EXPERIMENTAL AND MATHEMATICAL MODELS FOR *LISTERIA MONOCYTOGENES* TRANSFER BETWEEN DELICATESSEN MEATS AND CONTACT SURFACES

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Biosystems Engineering – Master of Science

2013

ABSTRACT

EXPERIMENTAL AND MATHEMATICAL MODELS FOR *LISTERIA MONOCYTOGENES* TRANSFER BETWEEN DELICATESSEN MEATS AND CONTACT SURFACES

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Currently, there is limited ability to follow *Listeria monocytogenes* through food-service environments, and there remain knowledge gaps concerning the underlying mechanisms that govern surface-to-surface transfer processes. Therefore, the objectives were to: (1) develop a method to quantify the spread of a physical surrogate, GloGerm™ powder (GGP), between delicatessen meats and contact surfaces, and mathematically relate that to *L. monocytogenes* transfer under equivalent conditions; and (2) aggregate existing transfer data and compare candidate models across different transfer scenarios to elucidate phenomenological differences attributable to contact surface or product type. GGP and *L. monocytogenes* inoculated pieces (8 x 8 cm), of turkey, ham, stainless steel, or high-density polyethylene were placed in contact with an uninoculated surface of an opposite material (i.e., meat vs. equipment surface) for 12 sequential transfers. The GGP curves (photographed and quantitatively analyzed via image processing) were fit to the corresponding *L. monocytogenes* data by a linear adjustment, noting that parameters for recipient surfaces did not differ significantly ($P > 0.05$). Linear, Weibull, and two-phase linear- Weibull models were fitted to transfer curves encompassing the major ready-to-eat meat products, equipment surfaces, and contact events. The most likely models were determined using the Akaike Information Criterion. For slicing data, model choice was product dependent, but static contact data yielded the same model regardless of the meat type or surface, indicating fundamental differences among transfer responses.

To my parents for their continuous support and never letting me give up

To my brothers for teaching me to be strong and to rise above all challenges

ACKNOWLEDGEMENTS

This work would not have been possible without Dr. Bradley Marks, my major advisor and mentor. I am truly appreciative of the time, advice, and support he graciously offered me regarding this project and overwhelming personal decisions. He kept me optimistic and gave me direction. His patience taught me patience. He pressed me to improve my problem solving and critical thinking skills. I recognize him for my professional and individual growth during my time at Michigan State.

I also want to give a special thanks to my committee members: Dr. Elliot Ryser and Dr. James Steffe. Dr. Ryser welcomed me into his lab with open arms and ensured that I had access to all necessary materials and equipment. His expertise in microbiology, past work, and unique suggestions, were invaluable throughout my project. His editing skills were also much appreciated. Dr. Steffe was the first professor I had during my graduate studies. His friendly and welcoming disposition helped me transition into my new home. I learned so much from both his courses and him as an individual. He is truly an educator. I am grateful for his door always being open.

I want to recognize Dr. Sanghyup Jeong for his time researching and programing algorithms as well as working with me to continuously tweak them. I also want to give a special thanks to Dr. Dan Guyer for sharing his expertise in imagery, and Dr. Lei Zhang for teaching me how to work in a microbiology lab especially with *Listeria*. Thank you to Dr. Ryser's lab team for allowing me to use your space and answering my endless questions and Dr. Marks' research team and graduate colleagues for their continuous support.

I can never thank the wonderful friends I have made here enough for sharing smiles, laughter, tears, and frustrations with me. You have consistently been there supporting for everything. You girls are the sisters I never had, and I am so grateful that I met and shared this time with each one of you.

And finally, my family, I would never have been able to do this without your love and support. You made me believe that I could be anything I wanted to be and accomplish anything I put my heart into. You made me strong when I was at my weakest, and would never let me quit.

This material is based upon the work supported by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, under Award No. 2010-51110-21004 and the United States Department of Agriculture, National Institute for Food and Agriculture, Agriculture and Food Research Initiative, Grant #2012-67017-3018.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

1.1 Background/problem statement

Listeria monocytogenes is a pathogen that originates in soil and water and is often found in various food products, which raises considerable public safety concern. Vegetables can become contaminated because of their contact with soil or improperly composted manure (CDC 2010). Animals can also carry *Listeria*, leading to contaminated raw meats and dairy products. Once the meat is cooked or milk is pasteurized, these products are essentially *Listeria*-free, but can be recontaminated at the manufacturing or food service level (CDC 2010).

Ingestion of *Listeria* contaminated food can lead to an illness called listeriosis in consumers. Pregnant women, newborns, persons with weakened immune systems, and the elderly are most susceptible to listeriosis (CDC 2010). Outbreaks and listeriosis cases have declined over the last two decades, with a 36% decrease from 1996 to 2006; however, there are still an estimated 1,600 illnesses and 290 deaths annually from listeriosis in the United States (CDC 2013).

Listeria monocytogenes is the number one target in ready-to-eat (RTE) meat and poultry products due to its ubiquitous presence. A risk ranking last completed in 2003, indicated that deli meats have the highest risk for illness and cases on a per serving basis, relative to other food categories, causing more than half the reported listeriosis cases each year (FSIS 2010a). In risk modeling, pathways of exposure are used as inputs including the retail, growth, consumption, and response stages (FSIS 2010a). Risk models predict the likelihood of illness or death resulting from *Listeria* contaminated deli meats based on industry data and consumer surveys. The risk assessment evaluating illness from manufacturer-packaged versus delicatessen-sliced meats

determined that 83% of cases resulted from deli meat sliced at the retail level (FSIS 2010a). Studies are still needed to assess how contamination occurs at the retail level, in order to create effective solutions to reduce the outbreaks of listeriosis.

An outbreak not only impacts public health, but also has large effects on the economy. Economic research has estimated that total sales of RTE deli meats each year in the United States are ~$25.2 billion, with 24.4% from pre-packaged meat and the remaining 75.6% sliced at retail establishments (Gombas and others 2003). From these two sources, about 0.4% and 2.7% of the product, respectively, contains *Listeria monocytogenes* (FSIS 2010b; Gombas and others 2003). If these products are recalled, the economic loss would be hundreds of millions of dollars.

Currently, predictive modeling tools and microbial-risk assessments are being used as guides to decisions relating to *Listeria monocytogenes* prevention and control. However, little is known about cross-contamination routes in food facilities leading to contaminated products (Ivanek and others 2004). Food processing and food service equipment and contact surfaces are recognized harbors for pathogens, which increase the risk of transfer to RTE foods and the resulting potential for foodborne illness. Very few tools and studies are available to accurately and quantitatively evaluate cross-contamination; therefore, several assumptions are being used to fill gaps, making it difficult to validate risk models.

1.2 Goals and objectives

With the importance of food safety, and the knowledge of problems related to cross-contamination of RTE deli products, the research presented in this thesis was conducted to

improve tools to quantify and describe *Listeria* transfer during handling and slicing of RTE meat products. The objectives were to:

1. Develop a method to quantify the spread of a physical surrogate, GloGerm™ powder (GGP), between delicatessen meats and contact surfaces.
2. Mathematically relate GGP to *Listeria monocytogenes* transfer under equivalent conditions.
3. Compare multiple models across different transfer scenarios, to elucidate phenomenological differences attributable to contact or product type.

LITERATURE REVIEW

2.1 *Listeria monocytogenes* and food safety

Listeria monocytogenes is a Gram-positive, non-spore forming, rod-shaped bacterium (Swaminathan and others 2007). This pathogen is of particular concern because of its ability to attach to surfaces and persist in low temperature environments. It is also resistant to acidic and low moisture conditions. The capability of this pathogen to survive in harsh conditions, and its potentially low infectious dose has led to RTE food safety concerns (Lunden and others 2002).

2.1.1 Listeriosis outbreaks and recalls

Numerous outbreaks and recalls have put *L. monocytogenes* in the headlines. For example, in 2007, there was a recall of ready-to-eat chicken products due to the presence of *Listeria monocytogenes* (FSIS 2010b). This confirms that sanitary procedures were not being practiced in this particular establishment. In 2000, ~17 million pounds of country ham, turkey, and chicken were recalled in Texas; over 900,000 pounds of wieners and deli meats were recalled in Tennessee, 19,000 pounds in New York, and 13,500 pounds of roast beef recalled in Utah (FSIS 2010b). In 2010, several ready-to-eat turkey breasts and smoked ham were recalled in Pennsylvania and Minnesota (FSIS 2010b). The continuing outbreaks and recalls over recent years support the need for preventative procedures to be established and enforced.

2.1.2 Regulatory agency guidelines

In 2003, the Food Safety and Inspection Service (FSIS) amended regulations to include *Listeria* as a hazard to address through HACCP plans. New regulations require that all establishments that produce RTE meat products must prevent any *Listeria monocytogenes* contaminated products or direct contact with *Listeria* contaminated surfaces (zero-tolerance) by adhering to one of three USDA alternatives (FSIS 2009). To prevent outbreaks, FSIS issued guidance to establishments, including resources and recommendations on: (1) validating and applying microbial agents, (2) effective control processes, and (3) sanitary practices (FSIS 2009). These compliance guidelines include information regarding challenge studies, shelf life assessments, and critical factors affecting microbial growth (time, temperature, pH, and concentration), as well as *Listeria* reduction due to different interventions (FSIS 2009). It also includes a *Listeria* sanitation program and procedures that provide information on water exposure challenges, and the importance of personal hygiene, separation of products, temperature, facilities, and equipment (FSIS 2009). FSIS also provided an updated list of microbial agents that are accepted for use in the production of meat and poultry products. Although the FSIS made efforts to regulate, inform, and educate these establishments, *Listeria* continues to persist and affect consumers.

2.2 Obstacles with *Listeria monocytogenes* and RTE products

The present obstacles related to RTE meat consumption are largely due to *L. monocytogenes* causing product recalls, foodborne illness, and challenges with control and

regulatory compliance (Sofos and Geornaras 2010). Over time, pathogens can increase in virulence and become are more resistant to antimicrobials and environmental stresses. They can emerge from cross-contamination from other foods, surfaces, water, and soils. To reduce *Listeria* outbreaks, meat safety must be improved through plant and retail establishment sanitary practices, and education to service workers and consumers (CDC 2010). Safety and training can be significantly improved once bacterial prevalence, adhesion, and transfer characteristics are further understood.

2.2.1 Prevalence and viability

The presence of bacteria on RTE meat and poultry products is likely a result of recontamination after processing. Foods and materials that support pathogen growth should be a focus when studying food safety (Chen and others 2003; Norrung and others 1999). The prevalence of bacteria appears to be dependent on the attachment, food type, manufacturing process, storage, and intrinsic factors. One strain may be capable of adapting and surviving on a specific surface, while others may not (Johnson and others 1990).

It is well known that planktonic bacteria are more susceptible to sanitizers, antimicrobials, and temperature treatment than attached bacteria biolfilms, which tend to be much more resistant (Krysinski and others 1992; Mosteller and Bishop 1993; An and Friedman 1996; Gray and others 1984). For example, *Staphylococcus epidermidis* secretes slime after adhering to implant surfaces making it more resistant to the body's defense system as well as antibiotics (Gristina and others 1987; Gristina and others 1989; Naylor and others 1990; Pascual

and others 1993; Sheth and others 1985). Attached biofilms of *L. monocytogenes* and *Salmonella* Typhimurium showed high tolerance when treated with trisodium phosphate, while their planktonic cells showed much lower resistance (Somers and others 1994). In addition to sanitizer resistance, studies have found that attached cells of *Listeria* (Oh and Marshall 1995) and *Salmonella* Enteritidis (Dhir and Dodd 1995) are less susceptible to heat.

Foods characteristics affect the growth and survival of bacteria. Higher *L. monocytogenes* populations have been documented in ready-to-eat products with high pH, water activity, and low nitrite concentration; however, such populations are seldom found in fermented meats (Grau and Vanderlinde 1992; Wang and Muriana 1994). This is supported by Lin and others (2006), who found that *L. monocytogenes* can survive and grow better on oven roasted turkey than on fermented salami or bologna, because of the lower moisture content and pH compared to turkey. High salt concentration also slows growth (De Reu and others 2002; Vorst and others 2006a). However, products that do not support the growth of pathogens can still serve as a means for transmission (Vorst and others 2006a).

Environmental factors also affect the viability and growth of a pathogen. Elevated temperatures and increased storage time often favor proliferation (Perez-Rodriguez and others 2008; Norrung and others 1999). However, *Listeria* has the ability to proliferate during cold storage (Lin and others 2006). In one study, the number of *L. monocytogenes* positive samples increased during storage of turkey, but, decreased during storage of salami and bologna(Lin and others 2006).

Low relative humidities limit growth and injure cells (Jackson and others 1993). The effect of dryness on viability differs between Gram-negative and Gram-positive bacteria. Gram-positive bacteria, such as *L. monocytogenes*, have a thicker peptidoglycan layer that enhances resistance to drying (Takahashi and others 2010). *L. monocytogenes* populations have been found to be the highest when on wet surfaces, including sponges, dish clothes, and faucets (Chen and others 2001); however, bacteria were found to survive for hours or days on dried surfaces (Pether and Gilbert 1971; Scott and Bloomfield 1990; Kusumaningrum and others 2003). Survival of *L. monocytogenes* on stainless steel with food residues was greater than without food residues (Takahashi and others 2010). The pre-slicing of meat also has shown greater populations compared to unsliced chubs during refrigerated storage (Elson and others 2004; Gillespie and others 2000).

Bacteria can be injured due to environmental factors, such as low relative humidity and cold temperatures, but mechanical slicing may also cause injury. Mechanical shear during slicing has been determined to injure *L. monocytogenes* due to the edge of the blade having greater friction and therefore increasing stress (Sheen and others 2010). The number of dead cells was found to increase with slicing. During slicing, most microbes were found on the receiving surface under the slicer blade (dead or alive). Although the mechanism was not elucidated, mechanical shear was reported to have a significant impact on bacterial death during slicing (Sheen and others 2010; Hoffman and Wiedmann 2001).

2.2.2 Adhesion

Use of additional mechanical equipment increases food production but also increases instances where food can come into contact with surfaces (McEldowney and Fletcher 1988). If these surfaces are not sanitized properly (with approved microbial agents) and regularly, bacterial attachment can occur, which can lead to cross-contamination during processing and handling. Reducing bacterial attachment on equipment has been a major concern due to the risk of foodborne illnesses that can occur when bacteria come into contact with food (Gibbs and others 1978; Adams and Mead 1983; Franco and others 1995; Smith and Fratamico 1995).

It has been noted for several decades that bacteria prefer to grow on a nutrient rich surface-liquid interface rather than moving freely in nutrient poor liquids (Costerton 1995; Zobell 1943). Adhesion is described as a two-phase process in which the first phase is physical, thus making it instantaneous and reversible, while the second phase is molecular and cellular, which is time-dependent and irreversible (Marshall and others 1971). In addition to modes of cellular motility, freely moving planktonic bacteria can move by physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, surface electrostatic charge, ionic forces, chemical bonds, and hydrophobic interactions (Dankert and others 1986; Krekeler and others 1989; van Oss 2003; McEldowney and Fletcher 1988; Arnold and others 1993; Ong and others 1999). Once these interactions occur, initial attachment has been completed and phase two can begin, in which bridging between the bacteria and surface occurs by bacterial structures, such as capsules, fimbriae, or pili and slime (An and Friedman 1998). The anchored bacteria produce exopolysaccharides to further anchor the bacteria and create a

favorable environment to allow more growth, attachment, and potential biofilm formation (Mafu and others 1990a).

Adhesion of bacteria to a surface is a complex process that depends on several factors including bacterial species, the environment, bacteria and material surface characteristics, and intrinsic physicochemical factors. Having a deeper understanding of each of these factors can assist in finding solutions to reduce product contamination, in many food service industries (Zottola 1994; Wirtanen and others 1996).

2.2.2.1 Environmental factors

Several environmental factors affect bacterial adhesion, including temperature, time of exposure, pH, bacteria concentration, nutrient availability, shear force, fat, proteins, and chemicals. For example, in one study, the number of bacteria adhering to a surface increased as time increased until a point of saturation was reached (Satou and others 1988). *Listeria* can attach to equipment surfaces and survive for extended periods, confirmed by positive meat and surface samples at the end of slicing (Hoffman and Wiedmann 2001). Higher inoculum levels of *E. coli* O157:H7 show stronger attachment to leafy greens and beef tissue, and *L. monocytogenes* has a strong attachment to glass when inoculum levels are high, due to nutrient availability and surface hydrophobicity. (Takeuchi and Frank 2000; Dickson and Daniels 1991; Fratamico and others 1996; Arnold and Silvers 2000). Attachment to surfaces has been found to be dependent on nutrient availability - the more available the nutrient, the greater the attachment strength (Takahashi and others 2010). High concentrations of electrolytes also increase attachment, while

the presence of protein can often inhibit adhesion (Abbott and others 1983). These factors all affect the adhesion of bacteria to surfaces by changing interactions that occur that allow adhesion, or, by changing bacterial or material characteristics.

2.2.2.2 Bacterial physicochemical factors

Physicochemical factors influencing adhesion are unique to each type and strain of bacteria. Two of the main factors are hydrophobicity and surface charge, which are both affected by growth medium, bacteria age, and bacteria surface structure (Krekeler and others 1989; Dankert and others 1986). It is well established that hydrophobic bacteria adhere more favorably to hydrophobic surfaces, and hydrophilic bacteria adhere better to hydrophilic surfaces (Hogt and others 1983; Satou and others 1988). Also, hydrophobic bacteria generally adhere to surfaces more strongly than hydrophilic bacteria (Vanloosdrecht and others 1987). This concept is supported by a study that found that *S. epidermidis* and *S. aureus* adhere better to polymers and metals, respectively (Barth and others 1989).

Listeria monocytogenes expresses low surface hydrophobicity and is classified as hydrophilic, but this is only apparent at low pH and high ionic strength (Mafu and others 1991). Often, a chemical treatment can change hydrophobicity and therefore attachment strength of bacteria (Hogt and others 1983; Westergren and Olsson 1983). The presence of proteins also can affect adhesion by changing bacteria surface physiochemistry (Reynolds and Wong 1983). When in solution, bacterial surface charge is negative (Hogt and others 1982) thus attracting ions of opposite charge (An and Friedman 1998). It has been determined that hydrophilic bacteria have a

high surface charge, while the surface charge is usually low for hydrophobic bacteria (Hogt and others 1982). However, adhesion of bacteria to surfaces could not always be attributed to the relative surface charge of the bacteria (Abbott and others 1983).

2.2.2.3 Material surface characteristics

The structure, chemical composition, physicochemistry, and electronic properties of material surfaces control reactivity that influences bacterial attachment. In a study by Arnold and Silvers (2000), susceptibility of many common equipment surfaces in a poultry processing plant to bacterial attachment and growth was tested. Results showed that, depending on the surface, the affinity for bacteria differed (Arnold and Silvers 2000). It was determined that a picker finger (made of rubber) inhibited bacterial attachment, while stainless steel, polyethylene, and conveyor belting had increased populations over time, stainless steel having the most attachment (Arnold and Silvers 2000). The study also discussed the effect of surface elemental composition, such as sulfur and zinc, two commonly known antimicrobials that are found in rubber and polyethylene (Arnold and Silvers 2000). This supports the picker finger results, but does not explain the susceptibility of polyethylene to bacterial contamination.

Chemical composition of surfaces affects the initial attraction forces as well as the strength and mechanism of adhesion between bacteria and surface materials. Several studies have shown that when a material surface is treated with microbial coating, attachment decreases (Bridgett and others 1992; Farber and Wolff 1992; Speier and Malek 1982). Coating surfaces

with protein alters surface hydrophobicity, also affecting attachment (Fletcher and Marshall 1982).

The hydrophobicity of surfaces that come into contact with bacteria has a large influence on attraction and strength of attachment. Metals are hydrophilic, have a negative charge, and a high surface energy, while polymers are hydrophobic, have a lower charge, and low surface energy (An and Friedman 1998). These low charged hydrophobic surfaces are however more susceptible to adhesion than hydrophilic materials, which resist adhesion (Hogt and others 1983; Ludwicka and others 1984). (Mafu and others 1990a) tested *L. monocytogenes* attachment ability on low energy surfaces of polypropylene and rubber and found no correlation, but in another study found *L. monocytogenes* to attach to both low and high energy surfaces after a short contact time. Another study came to the conclusion that large numbers of bacteria attached to hydrophobic plastics, a moderate number attached to hydrophilic surfaces with positive or neutral charge, and few to hydrophilic negatively charged surfaces (Fletcher and Loeb 1979). This result agrees with the idea that hydrophobic surfaces are more susceptible to contamination. Additionally, *L. monocytogenes* is more resistant to sanitizers when attached to hydrophobic polypropylene and rubber than when attached to hydrophilic stainless steel and glass, due to biofilm formation once multiple colonies are strongly attached (Mafu and others 1990b). This is in agreement with Fletcher and Leob's findings that greater numbers, and thus larger biofilms, are supported by hydrophobic materials, making the bacteria more resistant to chemical treatment (Fletcher and Loeb 1979).

Surface roughness and physical configuration also are contributing factors to adhesion after bacteria come in contact with a material surface. A medical implant study found that the

ultra-smooth surface Vivathane supported minimal bacterial attachment throughout the duration of the study, while other tested surfaces with irregularities promoted adhesion, biofilm formation, and accumulation (McAllister and others 1993). When surfaces of glass and polystyrene were roughened with a grindstone and placed on a river bottom, increased rates of colonization were also found (Baker and Greenham 1988). Rough surfaces increased effective surface area for attachment to occur, and surface depressions are favorable growth sites for bacteria (Baker and Greenham 1988; Arnold and Bailey 2000; Rodriguez and McLandsborough 2007). However, An and others (1995) found that roughening commercially pure titanium with sandpaper did not affect attachment of *S. epidermidis*. Sandpaper was also to treat stainless steel surfaces in a study by Arnold and Bailey (2000), who found that sanding, sandblasting, and electro-polishing smoothed the surface, thus increasing resistance to bacterial contamination, while the same finishes tested by Hilbert and others (2003) showed no differences in *Listeria* attachment.

Additionally, porous and grooved surfaces support greater bacterial growth and attachment (Merritt and others 1979; Locci and others 1981; Vorst and others 2006b). For example, braided medical sutures have increased attachment compared to non-braided sutures (Sugarman and Musher 1981). With non-absorbable materials, the physical configuration contributes more to bacteria attachment than does surface finish (An and Friedman 1998). Stainless steel grade (304/316) also has an effect on attachment due to the difference in pore number and size (Vorst and others 2006b). These findings agree with the idea that irregularities on material surfaces promote adhesion.

2.2.3 Transfer and cross-contamination

Multiple food-to-surface contacts during industrial processing of food are inevitable. Other sources of contact in the retail environment include workers, customers, gloves, utensils, slicing equipment, other food products, counter tops, and cutting boards. Often, many of these surfaces can be overlooked or poorly sanitized, leading to the potential for cross-contamination. Knowledge of bacterial sources, transmission routes, and rates (even at low levels) can help in finding effective methods for control in food service and retail environments.

2.2.3.1 Inoculum size influence

The number of bacteria on a surface may influence the number of bacteria transferred and the amount of time bacteria remain on the surface. Some authors determined that higher inoculation levels result in lower transfer rates, and lower inoculation levels result in higher transfer rates (Montville and Schaffner 2003), where "rates" are defined as the fraction of bacteria transferred from a donor to a recipient surface during a contact event. However, the number of bacteria transferred may remain constant as inoculum levels increase (Montville and Schaffner 2003). Some authors, however, have reported more *L. monocytogenes* positive deli meat slices when inoculum size on the blade was larger (Lin and others 2006; Rodriguez and others 2007b, Vorst and other 2006a). However, the efficiency of transfer, as calculated by Rodriguez and others (2007b), was not affected by initial inoculation size. It has also been noted that the extent of contamination depended on bacteria levels, as well as the product being sliced

(Lin and others 2006). Because of these contradicting results, more research must be conducted to further understand whether inoculum size does in fact affect transfer rates of bacteria.

2.2.3.2 Physicochemical factors

Meat composition, including fat, moisture, preservatives, pH, and nitrite concentration, affects the number of bacteria transferred, as well as growth and survival. There was greater *L. monocytogenes* cross-contamination when processed meats had a high pH, high water activity, and low nitrite concentration (Wang and Muriana 1994; Grau and Vanderlinde 1992). This is supported by the finding that there is more bacterial transfer from an inoculated blade to turkey with high moisture, no nitrite, and high pH than to bologna and salami with lower pH and moisture (Lin and others 2006). A film of fat was left on the slicer blade after cutting salami, while a clear-liquid film was left after cutting turkey (Lin and others 2006). Therefore, although salami is less supportive of *L. monocytogenes* growth, its fat transfer to the slicer may prolong cross-contamination (Vorst and others 2006a).

2.2.3.3 Material characteristics

Transfer rates are also affected by hydrophobicity, surface roughness, porosity, texture, finish, and hydration level of the surface material. One study found that although stainless steel has weaker attachment strength to bacteria compared to polyvinylchloride and polyurethane, stainless steel had a lower transfer rate (Midelet and Carpentier 2002). However, greater

numbers of *L. monocytogenes* were transferred from bologna to stainless steel than from bologna to polyethylene (Rodriguez and others 2007b). These two findings suggest that transfer between two surfaces depends on which surface contains the pathogen. This is supported by Harrison and others (2003), who reported that transfer from contaminated hands to a paper towel dispenser was lower than from a contaminated dispenser to hands. Additionally, the strength of hydrophobic interactions affects transfer, and conditioning of surfaces can alter hydrophobicity and thus attachment strength and transfer ability (Midelet and Carpentier 2002). Chen and others, assessed the transfer between many common surfaces in a kitchen and found that the transfer rates from highest to lowest were: chicken to hand, cutting board to lettuce, spigot to hand, hand to lettuce, , and hand to spigot (Chen and others 2001). Although both inoculation level and material affect transfer, Rodriguez and others (2007b) did not detect interactions between inoculation level and material type.

The effects of surface configuration and roughness on transfer are topics that have shown conflicting results across studies. In one instance, surface roughness and finish on stainless steel were reported to have no effect on *L. monocytogenes* transfer (Rodriguez and others 2007b); however, several other studies determined that finishes had an impact on transfer (Arnold and Silvers 2000; Whitehead and others 2006). These differing results could be due to the direction and amount of pressure applied when simulating the transfer event. In a study of wood and plastic cutting boards and their ability to transfer *Salmonella*, Cliver (2006) reported that results depended on the experimental method, varying from: plastic having more transfer, wood having more transfer, and both having the same amount of transfer. Variation also likely was due to the condition of the cutting boards.

Material hydration or wetness of a surface and relative humidity are factors that may need consideration in transfer studies. However, Rodriguez and others (2007a) reported that transfer to a wetted surface did not differ from that of a dried surface. A study comparing transfer of wet attached and dry attached cells of *L. monocytogenes* on stainless steel to bologna also found no difference in transfer (Montville and Schaffner 2003). When decreasing relative humidity of *Listeria* biofilms on stainless steel, transfer was shown to increase to bologna and hard salami (Rodriguez and others 2007a). Transfer was however greater to bologna, probably due to physicochemical properties of the meat. Rodriguez and McLandsborough (2007) suggested that adhesion forces between cells weaken during drying; therefore, increasing their chances for transfer upon contact.

2.2.3.4 Transfer prevention

Transfer prevention techniques, such as the use of gloves or hand washing, must be done appropriately to prevent contamination adequately. When working with raw meat and continuing onto to RTE products, high transfer is observed when gloves are not changed (Perez-Rodriguez and others 2008). It was determined that transfer from chicken to hands is reduced by using gloves, but not prevented (Montville and Schaffner 2003). Transfer from contaminated hands in gloves to food has been observed as well (Montville and Schaffner 2003).

In many transfer studies, high variability is reported between runs and often depends on the volunteer and hand contacts. Chen and others (2001) reported greater variability in cross-contamination rates and in levels of contamination when hands were included in a transfer study,

with the greatest variability in post-washed hands to surface. Montville and Schaffner (2003) also found large variability in results when studying transfer from hand to lettuce through a glove. In a model by Ivanek and others (2004), it was found that contamination within a slicing area is influenced mainly by contacts that employees' gloves make with the food and the duration the glove is worn.

2.2.3.5 Modeling transfer

Quantitatively modeling *L. monocytogenes* transfer can be useful in creating microbial risk-assessments for improved food safety. To date, most such models are empirical, created simply by plotting variables and fitting that data with an arbitrary function (Sheen 2008; Sheen and Hwang 2010; Aarnisalo and others 2007). Many also use the transfer-ratio based model, TR(%)/100×N colony-forming units (CFU), to determine the number of bacteria transferred (Perez-Rodriguez and others 2008). A simple model based on a contaminated source, intermediate source, and food that contacts the intermediate source has been established by (den Aantrekker and others 2003). The transfer ratio can easily be determined for each step. The problem with this model is that transfer rates vary greatly due to multiple factors that affect bacterial transmission as well as experimental differences and recovery method used (Perez-Rodriguez and others 2008). Because of this, probability distributions should be created to account for variability in data. Often, transfer rates are log transformed to create a normally distributed bell-shaped curve (Chen and others 2001; An and Friedman 1998). Hoelzer and others (2012) analyzed a broad set of *Listeria* transfer data by creating empirical coefficient-

based mathematical models and determining the shape of the distribution of transfer coefficients, finding that transfer coefficients varied considerably and after a $\log_{10}$ transformation were often best described by normal distributions.

Studies that use transfer ratios assume independence between cross-contamination events, allowing for transfer events to be multiplied to obtain a final number of bacteria once a cross-contamination process is complete (Perez-Rodriguez and others 2008). However, high inoculation levels are used for transfer studies to allow for detection, which can affect transfer rates leading to less accurate results, especially because in reality, when inoculum levels are very low, fewer transfer events may occur (Vorst and others 2006a). Another assumption in modeling is the state of bacteria being used in the transfer events. Stress, biofilms, and survival ability due to these can greatly affect transfer and thus should be considered when modeling (Perez-Rodriguez and others 2008, Keskinen et al, 2008).

Transfer ratio simulations, which are used to quantitatively describe microbial transfer, have been created that consider data gathered from other studies. Schaffner (2004) used a Monte-Carlo simulation to model *Listeria* cross-contamination in food-processing plants by tracking sub-types and prevalence. The model was based on prevalence of *L. monocytogenes* entering the plant on raw products, which contaminate the environment at a specific rate and then become a part of the environmental reservoir (Schaffner 2004). The strains that persist continue to be transferred to a contact surface, become part of the reservoir and, if persistent, continue to the finished product (Schaffner 2004). Strains with low numbers, but ability to persist, are dominant in the final product; however, different strains can have greater persistence in different areas of

the plant (Schaffner 2004). A beta-distribution was used to represent prevalence uncertainty in a population based on the number of positive samples (Schaffner 2004).

Growth models are also necessary to determine whether low numbers of pathogens can proliferate to levels unsafe for consumption. Ivanek and others (2004) quantified transfer and proliferation of *L. monocytogenes* through a fish processing plant by simulation. A compartmental model was used that included food, gloves, surfaces, and the environment as reservoirs. A difference-equation system created from the Reed-Frost model to simulate transfer was based on assumptions and several parameters, which included durations, rates, frequencies, contaminated units, and prevalence. The simulation showed that prevalence increased over a work shift and that the confidence intervals became wider over time as a result of uncertainties in the system; however, it did not model loss of contamination. Therefore, numbers were higher than expected, leading to a conservative estimate. Their simulation also indicated that cross-contamination in a slicing area is greatly influenced by glove contact and the duration of wearing gloves (Ivanek and others 2004). This model, as well as many others, is limited in application due to many assumptions that must be made due to the current lack of sufficient data in this field.

Sheen (2008) empirically modeled *L. monocytogenes* transfer to salami during slicing, with two cases: 1) when the blade was inoculated and 2) when the chub was inoculated and sliced, followed by slicing of an uncontaminated chub. Plots were created using CFU per slice as the dependent variable and slice number as the independent variable (Sheen 2008). When inoculation levels were high (>log CFU/g), exponentially decaying empirical models fit the transfer curves relatively well; however, when the inoculation levels were low, there was a random transfer pattern, but the same empirical models were used (Sheen 2008). Sheen and

Hwang (2010) used similar procedures to model transfer of *E. coli* O157:H7 on ham during slicing. However, they reported that when the blade was inoculated directly, a power law model fit the data best. But, when the inoculated chub was sliced, followed by an uncontaminated chub, the exponential decay model was the best fit (Sheen and Hwang 2010). This showed that for *E.coli* O157:H7 on ham, case 1 results in a higher probability of transfer when the inoculation level is low (< 5 log CFU/g), but case 2 shows longer persistence of transfer (Sheen and Hwang 2010). (Aarnisalo and others 2007) ran similar tests, but also considered slicing temperature and attachment time. Transfer of *L. monocytogenes* from an inoculated blade to uncontaminated gravad salmon and from inoculated gravad salmon showed exponential decay as the slice number increased (Aarnisalo and others 2007). Low inoculation level, colder temperatures, and longer attachment time led to lower total *L. monocytogenes* transfer (Aarnisalo and others 2007).

2.2.3.6 Aggregated transfer data

The number of papers published on bacterial transfer to/from food has increased approximately ten-fold over the past two decades, reflecting increasing attention to this important issue. However, there has been no standardization of methods or aggregation of data in this field. In order to quantitatively evaluate the research trends in this area over the past 40 years, a comprehensive keyword search of the literature was conducted via the ISI Web of Science database, to identify all studies including transfer data for key foodborne pathogens (*Listeria*, *Salmonella*, *Escherichia coli*, and *Campylobacter)*. Published transfer data were characterized in terms of pathogen, product type, surface, and other variables. The total analysis yielded ~55 distinct publications on foodborne pathogen transfer, of which 43 contained

numerical data, with a total of 756 data sets, ~1,194 individual replicate curves, and over 14,456 individual observations quantifying pathogen transfer between food products and contact surfaces, including: meat/poultry (n = 29) and produce (n = 11), with transfer to metals (n = 19), plastics (n = 16), hands/gloves (n =10), and several other surfaces (Table 2.1).

Table 2.1 List and approximate numbers of prior publications including each variable in food pathogen transfer studies (References categorized here are listed in Appendix, Table 6.16).

Variable	**Number of Papers**
Contact/Attachment time	10
Inoculation level	6
Temperature	12
Inoculated surface	7
Wet vs. dry	6
Pressure	6
Weak/strong biofilm	5
Friction	2
Hydrophobicity	4
Roughness	4
Sample size	2
Relative humidity	2
Composition	9

This growing body of literature is almost exclusively empirical, without any theoretical connection to the underlying mechanisms that govern these processes. There is a critical need

for a modeling framework that bridges micro- and macro-scale knowledge in order to move toward a unifying theory of bacterial transfer between foods, processing media, and relevant food contact surfaces. A thorough analysis of existing published transfer data and aggregation of that data as such, is an important first step toward that overall long-term goal.

The papers described above have been organized into a preliminary database defining the characteristics of each study, which will serve as the foundation for a broader, publicly-available database in this domain. A unified database that aggregates pathogen transfer data, and becomes a repository for future data, will help advance linkages between fundamental research and the observed transfer outcomes, while also improving the design of future studies to fill critical data gaps.

2.2.4 Effect of experimental design and recovery methods on quantitative transfer

When performing transfer studies, the experimental design and recovery methods are crucial to the results. Transfer studies will yield different results based on the variables considered during transfer and the methods used. Some researchers use single strain cultures, while others use multi-strain cocktails (Arnold and Silvers 2000). However, bacterial behavior can change due to interspecies competition, thus affecting study results (Okabe and others 1995). Likewise, in prior studies on the effects of surface roughness, some protocols used perpendicular force, while others used shear force, and the amount of force varied as well. The wood and plastic cutting board studies also differed due to the methods of inoculation, preparation, disinfection, and how samples were recovered (Cliver 2006). In another study, a sample of 25 g

and an entire package of sliced turkey were assayed and found that a higher percentage of samples were positive for *L. monocytogenes* when whole packages were analyzed (Lin and others 2006). This result, however, was material specific because the same test was performed on bologna and yielded approximately the same percentage in both cases.

The method of recovery also greatly influences the number of pathogens found on a surface. There are multiple methods used to assess surface contamination; however, there is not an accepted standard (Moore and others 2007). Often, sporadic data collection is documented due to the insensitivity of enumeration methods, which can yield misleading results (Kang and others 2007). Techniques to study bacteria attachment and biofilm formation need to be improved by finding ways to accurately quantify counts on a surface (Arnold and Bailey 2000). A challenge is that characteristics of each bacterium, food surface, and contact surface are different; therefore, each method has a different efficiency depending on the case (Kang and others 2007). Efficiency is also affected by the method and mechanics the researcher uses during sampling (Kang and others 2007). The most common recovery methods are swabbing, rinsing, and direct agar contact. It has been determined by several authors that swabbing does not give an accurate assessment of bacteria population on a surface (Kang and others 2007; Harrison and others 2003; Richard and Piton 1986). Kang found that rinsing had the highest recovery out of the three common methods and introduces the idea of using a sonicating toothbrush, which was found to show the greatest recovery. Recovery is important in quantifying bacteria transfer and the method used must be accurate and suitable to the equipment surface being evaluated so that data can be validated and models created.

2.3 Conclusion

Although there has been significant recent work conducted in the area of bacterial transfer and modeling in food facilities, this field is still in its infancy, and several critical knowledge gaps exist. These topics need to be addressed so that outbreaks can be further understood and prevention measures can be improved. Bacteria attachment and biofilm formation is well understood; however, knowledge concerning how adhesion and other factors affect transfer is lacking. Several studies have tested factors influencing transfer but have found differing results. Various models quantifying transfer have also been created, but nearly all are empirical, and others are simulations that have limitations.

In identifying research priorities related to *L. monocytogenes* and public health, the USDA previously highlighted food safety education and training, filling knowledge gaps, applying control measures, and monitoring the impact on food safety (USDA-NIFSI 2010). Consequently, the overall goal of this work is to understand cross-contamination by: identifying variables and potential phenomenological factors affecting transfer characteristics, as well as to improve the means to evaluate routes of *Listeria* transfer in a food service environment. In doing so, new control measures can be developed, and sources and incidence ultimately can be minimized through improved worker training interventions.

UTILIZING IMAGE ANALYSIS OF A FLUORESCENT PHYSICAL SURROGATE TO QUANTIFY *LISTERIA MONOCYTOGENES* TRANSFER BETWEEN COMMON DELICATESSEN MEATS AND CONTACT SURFACES

3.1 Introduction

Listeria monocytogenes is a ubiquitous pathogen found in various food products, including vegetables, raw meats, and dairy products. Once fully cooked, meat becomes essentially *Listeria*-free, but can be recontaminated during packaging or slicing in manufacturing facilities and retail establishments (CDC 2010). Outbreaks and listeriosis cases have declined over the last two decades; however, there are still an estimated 1,600 illnesses and 290 deaths annually from listeriosis in the United States (CDC 2013). This has raised a considerable public safety concern, leading to regulations created by the U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS). These include the requirement that all establishments that produce ready-to-eat (RTE) meat products must: (1) apply a post-lethality treatment and an anti-microbial agent or process, (2) apply a post-lethality treatment or an anti-microbial agent or process, or (3) follow sanitary practices so that direct contact surfaces are tested *Listeria*-free (FSIS 2009).

A risk ranking, last completed in 2003, indicated that deli meats are the highest risk food category for illness and cases on a per serving basis, being potentially responsible for more than half the reported listeriosis each year (FSIS 2010b). The risk assessment compared illness from RTE meats prepared in processing plants to those in retail establishments and determined that more cases resulted from deli meat sliced at the retail level than from pre-packaged meat (FSIS 2010b). The food industry, together with regulators, are continuously working to reduce the

incidence of *Listeria*, through improved plant and retail establishment sanitary practices, and education of processing workers, service workers, and consumers.

Currently, predictive modeling and microbial risk assessments are used as tools in evaluating food safety policies and practices. However, little is known about cross-contamination routes in food facilities leading to contaminated products (Ivanek and others 2004). This is due to the limited ability to follow specific pathogens through food processing facilities. Multiple factors, such as inoculum level, physicochemical factors, and material characteristics, also make predictive modeling inherently difficult by influencing transfer dynamics (Lin and others 2006; Montville and Schaffner 2003; Chen and others 2001; Midelet and Carpentier 2002; Rodriguez and others 2007a). Researchers who have studied *Listeria* transfer therefore have reported some conflicting results, likely due to variability in these factors across studies (Arnold and Bailey 2000; Rodriguez and others 2007b). A quantitative understanding could provide new insight and further reduce the risk of RTE contaminated products. Thus, mathematical cross-contamination models for *L. monocytogenes* transfer would be valuable tools that would enable assessment of interventions, particularly those related to sanitation and working training practices.

Previous work has assessed *L. monocytogenes* transfer between product and equipment surfaces in controlled settings (Sheen and Hwang 2010; Vorst and others 2006b; Vorst and others 2006a; Sheen 2008). These data, however, are difficult to translate into actual cross-contamination events that occur in food processing and food service environments where there is a more complex network of surfaces, including the contribution of human decisions and contacts. The use of a non-pathogenic surrogate would allow for evaluation of cross-contamination events in a “real-world” setting, as well as rapid assessment of transfer without the risk of foodborne

illness. Physical surrogates have been used previously to qualitatively identify high risk locations on slicers (Vorst and others 2006a) and leafy green processing lines (Buchholz and others 2012); however, a quantitative evaluation of a surrogate would be valuable in validating environmental cross-contamination models and quantifying transfer risk in facilities where RTE foods are handled/served.

In identifying research priorities related to *L. monocytogenes* and public health, the USDA previously highlighted food safety education and training, filling knowledge gaps, applying control measures, and monitoring the impact on food safety (USDA-NIFSI 2010). In order to create a useful and novel tool for studies in this area, the specific objective of this study was to develop and validate an image-based analysis method for a physical surrogate (Glo Germ™ Powder, GGP) to simulate the movement and population changes of *L. monocytogenes* to and from RTE delicatessen meats and common food contact surfaces.

3.2 Materials and Methods

GGP (Glo Germ™, Inc., Moab, UT) was chosen as a physical surrogate to simulate *L. monocytogenes* cross-contamination in a food service environment due to its small particle size (5 µm, compared to 1-2 µm for *L. monocytogenes*), fluorescence under ultraviolet lighting, and the potential to rapidly quantify the amounts of GGP transferred among surfaces. The materials used in this study are commonly found in delicatessens: stainless steel representing countertops and slicer surfaces, and high-density polyethylene representing typical cutting board materials. These materials also can exhibit high levels of bacterial attachment and growth over time

(Arnold and Silvers 2000). Delicatessen ham and turkey were chosen based on the amount of prior data available as well as their popularity with consumers. Each surface and meat served as a donor and recipient during separate tests.

3.2.1 *Listeria monocytogenes* preparation

Strain selection and preparation closely followed those reported by Vorst (Vorst and others 2006a). A six-strain cocktail of *L. monocytogenes* was used for surface transfer and cross-contamination studies. The strains were previously obtained from Dr. Catherine W. Donnelly (University of Vermont, Burlington). *L. monocytogenes* CWD 205 (source unknown), CWD 578 (dairy plant environment), CWD 701 (cheese), CWD 730 (dairy plant environment), CWD 845 (dairy plant environment), and CWD 1002 (pork sausage) were chosen based on their ability to form biofilms in a microtiter plate assay (Keskinen and others 2008). Strong (CWD 730 and CWD 845), medium (CWD 701 and CWD 1002), and weak (CWD 205 and CWD 578) biofilm formers were selected to consider all possible biofilm scenarios that may affect attachment and transfer. All stains were kept at –80°C in trypticase soy broth (TSB; Difco, Becton Dickinson) with 10% (vol/vol) glycerol. A loopful of each frozen stock culture was inoculated in separate tubes of TSB containing 0.6% (wt/vol) yeast extract (TSB-YE; Difco, Becton Dickinson, Sparks, Md.) and incubated for 24 h at 37°C. After 24 h a loopful of each TSB-YE culture was transferred to new tubes of TSB-YE and incubated overnight at 37° C. The optical density at 600 nm was taken for each suspension to initially verify cell concentration, and equal volumes of each culture were mixed to create a six-strain cocktail containing ~10^9 CFU/ml. Cell

concentration was also quantified by plating an appropriate dilution on modified Oxford agar (MOX; Difco, Becton Dickinson), followed by incubation at 37°C for 48 h.

3.2.2 Physical surrogate preparation

A 1:10 w/v suspension of the physical surrogate GloGerm polymer powder (GGP) was prepared by adding 1 g of GloGerm to 10 ml of 70% ethanol, followed by vortexing for 1 min.

3.2.3 Delicatessen meat

A chub each of restructured roasted turkey breast and ham were purchased from a local food store (Gordon Food Service, Okemos, Mich.). The chubs were held at 4°C and used within 20 days of purchase. The package label listed the turkey ingredients as turkey breast, turkey broth, and less than 2% of salt, dextrose, and sodium phosphates; the ham ingredients included fully cooked ham, water, salt, sodium phosphate, sodium erythorbate, and sodium nitrite.

3.2.4 Stainless steel and high density polyethylene surfaces

A series of electropolished grade 304 stainless steel (8 x 8 x 0.2 cm) and HDPE (8 x 8 x 1 cm) coupons were fabricated by the Michigan State University Department of Biosystems and Agricultural Engineering Shop (East Lansing, MI) and used for transfer.

3.2.5 Stainless steel and high density polyethylene inoculation

For the tests where stainless steel or HDPE was the donor surface, the *L. monocytogenes* inoculum (300 μl) was spread evenly over the surface of one of the coupons with an inoculating needle, so as to yield ~10^8 CFU/cm^2 (subsequently verified). A duplicate coupon of the same

material was inoculated with 300 μl of the concentrated GGP suspension. The inoculated coupons were held at ambient temperature in a laminar flow cabinet for approximately 30 min until visibly dry.

3.2.6 Turkey and ham inoculations

For tests where the meat products were the donor surfaces, two turkey or ham pieces, ~1 cm thick, were aseptically sliced from the chub. The meat was cut into squares measuring 8 by 8 cm using a flame-sterilized knife blade. The *L. monocytogenes* inoculum (300 μl) was spread evenly over the surface of one of the meat surfaces with an inoculating needle, so as to yield ~10^8 CFU/cm^2 (subsequently verified). A second meat sample was surface inoculated with 300 μl of the GGP suspension. The inoculated deli meat slice cutouts were refrigerated at 7°C for 15 min prior to the transfer tests.

3.2.7 Transfer of *L. monocytogenes* from turkey/ham piece to stainless steel/ high-density polyethylene coupons

At room temperature (~27ºC), uninoculated coupons (n = 12) were placed side-by-side, and the inoculated meat sample (50-55 g) was placed, inoculated side down, on the first coupon. Another coupon (36 g) was placed on top of the meat sample, with a 280 g weight on top of this second coupon. This was done to add an equal and consistent normal contact force of ~5.8 g/cm^2 to each coupon. After 3 s of contact, the meat sample, coupon, and 280 g weight were removed and placed on top of a second uninoculated coupon. This process was repeated for a total of 12 consecutive coupons. Each recipient coupon was put into a plastic Whirl-Pack® bag (Nasco

Sterile Whirl-Pack® Sampling Bags) with 25 ml of Phosphate Buffered Saline (PBS). The bags were sonicated in a sonicating water bath (Model FS 20, 40 kHz sonic cleaner, Fisher Scientific, Pittsburgh, Penn) for 10 min at 30°C. The liquid was plated (100 µl) on MOX, and incubated at 37°C for 48 h. All *Listeria* colonies were counted to determine CFU per coupon. Each test series was conducted in triplicate.

3.2.8 Transfer of *L. monocytogenes* from stainless steel/ high-density polyethylene coupons to turkey/ham piece

At room temperature (~27ºC), uninoculated 8 x 8 x 1 cm meat samples (n = 12) were placed side-by-side, and a *Listeria*-inoculated coupon (36 g for SS and 81 g for HDPE) was placed, inoculated side down, on the first recipient meat sample. A 280 g weight was placed on top of the test coupon, to give a normal force of ~5 g/cm^2 or 5.6 g/cm^2 onto the sample. After 3 s of contact, the coupon and 280 g weight were removed from the first meat recipient surface and placed on top of a second uninoculated meat sample. This process was repeated for a total of 12 consecutive contacts. Each recipient sample was put into a plastic Whirl-Pack® Filter bag (Nasco Sterile Whirl-Pack® Filter Sampling Bags) with 25 ml of PBS. The bags were stomached for 1 min (Seward Stomacher 400, Worthing, West Sussex, England), and the liquid was then spiral plated (100 µl) on MOX, which was incubated at 37°C for 48 h. Each test series was conducted in triplicate. Populations were determined as the CFU per meat sample.

3.2.9 Cleaning and disinfecting coupons

Between tests, coupons were sanitized, rinsed with deionized water, and dried with a composite tissue. The coupons then were autoclaved, followed by washing with detergent and drying before use.

3.2.10 Transfer of GGP from turkey/ham piece to stainless steel/ high-density polyethylene coupons

At room temperature (~27ºC), uninoculated test coupons (n = 12) were placed side-by-side, and a GGP-inoculated meat sample was placed on the first recipient coupon. A 36 g coupon and 280 g weight were placed on top of the meat sample for a normal force of ~5.8 g/cm^2. After 3 s of contact, the meat sample, coupon, and 280 g weight were removed from the first recipient surface and placed on top of a second uninoculated surface. This process was repeated for a total of 12 consecutive contacts. These studies also were conducted in triplicate. All recipient coupons were photographed individually using a digital SLR camera positioned 31 cm above and normal to the sample surface, and set to manual settings (Nikon-D3100 14.2-Megapixel Camera,18-55 mm f/4-5.6 VR Image Stabilization Lens; shutter speed = 1/5 s, aperture = f-stop 5.6, ISO = 400). The only light source was a long-wave (365 nm) ultraviolet lamp (UVP Inc, Model XX-15, 15 W bulb) positioned 25 cm to the side and 10º above the sample surface plane being imaged. Settings were determined by trial for the most uniform and brightest results in preliminary tests.

3.2.11 Transfer of GGP from stainless steel/ high-density polyethylene coupons to turkey/ham piece

At room temperature (~27ºC), uninoculated meat samples (n = 12) were placed side-by-side, and a GGP-inoculated coupon was placed on the first sample with a 280 g weight on top of the coupon for a normal force of ~5 g/cm^2 or 5.6 g/cm^2. After 3 s of contact, the coupon and 280 g weight were removed and placed on top of a second uninoculated meat sample. This process was repeated for a total of 12 consecutive samples. Each recipient sample was photographed individually, as described above.

3.2.12 Processing images to determine amount of GGP on surfaces

Image processing tools in MATLAB® (v7.6, The MathWorks, Natick, MA) were used to quantify the amount of GGP (ppm) on each surface. The amount of GGP transfer was quantified by an algorithm that determined specific thresholds for each meat type or coupon material (changing the image to binary) to filter out background noise, followed by multiplying the binary image pixel values by the original image pixel values and summing the pixel intensity in the sample area. Based on preliminary testing, this method yielded the most accurate pixel count and a useful sum of the intensity values that included only those pixels encompassing the GGP visible in the image.

The images first were read and imported into the program and cropped to the size of the sample surface. To correct for background noise in the images, red, blue, and green components were evaluated. The red component was used to identify the GGP area, the blue component was

used to distinguish the background noise, and the green component was used to determine the concentration of GGP on the surface.

```
Rimg=I_crop(:,:,1);

Gimg=I_crop(:,:,2);

Bimg=I_crop(:,:,3);
```

The blue component in the background was chosen to best distinguish the background from the GGP, and therefore was converted into its binary form, using the following algorithm.

```
Bimg_bw=Bimg;

for i=1:(dx+1)

   for j=1:(dy+1)

      if (Bimg(i,j) == 255), Bimg_bw(i,j)=1; else Bimg_bw(i,j)=0;
```

The area of background illumination, sum of the pixels in the red component image, and threshold to identify the effective green image were then determined.

```
B_area=sum(sum(Bimg_bw));

R_sum=sum(sum(Rimg));

R_thresh=R_sum/B_area;
```

By using the red component to identify the area containing GGP, the green component could be isolated from the background surface. Doing so changed the image into its binary form.

```
Gimg_bkg=Gimg;

for i=1:(dx+1)

   for j=1:(dy+1)

      if (Rimg(i,j)>= R_thresh), Gimg_bkg(i,j)=0;
```

By blurring the green component image, as detailed below, the background noise was removed without compromising the GGP affected area.

```
se=strel('disk',3); %Create morphological structuring element

h=fspecial('average',3); % Create predefined 2-D filter

[row, col]=size(Gimg_bkg);

sImg=imresize(Gimg_bkg, 0.4); % image reduction 1=full

bkg=imopen(sImg,se);

blurred=imfilter(bkg,h,'replicate'); % blurring background

blurred=imresize(blurred, [row,col]); % back to original image size

nGimg=Gimg-blurred; % background corrected image
```

This binary green component was then multiplied by the original image intensity values in order

to yield an intensity value that functioned as a good representation of the amount and intensity of fluorescent area in the original image.

G_sum=sum(sum(nGimg));

I=G_sum;

The GGP intensity was related to the concentration by creating calibration curves for each recipient surface. This was done by spreading 7 known serially diluted GGP concentrations ranging from 49 to 100,000 ppm of GGP on the four surfaces (stainless steel, HDPE, ham, and turkey) and taking images as previously described. The computed intensities were plotted against concentration. By visual observation of the data, and preliminary tests, a second-order calibration equation yielded sufficiently good results for this application.

3.2.13 Statistical analysis and model development

The sequential *Listeria* transfer results (mean values, LM_{obs}, log CFU) and GGP transfer results (log ppm) were related via a linear adjustment by offsets and multipliers. The offsets and multipliers were determined by linear regression as follows:

$$LM_{pred} = GGP \times Multiplier + Offset \quad (3.1)$$

$$SSE = \sum_{i=1}^{n}(LM_{pred_i} - LM_{obs_i})^2 \quad (3.2)$$

$$RMSE = \sqrt{\frac{SSE}{n}} \; ; n = 12 \quad (3.3)$$

3.3 Results and Discussion

3.3.1 Sequential transfer of *L. monocytogenes* inoculated surfaces to their counterparts

Sequential transfer of *L. monocytogenes* from an inoculated surface containing ~10^8 CFU/cm^2 to uninoculated surfaces followed an approximately logarithmic decline in all cases (Figure 3.1). According to the student t-test, there were no significant differences ($P<0.05$) between the average recovery for the turkey and stainless steel surface transfer pair; however, other pairs are significantly different from one-another. There were, based on an analysis of covariance (ANCOVA), significant differences ($P<0.05$) between the average slopes of *L. monocytogenes* recovery from the four surfaces: stainless steel, HDPE, ham, and turkey; suggesting that knowledge of surfaces involved in a transfer event is critical to determining amount of transfer. The figure shows the mean of triplicate experiments, with all standard errors of replication $\leq$ 0.59 log CFU.

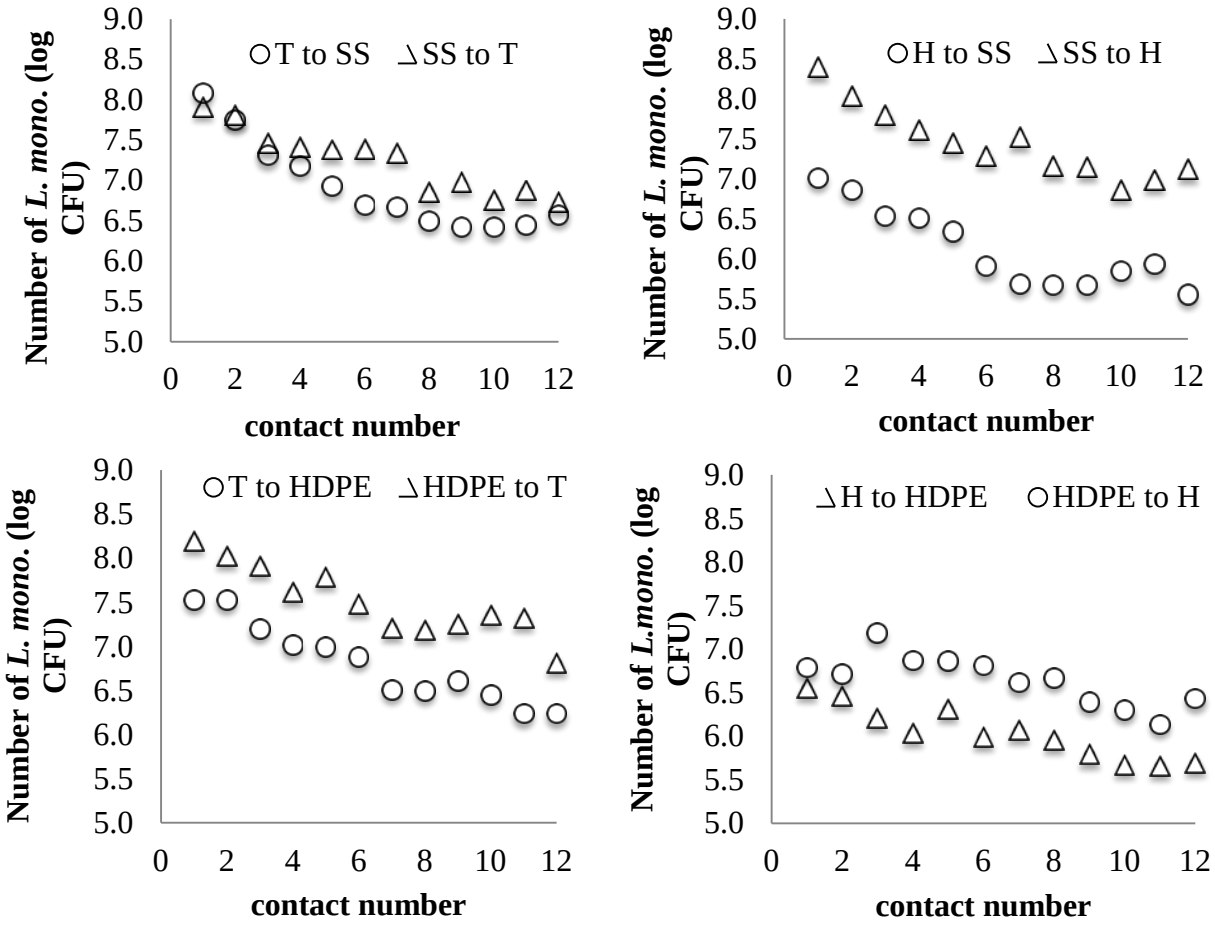

Figure 3.1 Population of *L. monocytogenes* on the receiving surface following sequential contacts when the donor surface was inoculated at ~10^8 CFU/cm^2 (data are means of triplicates).

In a similar study, *E. coli* O157:H7 was sequentially transferred between raw beef and identical stainless steel and high-density polyethylene coupons. Similar logarithmic trends were illustrated for each case; as well as surface pairs exhibiting different average transfer rates depending on which served as the donor and recipient (Campos and others 2007). Zhinong and

others (2006c) also performed experiments evaluating sequential transfer from *L. monocytogenes* inoculated ham to common conveyor belt materials: high density polyethylene, acetyl, and polypropylene. Trends were logarithmic as well; however, there were no differences in the transfer rates between materials, which was inconsistent with the data in this study. This could be due to the relative similarity in properties between the conveyor materials within that study. Both of these prior studies also reported more rapid reduction in transferred populations (3.0-4.0 log and 2.0-2.5 log in 10 contacts) than in this study, likely due to the use of a greater normal force (1800 g and 340 g versus 280 g used in this study) (Campos 2007; Zhinong. 2006).

3.3.2 Calibration curves for GGP on all surfaces

Cross-contamination studies have increased over the past few decades; however, quantification of bacterial transfer has not been well reported due to the inability to intentionally contaminate a food facility with a pathogen to follow its transfer in an actual food handling environment. The use of GGP as a quantitative transfer surrogate could make these types of studies feasible, at least within pilot-scale or simulated processing and retail environments. In order to quantify the amount of GGP (Fig. 3.2), calibration curves were created for each unique surface (Fig. 3.3, Table 3.1) by depositing dots of known concentrations on each surface and photographing under controlled ultraviolet light. This allowed for quantification of GGP regardless of image background. The intensities determined by image processing were related to the known concentrations on each surface to create the curves.

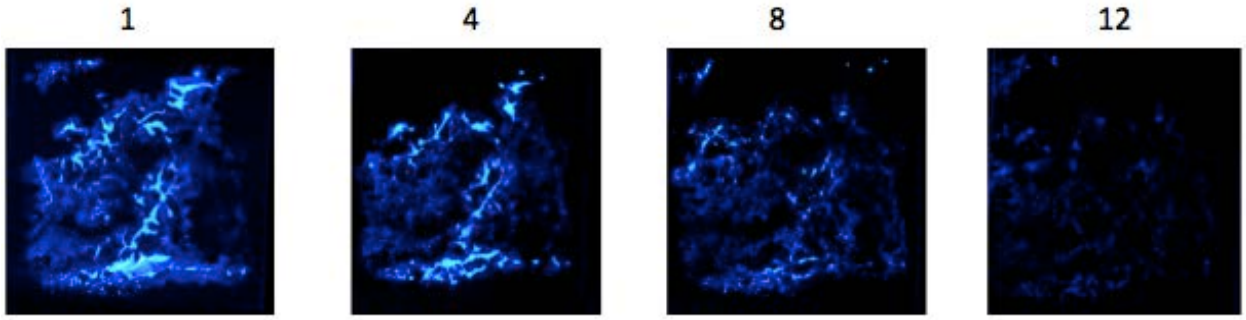

Figure 3.2. Stainless steel coupons, under ultraviolet light, after sequential contacts of GGP inoculated turkey samples.

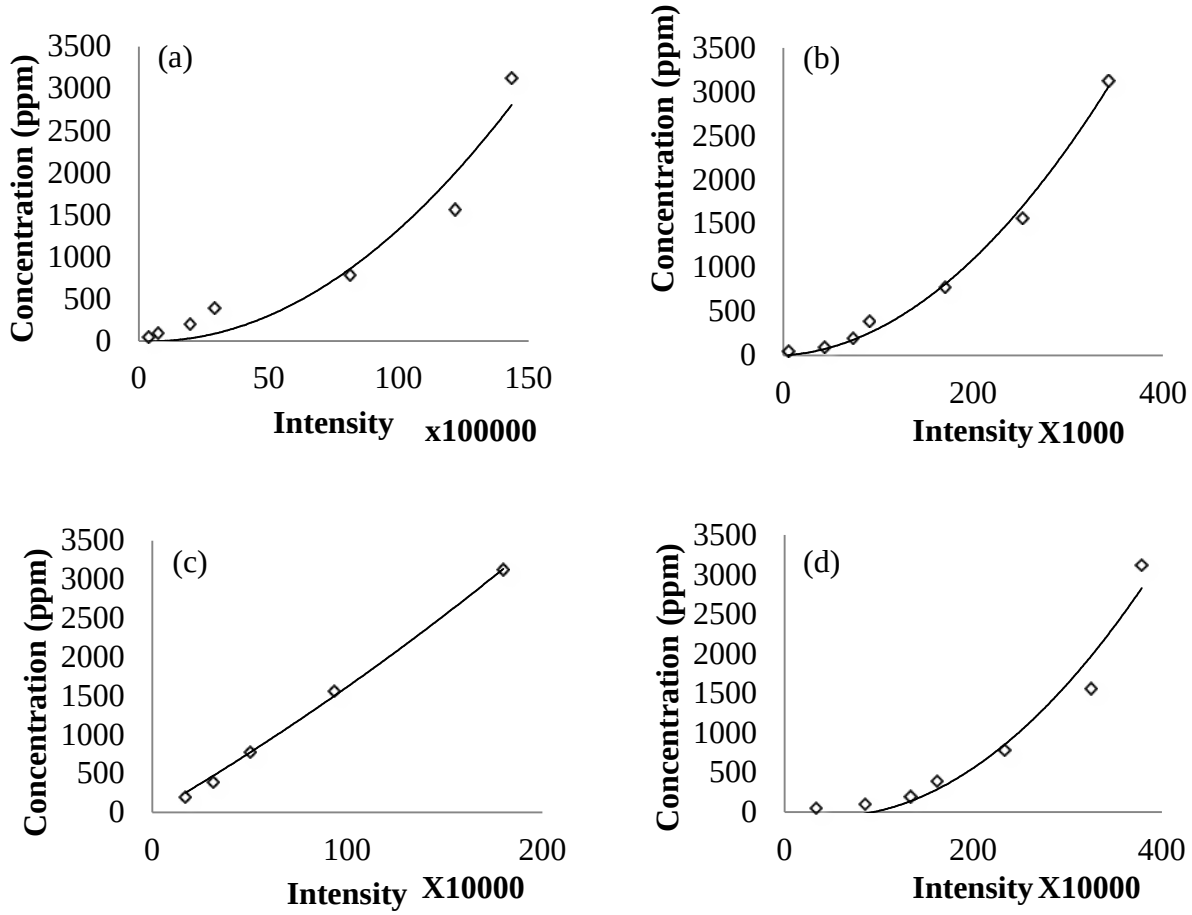

Figure 3.3 Calibration curves for image intensity vs. concentration of GGP for (a) Stainless steel, (b) Turkey, (c) Ham, (d) HDPE.

Table 3.1. Intensity vs. concentration calibration curve equations and goodness of fit for stainless steel, high density polyethylene, turkey, and ham recipient surfaces (y = concentration in ppm; x = sum of pixel intensities).

Recipient Surface	Equation	R^2
Stainless Steel	$y = 1.1 \times 10^{-11}x^2 - 1.4 \times 10^{-05}x$	0.94
High Density Polyethylene	$y = 3.7 \times 10^{-12}x^2 - 2.4 \times 10^{-05}x$	0.96
Turkey	$y = 2.4 \times 10^{-12}x^2 + 6.2 \times 10^{-06}x$	0.99
Ham	$y = 1.1 \times 10^{-12}x^2 + 7.3 \times 10^{-05}x$	0.99

Factors that will affect the feasibility of this image-based method for quantifying GGP include imaging distance, angle, light, and camera settings, because the amount of GGP being quantified is based solely on the images. Therefore, it is critically important to control the image acquisition variables

3.3.3 Relating transfer of *L. monocytogenes* and GGP to and from delicatessen meats and surfaces

Sequential transfer of *L. monocytogenes* from an inoculated material containing ~10^8 CFU/cm^2 to uninoculated material followed an approximately logarithmic decline in most cases.

GGP also exhibited a logarithmic trend that was adjusted upward, with offsets and multipliers determined by minimizing the root mean square error for each case; this was done first for each individual donor-recipient surface pair and then by aggregating the data based on just the recipient (i.e., imaged) surface. When treating each donor-recipient pair separately (Figure 3.4, a-h), the offset and multiplier values (Table 3.2) were significantly affected by the pairing ($P < 0.05$; by ANCOVA); however, when comparing the values among different surface pairings with a common recipient surface, there were no significant differences ($P > 0.05$). When analyzing the data pooled by recipient surface (Table 3.3), RMSE generally increased, and R^2 generally decreased, as would be expected; however, the predictive ability of the method (Figure 3.5) was still reasonable. Clearly, predictions based on specific donor-recipient pairs (Table 3.2 and Figure 3.4) were statistically superior to those based solely on the recipient surface (Table 3.3 and Figure 3.5). However, in practical application, the image will be taken only of the recipient surface, with the donor surface not necessarily known; therefore, the utility of the latter method is the more important analysis.

Table 3.2. Model parameters and statistics for predicting *L. monocytogenes* transfer with GGP for each transfer case.

Transfer surfaces	Offset (log CFU)	Multiplier (log CFU/ log ppm)	RMSE (log CFU)	R^2
T to SS	2.29	1.49	0.156	0.91
SS to T	5.01	0.768	0.216	0.68
T to HDPE	4.99	0.504	0.107	0.94
HDPE to T	5.07	0.997	0.208	0.71
H to SS	2.18	1.27	0.207	0.81
SS to H	3.07	0.848	0.201	0.79
H to HDPE	4.74	0.512	0.149	0.74
HDPE to H	3.63	0.653	0.237	0.29

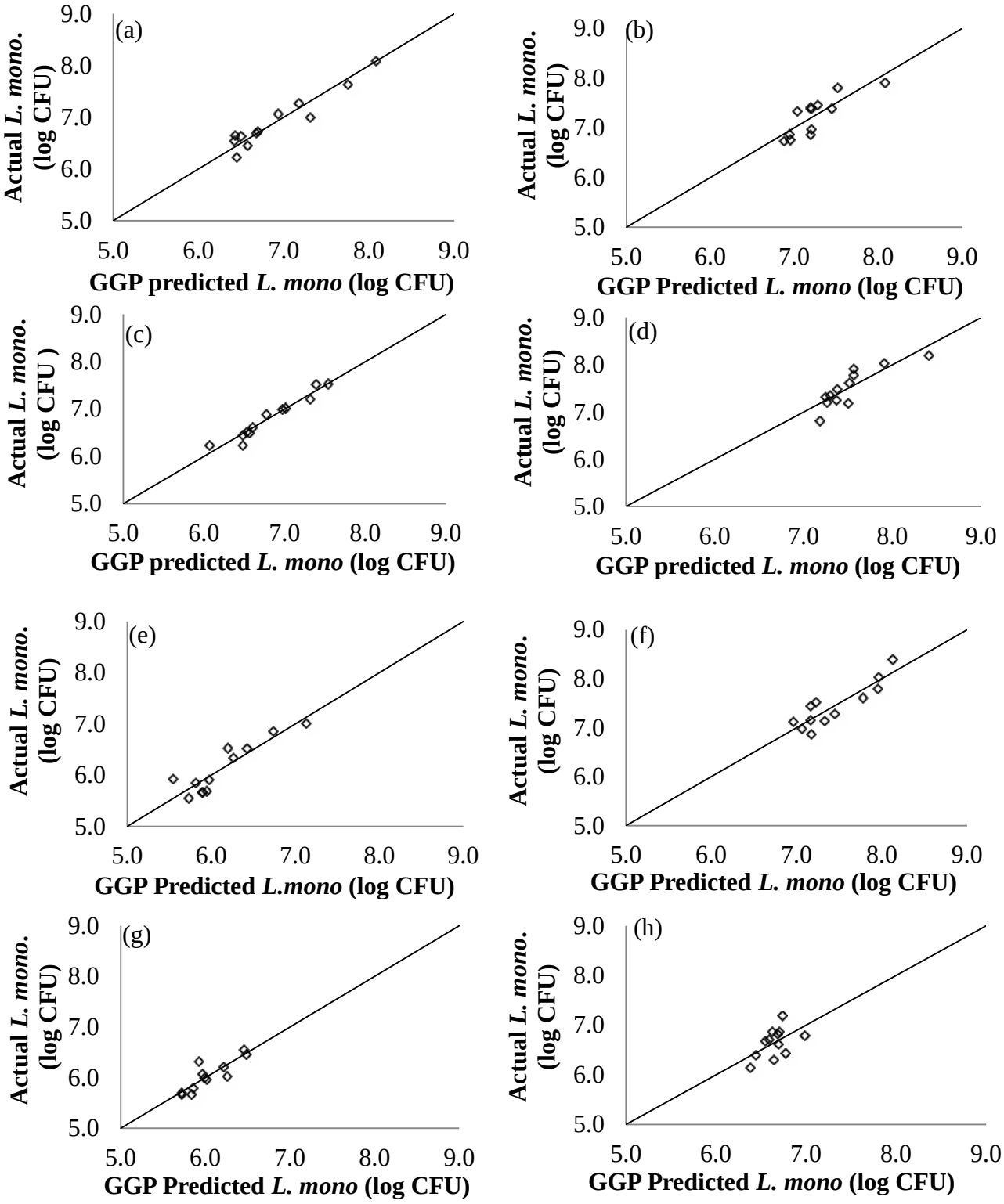

Figure 3,4 Predicted vs. actual *L. monocytogenes* surface counts for: (a) Turkey to stainless steel, (b) Stainless steel to turkey, (c) Turkey to HDPE (d) HDPE to turkey, (e) Ham to stainless steel, (f) Stainless steel to ham, (g) Ham to HDPE, and (h) HDPE to ham.

Table 3.3. Model parameters and statistics for predicting *L. monocytogenes* transfer by GGP for each recipient case.

Recipient Surface	Offset (log CFU)	Multiplier (log CFU/ log ppm)	RMSE (log CFU)	R^2
Stainless Steel	2.23	1.38	0.436	0.54
High Density Polyethylene	4.62	0.59	0.157	0.91
Turkey	5.98	0.53	0.345	0.29
Ham	1.91	1.05	0.265	0.76

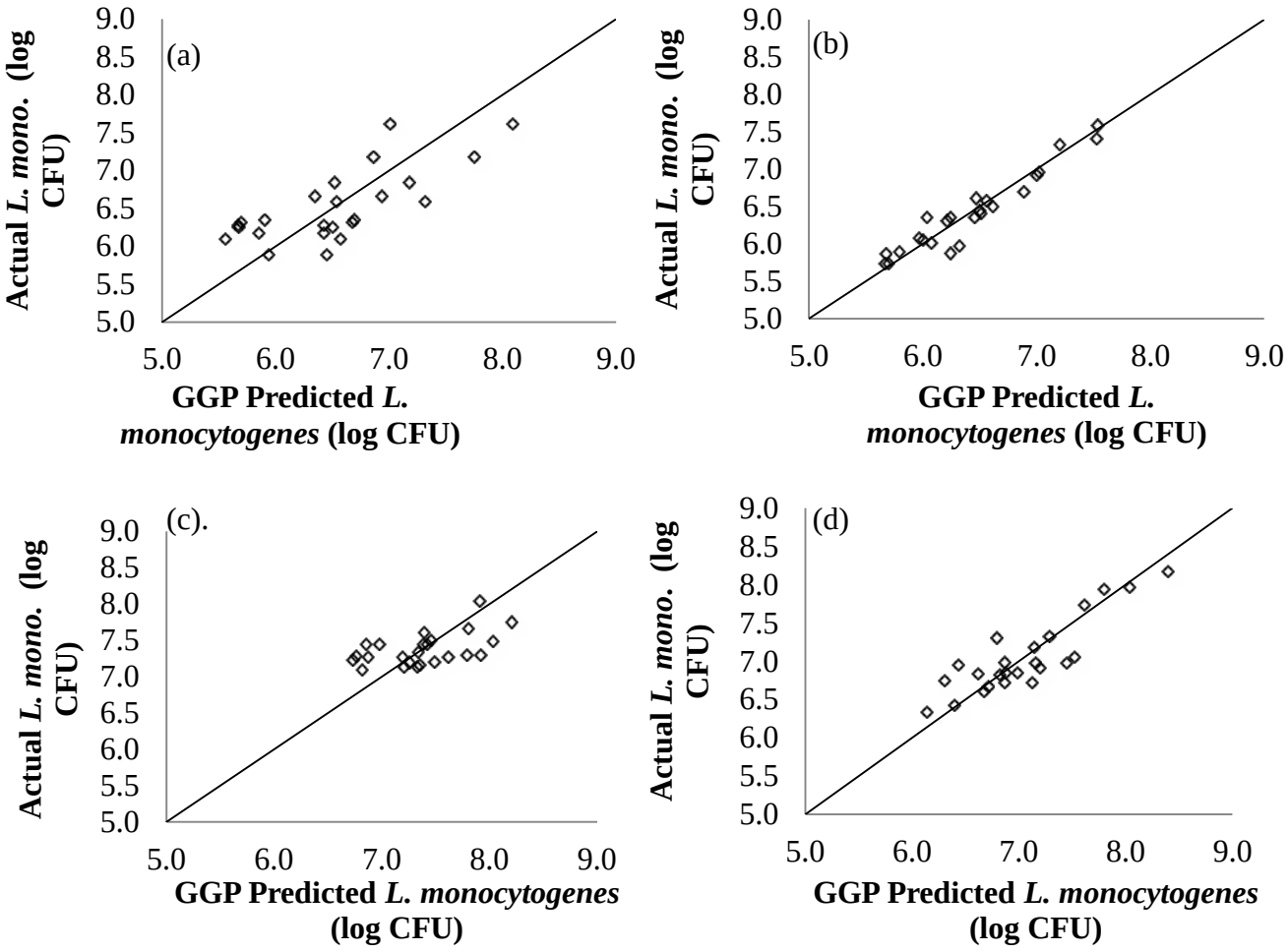

Figure 3.5 Image-predicted vs. actual *L. monocytogenes* surface counts for recipient surfaces: (a) Stainless steel, (b) HDPE, (c) Turkey, and (d) Ham.

Although *Listeria* numbers in actual food handling environments would likely be much lower, very high concentrations of both *L. monocytogenes* and GGP were used in this study so that numbers were easily detectable. GGP transfer curves were therefore compared to *L. monocytogenes* transfer data from a prior study (Zhinong and others 2006a) that evaluated the

sequential contact from ham to HDPE surfaces at lower inoculation levels of 10^7 and 10^4 CFU/cm^2. To accomplish this, the model slope determined above (for GGP transfer from ham to HDPE, 0.512 log CFU/ log ppm) was held constant, but the model intercept determined above was reduced by the appropriate value to compensate for the difference in inoculation levels, as compared to the 10^8 used in this study (i.e., reduced by 1 for the 10^7 data and by 3 for 10^4 data. The resulting prediction errors were < 0.55 log CFU/cm^2. Although greater than the calibration errors (Table 3.2), this is a reasonable value, relative to the desired application, thereby validating the utility of GGP as a surrogate for *L. monocytogenes* transfer when initial concentrations are known.

Table 3.4. Prediction error (RMSE) when comparing GGP based predictions to LM transfer from ham to HDPE at two different inoculation levels (10^4 and 10^7 CFU/cm^2) (Zhinong and others, 2006a). Model parameters and statistics for predicting *L. monocytogenes* transfer by GGP for ham to HDPE at different inoculation levels.

	multiplier (log CFU/ log ppm)		offset (log CFU/cm^2)		RMSE (log CFU/cm^2)	
	10^7	10^4	10^7	10^4	10^7	10^4
51% mc	0.512	0.512	3.74	0.74	0.49	0.54
62% mc					0.39	0.51
73% mc					0.53	0.47

In the present study, transfer of *L. monocytogenes* yielded a smaller slope than did GGP in all cases, meaning that the bacteria persisted longer on the donor surface, which was

accounted for by statistically relating *L. monocytogenes* to the corresponding GGP transfer data. This demonstrated that GGP could be used as a physical surrogate for *Listeria* transfer when considering the change in populations from an initial contamination level (rather than an absolute value).

As an image-based method for using physical surrogate, GGP, for the transfer of *Listeria monocytogenes* will enable subsequent studies that will contribute to the improvement of risk assessments as well as worker training. Currently, microbial risk in a commercial facility is based on probabilities, assumptions, and computational simulations that rarely can be validated with *in situ* observations (Ivanek and others 2004; Schaffner 2004). A simple model based on a contaminated source, intermediate source, and food that contacts the intermediate source has been established by (den Aantrekker and others 2003). In that work, transfer rates varied greatly due to multiple factors that affect bacterial transmission, as well as experimental differences and recovery methods used (Perez-Rodriguez and others 2008). Ivanek and others (2004) quantified transfer and proliferation of *L. monocytogenes* through a fish processing plant by simulation. That simulation showed prevalence increased over a shift, and the confidence intervals became wider overtime as a result of uncertainties in the system; however, it did not model loss of contamination; therefore, numbers were higher than actual numbers, making it an overly conservative estimation. Using a physical surrogate and the associated technique described here for microbial transfer is a relatively simple and rapid method to predict the change of *L. monocytogenes* populations in an actual food handling system after multiple contacts, which was the goal of this project.

3.4 Conclusions

Surface-to-surface transfer of *L. monocytogenes* and the proposed physical surrogate, GGP, was related by regression analysis, based on image analysis of GGP transfer. This was shown to be a feasible method to rapidly quantify risk, based on the change in populations of *L. monocytogenes*, given a known initial concentration and surface type.

Quantifying this surrogate during experimental food handling scenarios and using it to predict pathogen transfer risk provides the capability to quantitatively map actual cross-contamination routes in food facilities, thus giving a method to fill gaps in the data for risk assessments and validate risk models. This can be used as a tool to educate food service workers in food safety while determining the effects of worker training interventions in a retail food-handling environment. GGP image analysis is a valuable approach to assess the strength of control measures, and monitor their impact on food safety.

APPLYING MULTIPLE MODELS TO IDENTIFY COMMON CHARACTERISTICS IN *LISTERIA MONOCYTOGENES* TRANSFER DATA BETWEEN READY-TO-EAT MEAT PRODUCTS AND CONTACT SURFACES

4.1 Introduction

The number of papers published on bacterial transfer to/from food has increased approximately ten-fold over the past two decades (Benoit 2013), reflecting an increasing attention to this important issue. *Listeria monocytogenes* is of particular concern for prepared foods, due to its ability to survive and proliferate in cold temperatures (Lunden and others 2002). A recent risk assessment determined that listeriosis resulting from delicatessen products sliced in retail establishments is a significant concern, and therefore a high priority target for improvement (FSIS 2010b).

Multiple contacts between food and equipment/environmental surfaces are inevitable in processing, retail, and/or foodservice environments. At the retail level, sources of contact can include workers, utensils, slicing equipment, other food products, countertops, and cutting boards, which are often overlooked or poorly sanitized (Perez-Rodriguez and others 2008). Many factors have been suggested to affect transfer rates between these surfaces, including inoculum size, physicochemical factors, and material characteristics (Lin and others 2006; Montville and Schaffner 2003; Chen and others 2001; Midelet and Carpentier 2002; Rodriguez and others 2007a). However, since most research studies are conducted independently, using different methodologies and test variables, conflicting results and conclusions have been reported (Arnold and Bailey 2000; Rodriguez and others 2007b). This approach inherently leaves uncertainty and therefore knowledge gaps in this domain.

There is a critical need for standardization of methods and/or aggregation of data in this field. Literature addressing bacterial transfer to/from food products and contact surfaces is almost exclusively empirical, without any theoretical connection to the underlying mechanisms that govern these processes. Vorst (2005) and Sheen and Hwang (2008) modeled *Listeria* transfer between delicatessen slicers and ready-to-eat meat products, mentioning a variety of variables that impact transfer rates, including: direction of transfer, inoculation level, product composition, product surface characteristics, blade characteristics, and blade speed/force. Although transfer curves were analyzed differently based on some of these variables, phenomenological explanations for how they affect rates of transfer were not discussed (Sheen and Hwang 2008; Vorst 2005). These empirical models consider few parameters for transfer rate estimation, and each author noted that additional parameters that affect bacteria transfer are needed. Hoelzer and others (2012) aggregated a broad set of transfer data including the data described above and applied similar coefficient-based mathematical models, but additionally assessed probability distributions of transfer coefficients across studies. This is an important first step in evaluating cross-contamination dynamics; however, underlying factors affecting transfer still must be understood to explain why transfer coefficients and probability curves differ with differing parameters.

Utilizing a modeling framework that bridges micro- and macro-scale knowledge would support a unifying theory of bacterial transfer between foods and relevant food contact surfaces. Biologically meaningful parameters that help elucidate phenomenological explanations are necessary to further understand and analyze transfer occurring between meat and contact surfaces. Studies that generally characterize surfaces are limited and only briefly discuss the

possible effects on bacteria transfer. For example, Aarnisalo and others (2007) and Vorst and others (2006a) characterized roughness and wear of slicer blades throughout experimentation, and Rodriguez and others (2008) used atomic force microscopy to evaluate topography of different finishes on stainless steel coupons. Midelet and Carpentier (2002) measured contact angles of all surfaces used to determine material hydrophobicity. Identifying these material characteristics and if/how and the extent to which they affect bacterial transfer is an important part of studying cross-contamination and must be done to properly establish transfer models.

Given that the public safety concerns surrounding *Listeria* in delicatessen environments and the limited published data assessing its transfer, gaps in phenomenological understanding need to be assessed. Therefore, the objectives of this work were to: (i) develop a foodborne pathogen transfer database for comparisons across studies, and (ii) quantitatively compare three candidate models in order to identify phenomenological differences in pathogen transfer response between common meat products and equipment surfaces, including: mechanical slicers, kitchen knives, cutting boards, conveyor belts, and countertops. The overall goal is to demonstrate that aggregation of foodborne pathogen transfer data from many sources can significantly improve current understanding of the factors affecting transfer.

4.2 Materials and Methods

The general approach was to compile *Listeria* transfer data from prior studies that evaluated static contact, slicer, and knife slicing, and then analyze the aggregated data sets. The analysis (detailed below) entailed fitting three different models to each transfer curve, and quantitatively

evaluating the resulting parameters and goodness of fit. The core purpose was not to simply fit multiple models, but rather to use the models to identify any phenomenological characteristics of the transfer curves that were consistent for a particular transfer scenario (i.e., product and contact type) across multiple studies.

4.2.1 Data classification

Published bacterial transfer data were characterized in terms of product type, contact surface, and means of transfer, and included only data for ready-to-eat (RTE) meat products and common food contact surfaces. The variables considered included: means of transfer (mechanical slicing, knife slicing, sequential static contacts) and meat type (RTE ham, turkey, salami, and bologna). The available data encompassed approximately 75 data sets, with 253 total replications, and 5,838 individual observations from 6 publications, by 5 different authors in a single laboratory, distributed as described in Table 4.1. Although additional published transfer studies exist, the ability to access the associated data is currently limited. The data acquired were organized into a database (TranBase, Figure 4.1), reflecting the construct of ComBase, a well-known database for microbial response (i.e., growth, survival, inactivation) data (Baranyi and Tamplin 2004).

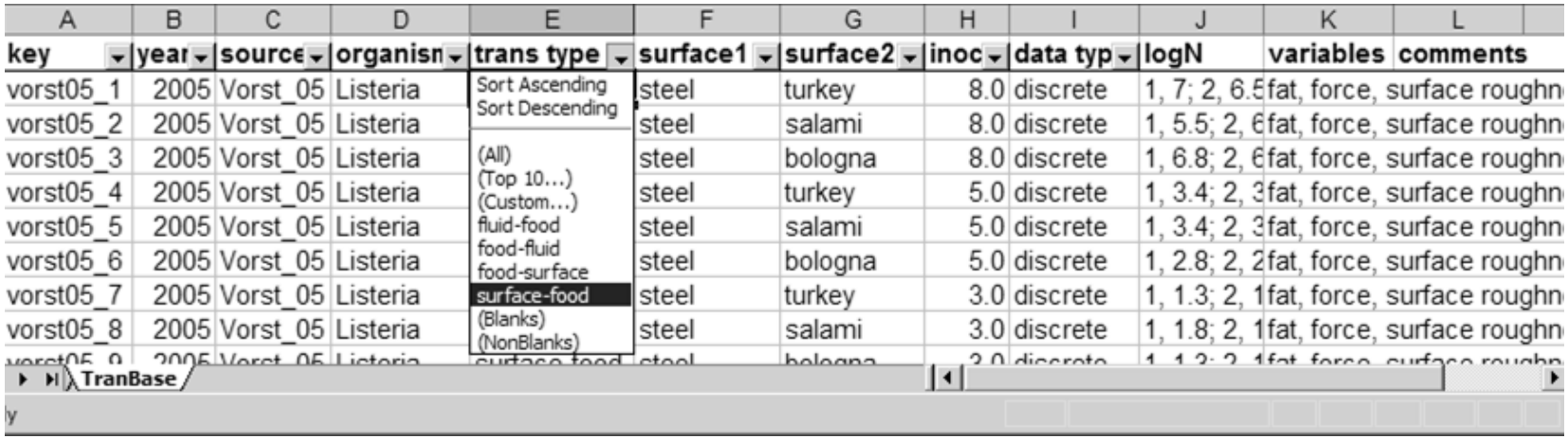

Figure 4.1 A screen-shot of the prototype database (TranBase) cataloging bacterial transfer data. Note the data fields and drop-down menus for sorting (e.g., as shown for choosing the type of transfer process).

Table 4.1 Classification of *L. monocytogenes* data aggregated and considered for transfer analysis based on transfer method and meat product type.

Contact type:	**n**
Sequential Static Contacts	30
Kitchen Knife	8
Mechanical Slicer	37
Product type:	
Turkey	30
Ham	29
Salami	14
Bologna	2

4.2.2 Modeling pathogen transfer between meat and surfaces during mechanical slicing, knife slicing, and sequential static contacts

In the previously published transfer studies, a range of experimental and analytical methods were used, and different test variables were considered. Additionally, each study tended to apply different models to describe "transfer curves" (i.e., number of bacteria on receiving surfaces, such as meat slices, after repeated contacts with a donor surface). Consequently, it is difficult to draw broad inferences across these prior studies. Therefore, using the data available, three empirical models were chosen as follows:

Log-Linear: $\log N = -(k \times n) + \log N_o$ (4.1)

Weibull: $\log N = -(k \times n^p) + \log N_o$ (4.2)

Linear-Weibull: if $n < n_c$, then $\log N = \log N_o - k \times n$, (4.3)

$$\text{else } \log N = \log N_o - (k/(p \times n_c^{p-1}) \times n^p + (1-p)/p \times k \times n_c$$

Where *n* is the slice or contact number; *N* is the bacterial concentration of slice *n;* N_o is a regression parameter (the intercept); *k* is a regression parameter related to the slope of the Log *N* versus *n* curve; *p* is a shape factor, *c* is a second regression parameter related to the slope of the Log *N* versus *n* curve; and n_c is the critical value where the curve shape changes for the two-phase model (described below).

The log-linear model has been used in previous studies to describe attachment strength of bacteria on surfaces during successive transfers to plates by contact (Eginton and others 1995). The Weibull curve was also used to describe pathogen cross- contamination during common food service tasks (Chen and others 2001) . Perez-Rodriguez and others (2007) used both a log-linear model and a Weibull model to describe transfer of *Escherichia coli* during slicing at two different inoculation levels. To our knowledge, a linear-Weibull two-phase model has not been previously used to explain bacterial transfer. However, Vorst and others (2006a) noted that, during mechanical slicing, most transfer occurs during the first 10-15 slices, with the transfer rate decreasing thereafter. Campos and others (2009) previously noted that such transfer curves had a distinctly log-linear decline for the first ~10 slices, followed by tailing. They theorized that this

distinct change in curve shape was due to the accumulation of meat residues on the slicer blade (Campos and others 2009); however, this hypothesis was not previously tested quantitatively. Therefore, a two-phase linear-Weibull curve with a distinct critical value (i.e., slice number) where the transfer response curve changes from linear to non-linear was considered for this analysis. In cases where the linear-Weibull fit was forced via regression and yielded a low critical value (e.g., 2), that result indicated that the two-phase model should not be considered to be the most likely model, as such a result actually indicates a rejection of this model in describing the response curve.

Several publications have generated transfer coefficients based on empirical models describing transfer during slicing of ham, salami, and 'gravad' salmon under exclusive conditions (Aarnisalo and others 2007; Sheen 2008; Sheen and Hwang 2010), but were not considered here due to the specificity of certain parameters on transfer rates. By applying quantitative, statistical comparisons of the three models across the aggregated data sets, the goal was to elucidate inherent differences in the general nature of transfer for the various cases, not just re-fit curves to the transfer data.

4.2.3 Fitting the equations to experimental data

Replicate data from individual studies were pooled, and the three models were fit by minimizing the root mean squared error (RMSE) in Excel using Solver (Version 14.2.4, Frontline Systems, Incline Village, NV), where:

$$SSE = \Sigma(\text{Model predicted value} - \text{Experimental value})^2 \quad (4.4)$$

$$RMSE = \sqrt{\frac{SSE}{n}} \quad (4.5)$$

where *n* is the total number of observations in the analyzed set.

The Akaike Information Criterion (AIC_C) (Motulsky and Christopoulos 2004) was used in addition to the RMSE to determine the best model fit. AIC_C , which quantifies model correctness by taking into account both the error and the number of parameters, includes the models' sum of squared residual errors (SSE), number of parameters (K), and number of data points (n). The Akaike model used for small samples (n/K<40) is:

$$AIC_C = -n \times \ln(SSE/n) + 2 \times K + (2 \times K \times (K+1))/(n-K-1) \quad (4.6)$$

The Akaike values for the three models were normalized based on measuring distances from the minimum Akaike value of the set (Eq. 4.7), which is the best fitting model, followed by determining the Akaike weights, w_i , of each candidate model (Eq. 4.8). These weights represent the probability that a model is the 'most likely' fit out of the tested candidate models and is based on the absolute difference between AIC_C scores, Δ_i:

$\Delta_i = AIC_{c,i} - \min AIC_c$ (4.7)

$$w_i = \frac{e^{-0.5*\Delta_i}}{\sum e^{-0.5*\Delta i}} \quad (4.8)$$

Δ_i = AIC_c difference between best fitting model and other candidate models *i*

$AIC_{c.i}$ = AIC_c for candidate model *i*

min AIC_c = minimum AIC_c when all candidate models are compared

Having done this for each pooled data set and each model, the most likely model was chosen for each data set based on the minimum RMSE and the highest weighted AIC_c. Subsequently, the fraction of data sets for which a given model was selected as best was determined for each transfer scenario and product type.

4.2.4 Statistical comparisons

All *Listeria* transfer curves considered had a minimum of three replicate datasets. Data were evaluated by pooling the replications and fitting a single curve in each model case.

Analyses of variance (ANOVA) were conducted to determine significant differences between two-phase critical contact values (n_C) due to product type, transfer scenario, and contact surface material, to establish whether there were consistent characteristics of the transfer curves for a given scenario that might relate to inherent, phenomenological explanations for the observed transfer responses.

4.3 Results and Discussion

4.3.1 Interpretation of models on transfer data

A total of 225 curve fits were performed, fitting each of the three models to the pooled replicate data sets. Utilizing replicates accounted for experimental errors as well as model errors when selecting the most likely model to explain the curve. A summary of the RSME and AIC_C results for each curve fit are organized in Table 4.2. Examples of most-likely model curve fits are illustrated in Figures 4.2 and 4.3.

Table 4.2 Summary of *L. monocytogenes* transfer and model statistics for (a) slicer data, (b) kitchen knife, and (c) static contact data considered in this study.

(a)	Surf. 1	Surf. 2	RMSE			Weighted AIC_C			n_C
			Weibull	Linear	Linear-Weibull	Weibull	Linear	Linear-Weibull	
1	Slicer	Ham	*0.53	0.54	0.54	*0.78	0.20	0.02	6
2	Slicer	Ham	0.48	0.49	*0.48	*0.72	0.03	0.25	11
3	Slicer	Ham	0.49	0.56	*0.49	0.33	0.00	*0.67	12

Table 4.2a (cont'd)

4	Slicer	Ham	0.56	0.57	*0.56	*0.68	0.07	0.25	5
5	Slicer	Ham	0.51	0.54	*0.51	*0.50	0.00	0.50	14
6	Slicer	Ham	0.50	0.53	*0.49	0.28	0.00	*0.72	8
7	Slicer	Ham	0.53	0.54	*0.53	*0.57	0.16	0.27	6
8	Slicer	Turkey	0.53	0.63	*0.41	0.00	0.00	*1.00	6
9	Slicer	Turkey	0.51	0.54	*0.51	*0.73	0.00	0.26	11
10	Slicer	Turkey	0.58	0.62	*0.57	*.63	0.00	0.37	16
11	Slicer	Turkey	0.60	0.70	*0.58	0.05	0.00	*0.95	5
12	Slicer	Turkey	0.66	0.67	*0.65	0.16	0.30	*0.54	18
13	Slicer	Turkey	0.48	0.55	*0.45	0.02	0.00	*0.98	8
14	Slicer	Turkey	0.63	0.68	*0.61	0.22	0.00	*0.78	8
15	Slicer	Turkey	0.56	0.63	*0.56	*0.61	0.00	0.39	4
16	Slicer	Turkey	*0.53	0.59	0.53	*0.82	0.00	0.18	3
17	Slicer	Turkey	*0.43	0.45	0.43	*0.65	0.14	0.20	4
18	Slicer	Turkey	0.50	0.57	*0.49	0.31	0.00	*0.69	17
19	Slicer	Turkey	0.62	0.69	*0.59	0.09	0.00	*0.91	5
20	Slicer	Salami	0.34	0.37	*0.34	0.33	0.00	*0.67	2
21	Slicer	Salami	0.59	0.60	*0.58	0.23	0.36	*0.41	2
22	Slicer	Salami	0.47	0.52	*0.46	0.26	0.00	*0.74	2
23	Slicer	Salami	0.73	0.79	*0.62	0.00	0.00	*1.00	2
24	Slicer	Salami	0.45	0.44	*0.44	0.08	*0.83	0.09	0
25	Slicer	Salami	0.46	0.46	*0.46	0.22	*0.69	0.10	2
26	Slicer	Salami	0.56	0.56	*0.56	0.23	*0.69	0.08	3

Table 4.2a (cont'd)

27	Slicer	Salami	0.60	*0.59	0.59	0.10	*0.81	0.09	2
28	Slicer	Salami	0.69	0.69	*0.69	0.21	*0.64	0.15	2
29	Slicer	Salami	*0.51	0.51	0.51	0.47	*0.49	0.04	2
30	Slicer	Salami	0.60	0.61	*0.60	*0.51	0.24	0.26	2
31	Slicer	Salami	0.52	0.52	*0.52	0.22	*0.70	0.08	0
32	Slicer	Turkey	*0.75	0.79	0.75	*0.76	0.02	0.21	9
33	Slicer	Salami	0.35	0.35	*0.34	0.22	*0.61	0.18	0
34	Slicer	Bologna	*0.69	0.69	0.69	0.24	*0.69	0.07	2
35	Slicer	Turkey	0.69	0.71	*0.68	0.37	*0.37	0.26	4
36	Slicer	Salami	0.92	0.93	*0.92	0.27	*0.62	0.10	1
37	Slicer	Bologna	0.47	0.47	*0.47	0.21	*0.72	0.07	3

Table 4.2 (cont'd)

(b)	**Surf. 1**	**Surf. 2**	**RMSE**			**Weighted AIC_c**			**n_c**
			Weibull	**Linear**	**Linear-Weibull**	**Weibull**	**Linear**	**Linear-Weibull**	
38	knife	Turkey	0.77	0.80	*0.74	0.28	0.19	*0.53	10
39	knife	Turkey	*1.06	1.12	1.06	*0.66	0.17	0.17	7
40	knife	Turkey	1.47	1.53	*1.46	*0.52	0.26	0.23	9
41	knife	Turkey	1.01	1.08	*1.00	*0.59	0.08	0.33	7
42	knife	Turkey	0.74	0.82	*0.71	0.31	0.01	*0.69	7
43	knife	Turkey	0.95	0.99	*0.94	*0.52	0.24	0.25	8
44	knife	Turkey	0.66	0.72	*0.66	*0.66	0.04	0.30	9
45	knife	Turkey	0.95	0.98	*0.94	*0.54	0.25	0.21	5

Table 4.2 (cont'd)

(c)	**Surf. 1**	**Surf. 2**	**RMSE**			**Weighted AIC_c**			**n_c**
			Weibull	**Linear**	**Linear-Weibull**	**Weibull**	**Linear**	**Linear-Weibull**	
46	T	SS	0.67	0.86	*0.58	0.00	0.00	*1.00	1
47	SS	T	*0.66	0.74	0.66	*0.77	0.01	0.23	1
48	H	SS	*0.20	0.50	0.21	*0.91	0.00	0.09	1
49	SS	H	0.60	0.63	*0.60	*0.68	0.11	0.21	1
50	T	HDPE	0.58	0.77	*0.39	0.00	0.00	*1.00	0
51	HDPE	T	0.59	0.67	*0.52	0.01	0.00	*0.99	0
52	H	HDPE	*0.23	0.62	0.25	*1.00	0.00	0.00	0
53	HDPE	H	0.39	0.39	*0.32	0.00	0.00	*1.00	0
54	SS	T	0.38	0.43	*0.38	*0.74	0.03	0.23	0
55	T	SS	0.31	0.31	*0.30	0.19	*0.72	0.09	0
56	T	HDPE	*1.46	1.49	1.46	0.33	*0.58	0.08	1
57	HDPE	T	0.22	0.25	*0.22	*0.92	0.02	0.06	0
58	Ham	PP	0.23	0.24	*0.23	*0.61	0.25	0.14	0
59	Ham	PP	0.21	0.22	*0.21	*0.53	0.32	0.15	0
60	Ham	HDPE	1.07	1.07	*1.07	0.23	*0.71	0.06	0
61	Ham	HDPE	0.29	0.29	*0.29	0.29	*0.65	0.06	0
62	Ham	AC	*0.32	0.33	0.33	*0.52	0.40	0.08	0
63	Ham	AC	0.43	0.46	*0.43	*0.69	0.09	0.23	0
64	Ham	PP	0.38	0.41	*0.38	*0.74	0.04	0.22	2
65	Ham	AC	0.27	0.27	*0.27	0.32	*0.50	0.18	0
66	Ham	HDPE	0.40	0.40	*0.40	0.22	*0.71	0.06	2

Table 4.2c (cont'd)

67	Ham	PP	0.32	0.45	*0.30	0.05	0.00	*0.95	0
68	Ham	AC	0.74	0.74	*0.73	0.24	*0.67	0.10	0
69	Ham	HDPE	0.69	0.70	*0.48	0.42	*0.44	0.14	0
70	Ham	AC	*0.53	0.55	0.53	0.03	0.02	*0.96	1
71	Ham	HDPE	0.20	0.37	*0.17	0.00	0.00	*1.00	0
72	Ham	PP	0.28	0.45	*0.25	0.00	0.00	*1.00	0
73	Ham	AC	0.46	0.67	*0.40	0.00	0.00	*1.00	3
74	Ham	HDPE	0.32	0.46	*0.30	0.00	0.00	*1.00	21
75	Ham	PP	0.43	0.46	*0.43	*0.63	0.05	0.33	4

*Most likely model based on lowest RMSE or highest weighted AIC_c.

Datasets 1-7 from (Zhinong and others 2006b), 8-31 from (Keskinen and others 2008), 32-37 from (Vorst and others 2006a), 38-45 from (Keskinen and others 2008), 46-57 from (Benoit 2013), 58-75 from (Zhinong and others 2006a).

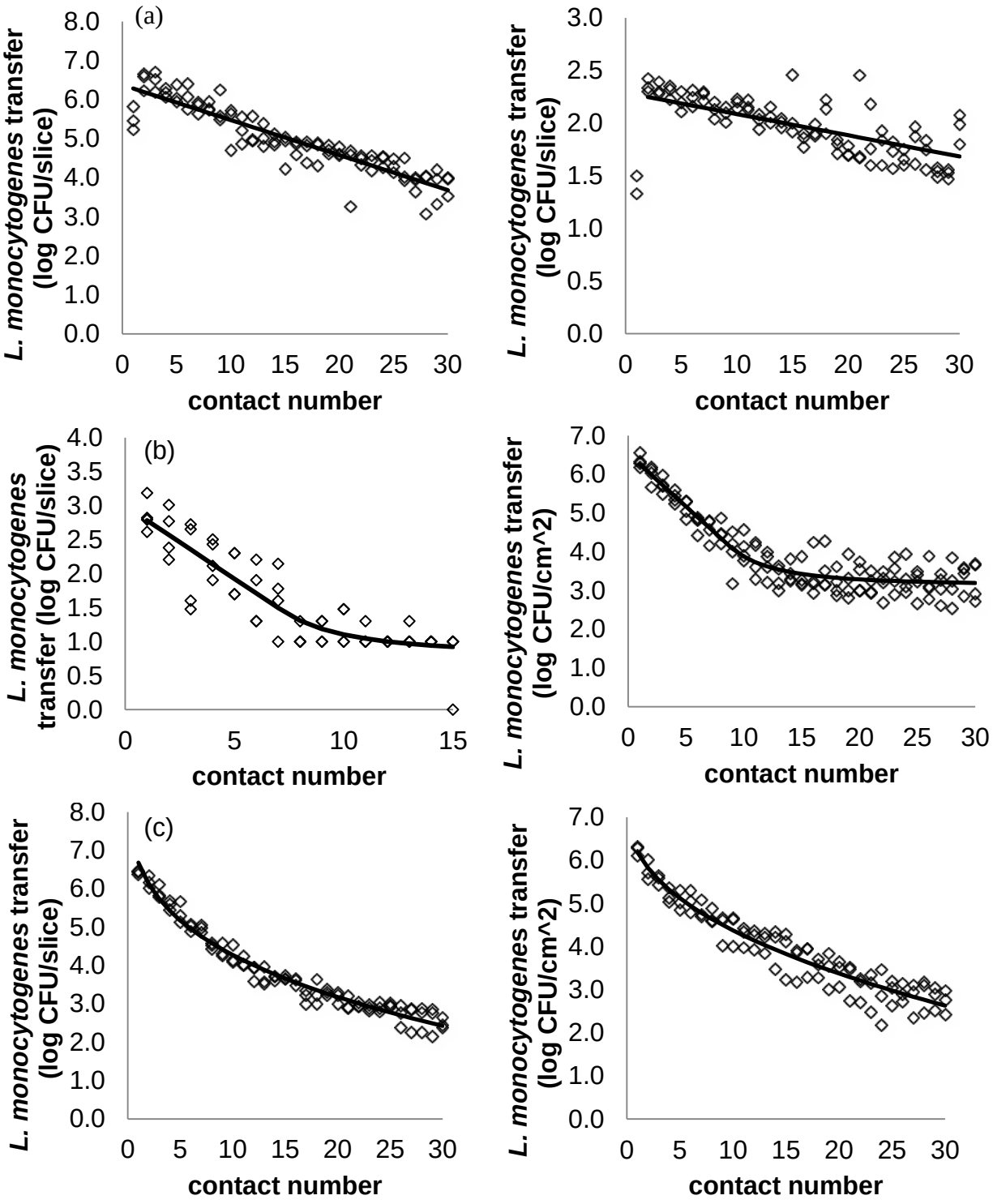

Figure 4.2 Examples of evaluated *L. monocytogenes* data sets and fits of the most likely models: (a) Linear, (b) Linear-Weibull, and c) Weibull.

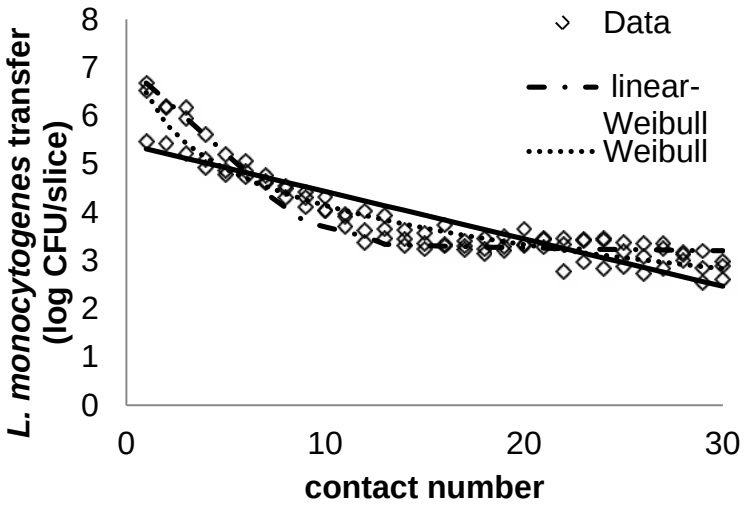

Figure 4.3 An example of a single evaluated dataset fitted to all three candidate models.

4.3.2 Mechanical slicing transfer characteristics

The two-phase (linear-Weibull) model has the lowest (best) RMSE for ~80% of the slicer data (Table 4.3b). However, when considering the AIC_C of the aggregated slicer data, no model was the superior choice for the majority of the cases (Table 4.3a). Because the two-phase model would be expected to have the lowest RMSE due the greater number of parameters, the AIC_C is the better criterion for evaluating correctness of the models, and will be the primary measure considered throughout the analysis.

Table 4.3 The number of datasets in each transfer scenario most likely to be correct in each model case based on (a) AIC_C and (b) RMSE.

(a) **AIC_C**

	Knife	Slicer	Contacts
Weibull	6	12	17
Linear	0	12	8
Linear-Weibull	2	13	13
SUM	8	37	38

(b) **RMSE**

	Knife	Slicer	Contacts
Weibull	1	6	9
Linear	0	1	2
Linear-Weibull	7	30	29
SUM	8	37	38

Inconsistency in selection of the best model for the slicer data, based on AIC_C**,** suggests that other factors may affect bacterial transfer during the mechanical slicing of delicatessen meats. The authors of the original studies noted that the amount of soiling on the slicer blade was dependent on product composition, thus affecting the transfer results. (Vorst and others 2006a; Lin and others 2006; Keskinen and others 2008). Vorst (2006a) reported the percent moisture and fat of each product before slicing, and noted that salami, which was relatively high in fat,

created a visible film, while the low fat turkey had a washing effect. Consequently, sliced products were categorized to determine whether there was a noticeable trend between transfer curves and product characteristics (Table 4.4). According to the AIC_C, the Weibull model best described turkey and ham transfer, while the linear curve best described salami and bologna transfer, thus supporting the hypothesis that product composition affects the overall transfer response of *Listeria* from product to slicer.

Table 4.4 The number of mechanical slicer datasets for each product type most likely to be correct in each model case based on (a) AIC_C and (b) RMSE.

(a) **Slicer AICc**

	Turkey	**Ham**	**Salami**	**Bologna**
Weibull	12	5	1	0
Linear	1	0	9	2
Linear-Weibull	7	2	4	0
SUM	20	7	14	2

(b) **Slicer RMSE**

	Turkey	**Ham**	**Salami**	**Bologna**
Weibull	7	2	2	0
Linear	0	0	3	0
Linear-Weibull	13	5	9	2
SUM	20	7	14	2

Although the Weibull model was the most likely for most of the mechanical slicer data, the linear-Weibull critical value (i.e., critical slice number) where the shape of the transfer response changes from linear to non-linear was analyzed to determine whether the value was affected by product type. The critical value for the aggregated slicer data ranged from 0 (for linear data) to 18, with an average of 7.4 and standard deviation of 4.9. The variability was largely due to the salami data that were more linear, having a low critical value of ~2, meaning that the two-phase model was forced, and therefore the simpler linear model better describes this type of transfer. This linear trend (Figure 4.2a) in salami data has been previously recognized in work that commented on the layer of fat the salami left on the slicer blade surface, which contributed to constant but lower transfer rates (Vorst and others 2006; Lin and others 2006). Ham and turkey, on the other hand, had statistically similar critical values ($P > 0.05$), with an average of ~9, which, due to their compositional similarities, as compared to salami, is plausible (Figure 2b). Previous work considered the effect of product residue on the blade as a contributing factor of curve-shape change. Campos and others (2009) qualitatively observed a bi-phasic tendency that began tailing after ~10 slices, which roughly corresponded to meat product residue accumulation of ~5 μm thickness. They noted that this critical value was product-specific, decreasing with increasing fat content of the food (Campos and others 2009).

Some model fit variability in slicer data may be due to methodology differences between studies and laboratories, as well as variables tested within a given study, which included biofilm forming ability, culture health, inoculation level, desiccation time, force, blade type, and blade age/wear. Different limits of detection and the manner in which this was reported also may be a factor affecting the results. For example, Vorst (2006a) noted that at lower inoculation levels,

salami yielded significantly different transfer curves than at higher inoculation levels, unlike turkey and bologna.

Empirical (curve fitting) models previously have been used in several commercial delicatessen slicer studies, including some of the studies evaluated here. A coefficient-based model developed by Vorst (2005) predicted *L. monocytogenes* transfer during slicing of RTE delicatessen meats at different inoculation levels, assuming: (1) the number of *Listeria* cells transferred from the blade to the meat during slicing was a fraction (f_1) of the number of *Listeria* cells on the blade just before each sequential slice, (2) the number of *Listeria* cells transferred to surrounding areas was a different fraction (f_2) of the number of cells on the blade just before each sequential slice, and (3) the number of cells on the blade before any slicing begins was N_0. The resulting exponential decay model predicted the number of CFU transferred to any given slice, as well as the number of CFU lost to the environment (including potential aerosols and bacterial death), therefore predicting the number of CFU transferred to slice X. This model was validated by Keskinen (2007) to accurately describe transfer to RTE delicatessen meats when considering biofilm forming ability, time, and injury, noting changes in variance and total cumulative transfer between studies without explanation. Curve fits also differed based on meat and inoculation levels (Vorst 2005); however, both Vorst and Keskinen noted that most transfer to the meat occurred during the first 10-15 slices. Sheen and Hwang (2008) also used exponential empirical models to describe bacteria transfer during mechanical slicing of deli meats; however, they also suggested that transfer results depended on inoculation level and contamination route. These prior studies mentioned model limitations and recommended that other factors also be

included in the models; however, they did not investigate underlying factors that influence shape of the transfer curves or use the model forms to quantitatively evaluate such differences.

4.3.3 Knife slicing data

According to the AIC_C of the transfer data analyzed, when meats were sliced with a knife (i.e., single blade action, rather than the continual spinning action of a mechanical deli slicer), the Weibull model was the best model in 6 of 8 cases (Table 4.3a). These data could not be compared across meats, as done for the slicer studies, because insufficient datasets were unavailable for this comparison. Therefore, it is uncertain whether the curve would be different based on product composition. However, when the turkey data from the knife studies were compared to those of the slicer studies, a statistically similar critical value ($p > 0.05$) of ~8 was observed, as well as the same conclusions based on AIC_C and RMSE findings (Table 4.2), suggesting that product composition had a greater impact on the transfer than the means of cutting.

4.3.4 Sequential static contact data

Analysis of sequential static contact data between various types of meats and contact surfaces was best described by the Weibull model according to the AIC_C (Table 4.3a). Both the AIC_C and RMSE implied that selection of the best model was unaffected by product composition

and contact material (Table 4.5). Zhinong and others (2006) also observed no significant difference in transfer rate between high density polyethylene, polypropylene, and acetylene surfaces and ham. The average critical value from all of the static contact data analyzed in this study was 1.5, indicating that the linear-Weibull was forced, and that a single-phase curve better described static sequential static contact transfer. These critical contact values differed from those of ham and turkey subjected to mechanical slicing and contact surfaces; however, in those cases, there was likely accumulation of residue on the blade, causing the transfer curve to change shape. In contrast, during sequential static contacts, excessive accumulation of product residue is unlikely, and therefore does not affect the shape of the transfer curves.

Table 4.5 The number of sequential static transfer datasets for each product type most likely to be correct in each model case based on (a) AIC_c and (b) RMSE.

(a) **Contacts AIC_c**

	Turkey	**Ham**
Weibull	4	7
Linear	2	6
Linear-Weibull	1	6
SUM	7	21

(b) **Contacts RMSE**

	Turkey	**Ham**
Weibull	1	5
Linear	0	0
Linear-Weibull	6	16
SUM	7	21

4.3.5 Effects on transfer due to product composition

When all slicer, kitchen knife, and sequential contact data were pooled by product type, the effect on transfer became apparent. According to the AIC_c, transfer to turkey and ham, which are similar in moisture and fat content, was generally best described by the Weibull model; while

transfer to salami and bologna, with much greater fat and lower moisture, were best described by the linear model (Table 4.6a).

Table 4.6 The number of datasets for each product type most likely to be correct in each model case based on (a) AIC_C and (b) RMSE.

(a) **AIC_C**

	Turkey	**Ham**	**Salami**	**Bologna**
Weibull	17	12	1	0
Linear	3	6	9	2
Linear-Weibull	7	11	4	0
SUM	29	28	14	2

(b) **RMSE**

	Turkey	**Ham**	**Salami**	**Bologna**
Weibull	8	17	2	0
Linear	1	1	3	0
Linear-Weibull	20	10	9	2
SUM	29	28	14	2

When comparing the critical contact values within products, the transfer response during cutting or slicing was different from that of sequential contacts. Turkey, for example, had an average critical contact value of ~9 for slicing, ~8 for kitchen knives, but ~1 for sequential static

contacts. This trend was similar for ham, which had a critical contact value of ~9 for slicing and ~1 for static contacts. This suggested that the build-up of product residue on the blade surfaces during mechanical slicing or cutting changed the transfer response as hypothesized above.

4.3.6 Future utility of database

This preliminary database provides a compilation of published transfer data that will be useful to scientists and risk assessors evaluating food pathogen cross-contamination. It contains data published over the past 8 years in an accessible format and provides further details concerning each study. This information previously was not available in this format, and offers a baseline for understanding the mechanisms that govern these processes. Currently, models and simulations for bacterial transfer through food processing environments are of macro-scale, focused on probabilities of contact events and/or amount of transfer.

Schaffner (2004) studied cross-contamination by using Monte Carlo simulation to track the number and prevalence of certain *Listeria* strains through a food processing plant. This method is probabilistic and based on transfer rate distributions that are assumed to be related to persistence and prevalence only. Ivanek and others (2004) described a compartmental model for *Listeria* cross-contamination that used a difference equation system to model transfer through a fish processing plant. This simulation focused on prevalence, frequency, number of contaminated units, and duration probabilities and assumptions. These models were not based on mechanisms of attachment and transfer, which leaves several uncertainties remaining

A comprehensive database compiling foodborne pathogen transfer data would be a valuable addition for the scientific community, enabling easier comparisons across studies, and broader analyses of transfer phenomena. The long-term goal is to build the current database into a publically-available tool. Such a database could then serve as a valuable resource for model development and risk assessments.

4.4 Conclusion

The goal regarding common types of food pathogen transfer events was to compare simple general models to identify phenomenological attributes of particular transfer scenarios, consistent across multiple studies. Comparing models based on an equivalent criterion (i.e., AIC_C) enabled quantitative evaluation of transfer response curve characteristics, and illustrated that the effects of product and contact type were consistent across multiple studies.

This analysis illustrated that there are considerable data already published in this area, but most such studies have been designed, executed, and reported independent of one another, without connecting data across studies. This is consistent with a recent report by Hoelzer and others (2012), which aggregated published transfer data from 37 studies (825 data points) to determine the probability distributions of transfer coefficients, finding that transfer coefficients varied widely across studies. Studies that have proposed and/or developed system-level modeling frameworks require prior knowledge or assumptions about the rates of transfer; however, given the current state of knowledge, such information is available only as product/pathogen/process-specific data or parameters. There is a significant need and opportunity for a unified database that aggregates all of these prior data and becomes a repository for future data. Such a database

will help advance linkages between fundamental research on factors controlling attachment and the observed transfer outcomes, while also improving the design of future studies to fill data gaps.

OVERALL CONCLUSIONS

5.1 GGP comparisons by means of image analysis

- Image-based analysis of GGP concentration on food contact surfaces is a means to rapidly quantify the risk of *L. monocytogenes* transfer occurring in a given scenario, for a known recipient surface and initial contamination level.
- An ANCOVA was performed to compare the slopes of the different GGP regressions. There was a significant difference among the slopes between surface transfer pairs; therefore, different thresholding levels were necessary for each surface, indicating that use of this approach likely requires knowledge of the surface type. However, when an ANCOVA was performed on recipient surfaces, there was no significant difference ($P > 0.05$) between the slopes; therefore, only knowledge of the recipient surface type (i.e., the surface being imaged) is necessary to quantify the amount of transfer.
- Transfer of *L. monocytogenes* and the proposed physical surrogate, GGP, by sequential contacts to and from ready-to-eat roast turkey, ham, 304 stainless steel, and high-density polyethylene can be related by regression analysis.

5.2 Aggregation of foodborne pathogen transfer data

- There is a growing body of literature addressing bacterial attachment and transfer to/from food products and contact surfaces, but most of the reports are essentially observational.

- Meta-analysis illustrates that there are considerable data already published in this area, but every study has been designed, executed, and reported with little consideration for its role in the larger body of data. This is consistent with a recent report by Hoelzer and others (2012), which aggregated published transfer data from 37 studies (825 data points) and showed that transfer coefficients varied widely across studies.
- Most transfer models are probabilistic or empirical curve fitting and do not consider underlying mechanisms that govern these processes.
- Studies that have proposed and/or developed system-level modeling frameworks require prior knowledge or assumptions about the rates of transfer; however, given the current state of knowledge, such information is available only as product/pathogen/process-specific data or parameters.
- There is a significant need, and opportunity, for a unified database that aggregates all of these prior data and becomes a repository for future data. Such a database (here described as TranBase) will help advance linkages between fundamental research on factors controlling attachment and the observed transfer outcomes, while also improving the design of future studies to fill data gaps that are revealed by meta-analyses of the compiled data.

5.3 Analysis of aggregated delicatessen test data

- Analysis revealed that the action of slicing and chopping gives similar critical contact values (~9), and therefore similar curves for ready-to-eat turkey.
- During slicing, high moisture and low fat turkey and ham transfer *Listeria* in a similar Weibull manner, while transfer to salami is more typically linear due its high fat and low moisture creating a product residue film on the blade.
- Sequential static contacts contrarily have the same shape of transfer regardless of the meat or surface.
- Transfer curves are dependent on several variables that must be reported for accurate comparisons.
- In this case, several differing transfer rates were observed, but the shape of the curve between the different types of transfer and food was the factor evaluated and compared.
- Aggregating data from different studies and research groups revealed transfer characteristics that may not have been evident within individual studies.
- Critical variables, such as temperature, inoculation methods, and sampling methodology, should be controlled when conducting transfer experiments, so that resulting data can be better compared across studies.

5.4 Utility of a Pathogen Transfer Database

- This preliminary database (i.e., TranBase) provides comprehensive, published transfer data that will be useful to the scientific community and risk assessors for evaluating food pathogen cross-contamination.
- This information previously has not been available in a compiled form that offers a baseline for exploring the factors that govern these processes.
- The information within such a database can be a valuable tool for the scientific community, in that it can allow for easier comparisons across studies, determination of the types of studies that are needed, and different or new types of analyses.
- The ability to analyze and identify problematic areas and understand transfer mechanisms will help improve risk models and safety measures, and thus prevent foodborne illness and outbreaks.

5.5 Future work

5.5.1 Standardization of image capturing

Quantification of GGP as a surrogate for *L. monocytogenes* in an actual delicatessen will require greater photographing technology than the one reported in this research. Since coupons were being photographed under controlled conditions, a standard tabletop setup was used; however, in a delicatessen it would be desirable to have an all-inclusive and mobile setup: a camera with an ultraviolet light attachment at a fixed distance and angle from the surface being imaged. This would allow for images to be rapidly taken of several surfaces without re-setup.

5.5.2 Creation of a simple analysis tool

In this research, a MatLab® image analysis code was used to quantify the amount of GGP surrogate on surfaces. This code requires manual input of individual images, cropped area x and y values, different thresholds for recipient surfaces, and different equations for transformation from intensity to concentration. If these steps were put into a simple format, where all images could be uploaded, the cropped area could be recognized, a selection box for recipient surface can be checked, and concentration on these surfaces were outputted into a file, the tool would be much easier for others to use. Automating the code as described would be possible and should be done to increase the utility of the tool.

5.5.3 GGP Model validation, use in other projects

Quantifying this surrogate during experimental food handling scenarios and using it to predict pathogen transfer risk provides the capability to quantitatively map actual cross-contamination routes in food facilities, thus giving a method to fill gaps in the data for risk assessments and validate risk models. This can be used as a tool to quantify the impact of worker training interventions in a retail food-handling environment. GGP image analysis is a valuable approach to assess the strength of control measures, and monitor their impact on food safety.

5.5.4 Enhancement of the TranBase database

This database was intended to be a baseline and serve as a depository for future data. It can then serve as a foundation for model building and risk assessments, which often note that assumptions have to be made because of data unavailability or inaccessibility.

5.5.5 Comparisons with other research groups

Aggregated existing food pathogen transfer data in an easily accessible format allows for easy data comparisons across research groups. This needs to be done on past and current work to maximize understanding of the information available, and should lead to standardizations of methods and reporting so that data comparisons will be simpler for future transfer studies. This important step will help advance linkages between fundamental research and the observed transfer outcomes, while also improving the design of future studies to fill critical data gaps.

5.5.6 The need for standardized testing methods and reporting results

Standard test methods and/or control measures should be used in pathogen transfer studies; in this way, data can be directly compared across studies without uncertainty due to differences in methods. A standard transfer data reporting method should also be suggested, to maximize the future utility of transfer studies.

APPENDIX

Trials for determining GGP concentration on surfaces

In attempt to model the transfer of *Listeria* through a food service environment, several physical surrogates and quantification methods were considered. The use of GGP polymer powder was first considered due to its size (5 um), approximately the size of a bacterium, and past work identifying high risk locations on slicers (Vorst and others 2006a). Tests to quantify GGP included creating calibration curves on several materials including stainless steel and RTE turkey. These were stomached/sonicated or swabbed, and absorbances were taken on a spectrophotometer. Both methods were unsuccessful either due to the presence of protein residues in the slurry or poor recovery. The use of a peanut allergen was then considered because of the high sensitivity of tests to determine its presence. Although there was high sensitivity, the recovery process of the protein was time-consuming and the protein was undesirable to spread. GGP had the qualities desirable: correct size, visibility, and rapid quantification. Therefore, capturing images of the indicator was attempted followed by image analysis algorithms.

GGP absorbance on surfaces

Methods and Results:

1. A GGP suspension containing 1g GGP in 10 mL 70% ethanol was created.
2. Six serial dilutions of 1:1 (vol/vol) were made from the suspension.
3. Six ten by ten square cut of turkey were inoculated with 100 µl of each of the dilutions of GGP suspension and spread with a hockey stick.
4. The suspension was allowed to soak in for 5 minutes at 7°C.

5. Each turkey piece was placed in a filter bag with 15 ml of 70% ethanol and massaged to release the GGP while keeping it in suspension.
6. Specimen from each bag was collected for absorbance readings with a spectrophotometer at 370 nm.

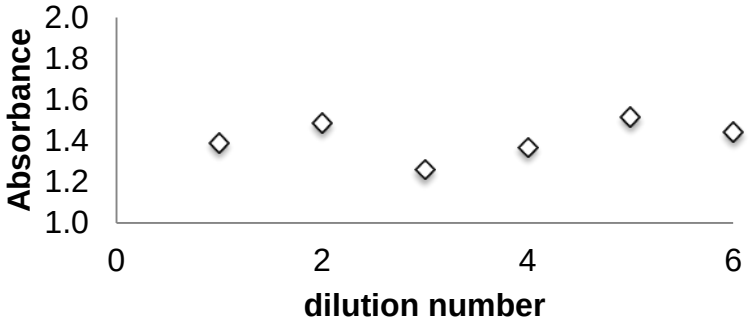

Figure 6.1 Absorbance readings of GGP serial dilutions on RTE turkey at 370nm.

7. Since this did not work due to soils in the solution from the turkey, the specimen was centrifuged for 10 minutes at 2000 rpm to separate the turkey solids.
8. It was visible that both the turkey pieces and GGP were stuck to the wall, not in solution, therefore absorbance could not be read.
9. The specimen was also filtered, however the filter caught mixtures of solids and GGP as well.

Conclusions: When attempting to determine absorbance readings from GGP on food surfaces, a spectrophotometer cannot properly estimate the correct value. This is likely due to the presence of meat pieces and/or fats and oils that alter the readings. Attempts to isolate the GGP for readings failed as well, therefore a new method for quantification of GGP on RTE surfaces must be considered.

Peanut allergen on RTE surfaces

Methods and Results:

1. A peanut allergen test-kit was obtained from Neogen Corporation. The kit including 5 solutions of different known concentrations of peanut allergen, as well an unknown spike solution, and extraction materials to determine concentrations in unknown amounts.
2. The vials of known concentrations were measured by pipetting 150 µl into a microtiter plate, and using the Microplate reader (Molecular Devices Corp. 2001) to relate readings to concentrations for a calibration curve.

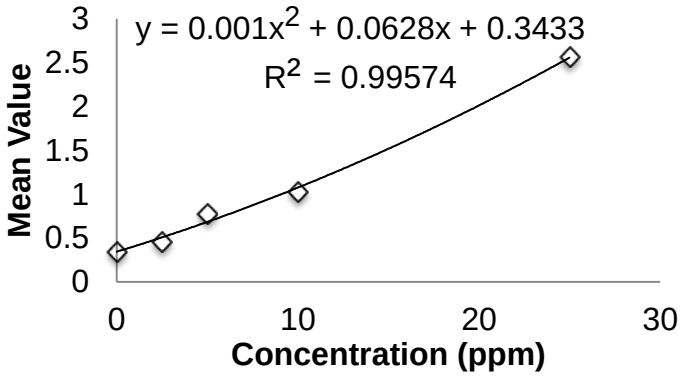

Figure 6.2 Calibration curve for known concentrations of peanut allergen using a Microtiter plate reader.

3. The unknown spike solution was diluted serially 5 times and was measured by pipetting 150 µl into the microtiter plate.

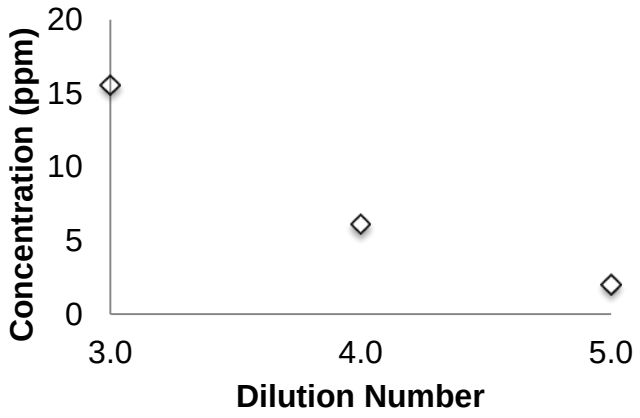

Figure 6.3 Comparing dilution number to concentration by use of calibration curve.

4. Once the curve was created, the spike solution was serially diluted 5 times and used to inoculate 5 stainless steel coupons with 100 µl of teach of the dilutions. The inoculated stainless steel coupons sat for 10 minutes to dry. A KimWipe was then wetted with 1 ml of PBS and half the plate was whipped, followed by putting each coupon in a Whirlpack bag with the addition of 25 ml of PBS. The bags were then sonicated. The extraction procedures of the Neogen Veratox kit were then followed and the final solution was measured using the Microplate reader.

Table 6.1 Recovery of known concentrations of peanut allergen by means of sonication or composite tissue.

Spike solution on Stainless steel and recovered				
	KimWipe	Sonication	KimWipe	Sonication
Dilution	Mean Value	Mean Value	Conc. (ppm)	Conc. (ppm)
0	1.62	1.73	16.1	17.3
1	0.757	1.58	5.86	15.6
2	0.614	0.710	3.97	5.25
3	0.659	0.337	4.57	0.166
4	0.300	0.503	-0.357	2.47
5	0.314	0.323	-0.159	-0.031
6	0.535	0.466	2.908	1.96

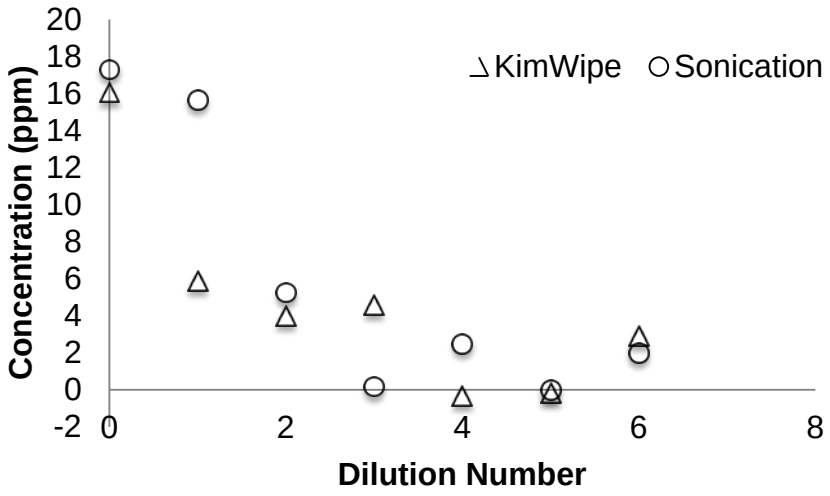

Figure 6.4 Concentrations of peanut allergen recovered from stainless steel by using KimWipe and sonication.

5. The method above was repeated for turkey. Five pieces were inoculated with dilutions of the spike solution and allowed to soak followed by additional of 25 ml of PBS in a whirlpack bag and stomached. The Neogen kit was used to extract the peanut allergen and and the final solution was measured using the Microplate reader.

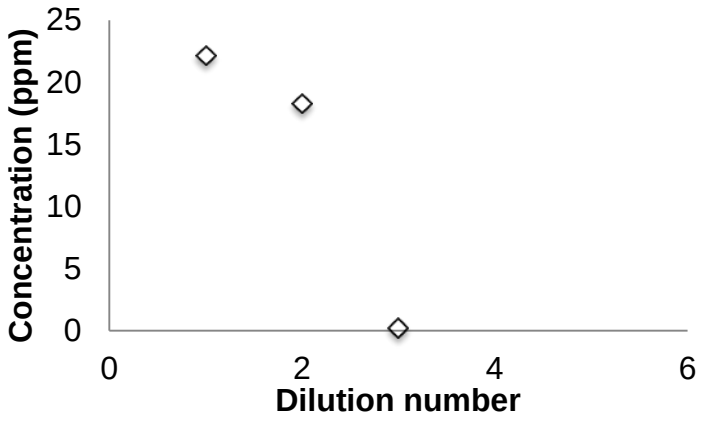

Figure 6.5 Concentrations of peanut allergen recovered from turkey by stomaching.

Conclusions:

Using peanut allergen as an indicator of *Listeria monocytogenes* on common RTE food and contact surfaces is not ideal. The extraction process is extensive and there is much room for error. As seen in the graphs above, simple recovery from stainless steel and turkey surfaces are not representative of serial dilutions inoculated onto the surfaces. Because these simple tests did not yield accurate results, furthering tests with actual transfer events was not attempted.

Trials and final protocol for imaging

Types of cameras considered

A point-and-shoot camera was utilized to take a single image of all coupons that served as transfer events. Since the camera could not be set to manual, capturing all the coupons at once would eliminate variability between photo, and therefore would give a more accurate account of indictor on the surfaces. Variability was found however due to the position of the coupon relative to the ultra-violet light source.

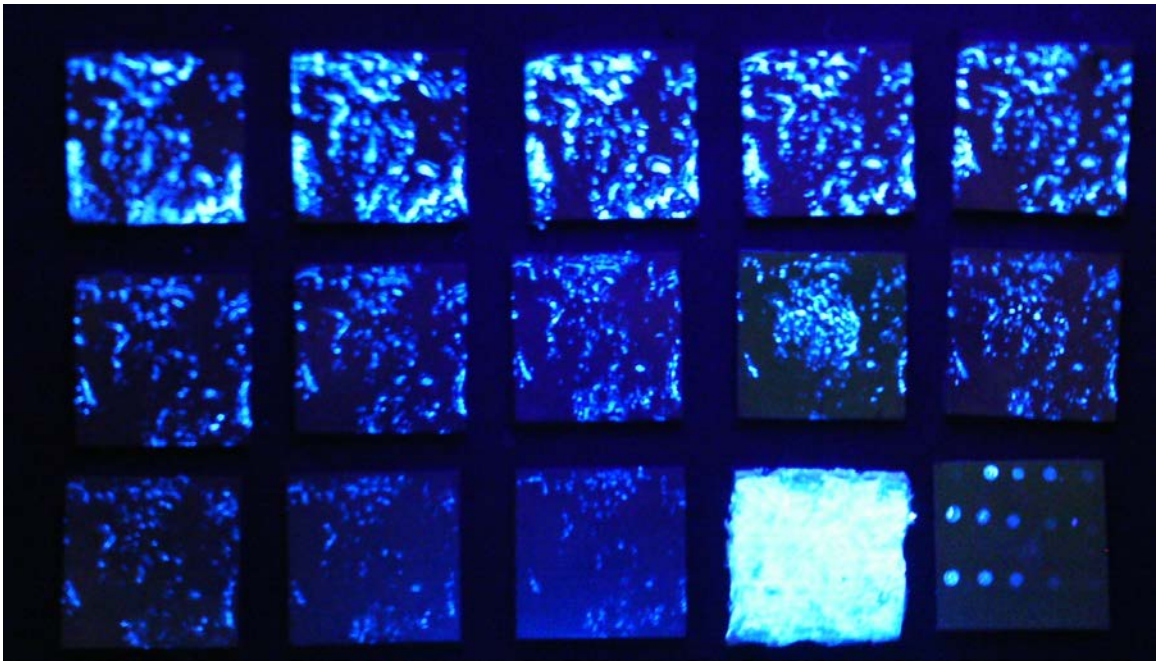

Figure 6.6 Photograph of GGP on stainless steel coupons taken with a point-and-shoot camera.

Because of the importance of consistent lighting, it was determined that a Digital SLR camera with manual settings should be used, and each coupon that served as a transfer event

should be photographed separately in the same location to the ultra-violet source to control variability.

Light sources considered

A bench-top 385 nm ultraviolet light was used for most of the images. The long wavelength UV, however, allows more background illumination than a shorter wavelength source, such as a 360 nm light. Background illumination/reflection can be a problem during image analysis because the algorithm may not recognize a difference between the indicator and background noise. Shorter wavelength LED flashlights were later considered due to this. The smaller size of the LED's also allowed for mobility in comparison to the bench top tube UV source.

Imaging protocol

Camera settings

Since GGP is sensitive to ultra-violet light controlled imaging is required. The digital SLR camera should have manual settings of the following:

Shutter speed: 1/5

Aperture: fsstop 5.6

ISO speed: 400

Each surface must be photographed separately but under the same conditions.

Tube lights

The black light should be at a constant or nearly constant distance from the surfaces being photographed. A distance of 10+-1 inch is preferred. The more variability in distance however, the less accurate the outcome, therefore choosing a distance is best. The light should also be angled at approximately 80 degrees from the camera (170 from the surface being photographed), which must be directly normal to the surface being imaged so that the surface appears to be 2-D. The distance from the camera lens to the surface being photographed was held consistently at 18 inches.

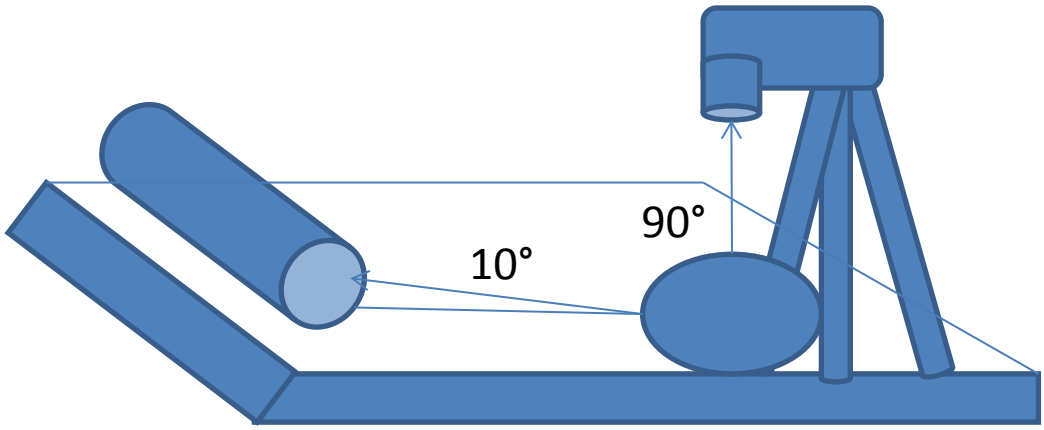

Figure 6.7 Set-up of digital camera with tube light. Camera stands on a tripod directly above the sample being photographed. The tube light is 10 degrees above the sample.

Bundle of UV LED flashlights

The UV- LED lights should be at a constant or nearly constant distance from the surfaces being photographed. A distance of 8+-1 inch is preferred. The more variability in distance however, the less accurate the outcome, therefore choosing a distance is best. The light should

also be angled at approximately 45 degrees from the surface being photographed. The distance from the camera lens to the surface being photographed was held consistently at 18 inches.

Figure 6.8 Tripod with camera directly above sample with bundle of UV-LED lights attached to tripod stand.

Comparing UV light sources

Both light sources with each of the mentioned conditions above demonstrated to not have a significant difference when determining the concentration of GGP on surfaces, therefore both methods can be used.

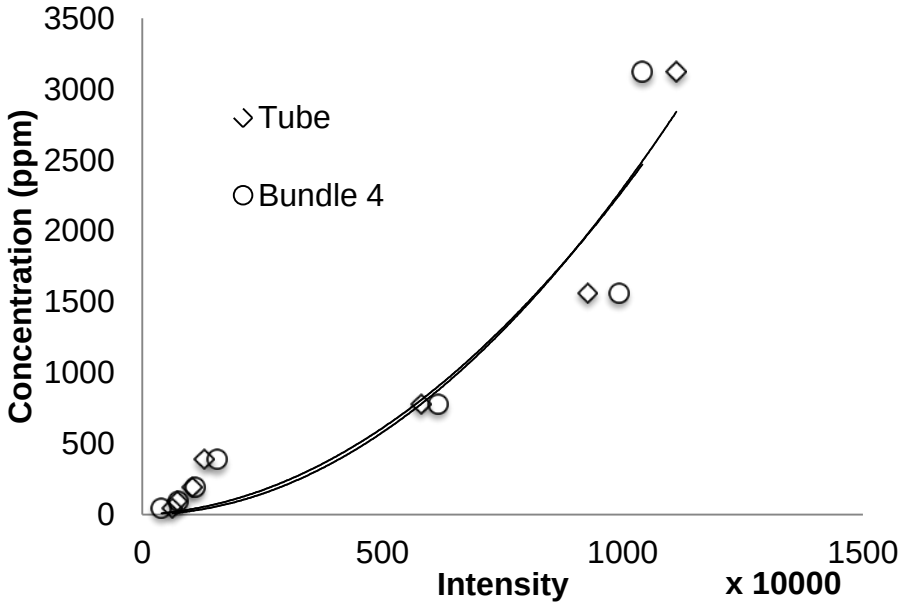

Figure 6.9 Calibration curve comparing intensity to concentration between the tube light and bundle LED's.

Calibration before imaging

For calibration before imaging, drip dots of the suspension at different known concentrations onto the surface and photograph with the settings described above. Take images of the surfaces without the GGP so that these can be used for calibration as well. Calibration will relate intensity to the known concentration.

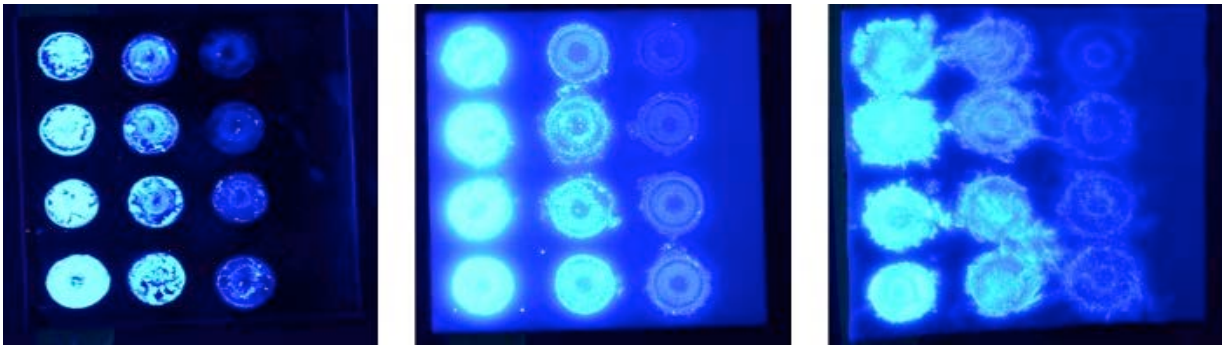

Figure 6.10 Known concentrations of GGP on stainless steel, high density polyethylene, and turkey surfaces.

Challenges with using different cameras:

Different cameras have different sensitivities. Therefore, a Nikon and a Cannon digital SLR Camera were used to photograph images under the same conditions and compared. The curves below demonstrate that with the same camera settings and conditions (distances, angles, light source etc.), the Nikon camera is more sensitive.

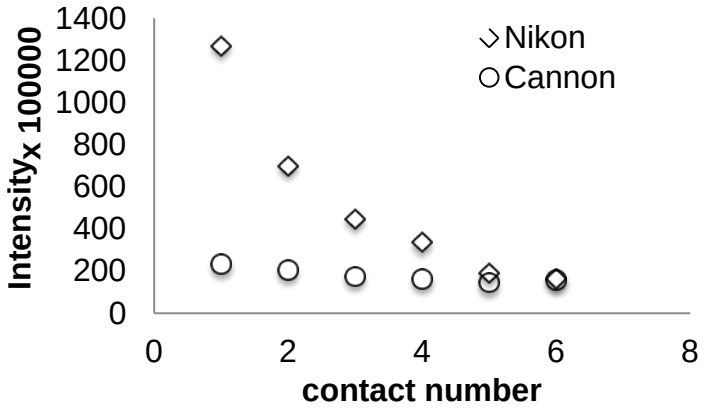

Figure 6.11 Graphical comparison of Cannon and Nikon digital SLR camera sensitivities during transfer from turkey to stainless steel surfaces.

MatLab trials and final code for image analysis

Trial Code 1:

The first algorithm considered filters a single or multiple color components: red, blue, green, or grey. The image is read and cropped to the size of the coupon, followed by calculating reference values of GGP on black and stainless steel surfaces for calibration. This calibration is manual however and has different wavelength cut-offs for each color and for each surface. The cropped image is then converted to grey, then to a binary image. The average amount of red, green, and/or blue pixels can then be determined.

From Figure 6.12, it is apparent that calibration curves for HDPE and turkey are limiting compared to stainless steel. The code also does not recognize the value of intensity of each color component, it is binary, simply counting if a pixel is illuminated (a value of 1), or not illuminated (a value of zero). It therefore, is not the most accurate account of amount of GGP on surfaces.

Table 6.2 Cut-off for background values for each surface and color.

Dilutions	**Stainless Steel**			**HDPE**		
ppm	r	g	b	r	g	b
100000	140	255	255	145	255	-
50000	133	240	255	140	255	
25000	125	228	255	135	255	
12500	115	215	255	120	255	
6250	91	195	255	90	235	
3125	72	170	255	77	220	
1562.5	61	155	255	75	190	
781.25	55	125	255	75	168	
390.625	39	80	202	65	143	
195.3125	25	61	195	53	116	
97.65625	10	30	180	34	77	
48.828125	10	15	166	25	60	

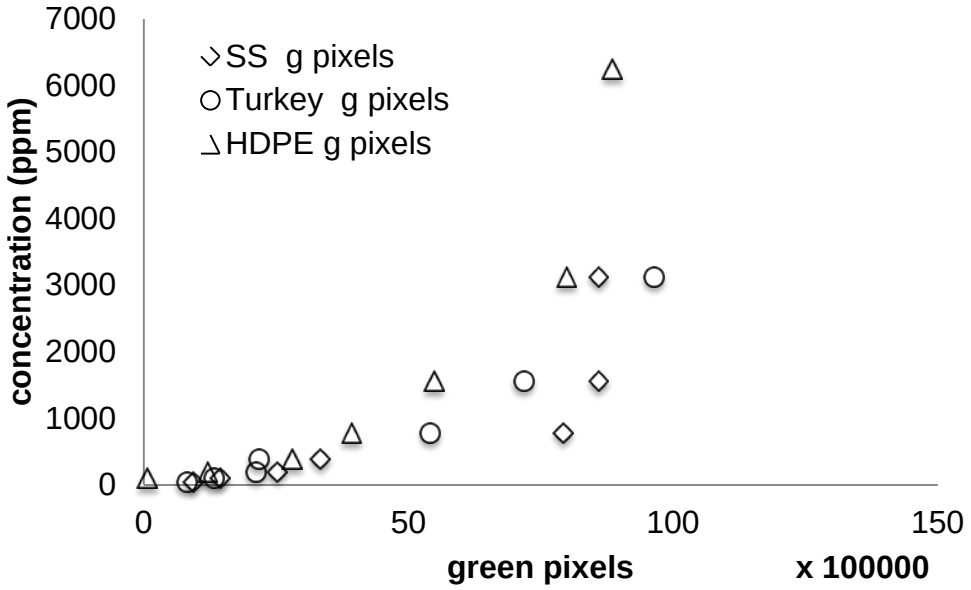

Figure 6.12 Average number of green pixels for each surface containing a known concentration of GGP.

Trial Code 2:

The second code uses black and white plates for calibration to bring background noise to zero. It only considers the green component of each surface. Then, depending on the surface, a threshold can be chosen. The problem with this code is that it is binary as before, and since the threshold is chosen by the user, it is subjective.

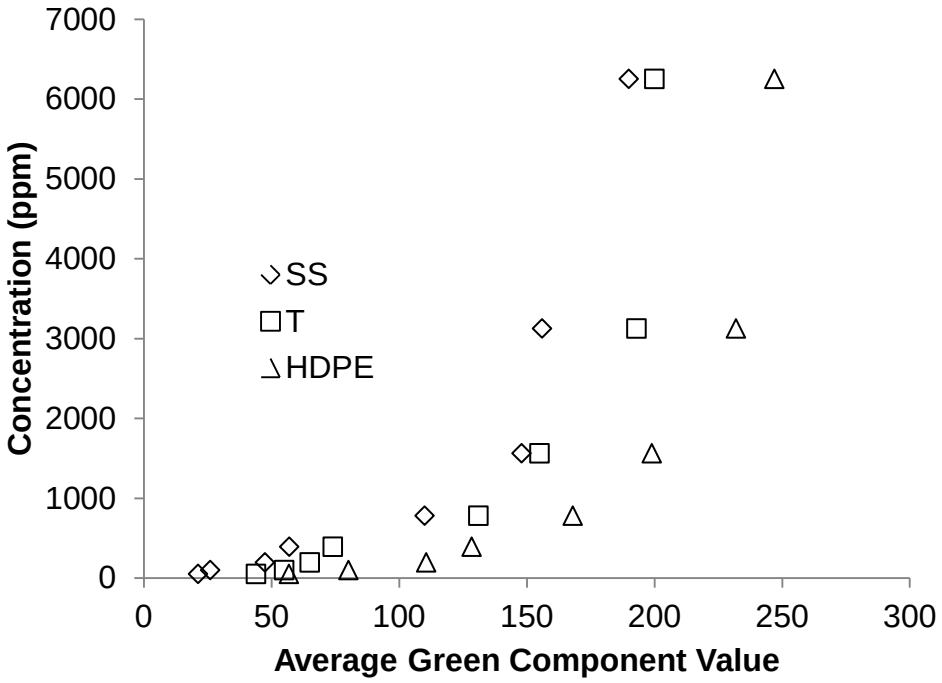

Figure 6.13 Comparing the green component value of known concentrations on different surfaces.

Trial Code 3:

The third code works similarly to the second code, however, it is more user friendly in that it downloads all the images in the file at once and crops them based on recognition. It also has a thresholding toolbox that can be used to appropriately choose the correct threshold for each surface. This however, as before, changes the images to binary when determining intensity and is subjective because the threshold is chosen by the user.

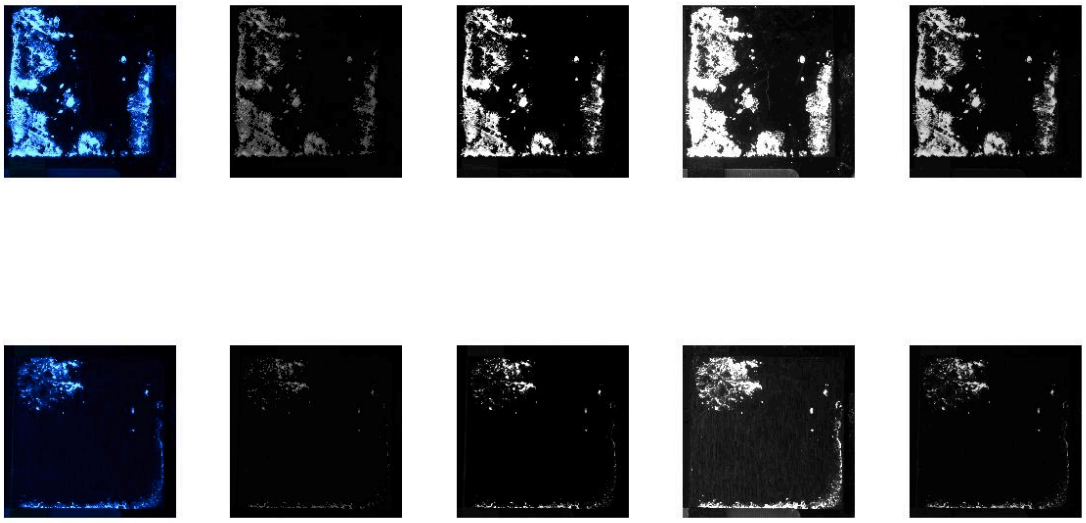

Figure 6.14 Images changed from original (left), to red, blue, green, and grey components.

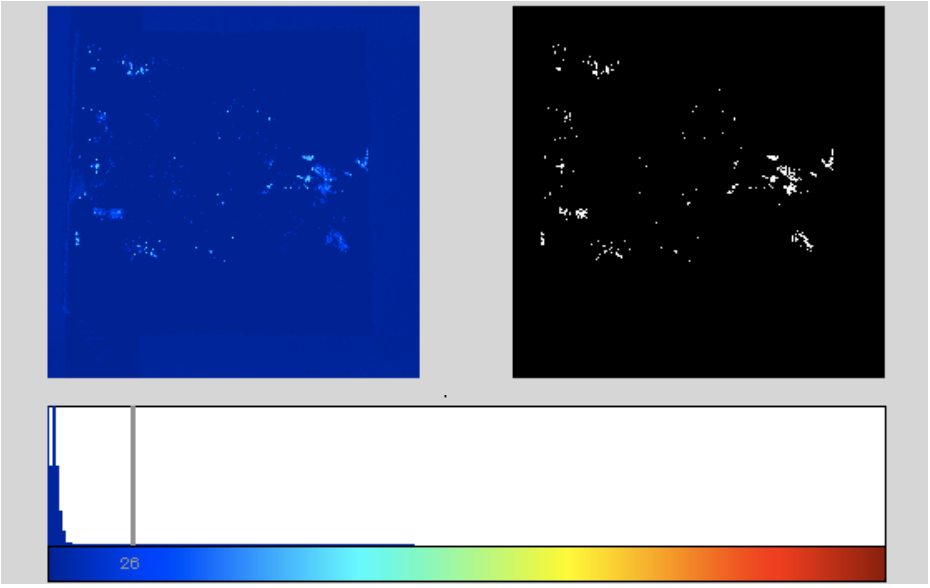

Figure 6.15 Threshtool that is used to subjectively choose a threshold for each image.

Trial Code 4, Final code:

The final code mixes ideas from each of the codes above. The images are imported into the program and cropped to the size of the surface. By using the red component, the green component, used to track the GGP, was isolated from the background surface. Doing so will change the image into binary, as before. This binary green component is then multiplied by the original image intensity values so as to give the correct intensity value. The intensity can then be related the concentration by creating a calibration curve and inputting it into the end of the code.

```
% Question to: Dr. Sangyup Jeong (Biosystems & Agricultural Engineering)
% December 22, 2011
% MICHIGAN STATE UNIVERSITY

clear all
%% Reading image file
% I=imread('C:\Documents and Settings\jeongsa1\My Documents\Academic-major\Research\Image Processing\Florescence\image_1.bmp');
fp='C:/Users/abenoit/Pictures/THESIS WORK/824 uv ss to turkey/';
% fp_Ref='C:\Users\jeongsa1\Documents\Academic-Active\Research\Topics\Image Processing\Image Processing\Amanda\Raw Files 100711\Reference Images\';
%% Variables to be CHANGED!
% File name
I_org=imread([fp,'DSC_0620.JPG']); % test image
% I_org=imread([fp_Ref,'DSC_0748_TURKEY.JPG']); % reference image
```

```
% Image crop parameters
dx=2200; dy=2200; % for test image
Xw=1054; Yw=358; % upper left corner of the crop image
figure, imshow(I_org)
%% Cropping image of interest
I_crop=imcrop(I_org,[Xw Yw dx dy]);
%% Nonuniform background correction
Rimg=I_crop(:,:,1);
Gimg=I_crop(:,:,2); % Extracting green component image
Bimg=I_crop(:,:,3);
% Identify backgroud illumination region
Bimg_bw=Bimg;
for i=1:(dx+1)
   for j=1:(dy+1)
      if (Bimg(i,j) == 255), Bimg_bw(i,j)=1; else Bimg_bw(i,j)=0; end; % convert blue image into binary
   end
end
B_area=sum(sum(Bimg_bw)); % area of background illumination
R_sum=sum(sum(Rimg)); % sum of red image
R_thresh=R_sum/B_area; % threshold for red image to identify effective green image area
% Isolate only background illumination in green image using red image and
% threshold
Gimg_bkg=Gimg;
```

```
for i=1:(dx+1)
    for j=1:(dy+1)
        if (Rimg(i,j)>= R_thresh), Gimg_bkg(i,j)=0; end; % 150 for steel, 100 for black
    end
end
% Compensating green image with blurred background illumination
se=strel('disk',3); %Create morphological structuring element
h=fspecial('average',3); % Create predefined 2-D filter
[row, col]=size(Gimg_bkg);
sImg=imresize(Gimg_bkg, 0.4); % image reduction 1=full
bkg=imopen(sImg,se);
blurred=imfilter(bkg,h,'replicate'); % blurring background
blurred=imresize(blurred, [row,col]); % back to original image size
nGimg=Gimg-blurred; % background corrected image
% Total effective gfp intensity
G_sum=sum(sum(nGimg));
I=G_sum;
% Plotting images
% figure, imshow(I_org)
% figure, imshow(Rimg)
% figure, imshow(Bimg)
% figure, imshow(Gimg)
% figure, imshow(blurred)
```

figure, imshow(nGimg)

%% Calculating intensity per unit area

%Different equation for each surface. Insert here:

C=

Minimizing differences between surface transfer data for average *L. monocytogenes* and GGP

Table 6.3 Minimizing differences between average *L. monocytogenes* and GGP transfer using of an offset and multiplier for (a) high density polyethylene to ham, (b) ham to HDPE, (c) turkey to stainless steel, (d) stainless steel to turkey, (e) turkey to HDPE, and (f) HDPE to turkey.

(a) HDPE to H				
	LM AVG	GGP AVG	NEW GGP	Resid^2
1	6.79131572	5.139312246	6.985257642	0.037613469
2	6.713408068	4.537693194	6.592683197	0.014574494
3	7.191851594	4.764728927	6.740830811	0.203419747
4	6.871166141	4.709566741	6.704835832	0.027665772
5	6.868944035	4.581027553	6.620960164	0.061496
6	6.814991337	4.678936994	6.684849006	0.016937026
7	6.61800081	4.691258358	6.692889065	0.005608251
8	6.67244164	4.471922154	6.549765624	0.015049405
9	6.395098585	4.308713777	6.443267271	0.002320222
10	6.300350348	4.609444995	6.639503396	0.11502479
11	6.136500636	4.219951086	6.385346958	0.061924492
12	6.434028181	4.806227472	6.767909854	0.111476972
	multiplier	**0.652529942**	**sum**	**0.67311064**
	offset	**3.631702522**	**RMSE**	**0.236838665**

Table 6.3 (cont'd)

(b) H to HDPE				
	LM AVG	GGP AVG	NEW GGP	Resid^2
1	6.556012097	3.347134783	6.457363794	0.009731488
2	6.463700195	3.396460892	6.482653304	0.00035922
3	6.209548107	2.876654053	6.21614819	4.35611E-05
4	6.035103518	2.955833693	6.256743614	0.049124332
5	6.318017991	2.303904611	5.922499388	0.156434965
6	6.00E+00	2.438738172	5.991628593	1.84876E-05
7	6.073417047	2.386074809	5.964628072	0.011835041
8	5.96E+00	2.48998954	6.017905184	0.003089151
9	5.791971182	2.173138173	5.855455398	0.004030246
10	5.671296687	2.13350889	5.835137453	0.026843797
11	5.664476339	1.90474529	5.717850291	0.002848779
12	5.696979437	1.910215527	5.720654883	0.000560527
	multipier	**0.512700285**	sum	**0.264919594**
	offset	**4.741286838**	RMSE	**0.148582074**

(c). T to SS				
	LM AVG	GGP AVG	NEW GGP	Resid^2
1	8.083955419	3.893289067	8.093640644	9.38036E-05
2	7.752157502	3.582813664	7.630566579	0.014784353
3	7.311162429	3.154543318	6.991801359	0.101991493
4	7.175896768	3.337652681	7.264908974	0.007923173
5	6.934995612	3.205546996	7.067873345	0.017656492
6	6.690734643	2.98044376	6.732131868	0.00171373
7	6.673660275	2.95944094	6.700806161	0.000736899
8	6.498355705	2.91220328	6.630351192	0.017422809
9	6.42541185	2.924923652	6.649323627	0.050136484
10	6.418436734	2.854844064	6.544799922	0.015967655
11	6.450024598	2.644208167	6.230636481	0.048131146
12	6.57281984	2.79275087	6.452187923	0.014552059
	multiplier	**1.4915**	**sum**	**0.291110097**
	offset	**2.2868**	**RMSE**	**0.15575357**

Table 6.3 (cont'd)

(d) SS to T				
	LM AVG	GGP AVG	NEW GGP	Resid^2
1	7.90894182	3.904892358	8.081945393	0.029930236
2	7.806806924	3.183940289	7.515277067	0.084989657
3	7.459382641	2.88193127	7.277897978	0.032936683
4	7.416234964	2.768141072	7.188458883	0.051881943
5	7.382543669	2.782166689	7.199483018	0.033511202
6	7.392241527	3.090896547	7.442144686	0.002490325
7	7.337994299	2.568376296	7.031443769	0.093973227
8	6.854554309	2.773319604	7.192529209	0.114227033
9	6.976585086	2.788876906	7.204757248	0.052062535
10	6.75916159	2.465928005	6.950919412	0.036771062
11	6.874445704	2.451283026	6.939408458	0.004220159
12	6.731794453	2.37160505	6.876781569	0.021021264
	multiplier	**0.786**	**sum**	**0.558015329**
	offset	**5.0127**	**RMSE**	**0.215641548**

(e) T to HDPE				
	LM AVG	GGP AVG	NEW GGP	Resid^2
1	7.531819117	5.051792022	7.537883696	3.67791E-05
2	7.527348411	4.759225895	7.390395751	0.018756031
3	7.201466365	4.611487331	7.315918034	0.013099185
4	7.020973768	3.999215007	7.007260339	0.000188058
5	6.997440278	3.928295183	6.971508357	0.000672465
6	6.885310961	3.532351468	6.771905876	0.012860713
7	6.51059465	3.045207688	6.526327772	0.000247531
8	6.494154036	3.117912611	6.562979655	0.004736966
9	6.611926365	3.193465856	6.601067431	0.000117916
10	6.452357773	2.956495704	6.481606436	0.000855484
11	6.241202285	2.952427242	6.479555449	0.056812231
12	6.240855646	2.138070371	6.069023231	0.029526379
	multiplier	**0.50411832**	**sum**	**0.137909738**
	offset	**4.991182787**	**RMSE**	**0.107202977**

Table 6.3 (cont'd)

(f) HDPE to T				
	LM AVG	GGP AVG	NEW GGP	Resid^2
1	8.202987532	3.349149808	8.411797267	0.043601505
2	8.031408464	2.840678345	7.904777242	0.016035466
3	7.918641835	2.496468054	7.561549505	0.127514932
4	7.617175143	2.448067728	7.513287338	0.010792676
5	7.792099727	2.496736576	7.56181726	0.053030014
6	7.49E+00	2.313830912	7.379433703	0.011498888
7	7.207499723	2.199385812	7.265315288	0.00334264
8	7.19E+00	2.434564713	7.499822867	0.096509759
9	7.255272505	2.305917712	7.371543091	0.013518849
10	7.353788045	2.233972253	7.299803001	0.002914385
11	7.325652471	2.181678112	7.247658135	0.006083116
12	6.8162413	2.113620898	7.179795191	0.132171432
	multiplier	**0.997145486**	**sum**	**0.517013663**
	offset	**5.072207654**	**RMSE**	**0.207567994**

<u>Replicate data for *L. monocytogenes* transfer between surfaces</u>

Table 6.4 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated turkey to stainless steel coupons.

	Log CFU on slice		
1	8.653212514	8.200713734	7.397940009
2	8.101231387	7.882239848	7.273001272
3	7.903089987	7.217483944	6.812913357
4	7.740362689	6.821185883	6.966141733
5	7.329906123	6.68797462	6.787106093
6	7.246129126	6.602059991	6.224014811
7	7.015988105	6.68797462	6.317018101
8	6.935759104	6.301029996	6.258278015
9	6.278753601	6.896250562	6.101231387
10	6.545616333	6.70969387	6
11	6.574031268	6.382467322	6.393575203
12	7.197280558	6.193820026	6.327358934

Table 6.5 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated stainless steel to turkey coupons.

	Log CFU on slice			
1	7.966141733	7.419129308	8.281601444	7.829303773
2	7.84509804	7.860338007	8.342422681	7.67669361
3	7.431363764	7.373371817	7.740362689	7.404406051
4	7.804480189	7.130333768	7.916453949	7.37794338
5	7.342422681	7.494850022	7.595220567	7.303735889
6	7.449092531	7.761551989	7.478927056	7.157607853
7	7.482516287	7.005395032	7.267171728	7.875061263
8	7.395763089	7.258278015	6.942008053	6.317018101
9	7.015988105	6.769007871	7.06069784	7.764362966
10	7.161368002	6.759667845	6.769007871	6.860338007
11	7.065392962	7.417056299	6.665111737	7.026328939
12	7.041392685	7	6.447158031	7.311753861

Table 6.6 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated ham to stainless steel coupons.

	Log CFU on slice		
1	6.942008053	7.329906123	5.641474111
2	6.916453949	7.126293791	5.278753601
3	6.146128036	6.942008053	5.079181246
4	6.375663614	6.84509804	5.667452953
5	6.70969387	6.146128036	4.966141733
6	6.23363058	5.829303773	4.413299764
7		5.983400738	4.328379603
8		5.960232873	4.432969291
9	6.070037867	5.317018101	4.390935107
10	6.287241711	5.249198357	4.298853076
11	6.210853365	5.966141733	4.334453751
12	5.916453949	5.373371817	4.190331698

Table 6.7 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated stainless steel to ham coupons.

	Log CFU on slice		
1	8.096910013	8.118099312	8.68797462
2	7.84509804	8.026328939	8.16879202
3	7.574031268	7.860338007	7.889301703
4	7.496583734	7.556302501	7.740362689
5	7.261262869	7.458637849	7.566732029
6	7.138302698	7.410777233	7.264227348
7	7.408663874	7.817069316	6.882239848
8	6.994537104	7.270096281	7.157607853
9	6.90982337	6.977723605	7.382467322
10	6.795880017	6.948168362	6.829303773
11	6.386944624	7.16879202	7.06069784
12	7.105510185	7.101231387	7.146128036

Table 6.8 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated turkey to HDPE coupons.

	Log CFU on slice	
1	7.210853365	7.852784869
2	7.172456974	7.882239848
3	6.90982337	7.49310936
4	6.821185883	7.220761654
5	6.837272703	7.157607853
6	6.804480189	6.966141733
7	6.477121255	6.544068044
8	6.347330015	6.640978057
9	6.45484486	6.769007871
10	6.342422681	6.562292864
11	6.157607853	6.324796718
12	6.092545208	6.389166084
13	5.787106093	6.239924813
14	5.896250562	6.433369747
15	6.227243782	6.146128036

Table 6.9 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated HDPE to turkey coupons.

	Log CFU on slice		
1	8.122215878	8.281601444	8.190331698
2	7.875061263	8.157607853	8.015988105
3	7.971971276	7.759667845	7.989004616
4	7.337459261	7.67669361	7.740362689
5	7.711807229	7.581209852	7.983400738
6	7.204119983	7.16879202	7.787106093
7	7.303735889	7.130333768	7.16879202
8	7.190331698	7.314393957	7.010723865
9	7.389166084	7.284430734	7.010723865
10	7.516865761	7.176091259	7.298307137
11	7.643452676	6.90982337	7.055951405
12	6.498310554	6.90982337	6.922984816
13	6.89279003	6.68797462	6.942008053
14	6.799340549	6.571126277	6.821185883
15	6.8162413	6.67669361	6.431363764
16	6.953034457	6.412880358	6.395763089

Table 6.10 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated ham to HDPE coupons.

	Log CFU on slice		
1	7.270096281	6.301029996	6.096910013
2	7.118099312	5.698970004	6.574031268
3	7.079181246	5.896250562	5.653212514
4	7.036429266	4.875061263	6.193820026
5	7.070037867	5.640978057	6.243038049
6	6.515211304	5.375663614	6.096910013
7	6.611457766	5.795880017	5.812913357
8	6.759667845	5.439332694	5.68797462
9	6.458637849	5.458637849	5.458637849
10	6.301029996	5.544068044	5.16879202
11	0	5.419129308	5.90982337
12	6.319626484	5.896250562	4.875061263

Table 6.11 Sequential transfer of *L. monocytogenes* from 10^9 CFU inoculated HDPE to ham coupons.

	Log CFU on slice	
1	6.954242509	6.62838893
2	7.051152522	6.375663614
3	7.088136089	7.2955671
4	6.929418926	6.812913357
5	7.193820026	6.544068044
6	6.989004616	6.640978057
7	6.67669361	6.559308011
8	6.867762025	6.477121255
9	6.769007871	6.021189299
10	6.462397998	6.138302698
11	6.574031268	5.698970004
12	6.48784512	6.380211242

Table 6.12 Sequential transfer of *L. monocytogenes* from 10^7 CFU inoculated stainless steel to turkey coupons.

	Log CFU on slice		
1	6.2955671	6.197280558	5.68797462
2	5.852784869	5.730378469	5.653212514
3	5.882239848	5.837272703	5.243038049
4	5.750122527	5.84509804	5.559308011
5	5.478927056	5.740362689	5.292809665
6	5.33243846	5.391376239	5.431363764
7	5.010723865	5.342422681	5.06069784
8	4.935759104	4.90982337	4.588271707
9	4.720159303	5.005395032	4.812913357
10	4.698970004	4.860338007	4.456745495
11	4.267171728	4.875061263	4.460521993
12	4.16879202	4.698970004	4.214181309
13	4.134336511	4.451018452	4.327358934
14	3.983400738	4.230448921	4.25224605
15	3.903089987	3.68797462	3.882239848
16	3.730378469	4.84509804	3.615423953
17	3.574031268	4.896250562	3.720159303
18	3.829303773	4.588271707	3.62838893

Table 6.13 Sequential transfer of *L. monocytogenes* from 10^7 CFU inoculated turkey to stainless steel coupons.

	Log CFU on slice		
1	5.640978057	6.264227348	5.837272703
2	5.278753601	6.065392962	5.435366507
3	5.079181246	5.867762025	5.421192468
4	5.667452953	6.139879086	6.01494035
5	4.966141733	5.105510185	5.77815125
6	4.412880358	5.031408464	5.267171728
7	4.327358934	4.942008053	5.070037867
8	4.433369747	4.916453949	4.309097617
9	4.391376239	4.67669361	4.456745495
10	4.298307137	4.740362689	4.04630002
11	4.334956116	4.948168362	5.339948062
12	4.190331698	4.994537104	5.412880358
13	4.787106093	4.339948062	5.015988105
14	4.462397998	4.698970004	5.45484486
15	5.005395032	5.031408464	4.339948062

Table 6.14 Sequential transfer of *L. monocytogenes* from 10^7 CFU inoculated turkey to HDPE coupons

	Log CFU on slice		
1	6.122215878	6.088136089	6.40654018
2	5.929418926	5.812913357	6.239924813
3	5.769007871	5.591064607	5.867762025
4	5.612783857	5.544068044	5.759667845
5	5.635986112	5.322219295	5.787106093
6	5.576916956	5.243038049	5.402261382
7	5.347330015	5.010723865	5.138302698
8	5.220761654	4.653212514	4.821185883
9	5.569666462	3.574031268	5.015988105
10	5.214181309	5.126293791	5.021189299
11	5.314393957	4.665111737	4.942008053
12	4.653212514	4.67669361	5.545616333
13	3.243038049	4.916453949	4.948168362
14	4.750122527	4.359361103	4.812913357
15	4.829303773	4.67669361	4.464265934
16	4.759667845	4.698970004	4.602059991
17	5.04630002	4.431363764	4.165095875
18	5.101231387	4.179695383	4.186815124
19	5.179695383	4.484299839	4.220761654
20	4.80106053	4.079181246	3.971971276

Table 6.15 Sequential transfer of *L. monocytogenes* from 10^7 CFU inoculated HDPE to turkey coupons.

	Log CFU on slice		
1	5.942008053	5.439332694	5.615423953
2	5.821185883	5.301029996	5.730378469
3	5.70969387	5.109747238	5.544068044
4	5.477121255	5.68797462	5.544068044
5	5.588271707	5.134336511	5.301029996
6	5.537819095	5.200713734	5.193820026
7	5.270096281	4.67669361	5.010723865
8	5.031408464	5.220761654	5.026328939
9	5.27588696	4.948168362	4.804480189
10	5.349763044	5.197280558	4.615423953
11	5.06069784	5.179695383	4.439332694
12	4.867762025	4.916453949	4.375663614
13	5.005395032	4.559308011	4.489606966
14	4.929418926	4.875061263	4.23363058
15	4.261262869	4.77815125	4.179695383
16	4.787106093	5.055951405	4.309097617
17	4.306425028	4.615423953	4.142232992
18	4.966141733	4.539389782	3.875061263
19	4.653212514	4.041392685	4.230448921
20	4.544068044	4.698970004	4.230448921

TranBase

Table 6.16 Bacterial transfer curve publications identified from the ISI Web of Knowledge.

Date	Author	Title	Bacteria	Data Sets	Data points	Rep	Equation	Surfaces
1971	Pether and Gilbert	The survival of salmonellas on finger-tips and transfer of the organism to foods	Salmonella				No curve	meat, hand
1990	Boer and Hahne	Cross-contamination with Campylobacter jejuni and Salmonella spp. From raw chicken products during food preparation	Campylobacter, Salmonella				No curve	cutting board, raw chicken, hands, beef
1990	Dickenson	Transfer of Listeria monocytogenes and Salmonella typhimurium between beef tissue surfaces	Listeria, Salmonella				No curve	cutting board, meat
1990	Scott and Bloomfield	The survival and transfer of microbial contamination via cloths, hands, utensils	Escherichia, Salmonella, Staphylococcus, Klebsiella	36	108	2	No curve	laminate, cloth, stainless steel, fingers, work surface
1994	Humphrey et al.	Contamination of hands and work surfaces with Salmonella enteritidis PT4 during the prepartion of egg dishes	Salmonella					fingers, egg, work surfaces
1996	Bradford et al.	The cross-contamination and survival of Salmonella enteritidis PT4 on sterile and non-sterile foodstuff	Salmonella	8	82	1		egg, melon, meat
1998	Zhao et al.	Development of a model for evaluation of microbial cross-contamination in the kitchen	Enterobacter					cutting board, vegetables, hand, meat
2001	Chen et al.	Quantification and variability analysis	Enterobacter	50	452	1	Beta,	chicken, hand,

Table 6.16 (cont'd)

		of bacterial cross-contamination rates in common food service tasks					Weibull, Gamma	spigot, lettuce, cutting board
2000	Montville et al.	Glove barriers to bacterial cross-contamination between hands to food	Enterobacter	91	480	1	No curve	chicken, lettuce, glove
2001	Sattar et al.	Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using Staphylococcus aureus as a model	Staphylococcus	32	64	1	No curve	finger pad, fabric
2002	Gill and Jones	Effects of wearing knitted or rubber gloves on the transfer of Escherichia coli between hands and meat	E. coli	44	88	4	No curve	meat, glove, hand
2002	Gorman	A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland	Escherichia, Salmonella, Staphylococcus, Campylobacter	13	90	1	No curve	meat, board, dishcloth, hands
2002	Midelet and Carpentier	Transfer of microorganisms, including Listeria monocytogenes, from various materials to beef	Listeria, Staphylococcus, Pseudomonas, Comomonas	12	144	2	No curve	stainless steel, PU, PVC, meat
2003	Harrison et al.	Bacteria transfer and cross-contamination potential associated with paper-towel dispensing	Micrococcus, Serratia	2	1000	1	No curve	paper towel dispenser, hand
2003	Kusumaningrum et al.	Survival of foodborne pathogens on stainless steel surfaces and cross-	Salmonella, Staphylococc	169	882	1	No curve	Stainless steel, vegetables, meat,

Table 6.16 (cont'd)

		contamination to foods	us, Campylobacter					sponges
2002	Mattick et al.	The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food	Salmonella, E.coli, Campylobacter	17	82	1	No curve	meat, sponge, kitchen surface
2003	Montville and Schaffner	Inoculum size influences bacterial cross contamination between surfaces	Enterobacter	1	1053	1	No curve	meat, cutting board, lettuce, hand, spigot, glove
2003	Moore et al.	Transfer of Salmonella and Campylobacter from Stainless Steel to Romaine Lettuce	Salmonella, Campylobacter	20	80	3	No curve	stainless steel to vegetables
2004	Kusumaningrum	A Quantitative Analysis of Cross-contamination of Salmonella and Campylobacter spp via Domestic Kitchen surfaces	Salmonella, Campylobacter	7	150	1	Monte carlo, beta-poisson	raw meat, stainless steel, vegetables, cutting board
2004	Vermeltfoort et al.	Phyicochemical Factors Influencing Bacterial Transfer from Contact Lenses to Surfaces with Different Roughness and Wettability	Pseudomonas, Staphylococcus	1	36	3	No curve	contact lens, glass, silicone
2006	Flores et al.	Transfer Coefficient Models for Escherichia coli O157:H7 on Contacts between Beef Tissue and High-Density Polyethylene Surfaces	E. coli	28	152	10	curve fit	beef, HDPE
2005	Lin et al.	Cross contamination between processing equipment and deli meats by	Listeria	3	61	1	No curve	meat, slicer

Table 6.16 (cont'd)

		Listeria monocytogenes						
2005	Luber et al.	Quantification of Campylobacter Species Cross-Contamination during Handling of Contaminated Fresh Chicken Parts in Kitchens	Campylobacter	27	168	1	No Curve	meat, hands, plate, sausage, cutting board, knife, cucumber, bread
2005	Vorst et al.	Transfer of Listeria monocyotgenes during Mechanical Slicing of Turkey Breast, Bologna, and Salami	Listeria	14	256	3	Curve fit	blade, meat
2006	Vorst et al.	Transfer of Listeria monocytogenes during slicing of turkey breast, bologna, and salami with simulated kitchen knives	Listeria	13	255	3	Curve fit	meat, knife
2007	Aarnisalo et al.	Modelling transfer of Listeria monocytogenes during slicing of 'gravad' salmon	Listeria	7	198	3	Exponential	slicer, fish
2006	Dawson et al	Residence time and food contact time effects on transfer of Salmonella Typhimurium from tile, wood, and carpet: testing the five-second rule	Salmonella	22	163	2	Curve Fit	Wood, tile, carpet, meat
2006	Knobben et al	Transfer of bacteria between biomaterials surfaces in the operating room-an experimental study	Staphylococcus, Propionibacterium	13	92	3	glove, broach, gown, light	hydrophobicity and roughness, wet vs. dry, friction
2007	Moore et al.	Recovery and Transfer of Salmonella Typhimurium from Four Different Domestic Food Contact Surfaces	Salmonella	8	49	2	curve fit	chicken, hand, gloves, lettuce,
2007	Perez-	Modeling transfer of Escherichia coli	E.coli,	6	115	3	Log-	slicer blade, meat

Table 6.16 (cont'd)

	Rodriguez et al.	O157:H7 and Staphylococcus aureus during slicing of a cooked meat product	Staphylococcus				linear and Weibull	
2007	Rodriguez et al.	Effect of Biofilm Dryness on the Transfer of Listeria monocytogenes Biofilms Grown on Stainless Steel to Bologna and Hard Salami	Listeria	1	8	1	No curve	food composition and water activity
2007	Rodriguez	Effects of inoculation level, material hydration, and stainless steel surface roughness on the transfer of Listeria monocytogenes from inoculated bologna to stainless steel and high-density polyethylene	Listeria	17	64	5		inoc. Level, topography, dry, wet
2006	Rodriguez	Evaluation of the Transfer of Listeria monocytogenes from Stainless Steel and High Density Polyethylene to Bologna and American Cheese	Listeria	1	64	3	No curve	stainless steel, HDPE, meat, cheese
2008	Keskinen et al.	Imapct of bacterial stress and biofilm forming ability on transfer of surface-dried Listeria monocytogenes during slicing of deli meats	Listeria	9	130	3	Curve fit	slicer, meat
2007	Keskinen et al.	Transfer of Surface-Dried Listeria monocytogenes from Stainless Steel Knife Blades to Roast Turkey Breast	Listeria	8	133	3	meat, knife	characterized n blade and cutting speed , time, RH, temp.
2008	S. Sheen	Modeling Surface Transfer of Listeria monocytogenes on Salami during Slicing	Listeria	4	192	3	Curve, changing variable	slicer, meat
2008	Sheen and	Modeling Surface Transfer of Listeria	Listeria	7	280	3	Curve fit	meat, slicing

Table 6.16 (cont'd)

	Hwang	monocytogenes from slicer to deli meat during mechanical Slicing						
2007	Verhoeff-Bakkenes et al	Quantification of Campylobacter jejuni Cross-Contamination via Hands, Cutlery, and Cutting Board During Preparation of a Chicken Fruit Salad	Campylobacter	31	150	3	No curve	meat, cutlery, cutting board, hand s
2009	Fravalo et al	Campylobacter transfer from naturally contaminated chicken thighs to cutting boards in inversly related to initial load	Campylobacter	4	109		Curve Fit	HDPE, cutting board, raw meat
2009	Jimene et al.	Survival of Salmonella on refrigerated chicken carcasses and subsequent transfer to cutting board	Salonella	10	40	2	Curve fit	meat, cutting board
2010	Sheen and Hwang	Mathematical modeling the cross contamination of E. coli O157:H7 on the surface of RTE meat product while slicing	E.coli	7	280	3	Curve fit, changing variables	meat, slicer

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