular areas within the slicer that continually became contaminated with GloGerm™ after slicing bologna. All five slicer components were contaminated to some degree during five of the six treatments (except floor drain) at levels that could potentially spread to sliced products. Previous research has found that when slicing turkey breast, salami, and bologna, contamination from the blade may spread to surrounding surfaces on both the slicer and a conveyor belt (Lin et al., 2006). These data may be used to highlight specific areas within the slicer that may need particular focus when cleaning and sanitizing in order to reduce the potential for pathogen growth and cross contamination.

Purchasing products from reputable sources is a crucial component of keeping contamination out of deli establishments. When the meat chub was the initial source of contamination, contamination was spread to all other studied surfaces. For this study, the entire outer surface of the meat chub was inoculated with GloGerm™ instead of the outer meat slice, in order to easily observe potential for cross contamination. Studies have shown that contamination introduced through the meat chub last longer on the blade and affects more slices than direct blade contamination (Sheen and Hwang, 2011). This trend may be explained by the thin residual film left on the slicer by the chub, which provides an optimal environment for contaminants to thrive and stay protected. The type of meat chub also affects the strength of this protective layer. For example, the low moisture (43%) and high fat (36%) of salami may leave a noticeable fat layer on blades that creates this optimal environment, while the high moisture (78%) and low fat of the turkey (<1%) may have the effect of washing off the blades and spreading the contaminant elsewhere. (Lin et al., 2006, Vorst et al., 2006). These data emphasizes the importance of ordering from suppliers with documented GMPs (Good Manufacturing Practices), GAPs (Good

Agricultural Practices), HACCP plans, and who are inspected by state or federal agencies. It is also crucial to educate employees on basic pathogen control in order for them to understand the need for appropriate product holding practices such as tracking inventory, temperature control, and product rotation (ILSIRF, 2005).

The potential for floor contamination spread to food has been observed in other studies and floor drains may be considered a niche, a hard to clean area where bacteria thrive (Lianou and Sofos, 2007; Penn State University, 2006; Tompkin, 2002). Although the contaminated floor drain in this study spread contamination other surfaces of the floor via a cart's wheels and employee's shoes, significant contamination was not spread to any food contact surfaces. Contamination, growth, and survival of bacteria in floor drains can be influenced by many factors including room temperature, foot traffic, drain material, and amount of fluid typically run through the drain (Zhao et al., 2005). Observational studies have noted deli food handlers failing to wash hands and change gloves after picking items up off the floor or using their hands to steady themselves while crouching down to get items out of a cooler or lower shelf (Robertson et al., 2010). Habits such as these (while not part of our study) are one pathway for contamination from the floor to spread to food contact surfaces.

When contamination began in the meat chub, employee's gloves, and the slicer blade, subsequent slices decreased in contamination. This trend has been observed in other studies as well (Perez-Rodriguez et al., 2007; Sheen and Hwang, 2011; Vorst et al., 2006). Populations of *L. monocytogenes* on a slicer blade inoculated with 10^5 CFU/cm² decreased to 10^2 CFU/slice after five slices and samples were generally negative after 27 slices (Vorst et al., 2006). Although the contamination level may be lower in some slices, there is still a need for concern due to the potential for some pathogens (most notably *L. monocytogenes*) to continue to grow once a

consumer has taken the product home. In this study, contamination originating with the employee's hands and prep table resulted in an increase in contamination in the subsequent slices. This is most likely due to the fact that these areas had more exposure to the product as the trials went on, instead of initially like the blade, gloves, and meat chub itself. Further research may be done to observe the trend of pathogen spread onto meat slices from areas other than the meat chub and slicer blade.

The current study has tracked the spread of contamination throughout food service environments using a fluorescent abiotic surrogate. The findings of this study reinforce the need for stricter compliance of equipment cleaning and personal hygiene practices among deli workers. The low cost, low risk, and easy to visualize nature of the GloGerm™ lends it to being an excellent tool for researchers and educators. The ability to visualize contamination spread is particularly beneficial because it gives researchers a more complete picture of where contamination spreads without the burden of extensive environmental testing. Further research may be done to compare the spread of GloGerm™ to the spread of a living organism and to develop successful training programs for both food service employees and consumers using these methods.

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Table 3.1. A reference for overall rating based on each panelist’s separate ratings for GloGerm™ coverage¹ and intensity² of every surface photo. Panelists provided three ratings, one for coverage, one for intensity, and then used this guide to determine an overall rating³.

Coverage	Intensity			
	None	Slight	Moderate	Heavy
None	None	None	None	None
Slight	None	Slight	Slight	Moderate
Moderate	None	Slight	Moderate	Heavy
Heavy	None	Moderate	Heavy	Heavy

¹“coverage” was considered the area in the photograph containing the GloGerm™ lotion

²“intensity” was considered the brightness of the GloGerm™ in the photograph.

³panelists were trained and the method was validated.

Table 3.2. The effect of starting contamination point on a) “coverage”¹ of spread and b) “intensity”² and c) overall spread of contamination to six common locations throughout the deli.³

Recipient of Contamination	Source of Contamination: Descriptive ⁴ , Numerical ⁵					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
Floor Drain	see Figure 4	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Gloves	none 0.00±0.00 ^a	heavy 3.00±0.00 ^c	moderate 1.69±0.79 ^b	moderate 2.19±0.66 ^b	moderate 2.38±0.62 ^b	heavy 3.00±0.00 ^c
Blade	none 0.00±0.00 ^a	slight 0.88±0.34 ^b	heavy 2.63±0.50 ^c	slight 1.19±0.40 ^b	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Meat Chub	slight 0.94±0.25 ^a	moderate 2.38±0.50 ^c	slight 1.25±0.45 ^a	heavy 3.00±0.00 ^d	moderate 1.88±0.34 ^b	moderate 2.31±0.8 ^{bc}
Prep Table	none 0.38±0.50 ^a	moderate 1.94±0.25 ^c	moderate 1.94±0.25 ^c	moderate 1.75±0.45 ^c	heavy 3.00±0.00 ^d	slight 1.00±0.00 ^b
Door Handle	none 0.44±0.63 ^a	heavy 3.00±0.00 ^d	moderate 1.75±0.77 ^b	slight 0.56±0.63 ^a	heavy 2.50±0.52 ^c	none 0.13±0.34 ^a

b)

Recipient of Contamination	Source of Contamination: Descriptive ⁴ , Numerical ⁵					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
Floor Drain	see Figure 4	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Gloves	none 0.00±0.00 ^a	heavy 3.00±0.00 ^d	moderate 1.50±0.73 ^b	moderate 2.06±0.57 ^b	heavy 2.81±0.40 ^c	heavy 2.94±0.25 ^c
Blade	none 0.00±0.00 ^a	slight 0.63±0.50 ^b	heavy 3.00±0.00 ^d	slight 1.13±0.34 ^c	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Meat Chub	slight 0.94±0.25 ^a	heavy 2.55±0.45 ^b	heavy 2.88±0.34 ^c	heavy 3.00±0.00 ^c	slight 1.25±0.45 ^a	slight 1.25±0.45 ^b
Prep Table	none 0.13±0.34 ^a	moderate 2.00±0.37 ^c	moderate 1.75±0.45 ^c	moderate 1.88±0.34 ^c	heavy 3.00±0.00 ^d	slight 1.19±0.40 ^b
Door Handle	none 0.38±0.50 ^a	heavy 3.00±0.00 ^c	slight 1.19±0.54 ^b	none 0.38±0.50 ^a	moderate 1.75±0.45 ^b	none 0.13±0.34 ^a

c)

Recipient of Contamination	Source of Contamination: Descriptive ⁴ , Numerical ⁵					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
Floor Drain	see Figure 4	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Gloves	none 0.00±0.00 ^a	heavy 3.00±0.00 ^c	slight 1.49±0.73 ^b	moderate 2.06±0.57 ^c	heavy 2.81±0.40 ^c	heavy 2.94±0.25 ^c
Blade	none 0.00±0.00 ^a	slight 0.63±0.50 ^b	heavy 3.00±0.00 ^d	slight 1.00±0.00 ^b	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Meat Chub	slight 0.94±0.25 ^a	heavy 2.50±0.52 ^c	moderate 2.13±0.62 ^c	heavy 3.00±0.00 ^d	slight 1.25±0.45 ^{ab}	moderate 1.69±0.70 ^b
Prep Table	none 0.13±0.34 ^a	moderate 1.88±0.34 ^c	moderate 1.75±0.45 ^c	moderate 1.88±0.45 ^c	heavy 3.00±0.00 ^d	slight 1.00±0.00 ^b
Door Handle	none 0.38±0.50 ^a	heavy 3.00±0.00 ^c	slight 1.25±0.68 ^b	none 0.38±0.50 ^a	moderate 2.31±0.70 ^b	none 0.13±0.34 ^a

¹“coverage” was considered the area in the photograph containing the GloGerm™ lotion

²“intensity” was considered the brightness of the GloGerm™ in the photograph.

³Within row, values with different letters are significantly different (based on the nonparametric-Behrens-Fisher $P < 0.05$)

⁴Descriptions translated into numerical form for statistical analysis as follows: None – 0, Slight – 1. Moderate – 2. Heavy – 3. An average rating for each of the surfaces was calculated (with values of 0.5 or above rounded up to the next integer, 0.49 and below rounded down to the next integer) along with standard deviation.

⁵the numerical value is the mean of all observations \pm standard deviation

Table 3.3. Additional locations contaminated throughout the mock deli: number of trials cross contamination was seen at each location (n=3). Because these were not consistent, they were not ranked for “coverage” or “intensity”

Recipient of Contamination	Source of Contamination					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
Hand wash sink faucet knobs	0	3	0	0	0	1
Top of glove box	0	3	0	0	0	3
Cart handles	0	2	0	0	0	0
Scale face	0	2	1	0	0	0
Surface of interior deli case shelf	0	0	2	3	0	0
Bottom of prep table sink, near drain.	0	0	0	2	3	0
Slicer table near slicer	0	0	1	1	0	0
Bottom of employee shoes	3	0	0	0	0	0
Cart Wheels	3	0	0	0	0	0

Table 3.4. Floor block ratings based on two photographs rated by each of the eight panelists (n=16).

Floor Block	1	2	3	4	5	6	7
Numerical rating	1.38±0.50 ^a	1.06±0.25 ^a	1.50±0.63 ^a	1.44±0.49 ^a	1.13±0.91 ^a	0.75±0.93 ^a	1.06±0.35 ^a
Descriptive rating ¹	slight	slight	moderate	slight	slight	slight	slight

¹Descriptions translated into numerical form for statistical analysis as follows: None – 0, Slight – 1, Moderate – 2, Heavy – 3. All values ≥ 0.50 were rounded up to the next integer for translation.

²Values within rows followed by the different letters are significantly different (based on the nonparametric-Behrens-Fisher $P < 0.05$)

Table 3.5. The effect of starting contamination point on a) “coverage”¹ of spread and b) “intensity”² and c) overall spread of contamination throughout the slicer components³.

a)

Recipient of Contamination	Source of Contamination: Descriptive ⁴ , Numerical ⁵					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
Blade	none 0.00±0.00 ^a	slight 0.88±0.34 ^b	heavy 3.00±0.00 ^c	slight 1.19±0.40 ^b	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Bed	slight 0.88±0.72 ^a	slight 0.50±0.52 ^a	heavy 2.56±0.70 ^b	moderate 2.25±0.45 ^b	moderate 1.50±0.34 ^a	slight 1.38±0.50 ^a
Shelf	none 0.00±0.00 ^a	slight 1.19±0.40 ^c	heavy 2.63±0.50 ^d	heavy 2.88±0.34 ^d	slight 0.63±0.50 ^b	slight 0.88±0.34 ^b
Handle	none 0.38±0.50 ^a	heavy 2.88±0.34 ^c	slight 0.63±0.50 ^a	heavy 2.88±0.34 ^c	heavy 3.00±0.00 ^c	slight 1.25±0.45 ^b
Carriage	none 0.00±0.00 ^a	heavy 2.75±0.45 ^d	slight 1.00±0.00 ^b	slight 1.38±0.72 ^{bc}	moderate 2.00±0.52 ^c	moderate 1.75±0.68 ^c

b)

Recipient of Contamination	Source of Contamination: Descriptive ⁴ , Numerical ⁵					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
Blade	none 0.00±0.00 ^a	slight 0.63±0.50 ^b	heavy 3.00±0.00 ^c	slight 1.13±0.34 ^b	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Bed	none 0.44±0.51 ^a	slight 0.63±0.72 ^a	heavy 2.69±0.70 ^c	moderate 2.06±0.85 ^c	slight 1.13±0.34 ^b	slight 1.25±0.45 ^b
Shelf	none 0.00±0.00 ^a	moderate 1.56±0.51 ^c	heavy 2.63±0.50 ^d	heavy 2.88±0.34 ^d	slight 0.63±0.50 ^b	slight 0.88±0.34 ^b
Handle	none 0.38±0.50 ^a	heavy 2.88±0.50 ^d	slight 0.56±0.51 ^a	moderate 1.88±0.34 ^b	moderate 2.38±0.50 ^c	slight 1.44±0.51 ^b
Carriage	none 0.13±0.34 ^a	moderate 2.38±0.50 ^c	slight 0.88±0.34 ^b	slight 0.88±0.34 ^b	moderate 2.13±0.34 ^c	slight 1.06±0.25 ^b

c)

Recipient of Contamination	Source of Contamination: Descriptive ⁴ , Numerical ⁵					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
Blade	none 0.00±0.00 ^a	slight 0.63±0.50 ^b	heavy 3.00±0.00 ^d	slight 1.00±0.00 ^b	none 0.00±0.00 ^a	none 0.00±0.00 ^a
Bed	none 0.43±0.51 ^a	slight 0.50±0.52 ^a	heavy 2.63±0.70 ^c	moderate 2.13±0.81 ^c	slight 1.13±0.34 ^b	slight 1.31±0.48 ^b
Shelf	none 0.00±0.00 ^a	slight 1.19±0.40 ^c	heavy 2.63±0.50 ^d	heavy 2.88±0.34 ^d	slight 0.63±0.50 ^b	slight 0.88±0.34 ^b
Handle	none 0.38±0.50 ^a	heavy 2.88±0.50 ^c	slight 0.59±0.50 ^a	heavy 2.75±0.45 ^c	heavy 3.00±0.00 ^c	slight 1.25±0.45 ^b
Carriage	none 0.00±0.00 ^a	heavy 2.88±0.34 ^e	moderate 0.94±0.34 ^b	slight 1.00±0.52 ^b	moderate 2.13±0.62 ^d	slight 1.13±0.34 ^{bc}

¹“coverage” was considered the area in the photograph containing the GloGerm™ lotion

²“intensity” was considered the brightness of the GloGerm™ in the photograph.

³Within row, values with different letters are significantly different (based on the nonparametric-Behrens-Fisher $P < 0.05$)

⁴Descriptions translated into numerical form for statistical analysis as follows: None – 0, Slight – 1. Moderate – 2. Heavy – 3. An average rating for each of the surfaces was calculated (with values of 0.5 or above rounded up to the next integer, 0.49 and below rounded down to the next integer) along with standard deviation.

⁵the numerical value is the mean of all observations ± standard deviation

Table 3.6. The effect of starting contaminant site on transfer to meat slices (third and ninth sliced consecutively) sliced during each of the six starting contamination locations¹.

Slice Number	Source of Contamination: Descriptive ² , Numerical ³ (mean±st. dev.)					
	Floor Drain	Gloves	Blade	Meat Chub	Prep Table	Hands
3	none	moderate	heavy	heavy	slight	slight
	0.38±0.52 ^a	2.38±0.74 ^c	3.00±0.00 ^d	3.00±0.00 ^d	1.25±0.46 ^b	0.63±0.52 ^a
9	none	Slight	heavy	slight	moderate	slight
	0.25±0.46 ^a	1.38±0.52 ^b	3.00±0.00 ^c	1.38±0.74 ^b	1.50±0.53 ^b	1.13±0.35 ^b

¹Within row, values with different letters are significantly different (based on the nonparametric-Behrens-Fisher $P < 0.05$)

²Descriptions translated into numerical form for statistical analysis as follows: None – 0, Slight – 1. Moderate – 2. Heavy – 3. An average rating for each of the surfaces was calculated (with values of 0.5 or above rounded up to the next integer, 0.49 and below rounded down to the next integer) along with standard deviation.

³the numerical value is the mean of all observations ± standard deviation

Figure 3.1. Layout of mock retail deli space including all equipment and dimensions (© floor drain).

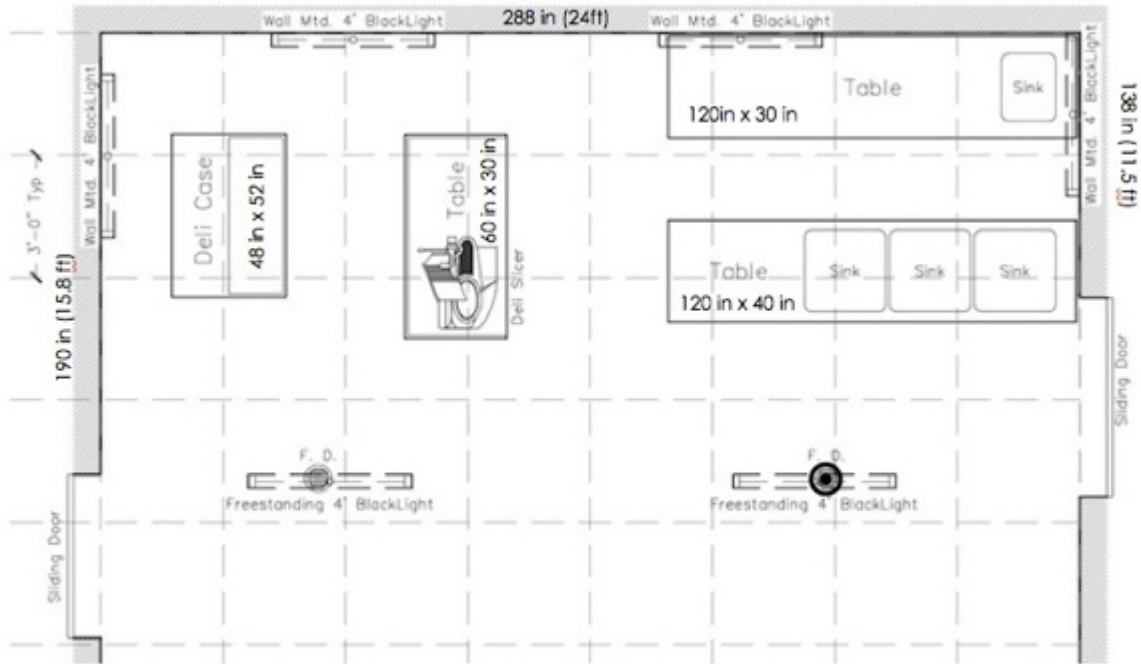
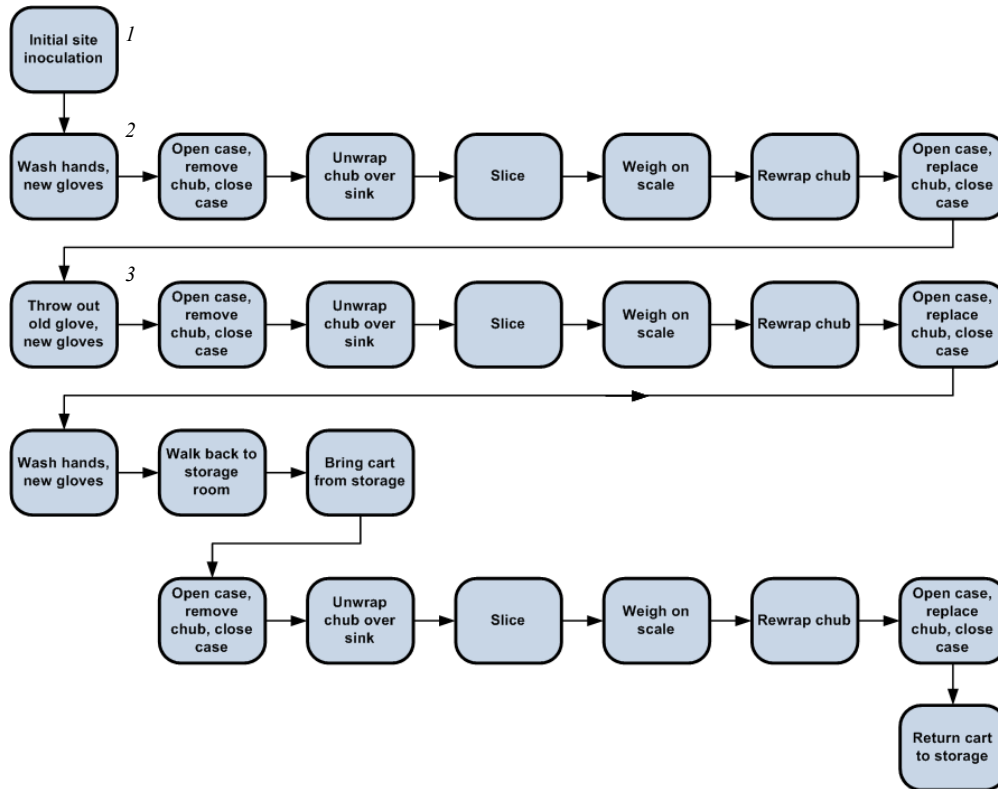


Figure 3.2. Series of standard deli operations performed (in this order) by researcher for each experimental trial replication. Adapted from Lubran et al (18).



¹For each of the six starting contamination sites (deli slice blade, rim of floor drain, surface of deli meat chub, employees bare hands, gloves, and the preparation table surface) 20 mL of GloGerm™ was applied. Each trial was replicated three times per starting contaminate site (18 replicates overall).

²“wash hands, new gloves” not performed during employee’s hands and gloves starting site replications respectively.

³“throw out old glove, new gloves” not performed during gloves starting site replication

Figure 3.3. Photographs and descriptions of the five slicer components (Hobart Electric Slicer model #2612C) analyzed for coverage, intensity, and overall contamination of GloGerm™.

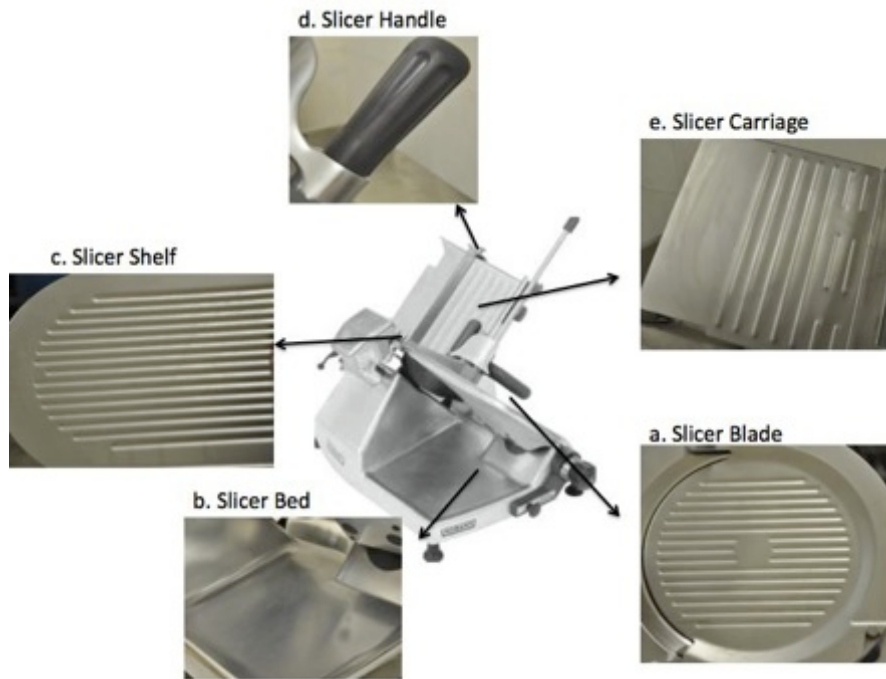


Figure 3.4. Example of photograph of slicer shelf (the angled surface used to hold the meat chub level and steady before coming in contact with the blade) analyzed by the eight panelists compared to control photograph (clean, no contamination). In this photograph GloGerm™ contamination was initiated on the slicer blade. Eight panelists' ranked photo 460 as having heavy contamination (2.75 ± 0.46)

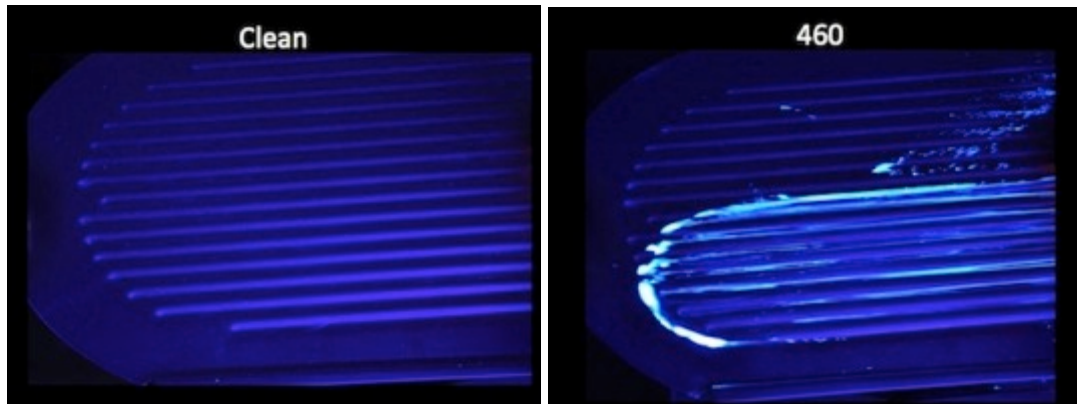


Figure 3.5. Map of floor drain contamination spread and intensity. Areas of floor separated into blocks (3' by 3'). Blocks on floor contaminated with GloGerm™ are designated by numerals 1-7. All other locations on the floor had no GloGerm™ transfer. (Key: ♦-slight, ♦♦-moderate, ♦♦♦-heavy, ⊙-floor drain)

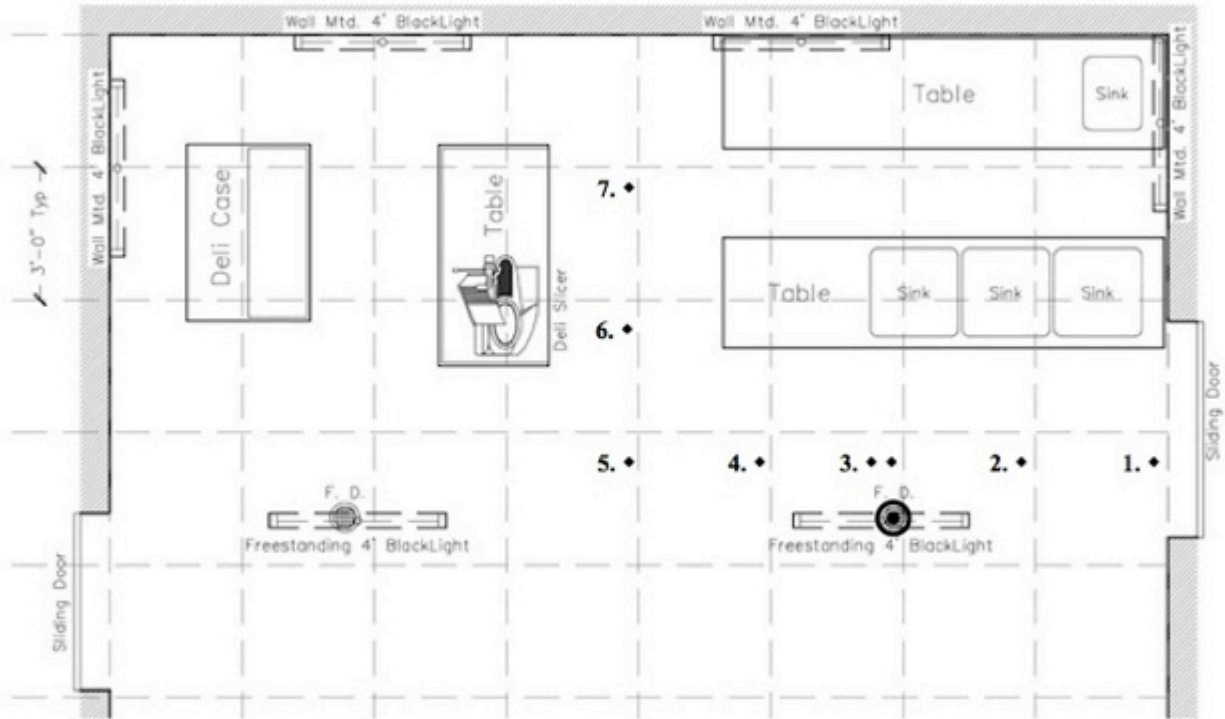
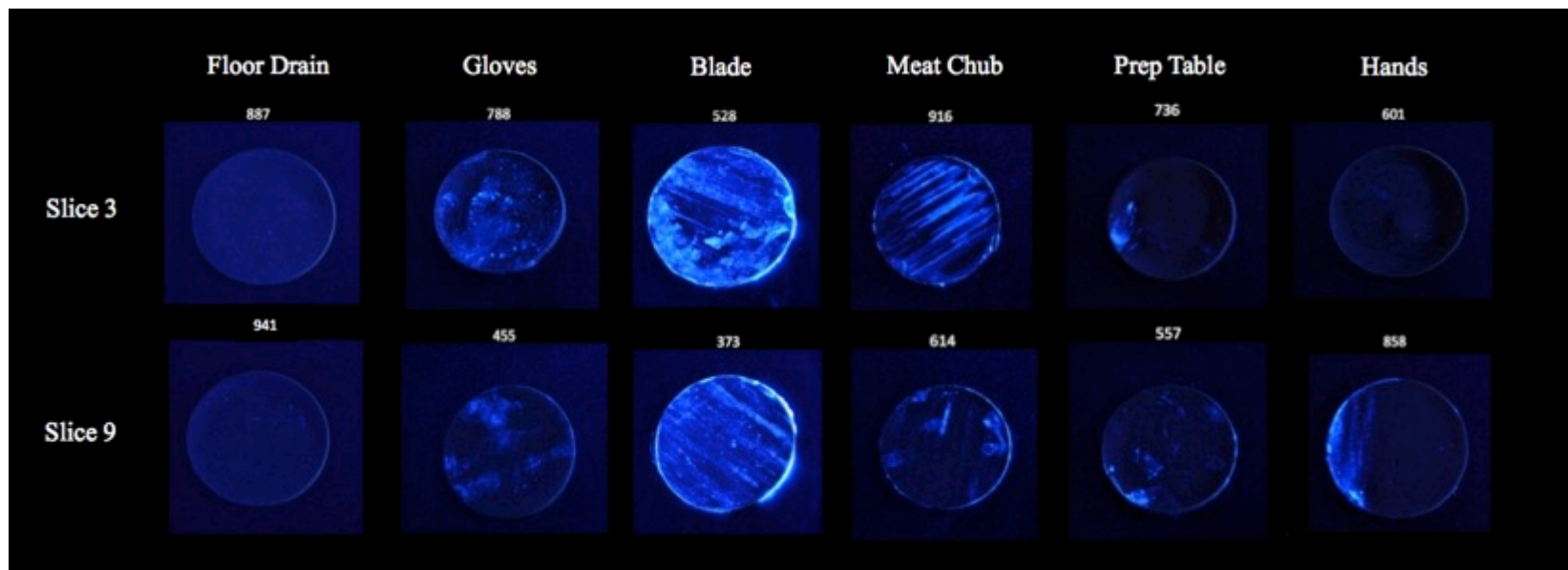


Figure 3.6. Example photographs of third and ninth meat slice sliced during each of the 6 starting contaminate trials.



Chapter 4.

Recovery of foodborne pathogens from stainless steel coupons when co-inoculated with GloGerm™

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Abstract.

GloGerm™ and similar fluorescent compounds have been used to visualize potential spread of microorganisms in a variety of settings (hospitals, labs, and food service environments). Inoculation of surfaces with a GloGerm™/pathogen cocktail may be beneficial because the fluorescence would help guide sampling locations for subsequent pathogen quantification. The purpose of this study was to determine if GloGerm™ and foodborne pathogens could be co-inoculated on to stainless steel and result in recoverable populations similar to when a pathogen is inoculated alone. Three common foodborne pathogens, (*E. coli* O157:H7, *Salmonella enterica* ser. Enteritidis, *Listeria monocytogenes*), and one potential surrogate organism (*L. innocua*) were inoculated (approximately 8 log CFU/cm²) on 2” by 2” stainless steel coupons alone and in combination with GloGerm™ (2:1, inoculum: GloGer^m™). Inoculated coupons were allowed to dry for 20 minutes and then sampled using polyester tipped swabs. The swabs were then placed in phosphate buffered saline, serially diluted, and plated on selective media. The experiment was replicated 3 times, sampling 3 coupons each trial, for a total of 9 samples per treatment/bacteria. The recovery rates of the GloGerm™/bacteria cocktail were compared to recovery rates of bacteria alone. Approximately 6.85, 7.01, 6.88, and 6.12 log CFU/cm² of *L. innocua*, *L. monocytogenes*, *S. Enteritidis*, and *E. coli* O157:H7 respectively were recovered from the stainless steel coupons when bacteria was inoculated alone. When co-inoculated with GloGerm™ 6.75, 7.02, 6.70 and 6.08 log CFU/cm² were recovered. There was no significant difference ($p > 0.05$) between bacteria recovered from the control coupons and

from the GloGerm™/bacteria cocktail coupons for all bacteria. Co-inoculating surfaces with GloGerm™/bacteria cocktails does not affect the recoverability of microorganism which may allow researchers to visually track bacteria through an environment using the fluorescence as a sampling guide.

Introduction.

Cross contamination plays a significant role in contributing to foodborne illness outbreaks. Transfer of pathogens from a contaminated surface to a non-contaminated surface can occur anywhere along the food production chain (harvesting, packing, processing, retail, consumer homes) (Kusumaningrum et al., 2003; Chen et al., 2001). Foodborne pathogens, including *Escherichia coli*, *Salmonella* and *Listeria monocytogenes*, can survive on food contact surfaces, equipment, floors, drains, and hands or gloves for hours or even days (Scott et al., 1990; Beresford et al., 2001; Blackman et al., 1996; Kusumaningrum et al., 2003). *Listeria monocytogenes* is a pathogen of particular concern because of its ability to persist in food processing and retail environments and the severity of illness it can cause in susceptible individuals (Lianou and Sofos, 2007). While the incidence and prevalence of bacteria within the retail environment has been documented (Hoeltzer et al., 2011; Saunders et al., 2004; Gombas et al., 2003), more work is needed in tracking potential cross contamination pathways in these environments, in order to reduce the potential of subsequent outbreaks.

GloGerm™ and similar fluorescent compounds have been used to visualize potential spread of microorganisms in a variety of settings (hospitals, labs, and food service environments) (Oberyszyn et al., 2001; Perfetto et al., 2003; Bergervoet et al., 2008; Snyder, 1999). A few studies have incorporated the use of GloGerm™ to specifically analyze cross contamination between food products and food contact surfaces (Vorst et al., 2006; Buchholz et al., 2012; Keskinen et al., 2007; Maitland et al., 2013). In order to determine the transfer of *L. monocytogenes* during slicing of various meat chubs, Vorst et al. (2006) sliced a turkey breast chub covered in GloGerm™ powder to determine the contact surfaces and surface areas throughout the deli slicer for identification in later trials. This method was repeated by Keskinen

et al (2007) to determine what locations on a deli slicer blade to inoculate when studying the effects of bacterial stress and biofilm formation on transfer of *L.monocytogenes* from blade to deli meat. Buchholz et al (2012) ran baby spinach leaves and iceberg lettuce heads, previously been soaked in a 0.35% (wt/vol) suspension of GloGerm™ /water, through a processing line to identify contact sites on the processing equipment for subsequent quantification of *E. coli* O157:H7 transfer. In our previous work, GloGerm™ lotion was used to track potential cross contamination pathways throughout a mock retail deli from several originating contamination sites (gloves, employees' hands, meat chub, slicer blade, preparation table, and floor drain) (Maitland et al., 2013). A trained sensory panel was used to rate fluorescence on photographs of the contaminated surfaces (from none to heavy) in order to produce quantitative data for statistical analysis (Maitland et al., 2013). While these previous studies have used GloGerm™ as a potential qualitative tracking device, it is unknown if the quantification of GloGerm™ alone correlates to concentrations of pathogens.

The objective for this study was to determine if co-inoculation of stainless steel surfaces with GloGerm™ and foodborne pathogens results in recoverable concentrations of the pathogen similar to when the pathogen is inoculated alone. If populations are recovered at similar concentrations when mixed with GloGerm™, then the use of a bacteria/GloGerm™ mixture may allow for simultaneous tracking and sampling of bacteria throughout a food manufacturing or processing environment.

Materials and Methods.

Culture maintenance.

Escherichia coli O157:H7 (994, isolated from salami) *Salmonella enterica* serovar Enteritidis, *Listeria innocua* (ATCC 33090), and *Listeria monocytogenes* serovar 4b (J1925,

sliced deli meat outbreak) were retrieved from the -80°C ultra-low freezer culture collection at the Food Science and Technology Department of Virginia Tech (Blacksburg, VA). After thawing, cultures were transferred separately into 10 mL of tryptic soy broth (TSB; Becton Dickinson, Franklin Lakes, NJ) and incubated at 35°C for 24 h. Each culture was then transferred into fresh TSB and incubated for another 24 h at 35°C successively for three days to activate cells.

Growth in GloGerm™.

All four strains were prepared and analyzed separately and their survival in GloGerm™ Gel (Moab, UT) was compared. GloGerm™ Gel is a nontoxic lotion made up of the following ingredients; 85% lotion (water, cetearyl alcohol, glyceryl stearate, glycerin, cetearth-20, mineral oil, coco caprylate/caprate, propylene glycol, isopropylene palmitate, triethanolamine, carbopol, diazolidinyl urea, methylparaben, propylparaben, disodium edta), 15% plastic (melamine copolymer resin). Each activated culture in TSB was diluted with 0.1% sterile peptone water (SPW) to approximate a 4-5 log CFU/ml concentration. Then, 10 mL of the diluted sample was added to 5 mL of either GloGerm™ lotion or the control (TSB) and incubated at 35°C. For each bacteria/GloGerm™ mixture and control mixture, six tubes were made in order to sample once every 4 hours for 24 hours. Every 4 hours, both a bacteria/GloGerm™ mixture and control mixture were vortexed, serially diluted into 0.1% SPW, and plated on Modified-oxford agar to isolate *Listeria* spp. (MOX; Becton Dickinson, Franklin Lakes, NJ), Sorbitol-MacConkey agar to isolate *E. coli* O157:H7 spp. (SMAC; Becton Dickinson, Franklin Lakes, NJ) and Xylose lysine deoxycholate agar to isolate *Salmonella* spp. (XLD; Becton Dickinson, Franklin Lakes, NJ) and incubated at 37°C for 48 h.

Survival and recovery from stainless steel.

Stainless steel 304 coupons were used as a test surface because they represent the most commonly found stainless steel surfaces in food processing environments. Stainless steel coupons (2" x 2"; Atlantic Stainless, North Attleboro, MA) were soaked and washed with Micro 90 cleaning solution (International Products Corp., Burlington, NJ) (prepared per manufacturer's standards; 2.3 ounces in 1 quart of water for a 2.0% solution). Coupons were rubbed using a clean disposable towel for 1 minute and then rinsed in sterile deionized water in accordance with common deli slicer cleaning protocol (Hobart, 2007). Following cleaning, coupons were sterilized by autoclaving for 15 min at 121°C (Crandell, 2010).

Preparation of GloGerm™/bacteria cocktail.

After the third transfer, cells from a 24-hr culture in TSB were centrifuged at 10,000 x g for 5 minutes using a Fisher Scientific AccuSpin 400 (FisherBrand, Pittsburgh, PA). Cells were washed with sterile 0.1% peptone water and re-suspended in sterile de-ionized water. A solution containing 1/3 GloGerm™ lotion (GloGerm, Moah, UT) (5mL) was combined with 2/3 re-suspended inoculum in sterile deionized water (10mL) to obtain an approximate 8 log CFU/ml concentration and then vortexed for 30 seconds to allow for uniform distribution of cells throughout the solution. The solution was serially diluted with 0.1% SPW, plated onto MOX, SMAC, or XLD, and incubated at 37°C for 48 h to determine initial inoculum levels.

Surface inoculation and enumeration.

Sterile coupons were placed into individual sterile petri dishes. One mL of GloGerm™/bacteria cocktail was deposited on the center of the coupon and spread over the entire surface using a sterile loop. The coupons were then allowed to dry for 20 minutes under a laminar-flow hood at room temperature (20±2°C). Coupons were then sampled with sterile polyester-tipped swabs (Fisher Scientific, Pittsburgh, PA) in a consistent back and forth motion, and the swabs

were placed in 10 ml sterile phosphate buffered saline (PBS), vortexed, serially diluted, and plated on MOX , SMAC , or XLD and incubated at 37°C for 24 h (Crandall et al., 2010). All conditions across all trials were kept consistent in order to successfully compare recovery of bacteria inoculated alone and in combination with GloGerm™.

Data analysis.

For the growth in GloGerm™ experiment, a one-way ANOVA was used to determine significant differences between samples taken every 4 hours ($p < 0.05$).

The coupon recovery experiment was replicated 3 times, sampling 3 coupons each trial, for a total of 9 samples per treatment/bacteria. The same number of control coupons inoculated with just the bacteria culture alone was also sampled, along with one coupon inoculated with just GloGerm™. The percent recovery of each bacteria and inoculum combination was determined by transforming the average of the initial inoculum in CFU/cm² into 100% and using this value to compare the percent recovery of the samples also in CFU/cm². The mean recovery values were calculated by transforming the CFU/cm² values to log₁₀ for statistical analysis and mean log (CFU/cm²) differences were calculated to account for differences in starting inoculum between the bacteria. The data was analyzed using a general linear model and ANOVA for least significant differences in mean recovery of bacteria compared to bacteria/ GloGerm™ cocktail at a significance level of $p < 0.05$. When a significant difference between means was indicated by ANOVA, Tukey's HSD was used to assess significant differences ($\alpha \leq 0.05$) between the mean difference comparisons using JMP v. 10.0 (SAS; Institute Inc., Cary, NC).

Results and Discussion

Growth Inhibition Determination.

All four bacteria, in nutrient rich broth (TSB), grew along a normal exponential growth curve (Figure 4.1). From hours 0 to 4 of sampling, no significant difference was seen in the growth of the bacteria in GloGerm™ mixture and bacteria grown in TSB ($p > 0.05$). In both mediums, populations of 3 of the 4 bacteria increased over 1 log CFU/mL in this time frame and populations of *E. coli* increased 0.2 log CFU/mL. All four foodborne pathogens mixed in GloGerm™ saw a significant difference in growth patterns than bacteria grown in TSB after hours 4 and 8 of sampling ($p < 0.05$). *L. monocytogenes*, *L. innocua*, and *S. Enteritidis* cells were below the limit of detection (<10 CFU/mL) by hour 12 of sampling, and *E. coli* samples were below the limit of detection (<10 CFU/mL) by hour 8.

There are a couple potential explanations for the growth and then gradual die off of the bacteria mixed in GloGerm™ lotion. The similar growth pattern from 0 hour to 4 hour may be due to a small amount of nutrients remaining in the mixture that had been transferred over with the initial inoculum. Once the bacteria exhausted these nutrients, however, the bacteria most likely began to interact with the antimicrobial preservative agent present in the lotion contents of the GloGerm™.

Diazolidinyl urea (DZU) is an antimicrobial preservative commonly found in cosmetics, skin care products, shampoos and conditioners, baby wipes and even household detergents, usually found in a concentration range of 0.1% - 0.5% (Orth et al., 1993; Zachariae et al., 2005; Llabres et al., 1985) The preservative acts on Gram negative and Gram positive bacteria, molds, and yeasts by releasing formaldehyde (Zachariae et al., 2005). Llabres et al (1985) found that at a concentration of 0.3% DZU, the Gram-positive *Staphylococcus epidermidis* was reduced to <1 CFU/mL after 5 hours and found to have a D-value of 42 minutes. While the bacteria in the Llabres study was exposed to pure sample of the compound, all four bacteria used in this study

began to die off after 4 hours when exposed to the compound in a lotion matrix. This antimicrobial effect was not considered a concern when comparing recovery rates from stainless steel coupons because sampling was taken within half an hour of exposing the bacteria to the GloGerm™ lotion.

Recovery of foodborne bacteria based on inoculum type.

There was no significant difference ($p>0.05$) between the recovery of the each bacterium based on inoculum type (alone or in GloGerm™), however there were differences between bacteria. In general, *E. coli* had the highest recovery of all four pathogens followed by *L. monocytogenes*, *S. Enteritidis* and *L. innocua* (Figure 4.2). *L. innocua* has been used as a non-pathogenic biotic surrogate for *L. monocytogenes* in a variety of food matrixes and environments (Goff, 1990; Kamat and Nair, 1996; Kozempel, 2002; Kim and Linton, 2008; Friedly, 2008). In this study however, significant differences were seen in the recovery of *L. innocua* in GloGerm™ as compared to *L. monocytogenes* (both alone and in GloGerm™) (Table 5.1). There was also a significant difference ($\alpha<0.05$) between recovery rates of Gram-negative bacteria (*S. Enteritidis*, and *E.coli*) and Gram-positive bacteria (*L. monocytogenes*, and *L. innocua*). When inoculated alone and with GloGerm™, Gram-negative bacteria were recovered at higher amounts than Gram-positive.

Although no research has been done to date investigating the recovery of a bacteria/GloGerm™ cocktail from stainless steel, the recovery rates of the bacteria from coupons when inoculated alone was comparable to some previously published studies. The recovery of *Salmonella* Typhimurium from a variety of food contact surface types using the swab technique has previously been reported (Moore et al., 2007a). From stainless steel, 2.15 CFU/25cm² of *S.*

Typhimurium were recovered after 1 hour of drying. Recovery in this study was approximately a 1.21 log CFU/cm² difference (6.2%) after 20 minutes of drying for *S. Enteritidis* alone.

Kang et al (2007) found that the swab method recovered on average 20% CFU/ coupon of a *L. monocytogenes* cocktail after 1 hour of drying. After 12 hours of drying, the swab method was responsible for less than a 2% recovery. Gomez et al (2012) compared different recovery methods on obtaining *L. monocytogenes* strains of human origin (4b) and food origin (1/2b) from a stainless steel table that had 10 years of use in a food preparation facility. The researchers found that when using the swab method 1.01 % of *L. monocytogenes* 4b was recovered, and 0.09% of *L. monocytogenes* 1/2b was recovered. Foschino et al (2003) recovered between 1 and 4% of *E. coli* cells from a variety of stainless steel surfaces using the swab method. While the percent recovery in this study of *L. monocytogenes* and *E. coli* was closer to 5 and 7%, respectively, the swabbing technique has been documented as producing varying results based on who is performing the method because of a high degree of variability in applied pressure and scrubbing action on the sample surface (Moore and Griffith, 2002, Moore et al., 2007b). For the scope of this study, one researcher performed the swabbing method across all trials to allow for consistency in comparing recovery of bacteria alone versus combined with GloGerm™.

Since no significant difference was found between recovery rates of bacteria alone and in combination with GloGerm™ and GloGerm™ has no bactericidal effect within 4 hour sampling period, further studies tracking potential cross contamination pathways originating from a certain source may be explored using a bacteria/ GloGerm™ cocktail. This UV fluorescing cocktail may be beneficial as an aid in visualizing previously unknown sampling locations within a variety of food manufacturing and retail environments.

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Table 4.1. Mean recovery (log CFU/cm²) of foodborne bacteria (*Listeria monocytogenes*, *L. innocua*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7) from 5.08cm by 5.08cm stainless steel coupons after inoculation alone and mixed with GloGerm™ (n=9).

Bacteria	Mean log(CFU/cm²) starting inoculum	Mean log(CFU/cm²) recovered	Mean log(CFU/cm²) difference¹
<i>L.innocua</i>			
alone	8.40±0.07	6.85±0.15	1.55±0.10 ^{c,d}
in GloGerm™		6.75±0.28	1.65±0.22 ^d
<i>L.monocytogenes</i>			
alone	8.36±0.09	7.01±0.11	1.35±0.15 ^{a,b,c}
in GloGerm™		7.02±0.17	1.35±0.17 ^{a,b,c}
<i>S. Enteritidis</i>			
alone	8.09±0.09	6.88±0.10	1.21±0.07 ^{a,d}
in GloGerm™		6.70±0.24	1.39±0.18 ^{c,d}
<i>E.coli</i>			
alone	7.28±0.07	6.12±0.09	1.16±0.06 ^a
in GloGerm™		6.08±0.06	1.19±0.07 ^{a,b}

¹values within the column not followed by the same letter were significantly different (Tukey's HSD ; $\alpha < 0.05$).

Figure 4.1. Growth of foodborne bacteria (*Listeria monocytogenes*, *L. innocua*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7) in tryptic soy broth and in GloGerm™ at 37°C over 24 hours.

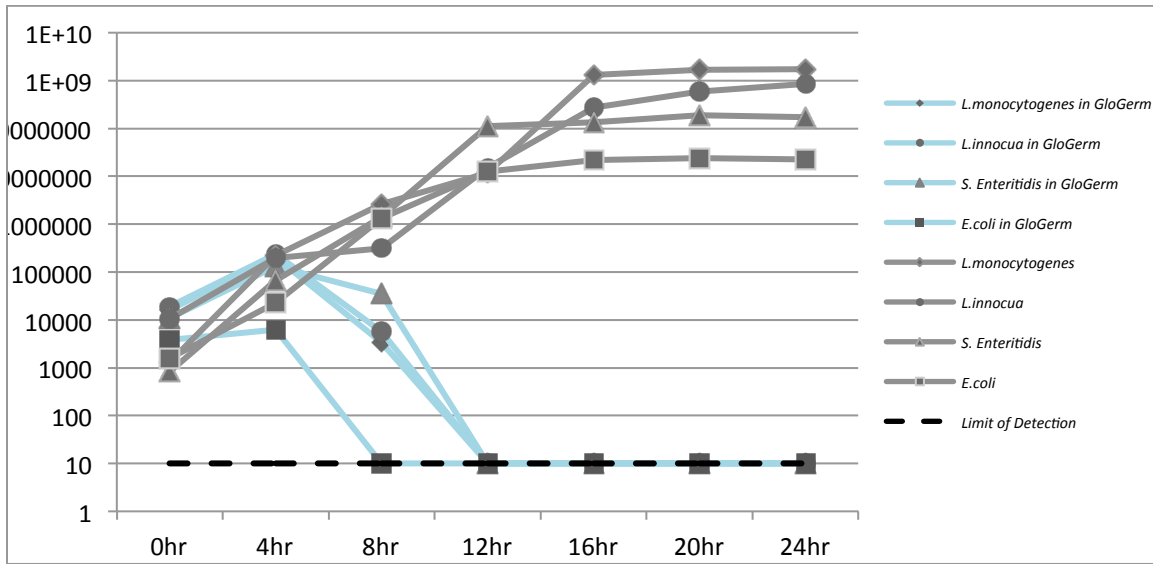
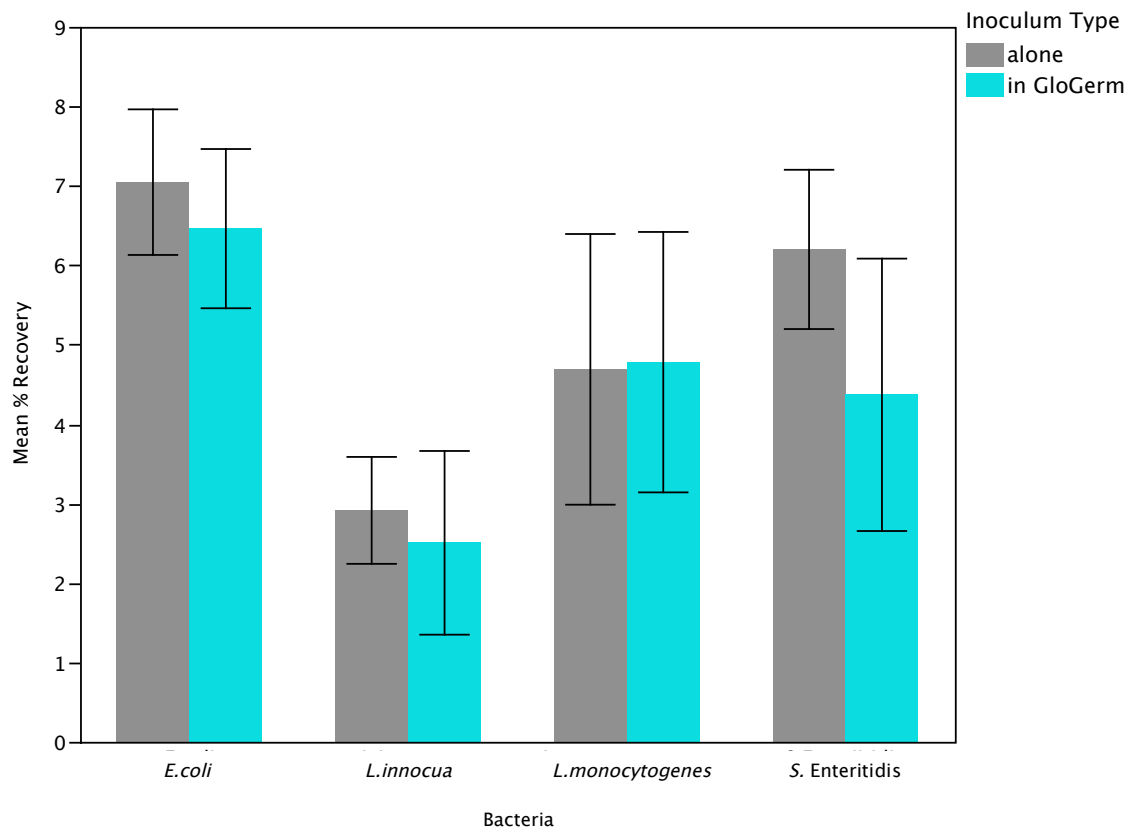


Figure 4.2. Mean percent recovery (CFU/cm²) of foodborne bacteria (*Listeria monocytogenes*, *L. innocua*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7) from 2” by 2” stainless steel coupons after inoculation alone and mixed with GloGerm™ (n=9).



Chapter 5.

Comparison between transfer rates of a GloGerm™/*Listeria* spp. cocktail and *Listeria* spp. alone during slicing of bologna with a deli meat slicer.

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Abstract

Recent risk assessments and case control studies have identified deli meats sliced at retail as a high-risk commodity for contamination of *Listeria monocytogenes*. A complete understanding of potential cross contamination pathways when contamination is introduced from a variety of sources is crucial to reducing this risk. A fluorescing abiotic surrogate can be used to visualize direct contact cross contamination within a space without timely and costly methods to culture potential pathogens, however the surrogate alone may not mimic pathogen spread. Co-inoculation of the abiotic surrogate with a pathogen may be useful to guide environmental sampling. This study was designed to determine if an inoculum comprised of either *L. monocytogenes* or *L. innocua* mixed with a fluorescing compound (GloGerm™ Gel) would spread throughout retail deli slicer and be recovered in a similar manner to when the organisms were inoculated alone. A bologna chub and the deli slicer blade was inoculated with one of the two pathogens and series of common deli procedures were completed. Following these actions, 5 locations on the deli slicer, the remaining bologna chub, and select meat slices were sampled. There were no differences ($p>0.05$) between the transfer of bacteria inoculated alone or with GloGerm™ to the selected sites, regardless of starting contamination source. Additionally, no differences ($p>0.05$) were found between the spread of *L. monocytogenes* and *L. innocua*. These findings suggest there may be potential in using *L. innocua* in combination with GloGerm™ to visually track and sample cross contamination from a known source throughout a retail deli environment and relate it to *L. monocytogenes* spread.

Introduction

The primary pathogen of concern in ready to eat (RTE) deli meats is *L. monocytogenes*. *L. monocytogenes* is one of the more deadly foodborne pathogens, responsible for an estimated 94% hospitalization rate and a 15.9% mortality rate (Scallen et al., 2011). *L. monocytogenes* is ubiquitous in nature and can be found throughout food processing environments. The pathogen's ability to grow at refrigeration temperatures and the lack of a final intervention step make RTE products handled in retail environments risky.

Deli meats sliced at retail are more likely to be contaminated with *L. monocytogenes* than those pre-packaged in processing plants (FSIS, 2010). Data from grocery store sampling in 4 different states and a variety of different store types revealed that 49 out of 3,518 samples of retail sliced deli meat were contaminated vs. 6 out of 3,522 samples of pre-packaged deli meat (Draughon, 2006). It has been concluded that approximately 85% of illness and deaths from listeriosis are attributed to deli meat sliced and packaged at retail facilities (Endrikat, 2010).

Identifying potential cross contamination pathways that may lead to contamination of deli meats in the retail setting is essential to reducing the number of listeriosis cases per annum in the United States (FDA, 2009). Several mathematical models have been developed and studies have been conducted to fully identify and examine pathways of cross contamination at the food processing plant level (Schaffner, 2004; Alessandria et al., 2010; Lappi et al., 2004). Until recently, studies at the retail level have been limited and only modeled hypothetical situations (Perez Rodriguez et al., 2006; Pradhan, 2011).

A complete view of potential cross contamination pathways is difficult to visualize without prior knowledge of which surfaces to sample. Several studies have used fluorescing surrogates as indicators of cross contamination in food processing environments (Ashby, 2003;

Burfoot, 2011; Nieto-Montenegro, 2008; Gibson, 2013). A previous study (Maitland et al., 2013), used a fluorescing surrogate (GloGerm™ lotion) to visualize sites within a mock retail deli that became contaminated during standard deli operation when six different sites were the initial contamination source. This study gave insight into where direct contact cross contamination may occur, however it is unknown if the transfer of bacteria correlates to the transfer of GloGerm™.

The objective of this study was to replicate select methods from Maitland et al (2013) within a BSL-2 lab and compare the transfer of a bacteria/GloGerm™ mixture to transfer of bacteria alone. If the mixture transfers similarly to bacteria alone, this mixture may be used in later studies to visualize all potential surfaces contaminated during mock retail deli operations and act as a guide for further sampling.

Materials and Methods

Culture selection and maintenance. A strain of *L. monocytogenes* serovar 4b strain J1815 was obtained from an environmental sample taken during the 2002 US multistate deli turkey outbreak (CDC, 2002). *L. innocua*, a possible surrogate for *L. monocytogenes*, was also used in order to determine potential for future research using the surrogate only. Both cultures were obtained from the -80°C ultra-low freezer at the Food Science and Technology department of Virginia Tech (Blacksburg, VA). The strains were serially activated (35°C for 24 h) in trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, MD.) over 3 days before each trial and confirmed by subsequent plating on Modified Oxford Agar (MOX; Difco, Becton Dickinson, Sparks, MD.).

Inoculum preparation. After being serially activated, cells from a 24-hr culture in TSB were centrifuged at 10,000 x g for 5 minutes using a Fisher Scientific AccuSpin 400

(FisherBrand, Pittsburgh, PA) to remove any residual nutrients. The cells were washed with sterile 0.1% peptone water and re-suspended in 10 mL sterile de-ionized water. The inoculum was prepared alone and in combination with a fluorescing compound, GloGerm™ GloGel (GloGerm Co., Moab, UT). The GloGerm™/bacteria inoculum was prepared by adding 5 mL of GloGerm™ to reach a final volume of 15 mL; for the inoculum containing only bacteria, an additional 5 mL of sterile deionized water was added to reach a final volume of 15 mL.

Source site inoculation. Two contamination sites, 1) meat chub or 2) deli slicer blade, were based on the work completed in Maitland et al (2013) to serve as the originating point of contamination for each trial.

Meat chub. A retail brand pork/ beef bologna chub was purchased from a local retailer (Kroger Company, Blacksburg, VA). Ingredients listed on the label were as follows: pork, water, beef, salt, less than 1.5% dextrose, sugar, sodium phosphate, paprika, sodium erythorbate, flavorings, monosodium glutamate, and sodium nitrate. The whole chub was cut into 8 cm segments to allow for consistency in application. Before each trial the chub segment was surface inoculated with 1 mL of either the bacteria alone or GloGerm™/bacteria mixture and allowed to air dry in a laminar flow-through hood for 15 minutes to obtain a final inoculation of ca. 6 log CFU/cm².

Deli slicer blade. The blade of an electric deli slicer (model 2612C, Hobart Corporation, Troy, OH) was removed and a 10 cm by 10 cm sterile aluminum template was used for consistency in applying 1 mL either inoculum. Both inocula were applied and distributed using sterile 1cc syringes without needles. The blade was allowed to dry under a laminar flow-through hood for 15 minutes before being re-attached. The final inoculation was ca. 6 log CFU/cm².

Recipient site determination and analysis. After the source site (either meat chub or deli slicer blade) had been inoculated, a series of procedures was performed, consisting of about 5 minutes worth of work. The procedures (Figure 5.1) were modified from the Maitland et al (2013) mock deli cross contamination study. Due to the need for a BSL-2 certified space to work with pathogens and space limitations within the BSL-2 lab the only contamination recipients that could be evaluated were the slicer (bed, blade, carriage, handle, and shelf; Figure 2), the remaining chub, and the third and ninth meat slices.

To enumerate *L. monocytogenes* or *innocua* from the meat slices, the bologna slices were placed into a sterile stomacher bag (FisherBrand Secure T, Pittsburgh, PA) containing a 1:5 (wt/vol) of phosphate- buffered saline (PBS) and homogenized for 2 min as described in Vorst et al (2004). The homogenate was then serially diluted and plated in duplicate on MOX.

To enumerate *L. monocytogenes* or *innocua* from the deli slicer, sites fluorescing were swabbed using the one-ply composite tissue (CT) recovery method developed by Vorst et al (2004). One- ply composite tissues (11.4 by 21.5 cm; Kim-wipe Ex-L 1-ply white tissue, Kimberly-Clark Corp., Roswell, Ga.) were folded to create 3.5 cm by 3.5 cm squares. The CT was hydrated with 10mL PBS in a sterile 7oz. Whirl-Pak bag (Nasco, Fort Atkinson, WI) and wrung out to remove excess liquid. The hydrated tissue was then used to swab the deli slicer sites, 10 times vertically and 10 times horizontally, within a sterile 10cm by 10cm aluminum template for consistency. The CT was then placed back in the Whirl-Pak bag and 40mL of PBS added. The bag was homogenized for 60 sec in a stomacher and hand massaged for 30 sec. The homogenate was then serially diluted and plated in duplicate on MOX. The procedure was repeated for each of the 5 deli slicer sites using a clean pair of gloves for each CT sample. The only variation occurred on the deli slicer handle, which was swabbed over its entire surface.

Cleaning and decontaminating the slicer. After each trial, the slicer was completely disassembled. The blade guard and blade were soaked in a Micro 90 solution for 30 minutes under the laminar flow hood while the other non removable areas of the slicer were cleaned with the same solution and air dried for 30 minutes. All surfaces were then sprayed with 70% ethanol and wiped clean. Sampling of all cleaned surfaces was done to ensure that no *L. monocytogenes* or *L. innocua* remained following decontamination.

Data analysis. For both *L. monocytogenes* and *L. innocua*, each source site trial and inoculum combination (meat chub and deli slicer blade; bacteria alone and with GloGerm™) was replicated three times. Control trials were run and sampled with nothing applied to the source sites as well as with only GloGerm™ applied to the source sites. Total bacterial counts were determined and appropriate transfer rates were calculated. For example, transfer rate from meat chub to slicer carriage was determined by: $(\text{CFU on slicer carriage})/(\text{CFU on meat chub}) * 100 = \text{Transfer Rate } (\%)$. When sites contained less than the detection limit (< 10 CFU per sample) the rates were calculated as if the concentration on the sites were at the detection limit and reported as less than (%) transfer. The bacterial counts were then converted to log CFU/cm² to approximate normal distribution for statistical analysis. Comparisons between bacteria and inoculum types were analyzed using a general linear model and one-way analysis of variance (ANOVA). Tukey-Kramer honestly significant different (HSD) was used for two or more mean comparisons within a data set ($\alpha \leq 0.05$, $p < 0.05$) using JMP v. 10.0 (SAS; Institute Inc., Cary, NC).

Results.

Control trials run with no inoculum as well as with GloGerm™ only, saw no contamination spread above the detection limit (10 CFU/cm²) to any of the sample sites. Also

samples taken after each trial of all cleaned surfaces reported no bacterial growth above the detection limit (10 CFU/cm²).

Starting inoculum levels on both the deli slicer blade and meat chub were, on average, ca. 6 log CFU/cm².

Transfer to slicer sites. Specific sites within the deli slicer (bed, blade, carriage, shelf, and handle) were evaluated separately. The mean populations recovered (CFU/cm²) from specific sites throughout the slicer and from the bologna chub after slicing are displayed in Figure 5.3 (when meat chub was the starting contamination site) and Figure 5.4 (when slicer blade was starting contamination site). In order to account for variation in starting inoculum levels per trial, for statistical analysis, bacterial counts were transformed into log values and the difference between the starting inoculum and populations recovered from each site were calculated. These results along with transfer rates (%) are displayed in Table 5.1 (when meat chub was starting contaminate) and Table 5.2 (when slicer blade was starting contaminate).

Overall, there were no significant differences found between the spread of bacteria to any of the slicer sites when inoculation was done using bacteria alone or with GloGerm™ lotion for either starting contamination site ($p > 0.05$). There were two exceptions including transfer of *L. monocytogenes* and *innocua* to the handle when the starting site was the slicer blade and meat chub respectively (Table 5.1 and 5.2). There was significantly greater transfer to the slicer handle when *L. innocua* was co-inoculated with GloGerm™ (Table 5.1) and when *L. monocytogenes* was co-inoculated with GloGerm™ (Table 5.2).

Transfer to meat slices. The transfer of the two inoculum types to selected meat slices was also compared. The recovery (CFU/cm²) from meat slice 3 and 9 after each trial is presented in Figure 5.5 (when meat chub was starting contaminant) and Figure 5.6 (when slicer blade was

starting contaminant). Again, bacterial counts were log transformed and differences between starting inoculum and populations recovered for statistical analysis. These results and transfer rates (%) for both starting contaminate sites are shown in Table 5.3.

When the meat chub was the starting contamination site, significantly more ($p < 0.05$) bacteria (*L. monocytogenes* and *innocua*) was spread to the 9th meat slice when inoculated alone. Additionally, when the slicer blade was the starting contamination site, significantly ($p < 0.05$) more transfer of *L. monocytogenes* and *innocua* was spread to the 3rd meat slice when the bacteria were inoculated alone. However, there was no difference in transfer to the 9th slice (Table 5.3).

Comparison between *L.monocytogenes* and *L.innocua*. There was generally no significant difference found between the spread of *L. monocytogenes* and *L. innocua* throughout the deli slicer ($p > 0.05$) (Table 5.1 and 5.2). The only exception occurred when contamination was transferred from the meat chub to the deli slicer bed. Significant more *L. innocua* was transferred to the slicer bed than *L. monocytogenes* (Table 5.1) This difference could be explained by compounding variation in transfer of bacteria between the meat chub and the slicer bed. For *L. monocytogenes*, the log recovery from the deli slicer bed was associated with the greatest error (Figure 5.4).

Discussion.

Comparison of spread between bacteria inoculated alone and with GloGerm™

Understanding cross contamination pathways within a retail deli environment is a crucial component of reducing foodborne illness contracted via contaminated RTE food products. Several studies have explored the transfer of foodborne pathogens throughout certain components of deli operations. These studies have focused mainly on direct transfer scenarios

between meat chub and deli slicer blade or knives (Perez-Rodriguez et al., 2007; Sheen et al., 2010; Sheen and Hwang, 2011; Aarnisalo et al., 2007; Vorst et al., 2006a; Vorst et al., 2006b; Rodriguez et al., 2007; Lin et al., 2006). Cross contamination studies tend to produce variable data. Many factors have been identified as having a significant influence on the transfer of bacteria from one surface to another, such as type of bacteria (Rusin et al., 2002), source and destination surface (Chen et al., 2001), inoculation level (Montville, 2002), time post-inoculation (Scott and Bloomfield, 1990), and moisture level (Chen et al., 2001). Since this study sampled more sites within deli slicer while also replicating more realistic deli operations, with breaks between slicing and wrapping/ unwrapping the meat chub, more direct comparisons may be made.

The areas on the deli slicer sampled in this study coincide with the areas found to be most contaminated by fluorescing surrogates in other studies. Vorst et al (2006a) bathed a chub in GloGerm™ powder and identified the carriage tray, back plate, blade guard, blade, and collection area as cross contamination surfaces. Gibson et al (2013) used an un-inoculated fluorescing compound powder to track cross contamination originating from a meat chub throughout a mock retail deli. Twenty-one participants came in and sliced 5 slices from 3 separate bologna chubs. The surfaces with highest concentrations after the slicing events were the carriage tray, collection area, gloves, and meat grip (Gibson, 2013). Although a different model deli slicer was used in the Gibson study, the carriage tray in their study is comparable to the slicer carriage used in this study, the collection area in their study was comparable to the slicer bed, and the meat grip in their study was comparable to the slicer handle.

When comparing to the data generated by Maitland et al (2013), where a sensory panel was used to rate transferred concentration levels of GloGerm™ alone, there are discrepancies. In

the previous study, the panel rated spread from the meat chub to the slicer components as follows: chub, shelf, and handle as heavy; bed as moderate; carriage and blade as slight. (Maitland et al., 2013). When the slicer blade was the origin of contamination the panel rated spread as follows: blade, bed, and shelf as heavy; chub and carriage as moderate; handle as slight (Maitland et al., 2013). Bacterial counts from this study showed that actual bacteria spread slightly more to initial contact surfaces, the shelf and carriage (for meat chub trials) and the shelf and bed (for slicer blade trials). In our previous study (Maitland et al., 2013), when the meat chub was the starting contaminate site, GloGerm™ spread heavily to the slicer handle. In this study, however, the slicer handle received the least amount of bacterial contamination. These findings suggest that bacteria alone may not spread as far as the GloGerm™ Gel.

Comparison of spread between *L. monocytogenes* and *L. innocua*

No significant differences were found between the spread of *L. monocytogenes* and *L. innocua* regardless of if they were inoculated alone or with GloGerm™. These results are supported by the fact that *L. innocua* is the *Listeria* species genetically closest to *L. monocytogenes* (Buchrieser et al., 2003). The two were not even recognized as separate species until 1981 (Seelinger, 1981). Although the two strains are different in terms of pathogenicity, they have been found to inhabit similar environmental niches (Lan et al., 2000). *L. innocua* has been widely used as a surrogate for *L. monocytogenes* in thermal inactivation studies for a variety of food matrixes and environments (Goff and Slade, 1990; Kamat, 1996; Kozempel et al., 2002; Kim and Linton, 2008; Friedly et al., 2008). In chapter 5, *L. innocua* was found to have similar growth characteristics to *L. monocytogenes* both in TSB and GloGerm™ lotion. This study found that *L. innocua* spread similarly to *L. monocytogenes* throughout a retail deli slicer and meat slices and could potentially be used as a nonpathogenic biotic surrogate in future studies.

It is important to note the limitations associated with the methods of this study. In order to ensure the consistency needed to compare the two inoculum levels, certain factors may limit the comparability of this data to real life transfer within a retail deli environment. First, this data does not take into account the variability caused by multiple operators. Only one researcher performed each replication and future studies may want to introduce multiple participants in order to produce data more comparable to actual spread. Second, to maintain consistency this study only sampled from a 100 cm² surface area of each contaminated area as opposed to sampling the entire fluorescing area. While this study found no real significant differences between the recovery of GloGerm™/ bacteria mixture and bacteria alone spread, standard deviation values were, on average, much larger for the GloGerm™ mixture recovery values. Future studies examining transfer to particular sites more frequently may need to be done to examine this variability. Also, this study chose to only analyze two starting sites of contamination

Conclusions.

Overall the findings of this study indicate that there is no significant difference between the way *L. monocytogenes* and *L. innocua* inoculated with GloGerm™ and bacteria inoculated alone transferred throughout a deli slicer and to certain meat slices. These findings suggest there may be potential in using *L. innocua* in combination with GloGerm™ to visually track and sample contamination from a known source throughout a retail deli environment. Using *L. innocua* prevents researchers or participants from being exposed to pathogenic bacteria while the fluorescing aspect of the GloGerm™ may help to simultaneously uncover previously unknown cross contamination areas while sampling.

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Table 5.1. Differences in recovery (log[CFU/cm²]) and transfer rates (%) of bacteria alone and in combination with GloGerm™ from a meat chub inoculated with ca. 6 log CFU/cm² throughout a deli slicer (n=3) after a series of deli procedures.

Inoculum:	Sample Site: Log CFU/cm ² (TR %)				
	Shelf	Carriage	Blade	Bed	Handle
<i>L.monocytogenes</i>					
Alone	3.66 ^a (0.0244)	4.05 ^a (0.0135)	4.22 ^a (0.0065)	4.89 ^{ab} (0.0015)	5.75 ^a (0.0003)
With GloGerm™	4.06 ^a (0.0154)	4.49 ^a (0.0109)	4.24 ^a (0.0081)	5.62 ^a (0.0003)	4.50 ^a (0.0167)
<i>L.innocua</i>					
Alone	3.68 ^a (0.0273)	3.47 ^a (0.0505)	3.34 ^a (0.0564)	3.38 ^c (0.0605)	5.19 ^a (0.0008)
With GloGerm™	3.30 ^a (0.0802)	3.54 ^a (0.0864)	3.36 ^a (0.1357)	3.91 ^{bc} (0.0205)	3.54 ^b (0.0864)

Values in the same column with different superscript letters indicate significant differences.

Table 5.2. Differences in recovery (log[CFU/cm²]) and transfer rates (%) of bacteria alone and in combination with GloGerm™ from a slicer blade inoculated with ca. 6 log CFU/cm² throughout a deli slicer (n=3) after a series of deli procedures.

Inoculum:	Sample Site: Log[CFU/cm ²] (TR %)				
	Shelf	Carriage	Bed	Chub	Handle
<i>L.monocytogenes</i>					
Alone	3.89 ^a (0.0205)	5.31 ^a (0.0008)	4.43 ^a (0.0056)	4.60 ^a (0.0047)	5.94 ^a (0.0001)
With GloGerm™	3.05 ^a (0.1482)	4.65 ^{ab} (0.0036)	3.99 ^a (0.0209)	4.43 ^a (0.0087)	4.09 ^b (0.0122)
<i>L.innocua</i>					
Alone	3.37 ^a (0.0454)	3.61 ^b (0.0250)	4.42 ^a (0.0042)	4.12 ^a (0.0110)	5.23 ^{ab} (0.0007)
With GloGerm™	3.57 ^a (0.0293)	4.60 ^{ab} (0.0037)	4.29 ^a (0.0091)	4.02 ^a (0.0120)	4.63 ^b (0.0030)

Values in the same column with different superscript letters indicate significant differences.

Table 5.3. Comparison of bacteria alone and GloGerm™/bacteria mixture mean population (log[CFU/cm²]) difference from initial inoculum (ca. 6 log[CFU/cm²]) from third and ninth meat slice (n=3) after a series of typical deli operations.

Inoculum:	Meat Chub as starting site: Log[CFU/cm ²] (TR %)		Slicer Blade as starting site: Log[CFU/cm ²] (TR %)	
	3 rd Slice	9 th Slice	3 rd Slice	9 th Slice
<i>L.monocytogenes</i>				
Alone	2.26 ^a (0.6204)	3.30 ^b (0.5700)	1.82 ^{bc} (5.1417)	4.01 ^a (0.0257)
With GloGerm™	2.77 ^a (0.2620)	4.53 ^a (0.0029)	3.19 ^a (0.1126)	4.46 ^a (0.0051)
<i>L.innocua</i>				
Alone	2.37 ^a (0.5466)	2.55 ^b (0.3705)	1.05 ^c (9.3238)	3.38 ^a (0.0464)
With GloGerm™	2.98 ^a (0.2605)	3.45 ^a (0.0037)	2.89 ^{ab} (0.1572)	4.33 ^a (0.0111)

Values in the same column with different superscript letters indicate significant differences.

Figure 5.1. Series of standard deli operations performed (in this order) by researcher for each experimental trial replication. Modified from Lubran et al, 2010 and Maitland et al., 2013.

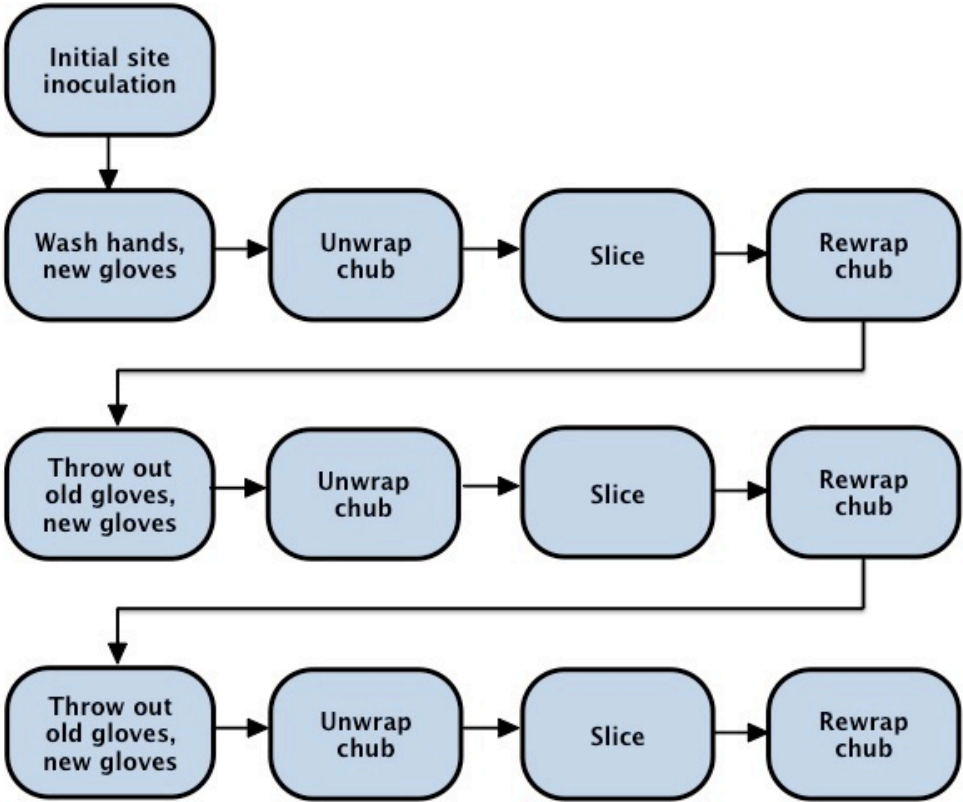


Figure 5.2. Photographs and descriptions of the five slicer components (Hobart Electric Slicer model #2612C) sampled for *L. monocytogenes* and *L. innocua*.

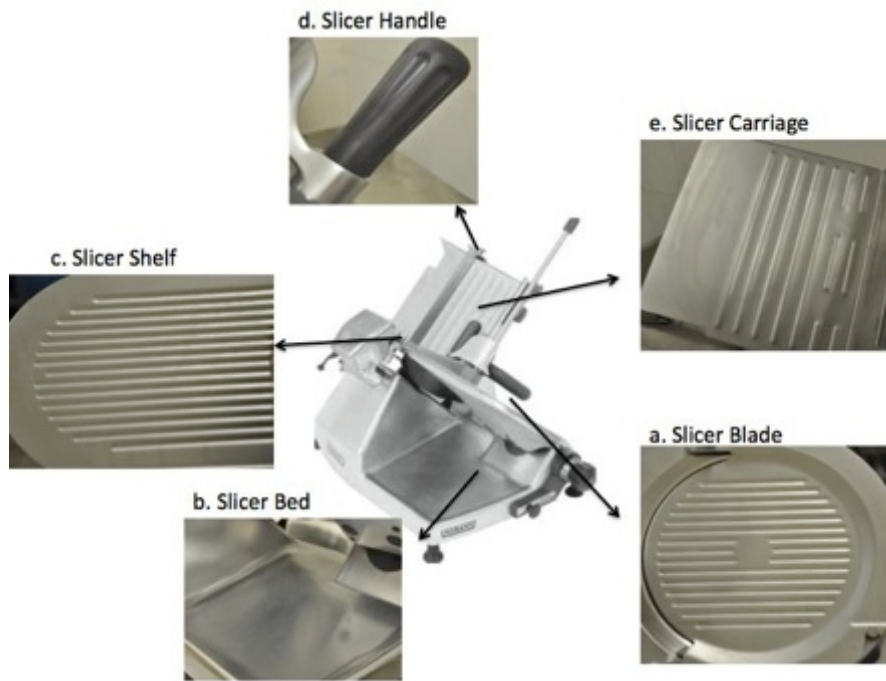


Figure 5.3. Comparison of bacteria recovered (mean populations log CFU/cm²) from slicer locations and meat chub following inoculation alone and in a GloGerm™/bacteria mixture (n=3) after a series of typical deli operations. The meat chub was the initial source of contamination (ca. 6 log CFU/cm²).

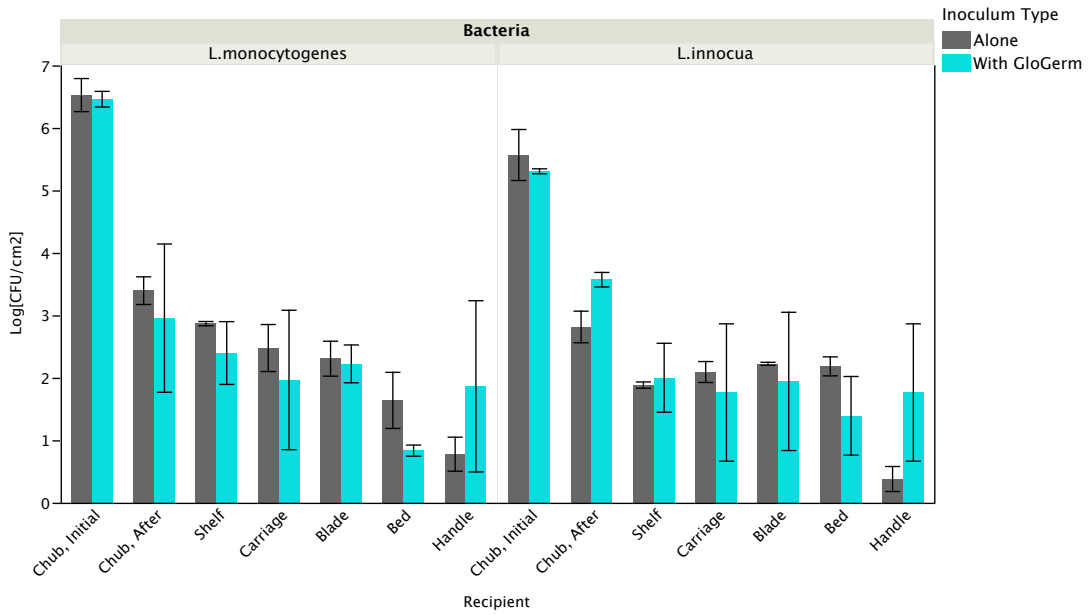


Figure 5.4. Comparison of bacteria recovered (mean populations log CFU/cm²) from slicer locations and meat chub following inoculation alone and in a GloGerm™/bacteria mixture (n=3) after a series of typical deli operations. The slicer blade was the initial source of contamination (ca. 6 log CFU/cm²).

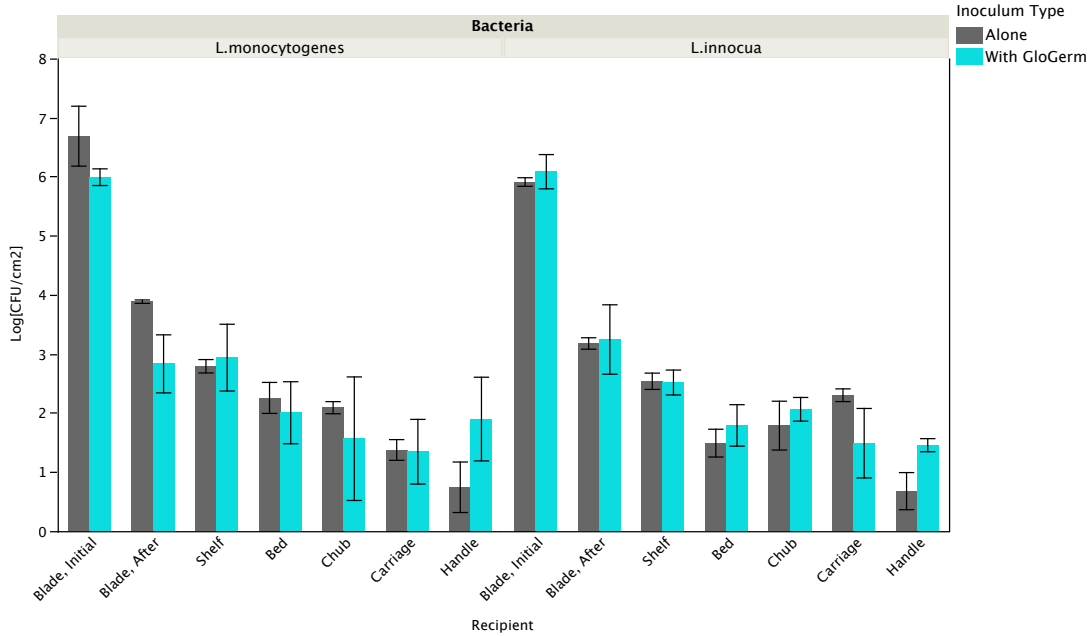


Figure 5.5. Comparison of bacteria alone and GloGerm™/bacteria mixture (log[CFU/cm²]) recovered from 3rd and 9th meat slices (n=3) when meat chub was source of contamination (ca. 6 log CFU/cm²).

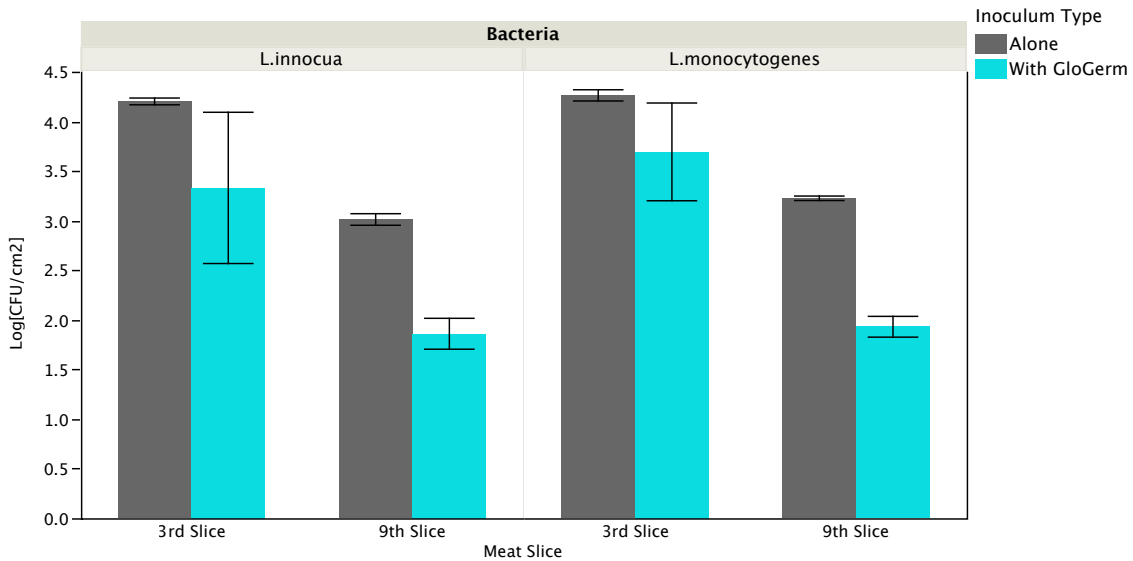
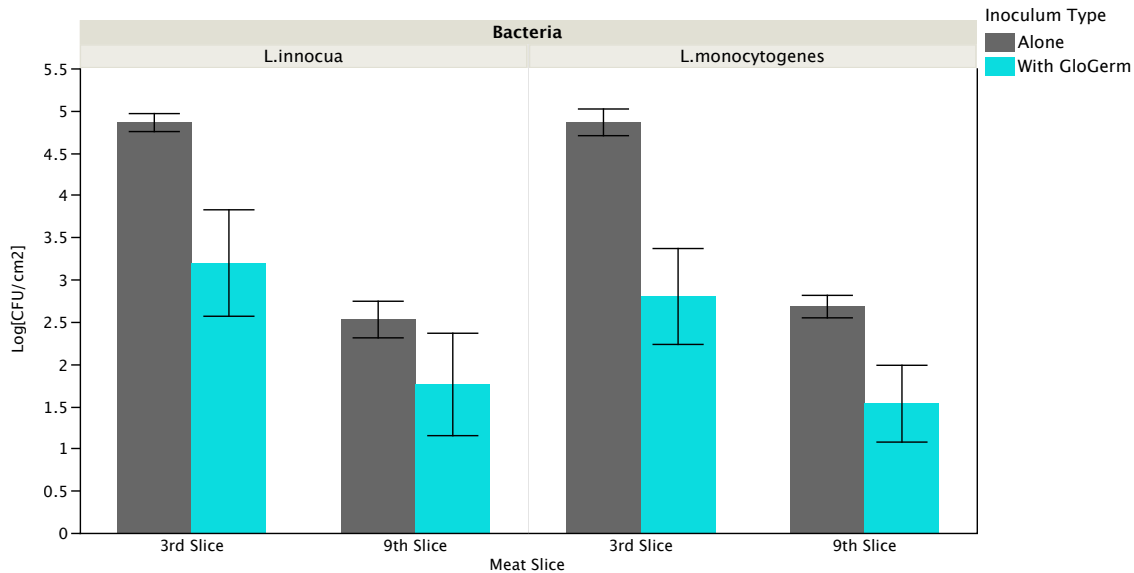


Figure 5.6. Comparison of bacteria alone and GloGerm™/bacteria mixture (log[CFU/cm²]) recovered from 3rd and 9th meat slices (n=3) when slicer blade was source of contamination (ca. 6 log CFU/cm²).



Chapter 6.

Conclusions.

The consequences of foodborne illness can range from fairly unpleasant to quite severe depending on the affected individual. These consequences include missed days at work, potential chronic conditions, and for those with weakened immune systems, even death. While *L. monocytogenes* is not responsible for as many reported cases as some bacteria, such as *Salmonella* and Enterohemorrhagic *E. coli*, hospitalization rates are much higher. Listeriosis is the third leading cause of death among foodborne infections. Ready to eat (RTE) foods are of particular concern as a vehicle for listeriosis because these products are consumed without a final intervention step. After the findings of several studies and risk assessments revealed deli meat sliced at retail was at higher risk for *Listeria* contamination than pre-packaged deli meat, there have been many studies done examining what may be happening behind the counter to contribute to this risk. The work from this study was conducted to track potential cross contamination pathways throughout a retail deli environment. Our approach was to use a fluorescing abiotic surrogate and, before the main objective was attempted, to confirm that the fluorescing abiotic surrogate could be used successfully.

A new method for rating photographs of GloGerm™ spread throughout a mock retail deli was developed through training session feedback from sensory panel members. After being validated, this method was used to produce quantitative data rating the potential spread of contamination from several different starting sites throughout a mock retail deli. The findings of this study were a part of a collaborative contribution to a current FDA/FSIS risk assessment, helping researchers to determine some inputs or events not previously considered in the model.

In order to further examine the use of GloGerm™ in tracking cross contamination and the potential of inoculating starting contaminant sites using a GloGerm™ and bacteria mixture for tracking purposes, methods were developed to compare the recovery of bacteria alone to the recovery of the mixture. For three different foodborne pathogens and one surrogate bacteria, no significant difference was seen between mean recoveries of the inoculum types from stainless steel coupons. When trials were run to determine how the two inoculum types transferred throughout a deli slicer and to meat slices, again, no real significant difference was found between mean recovery. The presence of an antimicrobial, diazolidinyl urea, in the GloGerm™ lotion limits the use of this product for studies that may require the bacteria to be in contact with the GloGerm™ for extended periods.

While the data generated was beneficial in visualizing potential areas of cross contamination occurring from a known starting contaminate source, the lotion component of the GloGerm™ Gel may have contributed to slightly exaggerated transfer quantities as compared to bacteria transfer rates, especially on areas like the deli slicer handle. These areas saw heavier spread of GloGerm™ alone, when analyzed by the sensory panel, than actual bacterial recovery when *Listeria* spp. were inoculated alone. GloGerm™ Gel was chosen as the only GloGerm™ product that was discernible throughout a space as large as the mock deli.

Further research may be done to determine the potential of using these methods to produce training materials. Currently, there is a recommendation for stronger education focusing on understanding potential cross contamination pathways for retail deli employees. Although the photographs may portray an exaggerated view of contamination transfer, *Listeria* is a hardy environmental organism, with the ability to grow in many different matrices at cold temperatures. In person trials or photographs may be used to visually demonstrate that

contamination of a surface or niche with even a small number of cells could eventually lead to high numbers if improperly sanitized.

Although cross contamination studies generally tend to have higher standard deviations, further research needs to be done to examine the higher variation consistently seen amongst the GloGerm™/bacteria mixture trials.

Appendix A.

Developing and validating a method to use a sensory panel to evaluate GloGerm™ concentrations for use as an abiotic surrogate for bacterial cross contamination studies.

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Abstract.

The use of a fluorescing abiotic surrogate to visualize the potential spread of bacteria throughout an environment could be beneficial to researchers. The aims of this study were to develop and validate a rating method for visualization of different fluorescence concentration levels (GloGerm™) in order to quantify and analyze surface to surface cross contamination during experimental trials in a mock retail deli. An 18-member panel ranked photographed concentrations of GloGerm™ in deionized water (ranging from 0% to 100% in increments of 25%) on common retail deli surfaces. Subsequently, 8 panelists were trained to rate photographs of the fluorescing surrogate on comparative deli surfaces using the terms none, slight, moderate, and heavy. Preliminary tests determined that a panel (n=18) could generally tell the differences between 5 concentrations of GloGerm™ gel on different surfaces, according to how brightly the gel appeared in photographs and the difference in dilutions when the gel had spread from an inoculated blade to meat slices and to a glove used by the operator. The trained panel (n=8) identified the necessity to rate photographs in terms of two attributes: intensity (fluorescent brightness in the photograph) and coverage (fluorescing area in the photograph). Researchers pre-defined an overall attribute rating scale based on the combination of rating categories for each attribute. Evaluating the two characteristics independently with a pre-defined strategy for decision-making allowed panelists to identify an overall rating with low standard deviations (less than ± 0.35). Incorporating an overall rating after evaluating intensity of specific characteristics

is debated. Pre-defined strategies for incorporating attribute ratings into decisions for overall rating provided categorization of cross contamination in a mock deli setting.

Introduction.

The Center for Disease Control and Prevention has estimated 9.4 million illnesses, nearly 56,000 hospitalizations, and more than 1,300 deaths are linked to foodborne pathogens in the United States every year (Scallen et al., 2011). Cross contamination within food production, retail, and consumer homes is a significant factor contributing to these illnesses, hospitalizations, and deaths. It is difficult to track cross contamination throughout a food preparation space because spread is not consistent and the labor involved in environmentally sampling all surfaces is prohibitive.

The use of a fluorescing abiotic surrogate to visualize the potential spread of bacteria throughout an environment via direct contact could be beneficial to those concerned with food safety. These surrogates would remove the potential risk and cost of environmental sampling and allow visualization of spread throughout the food production/preparation setting. Surrogates have already been used in research to visualize contamination within a variety of different biological systems (Sinreich et al., 2008; Renken et al., 2005; Baeza et al., 2004; Oberyszyn et al., 2001; Perfetto et al., 2003; Bergervoet et al., 2008; Snyder et al., 1999). Recently, the use of surrogates has been applied to studies within the food industry (Ashby et al., 2003; Burfoot et al., 2011; Buchholz et al., 2012; Keskinen et al., 2007; Gibson et al., 2012; Nieto-Montenegro et al., 2008). No studies to date, however, have used an abiotic fluorescent surrogate to visually track and quantify the potential cross contamination through various food processing/preparation settings.

One method to turn visual images of the fluorescent compound on various surfaces (as a result of cross contamination) into quantifiable data may be through the use of a trained sensory panel. Panels have previously been used to evaluate photographs in order to assess various characteristics and quality of foods (Garitta, 2013; O'Sullivan, 2003; Balaban, 2008;

Bruguapaglia and Destefanis, 2010). There are benefits in using photographs as opposed to viewing the actual food or surface including; the ability to permanently capture characteristics that may deteriorate over time and the possibility of collecting data from a large number and variety of individuals. This study was designed to develop and validate a ranting method for visualization of different GloGerm™ concentration levels on a variety of food contact surfaces. This method would subsequently be applied to quantify and analyze surface to surface cross contamination during experimental trials in a mock retail delicatessen (deli).

Materials and Methods.

Researchers chose to track contamination throughout a mock retail deli environment using an abiotic surrogate. The mock deli is outlined in Figure A.1. The space was created in the Food Science & Technology department at Virginia Polytechnic Institute and State University. The general layout was shaped following observations at local retail delis, as well as conversations with representatives from established retail deli operations. Six black light cases (containing two 48 in 40-watt bulbs each; GE, Lowes, Christiansburg, VA) were evenly spaced around the exterior of the setup to allow for complete illumination over the tables, drains, and into the deli case, producing adequate fluorescence for photography. Preparation of this mock deli took considerable time; therefore only photographs used in the final training were taken in this space, while preliminary training photographs were taken in a darkened lab.

The surrogate selected was a commercially available product called GloGerm™ Gel (GloGerm, Moab, UT). This product is well known for use in teaching proper hand washing techniques and aseptic procedures in schools, labs, and hospitals. GloGerm™ is invisible under incandescent light sources but will fluoresce under UVA or black light (400 – 360nm). Although GloGerm™ is also available in powder and liquid forms, the gel form was chosen for this study

because of its uniform coverage of surfaces and ease of photographing. It fluoresces a bright blue color under the UV light and was the easiest to distinguish on all meat, employee, and equipment surfaces in the mock deli environment. Photographs were taken using a Nikon D90 camera mounted on a tripod. The camera was set at ISO 400 with an exposure time of 1.3s at f/6.3. These settings were adapted from Blenkham et al (2008).

Part I: Preliminary Training. Eighteen participants (students, faculty, and staff from the Virginia Tech Food Science and Technology department) were recruited and trained (Institutional Review Board, IRB# 10-654) at Virginia Polytechnic Institute and State University (Blacksburg, VA). Recruitment was done by sending an email through the department's listserv and volunteers were offered a gift card for a local grocery store that they would receive at the end of all training sessions as incentive.

Session I. In the first training session, panelists met in a classroom on the Virginia Tech campus and were given background information on GloGerm™ lotion gel and its common uses. They were then shown photographs on a projection screen of different dilutions of GloGerm™ gel as photographed under UV light. The panelists were shown two photographs of different dilutions of GloGerm™ gel (dilutions in the photographs ranged from 10 to 100% lotion in de-ionized water; increments of 10%) on two common areas of contamination in a retail deli (glove and salami slice) and asked which one was brighter. Figure A.2 is an example of one of the photographs the panel evaluated. The comparisons shown in these photographs ranged from very different dilutions (10% Gel vs. 90% Gel) to very similar dilutions (50% Gel to 60% Gel). The panelists were also shown two sets of 4 photographs and asked to rank the photos from least to most bright. Figure A.3 is an example of one of the photographs the panel evaluated. These photographs contained samples with concentrations varying from 10% to 100% GloGerm™ .

Session II. The second training session, also held in a classroom, introduced panelists to photos that would more closely resemble the images taken during the proposed experimental study. GloGerm™ Gel was diluted into 5 different levels using deionized water (DW). These levels included the following: 1) 100% Gel, 0% DW; 2) 75% Gel, 25% DW; 3) 50% Gel, 50% DW; 4) 25% Gel, 75% DW; and 5) 0% Gel, 100% DW.

In order to replicate how the gel would spread in the deli environment, simulated trials were completed as follows. In 5 separate trials, 10 mL aliquots of the 5 different dilutions were applied onto the blade of the slicer (Hobart, model #2612C) and three slices from a meat chub (salami and turkey breast chubs) were sliced. To allow for consistency in application of the gel onto the slicer blade throughout the study two templates were created based on chub surface area. For salami, template area was 5 x 2 ½ inches while the turkey breast chub had a larger surface area and was sliced with a contaminated area of 5 x 4 inches. An example of the turkey breast template is shown in Figure A.4.

Photos were taken of the contaminated blade before slicing, the first and third meat slice, the blade after slicing, and one glove used throughout the whole process after each slicing event. The whole procedure, for both salami and turkey, was repeated three times. In the sensory training session, panelists were asked to rank 5 photos of the same contaminated surface exposed to 5 different concentrations of gel.

Session III. The third training session was held in the experimental sensory booths and simulated the sensory evaluation testing conditions for the experimental study. This session was used to familiarize the panelists with the individual touchscreen computer monitors and software, and required individuals to independently rank the pictures. Throughout the study, panelists viewed the photographs from the deli on individual 17” monitors in the sensory booths located in

the Food Science and Technology building at Virginia Tech. The tests were run using SIMS 2000 (Sensory Computer Systems software) that allows the panelists to complete the tests under a coded identity and compiles all the data in a final spreadsheet. For this training session, only one set of photographs showing the blade contaminated before slicing with the 5 different dilutions was ranked.

Validation of Preliminary Training Sessions. After completing all training sessions, the panelists were given a test in the individual sensory booths at the Virginia Tech Food Science department (Blacksburg, VA). Each panelist was shown 5 sets of photographs (Figure 3.5: contaminated blade before slicing; Figure A.6: blade after slicing salami; Figure A.7: blade after slicing turkey; Figure A.8: salami first slice; and Figure A.9: glove used during slicing) of 5 photographs (5 different concentration levels) each and asked to rank photos within each set from least to most bright. Panelists analyzed all 5 photographs for each set on one screen. Overhead lights in the room and in individual booths were turned off in order to reduce glare on the computer monitors and standardize the lighting conditions for viewing the images.

Part II: Final Training. Eight participants from the original validation studies were selected to serve on the sensory panel for this research based on availability and consistency of responses from the preliminary training. This panel of eight members was additionally trained (IRB# 11-014) to rate a single photograph instead of ranking a series of photographs as completed for the validation process. Again the panelists were offered a gift card to a local grocery store as incentive. Results from each training session were analyzed to determine effectiveness of training and to suggest necessary changes in methodology to ensure optimal data collection. The 8-member panel went through multiple training sessions (described below) until responses were consistent.

While photographs from previous training sessions had been taken within a controlled lab space only of the deli slicer, meat slices, and gloves, photographs used in these training sessions were collected from preliminary trials within a previously mentioned mock retail deli space (Figure 1) in the Food Science and Technology Department of Virginia Tech (Blacksburg, VA). A series of deli procedures was modified from previous research (see Figure A.10) and completed after inoculating six different starting contamination sites (the slicer blade, employee's gloves, employee's hands, meat chub, preparation table, and floor drain) with 20mL of GloGerm™. Taking photographs after these trials and in this larger environment allowed for spread of contamination that would be more realistic to the spread seen in the proposed study and allowed for the introduction of photographs of contamination spread to other surfaces, including the floor.

Session I. In the first training session, panelists were asked to rate the level of contamination from a single photograph. The panelists were shown 6 randomly selected photographs of sliced salami obtained from the preliminary slicer contamination trials. The concentration of GloGerm™ on the test sample salami slices was unknown. Panelists were given a guide with photographs of 5 slices of salami contaminated with each of the 5 previously described known concentrations (Figure A.12).

Panelists were shown 6 coded experimental salami slice photographs, one at a time, and asked to rate the slice from 1 to 5 on level of GloGerm™ brightness. They were told to compare the experimental photographs with the known concentrations in the guide (Figure A.12) in order to complete their rating.

Session II. In order to determine if the panelists could apply their knowledge of rating contamination levels on different surfaces, the panelist were shown 11 photographs selected

from a trial conducted within the mock deli where contamination originated from a floor drain. For this particular trial spread of contamination throughout the floor was evaluated. In order for consistency in photographs, 3' by 3' sections of the floor were marked, photographed, and analyzed separately. Additionally, the panelists evaluated contamination on some photographs taken after cleaning occurred, when no contamination should have been present. The panelists were again given the guide of salami slices and told to consider them while looking at photographs of different surfaces. The photographs were randomly coded, but for data collection purpose the photographs were labeled as number of 3'x 3' blocks away from the initial point of contamination (Figure A.13). Each photograph was presented one at a time and the panelists were asked to rate the GloGerm™ brightness on a level of 1 to 5. Examples of photographs and results are shown in Figures A.14 and A.15. After the data collection, the panel had a focus group discussion to discuss challenges with rating the photographs.

Session III. To address the panelists concerns that were brought up in the focus group, the researchers adjusted the testing protocol. The experiment was altered so that the panelist would now be asked to rate to photographs using descriptors. Descriptors chosen were: none (previously 1), slight (2), moderate (3), or heavy (4) contamination. The researchers instructed the panelists to consider the entire image for coverage and not just analyze the areas where GloGerm™ was present. In order to familiarize the panelists with photographs typical to the study and allow for comparisons, four photographs of four different surfaces from trial runs were shown together (an example of one set is seen in Figure A.16).

Validation of Final Training Session. A final training session was run to ensure consistency between panelists' responses and mimic the methods of the final evaluation to be used for the research project. Panelists were shown randomly coded photographs of GloGerm™

contamination spread from six starting inoculation points (slicer, prep table, meat chub, employee hands, employee gloves, and floor drain) to the same two surfaces (meat slices and slicer blade).

The panelists were asked to rate each photograph according to two factors: 1) intensity; or the brightness and 2) coverage, or surface area covered as either none, slight, moderate, or heavy. This was done in order to evaluate both bright spots of accumulated GloGerm™ and area covered in GloGerm™ as contamination. A chart was created to combine these two scores into one overall rating (see Table A.1). As a guide the panelists were given one control photograph of the same image without any contamination (see Figure A.17).

Results and Discussion.

Part 1: Preliminary Training.

Session I. In this training session, panelists were introduced to GloGerm™ and how it appears in photographs for the first time. They were also tested on their ability to differentiate between varying dilutions applied to surfaces such as gloves and meat slices. Fourteen of the 18 panelists (78%) correctly discriminated between the two dilutions shown. Ten (56%) were able to correctly rank both sets while the other 8 panelists correctly ranked at least 1 of the sets. This training session was important in introducing the panelists to GloGerm™ gel, illustrating the potential range of visible intensity of the gel that may be captured under the course of the study using a standardized approach, and showing panelists how certain volumes (1 mL) at different concentrations appeared when evenly spread on common areas in a deli.

The brightness of the gel was affected by dilution and there were differences in uniformity when inoculated onto product surfaces. The undiluted gel was thicker and showed more streaks compared to the diluted gels, which spread across the surfaces more completely and

uniformly. These standardized conditions, while valuable for training, did not capture differences in gel distribution or load, as would be evident under experimental conditions.

Session II. Seventeen panelists attended the second training session and were introduced for the first time to photographs that better represented those seen in the final study. Ten panelists (59%) correctly ranked at least 4 of the 5 sets of photos they were shown. After discussion of the correct ranking, all panelists agreed they could see the differences and recognize the correct order. This session was important in introducing the panelists to the types of photographs they would see throughout the whole study.

Session III. The third training session introduced panelists to the conditions in which they would be viewing the photographs during the main study. When viewing the photo sets in the sensory booths, seventeen (94%) panelists correctly ranked the 5 photographs.

Validation. During the validation of the preliminary training, all 18 panelists correctly ranked the first 3 concentrations on the inoculated blade photographs and five (28%) were able to correctly rank all 5 (Figure A.5). Nine (50%) correctly ranked the first two concentrations and only 1 panelist correctly ranking all 5 concentrations (Figure A.6). The photographs of the blade after slicing turkey were easier for panelists to rank with 12 (67%) of the panelists correctly ranking all 5 concentrations (Figure A.7). All 18 panelists (100%) correctly ranked all 5 concentrations spread to the salami slice from the contaminated blade (Figure A.8). All 18 (100%) panelists correctly ranked the first three concentrations and 4 (22%) could identify all 5 (Figure A.9).

Overall, these preliminary training sessions determined that a panel of 18 subjects could generally tell (\geq half of the panelists ranking correctly) the differences between 5 concentrations of GloGerm™ gel on different surfaces, according to how brightly the gel appeared in

photographs. The subjects could even tell the difference in dilutions when the gel had spread from an inoculated blade to meat slices and to a glove used by the operator. It was decided that these initial samples could be used as a control to aid panelists in rating future photographs in this study based on level of brightness.

Part II: Final Training.

In the final training sessions, the panelists group was reduced from 18 to 8 based on individual previous performance and availability.

Session I. The results from the first training session with the smaller group are shown in Table A.2. Variation in responses depended on the photograph shown. The panelists agreed more consistently on some slices (872) while other slices (982) had a wider variation of response ratings.

Session II. The second training session introduced photographs of surfaces that became contaminated as a result of cross contamination from the floor drain. This was the first time panelist viewed floor surface contamination. All results are shown in Table A.3. Contamination ratings from this session had a greater standard deviation than the first trial. Panelists had trouble using the guide for salami slices and translating it into a comparison for contaminated floor images. However, there was little deviation at the extreme of the rating spectrum such as the drain (initial point of contamination, rated 5 by panelists) and the clean control photographs (rated 1 by panelists).

The panelists mentioned that the term “brightness” was hard to understand because they did not know if they should be looking at the brightest point on the photograph or the largest amount of surface area covered. The panel discussed that it was important to take both these characteristics into consideration. Panelists also mentioned that some photographs seem to have

brighter backgrounds than others, even on the same surfaces, and that may be distracting.

Panelist's comments were taken into consideration for the next training session. Special considerations were taken to ensure all similar areas are receiving uniform light levels and the camera from then on was to be set on the same exposure.

Session III. In the third training session, the ratings were adjusted from numerical to a nominal scale consisting of 4 values; no, slight, moderate, and heavy. For statistical purposes in the results, none, slight, moderate, and heavy were changed to numerical values (1,2,3,4 respectively) before data analysis. Results are seen in Table A.4. Changing the terminology helped the panelists understand exactly what they were evaluating and lowered standard deviations.

Validation study. In the final validation study, based on feedback from the panel, panelists were asked to nominally rate each photograph based on two characteristics, coverage and intensity. The ratings for both characteristics were combined into one overall rating using the chart seen in Table A.1. For statistical purposes in the analysis, no, slight, moderate, and heavy were changed to numerical values (1, 2, 3, 4 respectively). The results are seen in Table A.5. Rating these two characteristics separately allowed for panelists to adequately incorporate both into the overall rating while still keeping standard deviations low (less than ± 0.35).

Conclusions.

Since there are no previously published studies that have used a sensory panel to evaluate photographs of this kind, feedback and interactions from preliminary training sessions were crucial to developing this new method. The panelists' comments were instrumental in leading researchers to identifying intensity and coverage of the GloGerm™ Gel contamination as two

different attributes to be analyzed separately. This pre-defined strategy allowed panelists to characterize the contamination seen in the photographs and reduced variability within responses.

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Table A.1. A guide for the eight sensory panelists to determine final rating based on each panelist’s separate rankings for GloGerm™ coverage and intensity of every surface photo. Panelists provided three ratings, one for coverage, one for intensity, and then used this guide to determine an overall rating.

Coverage	Intensity			
	None	Slight	Moderate	Heavy
None	None	None	None	None
Slight	None	Slight	Slight	Moderate
Moderate	None	Slight	Moderate	Heavy
Heavy	None	Moderate	Heavy	Heavy

Table A.2. Contamination rating (on a scale of 1 to 5) panelists assigned to 6 meat slices when contamination originated from the slicer blade during Part II: Session I.

Photo Code of Meat Slice	Mean Rating (from 1-5)	Standard Deviation
137	4.0	0.53
234	4.0	0.50
438	5.0	0.44
734	3.0	0.33
872	3.0	0.24
982	3.0	1.00

Table A.3. Rating (on a scale of 1 to 5) panelists assigned to deli locations when contamination originated from the floor drain during Part II: Session II.

Deli Location	Mean Rating (from 1-5)	Standard Deviation
Drain	5.0	0.00
Cart's Wheels	4.0	0.92
Bottoms of Shoes	4.0	0.52
-1 away ¹	4.0	0.64
+1 away ¹	4.0	0.64
-2 away ¹	3.0	0.70
+2 away ¹	3.0	0.71
-3 away ¹	3.0	0.89
-4 away ¹	3.0	0.89
Clean	1.0	0.35
Clean	1.0	0.74
Clean	1.0	0.35

¹Numbers (1, 2, 3 etc) represent 3'by 3' blocks of floor space. Sign (+/-) indicates direction away from drain.

Table A.4. Contamination rating (on a scale of 1 to 5) panelists assigned to photos of 4 surfaces with contamination transferred from one of six different contamination origination sites during Part II: Session III.

Floor Photos	Mean Rating (from 1-5)	Standard Deviation
A	3.0	0.52
B	3.0	0.53
C	1.0	0.00
D	2.0	0.35
Meat Slice Photos	Mean Rating (from 1-5)	Standard Deviation
A	3.0	0.00
B	3.0	0.46
C	2.0	0.00
D	3.0	0.00
Glove Photos	Mean Rating (from 1-5)	Standard Deviation
A	3.0	0.46
B	4.0	0.00
C	4.0	0.52
D	4.0	0.52
Blade Photos	Mean Rating (from 1-5)	Standard Deviation
A	2.0	0.46
B	4.0	0.00
C	3.0	0.00
D	3.0	0.46

Table A.5. Contamination rating panelists assigned to photos of meat slice and slicer blade contamination spread from one of six different contamination origination sites (n=8) during validation of final training.

Meat Slice Photo Code	Mean Rating (1-5)	Std. Dev.	Meat Slice Photo Code	Mean Rating (1-5)	Std. Dev
279	3.0	0.30	981	4.0	0.00
415	3.0	0.30	203	4.0	0.35
894	4.0	0.35	642	1.0	0.35
982	4.0	0.24	710	3.0	0.14
510	3.0	0.15	691	4.0	0.00
729	4.0	0.25	562	4.0	0.25
290	2.0	0.15	732	2.0	0.16
389	4.0	0.00	552	2.0	0.25
175	2.0	0.00	914	4.0	0.16
027	4.0	0.00	824	2.0	0.35

Blade Photo Code	Mean Rating (1-5)	Std. Dev.	Blade Photo Code	Mean Rating (1-5)	Std. Dev
125	4.0	0.35	703	2.0	0.00
800	2.0	0.16	987	4.0	0.16
901	2.0	0.00	743	1.0	0.00
377	2.0	0.00	843	1.0	0.00
772	2.0	0.25	289	4.0	0.25
804	4.0	0.16	200	4.0	0.00
882	2.0	0.00	669	1.0	0.00
992	4.0	0.25	891	4.0	0.15
210	2.0	0.25	228	4.0	0.15
202	2.0	0.00			

Figure A.1. Map of floor layout in mock retail deli (located at Virginia Tech Food Science and Technology department, Blacksburg, VA). Numbers (1, 2, 3 etc) represent 3'by 3' blocks of floor space. Sign (+/-) indicates direction away from drain.

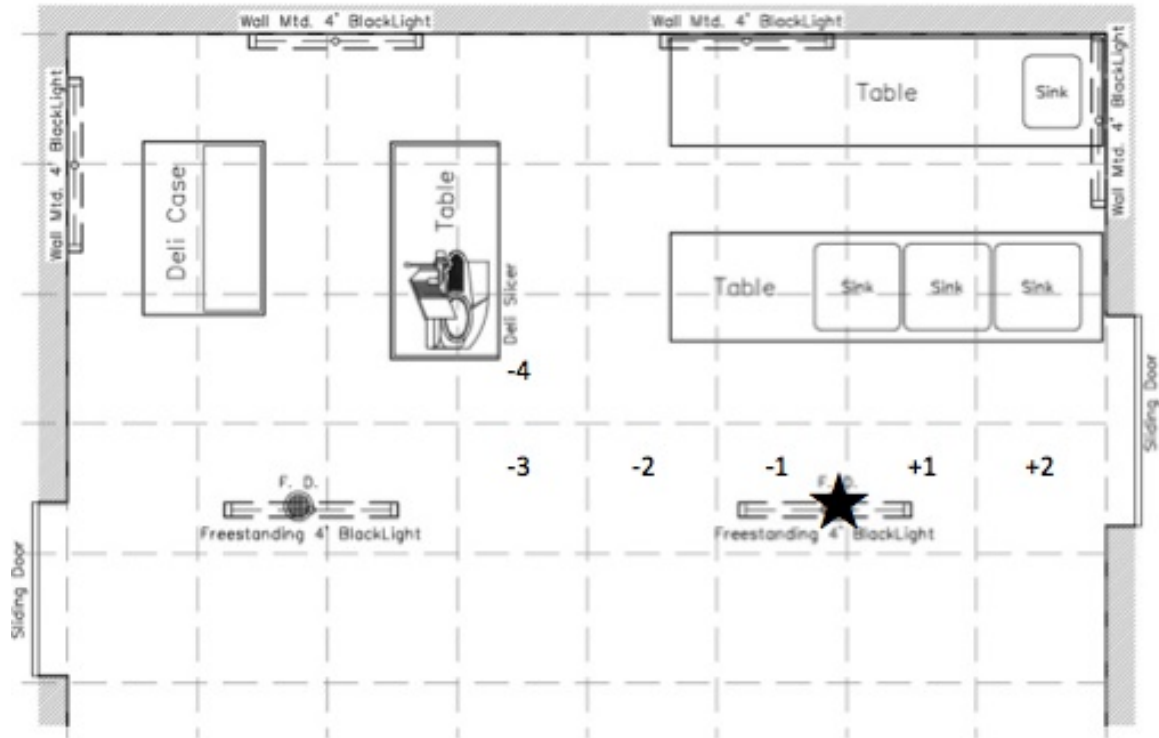


Figure A.2. Photograph of two different concentrations (511- 90%, 308-70%) of GloGerm™ gel inoculated onto a gloved hand. This photograph is an example of photographs used to train the sensory panel during the preliminary training session I.

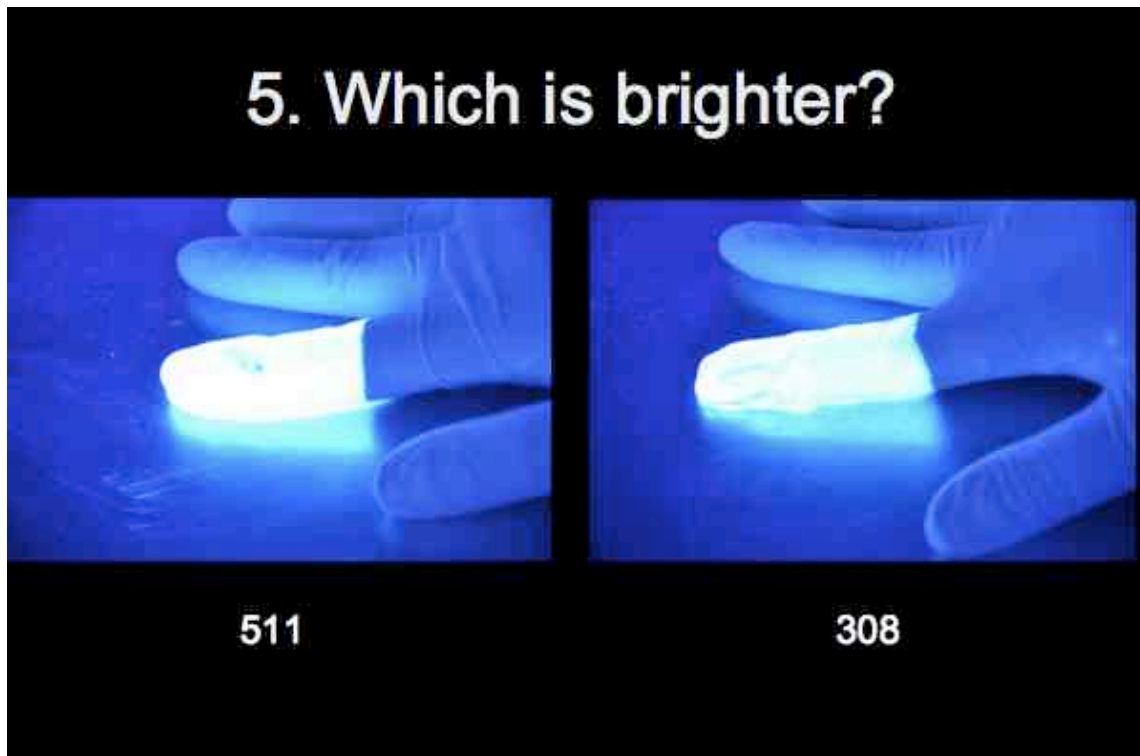


Figure A.3. Photograph of four different concentrations of GloGerm™ gel inoculated onto a turkey slice. This photograph is an example of photographs used to train the sensory panel during the preliminary training session I. Correct order 814, 868, 434, 744).

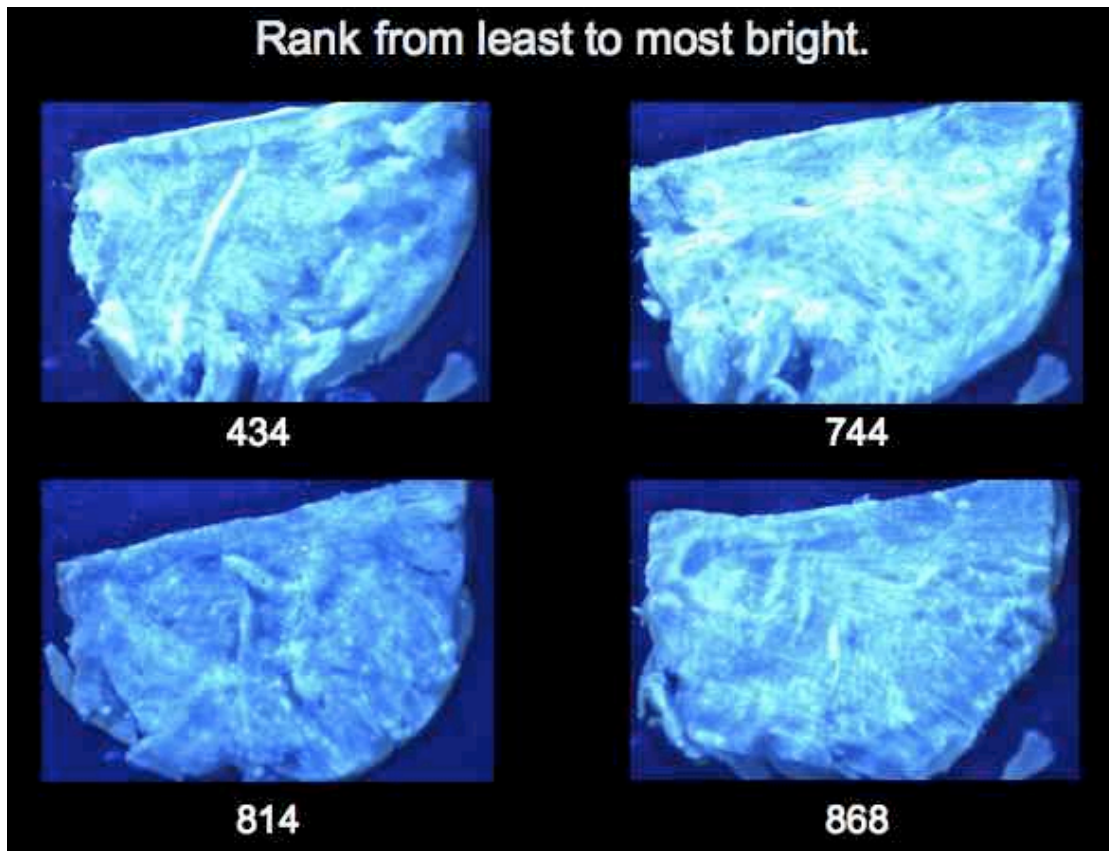


Figure A.4. The template (5 X 4) used for inoculation before slicing a turkey chub attached to the slicer blade. This template was used to insure consistency of application of the GloGerm™ gel onto the slicer blade.

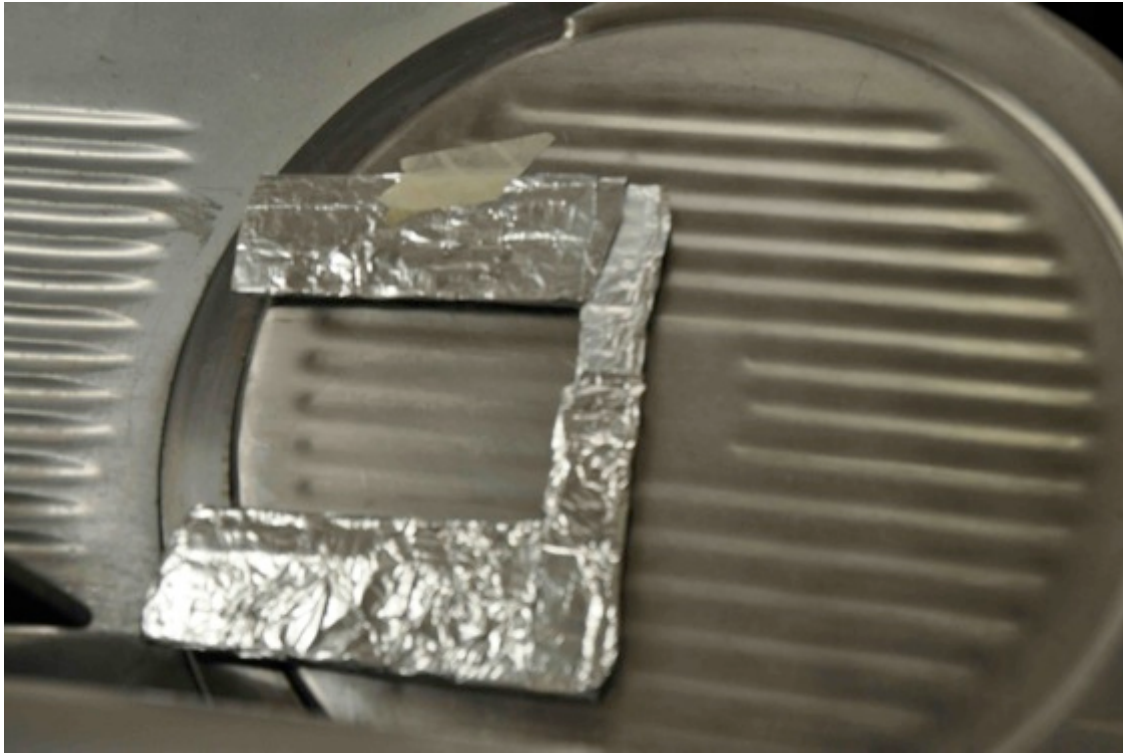


Figure A.5. Photographs of the inoculated meat slicer blade before slicing evaluated by the sensory panel during validation of the preliminary training. Correct Order (0%-100%GloGerm); 353, 381, 769, 920, 634.

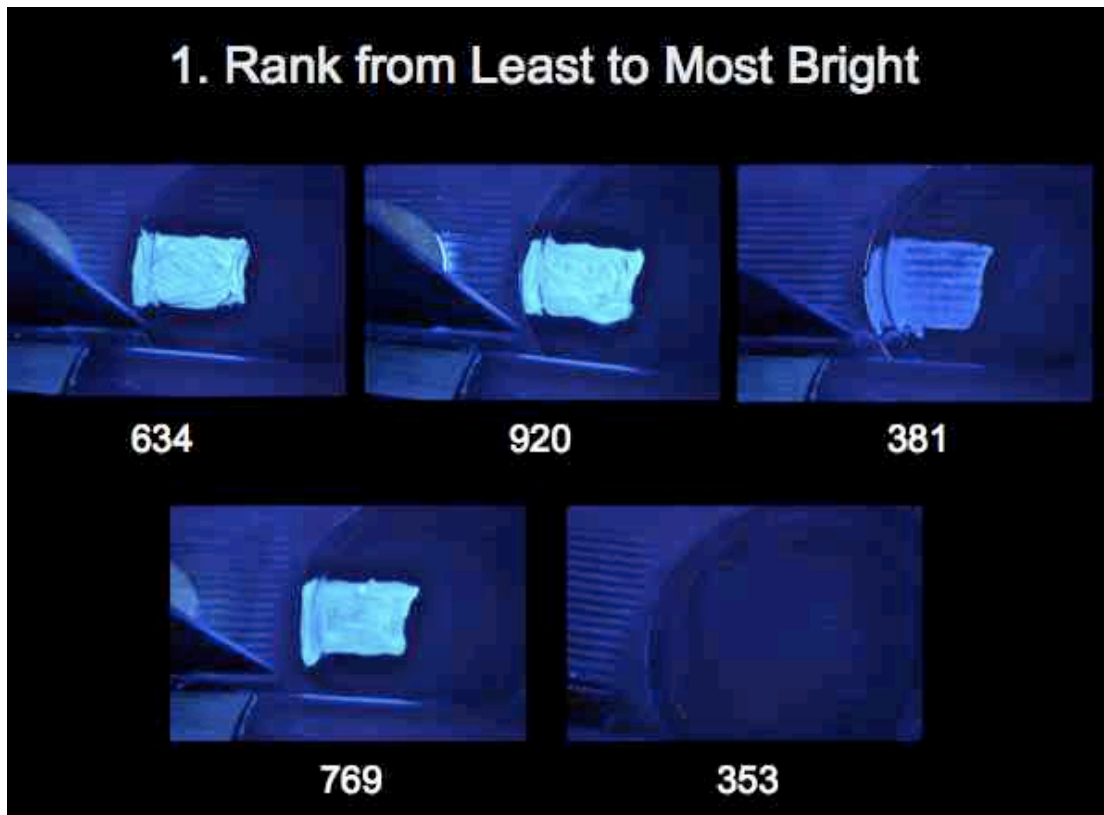


Figure A.6. Images of the meat slicer blade after slicing salami evaluated by the sensory panel during validation of the preliminary training.
Correct Order (0%-100%GloGerm); 823, 341, 955, 749, 652.

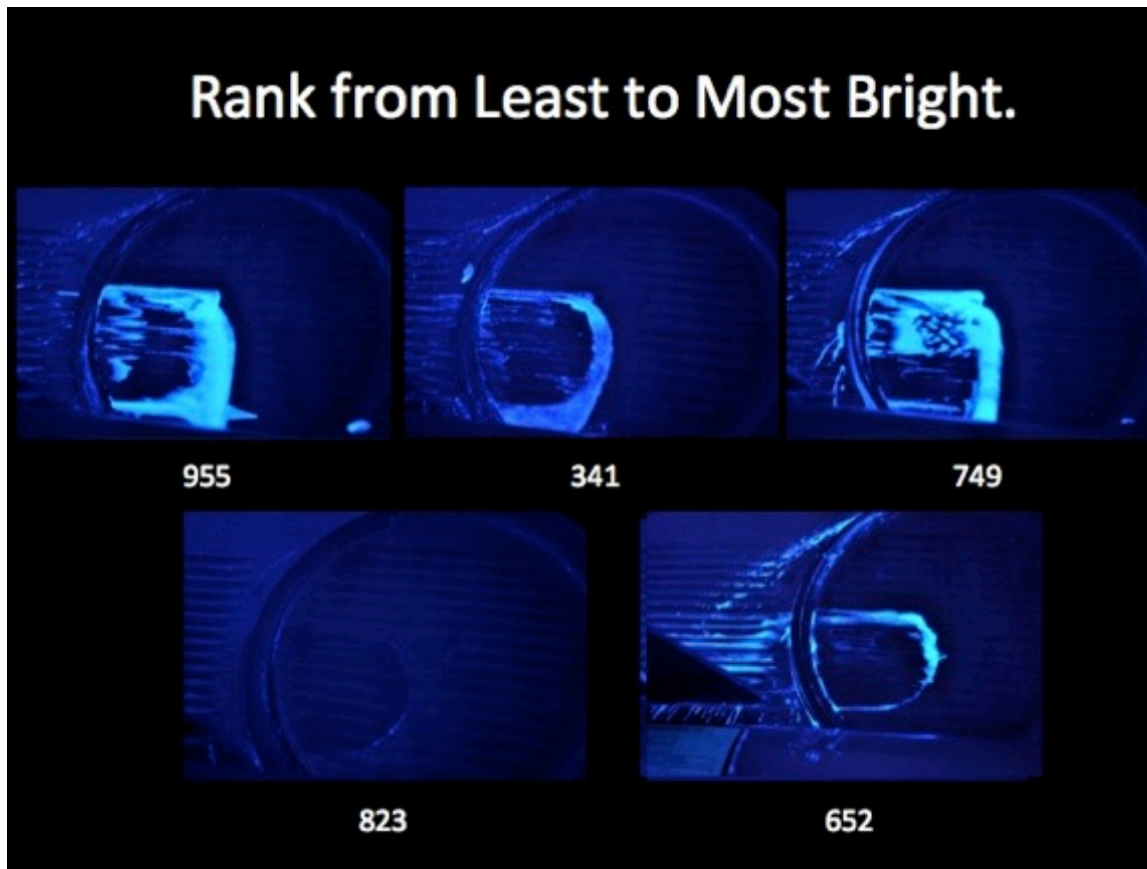


Figure A.7. Images of the inoculated meat slicer blade after slicing turkey evaluated by the sensory panel during validation of the preliminary training. Correct Order (0%-100%GloGerm); 209, 119, 852, 938, 245.

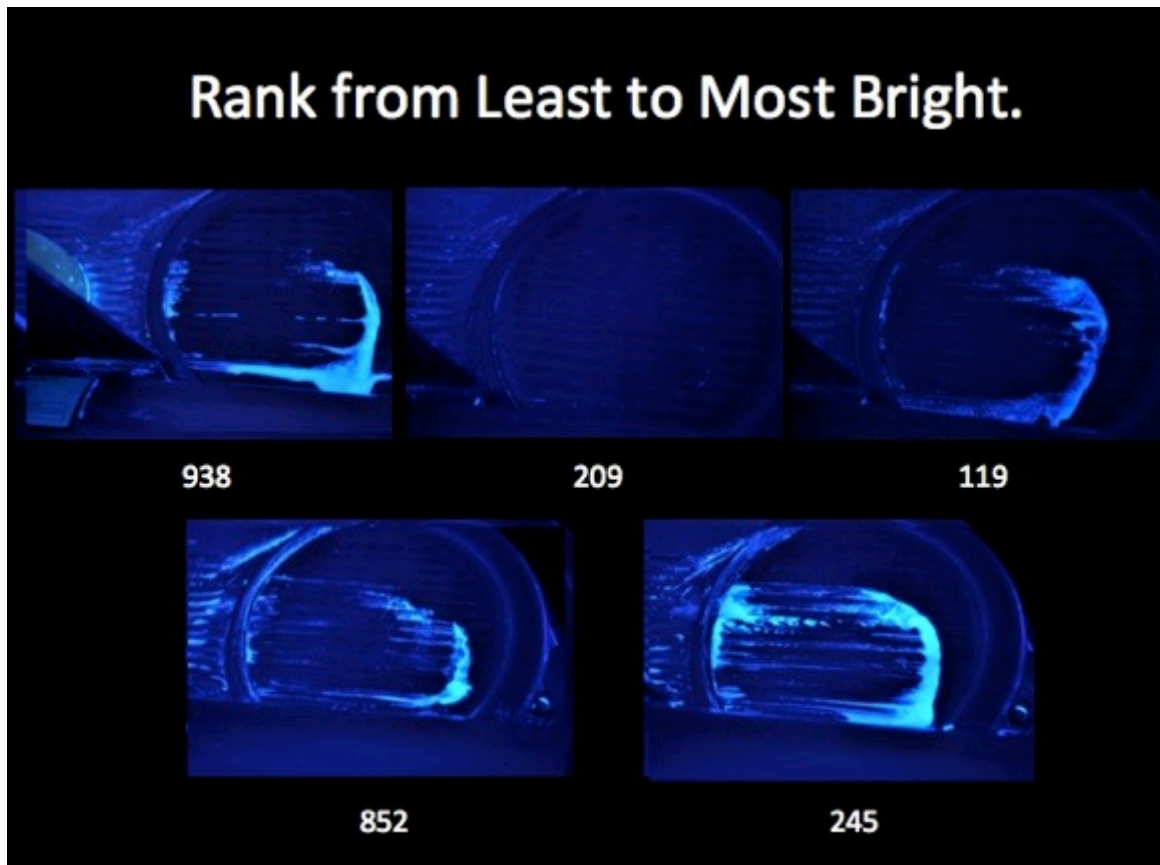


Figure A.8. Images of the first salami slice evaluated by the sensory panel during validation of the preliminary training.

Correct Order (0%-100%GloGerm); 953, 596, 578, 499, 937.



Figure A.9. Images of the gloves used during slicing evaluated by the sensory panel during validation of the preliminary training.
Correct Order (0%-100%GloGerm); 714, 653, 429, 128, 655.



Figure A.10. Series of standard deli operations performed (in this order) by researcher for each experimental trial replication. Adapted from Lubran et al (18).

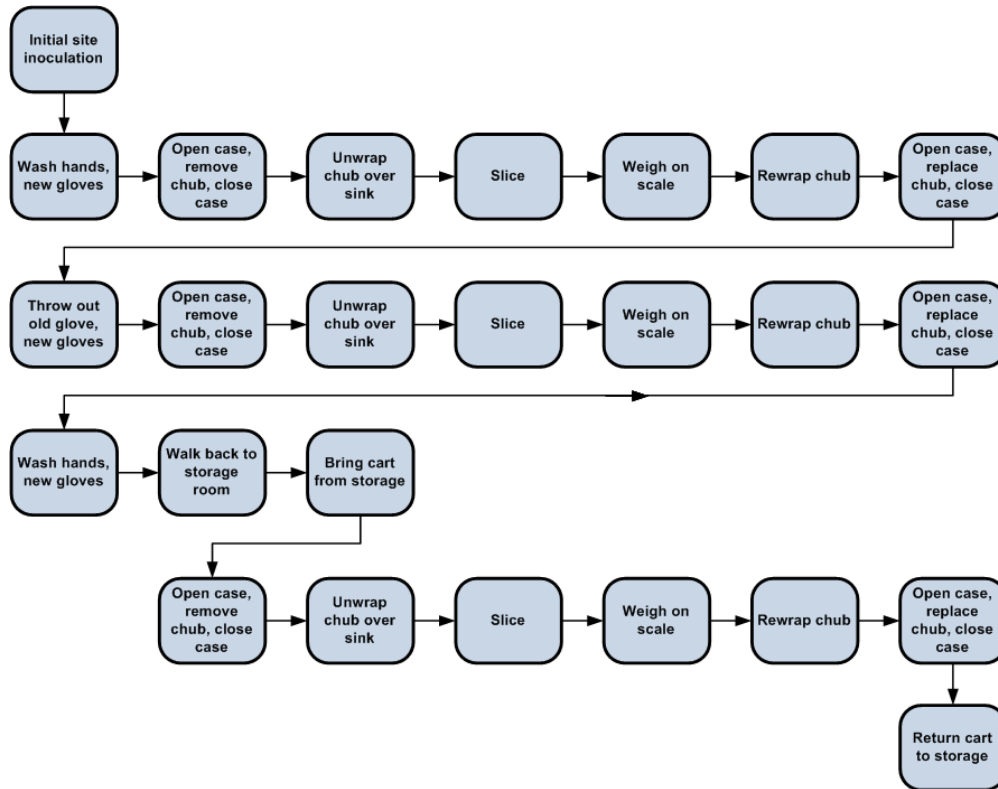


Figure A.11. A comparison of the GloGerm™ contamination spread from the contaminated slicer onto salami (A) and turkey (B). Each photograph shows the first slice from five initial (100, 75, 50, 25 and 0% GloGerm™) slicer contaminations. Starting l-r at top; 100% to 0% GloGerm™.

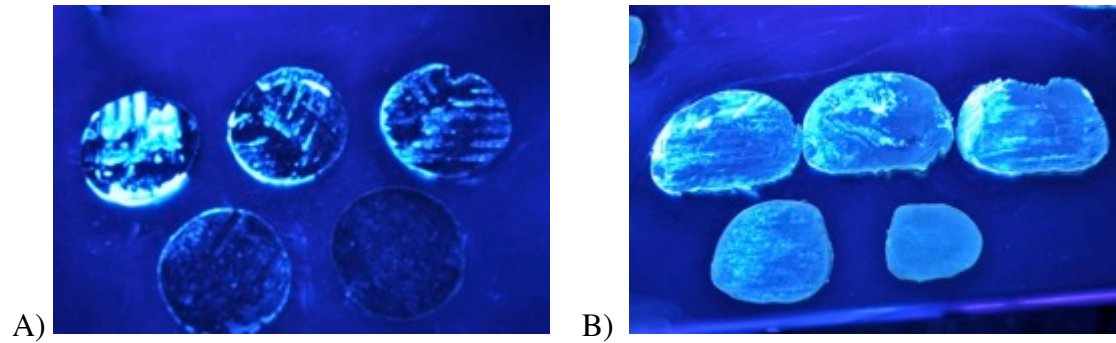


Figure A.12. Salami slices used by panelists in final training, sessions I, and II as a reference for rating other contaminated surfaces . (1: 0% GloGerm™; 2: 25% GloGerm™; 3: 50% GloGerm™; 4: 75% GloGerm™; 5:100% GloGerm™).

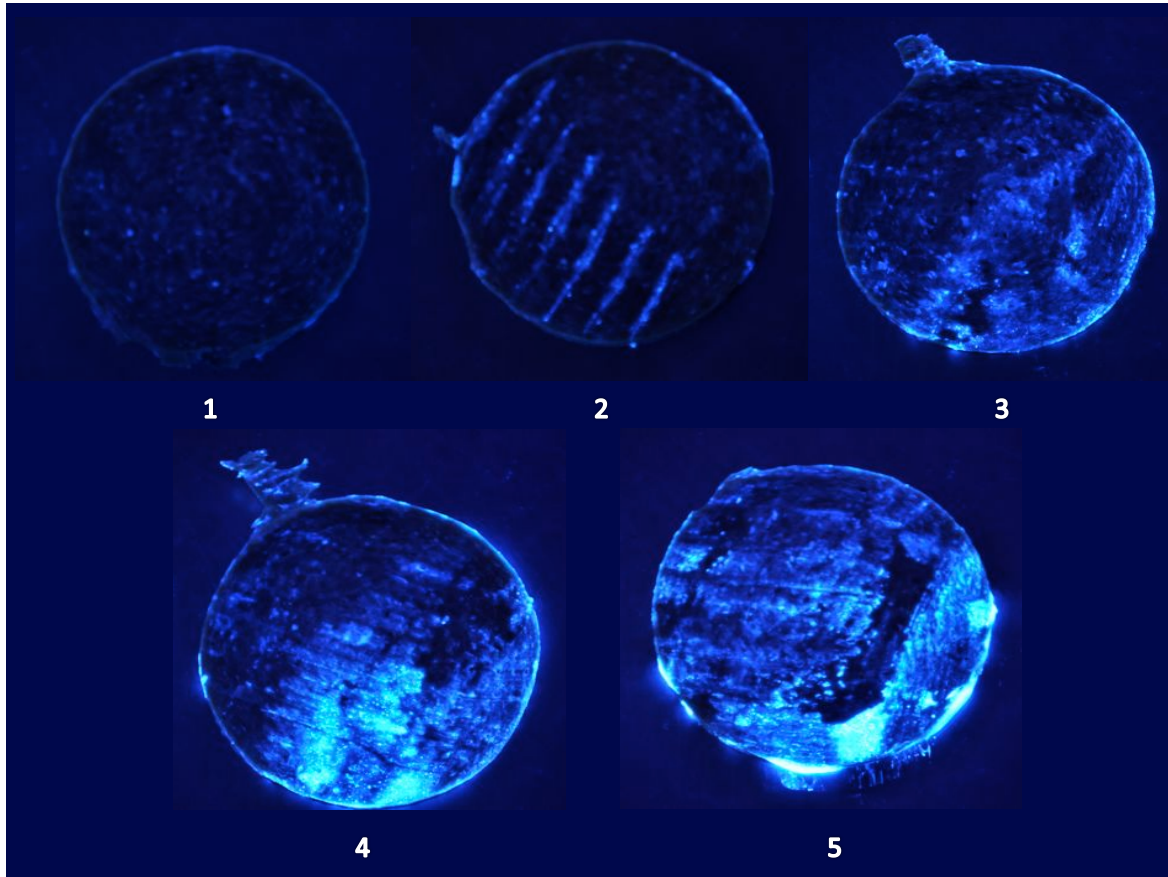


Figure A.13. Image of the floor, -1 location away from the floor drain taken after contamination spread from floor drain. Rated by panelists in Part II: session II. (2 panelists rated 3; 5 panelists rated 4; 1 panelist rated 5)

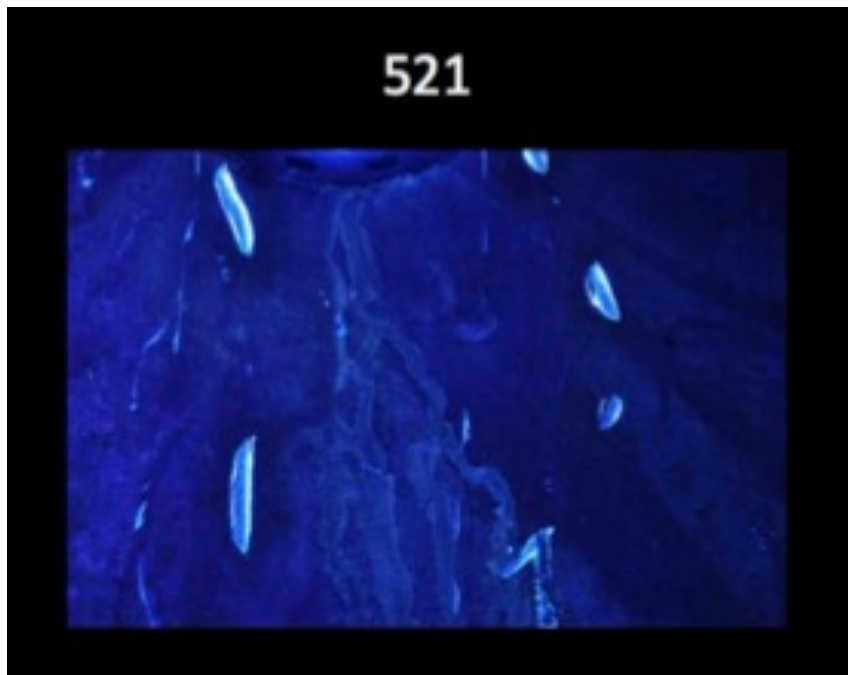


Figure A.14. Image of the floor, +2 location away from the floor drain taken after contamination spread from floor drain. Rated by panelists in Part II: session II. (1 panelist rated 2; 4 panelists rated 3; 3 panelists rated 4)

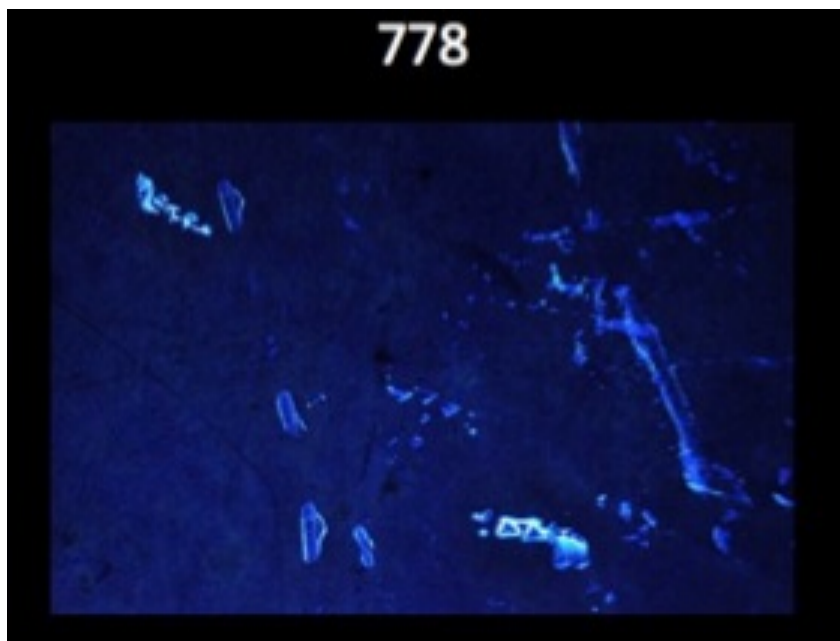


Figure A.15. Varying contamination spread to the slicer blade when meat chub was the starting contaminate site and rated by the panelists in final training session III.

- A. 6 panelists rated it slight, 2 panelists rated it moderate
- B. 8 panelists rated it heavy
- C. 8 panelists rated it heavy
- D. 6 panelists rated it moderate, 2 panelists rated it heavy.

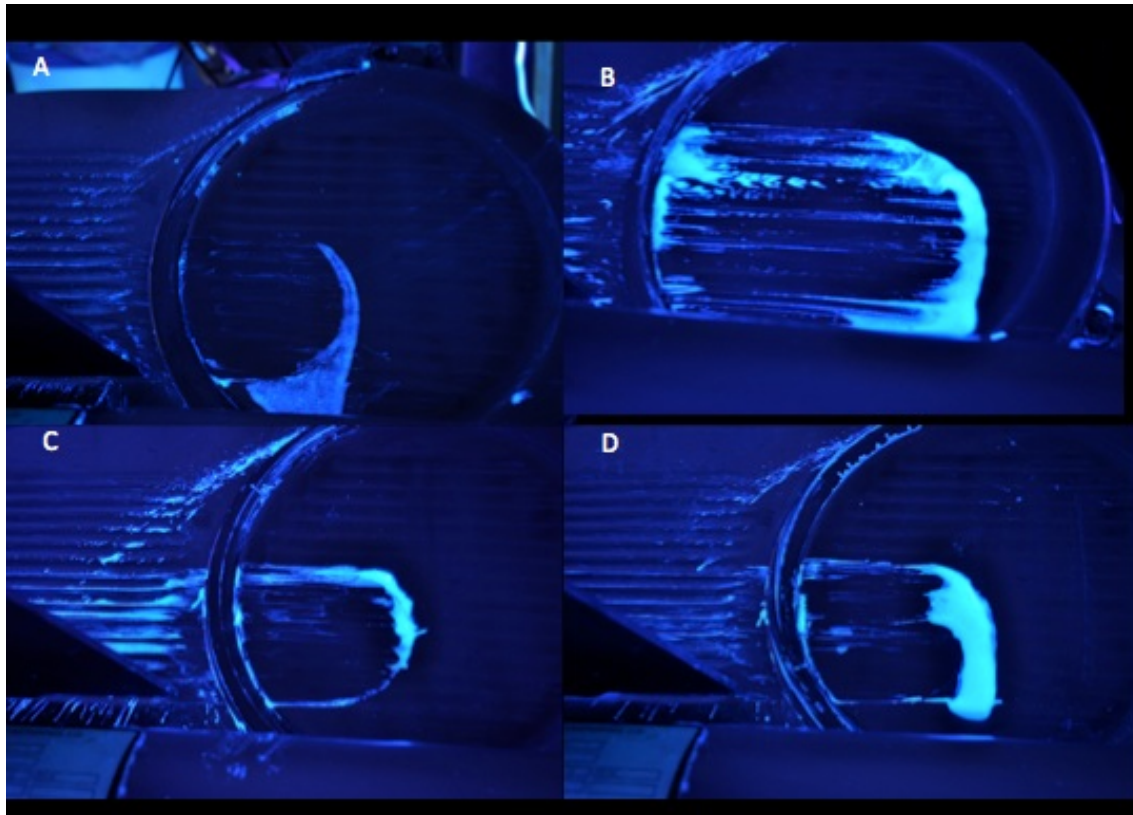


Figure A.16. Example of a meat slice control photo (A; clean) and meat slice experimental photo (B; 203) used in the validation of the final training.

