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TRANSFER OF *LISTERIA MONOCYTOGENES* DURING SLICING OF READY-TO-EAT DELICATESSEN MEATS

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TRANSFER OF *LISTERIA MONOCYTOGENES* DURING SLICING OF READY-TO-EAT DELICATESSEN MEATS

By

Keith Vorst

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ABSTRACT

TRANSFER OF *LISTERIA MONOCYTOGENES* DURING SLICING OF READY-TO-EAT DELICATESSEN MEATS

By

KEITH VORST

In response to continued concerns regarding *Listeria* cross-contamination of ready-to-eat meat and poultry products in both retail and home kitchens, a series of studies was conducted to: (1) optimize the quantitative recovery of *L. monocytogenes* from stainless steel surfaces, (2) determine direct and sequential transfer rates for *L. monocytogenes* from artificially contaminated ready-to-eat luncheon meats to a delicatessen slicer and vice versa, (3) determine the effects of cutting force, stainless steel grade, sharpness, and product composition on transfer of *L. monocytogenes* from artificially contaminated ready-to-eat luncheon meats to knives and vice versa, and (4) develop a mathematical model based on the transfer coefficients obtained from the previous three objectives that will predict the numbers of *L. monocytogenes* cells transferred during slicing of delicatessen meats.

Initially, four sampling devices: (1) sterile environmental sponge (ES), (2) sterile cotton-tipped swab (CS), (3) sterile calcium alginate fiber-tipped swab (CAS), and (4) 1-ply composite tissue (CT), were evaluated for quantitative recovery of *L. monocytogenes* from food-grade stainless steel. Recovery was 2.70, 1.34, and 0.62 log greater using CT compared to ES, CS, and CAS, respectively. The CT device, which is inexpensive and easy to use, represents a major improvement over other methods in quantifying *L. monocytogenes.*

Thereafter, a commercial delicatessen slicer blade and simulated kitchen knife blades were used as vectors for sequential transfer of *L. monocytogenes* from (a) an inoculated blade (~10^8, 10^5, 10^3 CFU/blade) to 30 slices of uninoculated delicatessen turkey, bologna, and salami, (b) inoculated product (~10^8 cm^2) to the blade and (c) inoculated product (10^8, 10^5, 10^3 CFU/cm^2) to 30 slices of uninoculated product via the blade with cutting force and product composition also assessed for their impact on *Listeria* transfer. Using slicer blades inoculated at 10^8 CFU/blade, *Listeria* populations decreased logarithmically to 10^2 CFU/slice after 30 slices. Findings for inoculated slicer blades and products (10^5 CFU/blade or cm^2) were similar with *Listeria* counts of 10^2 CFU/slice after 5 slices and enriched samples generally negative after 27 slices. Using 10^3 CFU/slicer blade, the first 5 slices typically contained ~10^1 CFU/slice by direct plating with enrichments negative after 15 slices. Knife blades containing 10^5 and 10^3 CFU/blade typically yielded direct counts out to only 20 and 5 slices, respectively, with "tailing" observed thereafter. Variables that enhanced *Listeria* transfer during slicing and cutting included higher fat and lower moisture content, application force, blade surface roughness, and stainless steel grade with greater transfer using 304 as opposed to 316.

These finding were then used to develop four fitted predictive models in the form [CFU (X) = ka^X] along with a program written in GWBasic. These models can be used if any two of the following three values are known: (a) initial inoculum level, (b) total bacterial transfer, (c) fraction of bacteria remaining on blade after consecutive slicing, solving for each model parameter CFU(X), k, or a. Based on our models, the greatest number of *Listeria* (>90%) will be found in the first 15 slices.

To my wife for never ever letting me give-up

To my son and future son or daughter may you build on my successes, learn from my failures, and rise above my weaknesses

To my parents for pushing enough but not too much

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This dissertation was not solely written by me but with the help of many influential people. Foremost among them is my wife who never let me give-up when all hope seemed lost. After receiving my Masters degree she constantly encouraged me to further develop my professional skills and use these to make my contribution to science and society. We share this degree on many levels and none more important than my love for her and my son.

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ABBREVIATIONS

AFM	Atomic Force Microscopy
AISI	American Iron and Steel Institute
AOAC	Association Official Analytical Chemists
B	Blade
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CAS	Calcium Alginate Swab
CDC	Center for Disease Control and Prevention
CFSAN	Center for Food Safety and Applied Nutrition
CFU	Colony Forming Units
CS	Cotton Swab
CT	Composite tissue
CY	Calendar Year
DS	Delicatessen
ES	Environmental Sponge
FAO	Food and Agriculture Association
FDA	Food and Drug Administration
FSIS	Food Safety Inspection Service
GLM	General Linear Model
GT	Growth Time
ICMSF	International Commission on Microbiological Specifications for Foods

LT	Lag Time
MOX	Modified Oxford
MS	Medium Sharp
mTPA	Modified Tryptose Phosphate Agar
mTPAN	Modified Tryptose Phosphate Agar Sodium Chloride (NaCl)
P	Product
PBS	Phosphate Buffered Saline
RH	Relative Humidity
RTE	Ready-to eat
S	Sharp
SAS	Statistical Analysis Systems
SEM	Scanning Electron Microscopy
TSA-YE	Trypticase Soy Agar-Yeast Extract
TSB	Trypticase Soy Broth
TSB-YE	Trypticase Soy Broth-Yeast Extract
USDA	United States Department of Agriculture
WHO	World Health Organization

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INTRODUCTION

Listeria monocytogenes is a serious bacterial foodborne pathogen that can reside in food processing facilities for many years (Tompkin, 2001). Not surprisingly, those strains that are most persistent in factory environments have been shown to possess greater capability to adhere to food contact surfaces (Lunden et al., 2000, 2002; Norwood and Gilmour, 1999) with attachment of *L. monocytogenes* to stainless steel occurring in as little as 20 min (Mafu et al., 1991). Such persistence of *L. monocytogenes* in food processing environments allows this pathogen to contaminate finished product and previously uncontaminated facilities via processing equipment and other food contact surfaces.

Processing equipment increases the risk of widespread dissemination of foodborne pathogens with bacterial transfer, reversible/irreversible attachment and biofilm formation influenced by stainless steel surface finish and composition of the food contact surface. In a comparative study of different food contact surfaces (Beresford et al., 2001), coupons of grade 304 (2B finish and 2B sand-blasted finish), 316 (electropolished) and 430 stainless steel (2BA finish) were immersed in a broth culture of *L. monocytogenes* for 2 h and then removed. After gentle rinsing, 25% of the *L. monocytogenes* cells were released from both types of grade 304 stainless with only 7 and 5% of the population shed from grades 316 and 430, respectively, thus suggesting involvement of stainless steel grade and surface finish in bacterial attachment. Arnold and Bailey (2000) assessed bacterial attachment rates for four different surface finishes of grade 304 stainless steel. When exposed to a mixed bacterial culture obtained from a poultry carcass rinse, bacterial attachment was at

least 1 log lower on electropolished stainless steel compared to the other three surfaces. These findings clearly have important ramifications in the manufacture of stainless steel knife blades, delicatessen slicer blades and other food contact surfaces found on food processing equipment as well as in commercial and retail food processing environments. However, the transfer rates for *L. monocytogenes* to and from contaminated ready-to-eat (RTE) meat products to stainless steels of different compositions and surface finishes remain poorly understood.

Transfer of *Listeria* from a contaminated product to a slicer blade or knife and then to a previously uncontaminated product is likely a major route of dissemination in retail delicatessens. However, adherence and subsequent release of any microorganisms from a blade during slicing are likely impacted by a multitude of factors that relate to the particular bacterial strain as well as the type of equipment (e.g., slicing machine, knife), type and condition of the blade (e.g., stainless steel grade, blade sharpness, extent of wear and corrosion) and cutting force. The aforementioned factors and the particular product being sliced will likely result in different transfer rates. Our work has shown that *L. monocytogenes* can transfer from artificially contaminated delicatessen slicer and knife blades to delicatessen meats and vice versa during slicing with the extent of transfer being product dependent. Additional findings suggest less prolonged transfer using grade 316 as opposed to grade 304 stainless steel knife blades with surface roughness and stainless steel grade impacting the rate of *Listeria* transfer.

Based on informational gaps identified in the current *Listeria* risk assessments (FAO/WHO 2004; FDA 2003; FSIS, 2003) specific information is needed concerning

the extent of *Listeria* transfer (a) from contaminated foods to soiled and unsoiled surfaces and (b) from contaminated surfaces (soiled and unsoiled) to foods. This transfer potential needs to be quantified and expressed in terms of transfer coefficients that can be incorporated into the various *L. monocytogenes* risk assessments to more accurately define the risks associated with consumption of ready-to-eat (RTE) foods. Based on the FSIS risk assessment for *Listeria* in RTE meat and poultry products (FSIS, 2003), of the approximately 500 listeriosis fatalities each year, an estimated 242 deaths are related to consumption of *Listeria*-contaminated delicatessen meats. These findings suggest that minimizing contamination at delicatessens will clearly have a major impact on reducing the incidence of listeriosis and in meeting and/or exceeding the goals identified in *Healthy People* 2010 (2004).

The research being reported in this dissertation was conducted in direct response to the identification of the *Listeria* transfer rate as an informational gap in the 2003 FDA *Listeria Risk Assessment* (FDA/FSIS/CDC, 2003). The approach was to first calculate a series of transfer coefficients for *L. monocytogenes* during slicing of various luncheon meats with a delicatessen slicer or knife blade and then develop a predictive mathematical model for *Listeria* transfer that can be used to refine the current *Listeria* risk assessments. The general hypothesis for this research is that the rate of *Listeria* transferred during slicing of delicatessen products is influenced by the type of slicer or knife blade and product composition.

The specific objectives of this three-year study were as follows:

Objective 1: Optimize the quantitative recovery of *L. monocytogenes* from stainless steel surfaces.

Objective 2: Determine the direct and sequential transfer rates for *L. monocytogenes* from artificially contaminated ready-to-eat luncheon meats to a delicatessen slicer and vice versa.

Objective 3: Determine the effects of cutting force, stainless steel grade, sharpness, blade wear and product composition on transfer of *L. monocytogenes* from artificially contaminated ready-to-eat luncheon meats to knives and vice versa.

Objective 4: Develop a mathematical model based on the transfer coefficients obtained in Objectives 1, 2, and 3 that will predict the numbers of *L. monocytogenes* cells transferred in delicatessens when either the slicer blade/knife or product is contaminated.

CHAPTER 1

LITERATURE REVIEW

1.1. *LISTERIA MONOCYTOGENES*

Listeria monocytogenes is a ubiquitous foodborne pathogen found in many raw foods and processing environments. The ability of this organism to attach to many different substrates and survive in harsh environments has prompted great public concern and resulted in strict regulatory policies including the currently enforced 'zero tolerance" policy for *L. monocytogenes* in cooked and/or otherwise processed ready-to-eat (RTE) foods.

1.1.1. Characteristics of *Listeria*

The genus *Listeria* comprises a group of gram positive, non-spore forming, short rod-shaped bacteria and contains the following six species: *L. monocytogenes, L. innocua, L invanovii, L. seeligeri, L. welshimeri,* and *L. grayi. Listeria monocytogenes* – the primary human pathogen of the aforementioned species, is of particular concern as a foodborne pathogen due its psychrotrophic characteristics and ability to grow in refrigerated foods (Rocourt, 1999). Other characteristics of importance to food manufacturers include the organism's resistance to acid, salt and low moisture environments. Thus, while typically found in soil and water as well as wild and domesticated animals, it is not surprising that *L. monocytogenes* is also common in many food-processing environments.

1.1.2. Manifestations of listeriosis

Listeriosis, the disease caused by infection with *L. monocytogenes*, is generally confined to high-risk groups including immunocompromised adults, pregnant women, and neonates. Such infections are relatively rare with an estimated 2500 cases occurring annually in United States, 500 of which are typically fatal (Mead et al., 1999).

Listeria monocytogenes primarily infects humans via contaminated food and invades the intestine resulting in multiplication and systemic spread via the circulatory system. Manifestations of listeriosis include flu-like symptoms, meningitis, spontaneous abortion, fetal death, neonatal septicemia and less commonly gastroenteritis (Slutsker and Schuchat, 1999). The incubation time for listeriosis typically ranges from 14 to as long as 70 days greatly complicating the investigation of potential foodborne outbreaks.

1.1.3. Susceptible populations

Based on relative risk of population subgroups for listeriosis, the Food and Drug Administration / Center for Food Safety and Applied Nutrition (FDA/CFSAN) has ranked food categories based on a per serving basis. Delicatessen meats ranked first in the predicted relative risk rankings for listeriosis among food categories for three U.S. age-based subpopulations (Table 1.1). These predicted risk rankings along with the increased number of meals consumed outside the home and the growing population of immunocompromised adults have resulted in heightened scrutiny of meat processors and retailers alike.

Table 1.1. Predicted relative risk rankings for listeriosis among food categories for three age-based subpopulations and the United States total population using median estimates of relative predicted risks for listeriosis on a per annum basis (FDA/FSIS/CDC, 2003)

Food Categories[a]		Subpopulation			
		Intermediate Age[b]	Elderly[b]	Perinatal[b]	Total[b,c]
SEAFOOD	Smoked Seafood	9	9	9	9
	Raw Seafood	17	21	17	18g
	Preserved Fish	19	17	19	19g
	Cooked Ready-to-Eat Crustaceans	8	8	8	8b,d,e
PRODUCE	Vegetables	12	12	12	12
	Fruits	10	10	10	10
DAIRY	Fresh Soft Cheese	14	18	14	14f
	Soft Unripened Cheese, >50% moisture	5	5	5	5b,c
	Soft Ripened Cheese, >50% moisture	16	16	16	16f
	Semi-soft Cheese, 39-50% moisture	15	15	15	15f
	Hard Cheese, <39% moisture	23	23	23	23
	Processed Cheese	20	20	21	21h
	Pasteurized Fluid Milk	2	2	2	2a
	Unpasteurized Fluid Milk	7	7	7	7d,e

Table 1.1. (Con't)

Food Categories[a]		Subpopulation			
		Intermediate Age[b]	Elderly[b]	Perinatal[b]	Total [b, c]
Dairy (con't)	Ice Cream and Frozen Dairy Products	21	19	20	20h
	Cultured Milk Products	22	22	22	22h
	High Fat and Other Dairy Products	3	3	3	3a
MEATS	Dry/Semi-Dry Fermented Sausages	13	13	13	13
	Deli Meats	**1**	**1**	**1**	**1**
	Pâté and Meat Spreads	6	6	6	6b,c,d
COMBINATION FOODS	Deli-type Salads	18	14	18	17f

[a] Food categories are grouped by type of food but are not in any particular order.

[b] A ranking of 1 indicates the food category with the greatest predicted relative risk of causing listeriosis and a ranking of 23 indicates the lowest predicted relative risk of causing listeriosis.

[c-h] Ranks with the same letter are not significantly different based on the Bonferroni Multiple Comparison Test (alpha=0.05).

Due to the high fatality rate (~20%) and hardiness of this organism, a regulatory policy of "zero tolerance" was established in the United States for all RTE foods in 1985 following the now infamous outbreak in California in which consumption of Jalisco-brand Mexican style-soft cheeses was linked to at least 300 cases of listeriosis including 85 fatalities (MMWR, 1985). Typical heat treatments given to RTE and processed foods will eliminate *L. monocytogenes* (Frye et al., 2002). However, many processed foods, including smoked salmon and turkey, ham, and beef luncheon meats, remain prone to *Listeria* contamination and have been the subject of numerous Class I recalls. These findings reaffirm the importance in minimizing post-processing contamination of RTE foods. While regulatory agencies and food processors struggle with the challenges of controlling *Listeria* contamination during processing, many retail delicatessen environments afford ample opportunity for cross-contamination and subsequent growth of *Listeria* in RTE products due to increased handling and reduced regulatory control.

1.2. Listeriosis Outbreaks

Listeria monocytogenes first emerged as a foodborne pathogen in 1981 when 17 of 41 people died of listeriosis after consuming coleslaw that was marketed in the Maritime Provinces of Canada (Schlech, 2000). Since 1979, a total of 13 listeriosis outbreaks have been reported with nine from contaminated RTE meat products (CDC, 2004). The California outbreak in 1985 led to numerous stillbirths from Mexican-Style cheese. More recent outbreaks (1998, 2001, and 2002) involving various processed meats have made *L. monocytogenes* a pathogen of heightened public

concern, most notably with the 1998 outbreak resulting in 21 fatalities from BallPark brand turkey hotdogs manufactured by BilMar Foods (Zeeland, MI). Since May of 2000, four major outbreaks of foodborne listeriosis have been documented in the United States; three were linked to consumption of delicatessen-sliced turkey breast (Table 1.2). The first of these outbreaks was responsible for 29 cases of listeriosis, including 4 deaths and 3 miscarriages/stillbirths, in 10 states and prompted the recall of 16.9 million pounds of product (MMWR, 2000). In June of 2001, another listeriosis outbreak characterized by 16 cases of acute febrile gastroenteritis (no fatalities) was identified in Los Angeles County, California (MMWR, 2001).

Precooked delicatessen-sliced turkey was identified as the vehicle of infection with *L. monocytogenes* serotype 1/2a of the same molecular fingerprint recovered from six of the victims and from some of the leftover turkey at levels of 1.6×10^9 CFU/g. The latest and largest of these three outbreaks involved 46 culture-confirmed cases of listeriosis, including 7 deaths and 3 miscarriages/stillbirths in eight primarily northeastern states and led to the recall of 27.4 million pounds of RTE turkey and chicken products (MMWR, 2002). In all cases, processed RTE meats (e.g., delicatessen turkey and turkey frankfurters), were the most common vehicle of infection.

Table 1.2. Listeriosis outbreaks in the United States 1979-2003 (CDC, 2004)

Year	State	Serotype	Vehicle
2003	TX	4b	Mexican-Style Cheese
2002	9 *	4b	Deli turkey meat
2001	CA	1/2a	Deli Turkey meat
2000	10 *	4b	Deli type turkey, chicken meat
2000	NC	4b	Mexican-Style Cheese
1999	3 *	1/2a	Pate
1999	4 *	4b	Mexican-Style Cheese
1998	22 *	4b	Hot Dogs
1994	IL	1/2b	Chocolate Milk
1989	CT	4b	Shrimp
1985	CA	4b	Mexican-Style Cheese
1983	MA	4b	Milk
1979	MA	4b	Produce

* Multistate outbreak

1.3. Incidence of *Listeria* in RTE Meats

Beginning in 1987, the USDA/FSIS setup a *Listeria* monitoring program for meat products (USDA/FSIS, 2003). Initial sampling included cooked beef products, but was expanded in 1993 to include meat/poultry products as well as meat/poultry spreads. Data from 1990-2000 (FSIS, 2005) shows the prevalence of *L. monocytogenes* to be highest in ham and luncheon meats compared to 7 other categories of RTE products (Table 1.3).

In the most recent comprehensive survey of sliced luncheon meats, 31,705 samples of ready-to-eat meat and cheese products were tested for *L. monocytogenes* over 14-23 months in retail markets across the United States. *L. monocytogenes* was identified in 0.4% of prepackaged vs. 2.7% of delicatessen-sliced luncheon meats with deli meats sliced on demand accounting for approximately 75% of all deli meat

sales (Gombas et al., 2003). Thus, consumer exposure to *Listeria* is approximately 7 times greater from luncheon meats sliced on-site at delicatessens as compared to pre-packaged commercially sliced products.

Table 1.3. Prevalence (%) of *L. monocytogenes* in RTE meat and poultry products, CY 1990-2000

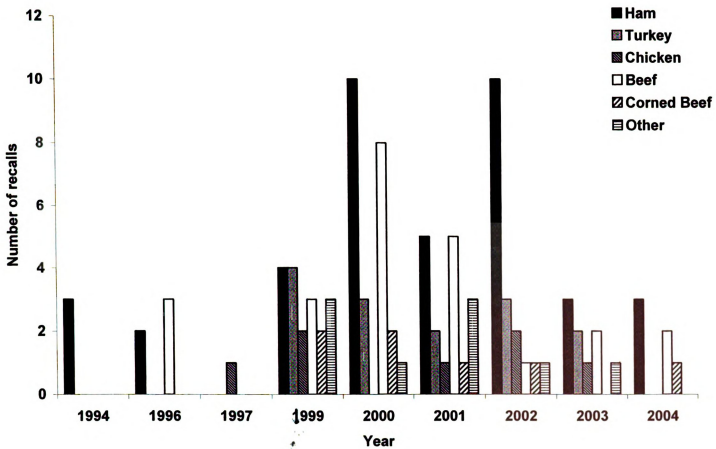
Year	Cooked, Roast, Corned Beef	Sliced Ham & Luncheon Meats	Small Cooked Sausages	Large Cooked Sausages	Jerky	Cooked Poultry Products	Salads/ Spreads/ Pâtés	Fermented Sausages	Other
1990	6.38	7.69	4.21	5.32	0.00	2.79	5.48	N/A[a]	N/A
1991	4.02	5.48	7.24	4.60	0.00	2.62	3.17	N/A	N/A
1992	3.86	7.89	6.03	0.42	0.00	2.01	3.32	N/A	N/A
1993	3.04	8.05	5.30	2.13	0.00	1.91	2.19	N/A	N/A
1994	2.09	5.46	4.81	1.14	2.22	2.37	2.41	N/A	N/A
1995	2.68	5.00	4.09	1.14	0.00	2.25	4.69	N/A	N/A
1996	3.35	7.69	3.74	0.95	0.00	3.17	2.17	N/A	N/A
1997	2.08	4.20	2.74	1.62	0.00	0.95	2.43	9.26	N/A
1998	2.15	4.18	3.49	1.19	1.56	2.22	3.11	2.87	N/A
1999	2.71	4.58	1.76	0.43	0.00	1.44	1.15	2.09	N/A
2000	2.24	3.05	1.26	0.51	0.75	1.24	0.98	1.49	0.00
Cumulative	2.95	4.47	2.97	1.09	0.58	1.97	2.83	2.67	0.00

1.4. *Listeria* recalls

Post-process contamination of cooked/RTE delicatessen products with *L. monocytogenes* has made this pathogen the leading cause of Class I microbiologically related recalls (Levine et al., 2001). Since 1994, *Listeria* contamination of delicatessen meats has resulted in 66 Class I recalls (Figure 1.1). Of these 66 recalls, ham was most frequently contaminated (44 recalls) followed by beef (24 recalls), turkey (14 recalls), and chicken (7 recalls). Although responsible for the greatest number of recalls, ham has not been implicated in large multi-state outbreaks unlike turkey and chicken (Figure 1.1). Within the last decade more than 50 million pounds of hot RTE hot dogs, chicken, and turkey luncheon meats have been recalled for *Listeria* contamination. These recalled RTE meat products were linked to more than 130 cases of listeriosis and 28 fatalities during two separate outbreaks in 1998 and 2001 (MMWR, 2000).

Products recalled during these outbreaks were suspected of becoming contaminated during packaging. While ham products have seen the highest total number of recalls (44) since 1994, refrigerated growth studies by Glass and Doyle (1989) demonstrated a greater growth of *Listeria* in processed poultry products as compared to ham, bologna, and bratwurst. When processed meats were inoculated with a five- strain cocktail of *L. monocytogenes* ($<10^2$ CFU/g) and stored at refrigeration temperatures, the pathogen grew to 10^3-10^4 CFU/g on ham after 6 weeks and to10^3-10^5 CFU/g on turkey and chicken after 4 weeks. Consuming low levels of *L. monocytogenes* in food is not uncommon and is not considered a significant risk to most people (Chen et al., 2003).

Figure 1.1. Class I recalls of delicatessen meat products 1994-2004 (USDA/FSIS, 2005)



However, the aforementioned studies illustrate the ability of *L. monocytogenes* to reach levels potentially hazardous levels (>10^3 CFU/g) in certain delicatessen products such as chicken and turkey luncheon meat. Delicatessen-sliced products continue to be a concern due to temperature abuse and varying degrees of regulatory scrutiny at the retail level. Previous studies have not addressed the differences in growth, transfer, and distribution of foodborne pathogens in retail delicatessens that result from different storage, handling, and food preparation practices.

In response to previously mentioned outbreaks that were traced to consumption of delicatessen-sliced turkey, quantitative transfer to and from commercial slicing machines, knives, and cutting boards in delicatessens was identified as both a major public health concern and a key informational gap in the 2003 FDA *Listeria Risk Assessment* (FDA/FSIS/CDC, 2003).

1.5. USDA and FDA guidelines

The public health significance surrounding *L. monocytogenes* has led to a regulatory policy of "zero tolerance" in the United States for this organism in cooked and/or otherwise processed RTE foods. The three previously discussed outbreaks involving delicatessen turkey have prompted the development of three USDA-mandated alternatives for controlling *Listeria* in delicatessen meats – (a) post-package pasteurization, (b) product reformulation to prevent *Listeria* growth and/or (c) increased product and environmental testing (FSIS, 2003b). While food processing environments remain major sources of contamination, the extent to which *Listeria* is

transferred from food contact surfaces and utensils to RTE products at retail delicatessens remains largely unknown. In one of two studies reported, Hudson and Mott (1993) collected various environmental swab samples from a supermarket delicatessen and isolated *L. monocytogenes* from a knife and slicing machine with the pathogen also found at most sites near a display case of processed meats. In the remaining study, Humphrey (1990) evaluated retail delicatessen meat slicers in the UK and found *L. monocytogenes* on 10 of 32 slicer blades, thus suggesting ample opportunity for *Listeria* transfer. These studies support the need for increased scrutiny at the retail level.

Many delicatessen meats have an estimated 10-30 day shelf-life at 1-5° C with growth estimates showing up to a 2 log increase during this period (Glass and Doyle, 1989) (Table 1.4). RTE products are exposed to wide fluctuations in temperature from the point of manufacture to the time of consumption allowing *Listeria* ample time to reach potentially infectious levels.

Table 1.4 Generation (GT) and Lag Times (LT) of *L. monocytogenes* in meats

Food	Temperature (C)	GT (h)	LT (h)	REF
Roast Beef	-1.5[a]	100	173.7	1*
	3[a]	26.7	59	1
	3[b]	80.9	477.1	1
Corned Beef	0	110		1
Cooked Meat	5	44-61		1
Ham	5	33.2		1
	10	13.4		
	15	6.1		
Cooked beef	5	18.6-22.6	80.6-83.4	1
	10	8.5-9	22.6-30.4	1
Sliced Turkey	4.4	15.6-37.8		2*
Pate	7	19.7	48	1
	23.5[a]	1.6	24	1
Pate	10[a]	9.12	27.6	1

1* – J. Farber and P. Peterkin, 1999

2* – Glass and Doyle, 1989

The wide temperature range in home refrigerators is more likely to promote the growth of *L. monocytogenes* in RTE foods to infectious levels than are the more tightly temperature controlled commercial refrigeration units with the former (Pinner et al., 1992) conditions more likely to adversely affect "at risk" consumers. Given the relatively short generation time (<16 h) for *Listeria* in some products at refrigerated temperature and the extended storage and distribution times for delicatessen meats (>10 days), the risk of contracting listeriosis from processed meats has become a major concern for pregnant women and the elderly (Table 1.5).

Table 1.5. Estimated storage temperature and duration between manufacture and retail for predicted median growth (FDA/FSIS/CDC, 2003)

Food Category		Temperature Range[a] (°C)	Storage Time[a, b] (days)		Median Growth [c]
			Min	Max	(log cfu)
SEAFOOD	Smoked Seafood	1 to 5	10	30	1.08
	Raw Seafood	1 to 5	1	3	0.11
	Preserved Fish	NA[b]	NA[b]	NA[b]	NA[b]
	Cooked RTE Crustaceans	1 to 5	1	3	0.28
PRODUCE	Vegetables	1 to 5	1	10	0.1
	Fruits	NA[b]	NA[b]	NA[b]	NA[b]
DAIRY	Fresh Soft Cheese	NA[b]	NA[b]	NA[b]	NA[b]
	Soft Unripened Cheese	NA[b]	NA[b]	NA[b]	NA[b]
	Soft Ripened Cheese	1 to 5	10	30	0.04
	Semi-Soft Cheese	NA[b]	NA[b]	NA[b]	NA[b]
	Hard Cheese	1 to 5	10	45	-0.94
	Processed Cheese	NA[b]	NA[b]	NA[b]	NA[b]
	Pasteurized Fluid Milk	1 to 5	1	3	0.2
	Unpasteurized Fluid Milk	NA[b]	NA[b]	NA[b]	NA[b]
	Ice Cream and Frozen Dairy Products	NA[b]	NA[b]	NA[b]	NA[b]

Table 1.5. (Con't)

			Min	Max	(log cfu)
Dairy (Con't)	Cultured Milk Products	NA[b]	NA[b]	NA[b]	NA[b]
	High Fat and Other Dairy Products	1 to 5	3	10	0.24
MEATS	Frankfurters	1 to 5	10	30	1.03
	Dry/ Semi-dry Fermented Sausage	NA[b]	NA[b]	NA[b]	NA[b]
	Deli Meats	1 to 5	10	30	1.86
	Pâté and Meat Spreads	1 to 5	1	7	0.34
COMBINATION FOODS	Deli-type Salads	NA[b]	NA[b]	NA[b]	NA[b]

[a] Rectangular distributions were used for both the temperature range and storage times.
[b] NA - Not applicable because none of the samples were collected at manufacture so growth between manufacture and retail was not calculated for these food categories.
[c] Median growth (log cfu) is calculated by multiplying the storage times and the exponential growth rates

1.6. *Listeria* post-process contamination

The transfer of foodborne pathogens from contaminated food to previously uncontaminated food via slicing machines was recognized over 40 years ago. During the 1960's, an outbreak in Aberdeen, Scotland led to 469 cases of typhoid fever from contaminated corned beef that was sliced at a retail delicatessen, thus providing evidence for delicatessen slicing machines as vectors for contamination of previously uncontaminated product (Howie, 1968). In a far more recent study, Lin et al. (2004a) showed increased *Listeria* transfer from contaminated slicer parts to turkey, salami, and bologna during slicing. In their study, a commercial delicatessen slicer blade was inoculated with a five-strain cocktail of *L. monocytogenes* (10^2 CFU) and then used to slice turkey, bologna, and salami. Packages containing five slices of each product were vacuum-sealed and assessed for *Listeria* growth after 1, 30, 60, and 90 days of storage at 4°C. While *Listeria* populations increased in roast turkey breast, numbers gradually declined in salami and bologna and fell below detectable limits after 60 and 90 days of storage.

1.6.1. Food processing environments

Listeria monocytogenes can reside in food processing facilities for many years (Tompkin, 2001) with those strains that are most persistent in factory environments possessing greater capability to adhere to food contact surfaces (Kim and Frank, 1994; Lunden et al., 2000, 2002; Norwood and Gilmour, 1999; Tiwari and Alenrach, 1990). Attachment of *L. monocytogenes* to stainless steel surfaces can occur in as little as 20 min allowing ample time for long-term transfer (Mafu et al., 1991). While

many bacteria are capable of producing biofilms, true biofilm-forming strains of *L. monocytogenes* are relatively rare (Kalmokoff et al., 2001) with this pathogen more commonly seen in biofilms containing a mixed microflora (Bremer et al., 2001). Adherence and subsequent transfer of *Listeria* is impacted by various environmental conditions including temperature, relative humidity and substrate composition. The persistence and spread of *Listeria* in food-processing environments (Lunden et al., 2002; Tompkin, 2001; Vogel et al., 2001), and ability to grow to populations of 10^4 to 10^6 CFU/g on many refrigerated RTE foods such as smoked salmon and luncheon meats (Miettinen et al., 2001, Pinner et al., 1992) has made this pathogen a great concern to the food industry.

Many studies have demonstrated the ability of *L. monocytogenes* to attach to various materials and transfer to subsequent food products (Ak et al., 1994; Arnold and Bailey, 2000; Beresford et al., 2001; Midelet and Carpentier, 2002, Schaffner et al., 2004). Results of these studies suggest increased scrutiny of food contact surfaces and development of new and innovative materials to reduce bacterial attachment and subsequent transfer.

The aforementioned persistence of *L. monocytogenes* in food processing plants allows this pathogen to enter previously uncontaminated facilities via processing equipment and other food contact surfaces. In one study, Lunden et al. (2002) demonstrated plant-to-plant transfer of *L. monocytogenes* via a dicing machine with the same strain of *Listeria* identified at three different facilities. Studies have also shown the attachment and transfer of bacteria during food preparation and handling in food service environments. A study by Ak et al., (1994) demonstrated that

the absorption and bactericidal activity of wooden cutting boards represented a significant advantage over plastic cutting boards with subsequent transfer more likely from plastic boards to other food products. In other work by Schaffner et al. (2004), plastic cutting boards were identified as a source of contamination when slicing various raw meats and vegetables with coliform counts ranging from 2-4 log CFU/g. These studies clearly demonstrate the impact of material composition, surface conditioning, and surface structure on attachment of bacteria and dissemination to foods.

1.6.2. Sampling and Recovery

Current methods relying on cellulose sponges and cotton swaps for environmental sampling of food processing facilities continue to be plagued by poor repeatability and efficacy. The medical and pharmaceutical industries have evaluated newer direct plating methods utilizing technologies such as adhesive sheets (Yamaguchi et al., 2003) and pads (Tominaga et al., 2001). These devices are reportedly superior to traditional swabbing in terms of both recovery and reproducibility. However, the food industry is faced with several major obstacles that make these sampling devices far less advantageous. Presence of food particulates, fat, and oil on food processing equipment and other food contact surfaces makes recovery with adhesive sheets and pads very inefficient. In addition to poor performance on heavily soiled food contact surfaces, these devices are costly when compared to traditional swabs and environmental sponges.

Thus far, recovery strategies that have been developed to assess presence or absence of *Listeria* in food processing environments lack the needed sensitivity to quantify *Listeria* on solid surfaces (Vogel et al., 2001). The efficiency and reliability of traditional environmental sampling devices such as the sponge and cotton swab have been debated since their introduction in the 1970's (Ware et al., 1999). Studies on meat surfaces have also shown that traditional destructive surface sampling methods such as excision yield higher bacterial recovery ($\geq$ 50%) than non-destructive swab and sponge methods (Tompkin, 2001). Direct agar contact plating, including the use of Rodac® plates, has been plagued by limited sampling area, cost, and difficulty with food particulates. While Rodac® plates have been successful for sampling aerosols (Crozier-Dodson and Fung, 2002), their relatively small size and their inability to withstand even a modest amount of mechanical energy during sampling make them inadequate for many food contact surfaces (Moore and Griffith 2002).

1.7. *Listeria* attachment and transfer

Given the hardiness and wide occurrence of *Listeria* in the environment, *L. monocytogenes* has been successfully recovered from many raw and processed foods. Attachment and subsequent transfer of *Listeria* to various materials can occur in a very short time. Bacterial attachment to stainless steel has been widely studied (Akier et al., 1990; Arnold and Bailey, 2000; Arnold and Silvers, 2000; Norwood and Gilmour, 1999; Vantanyoopaisarn et al., 2000). In addition to stainless steel, limited work using various polymers and fabrics (Ak et al., 1994; Beresford et al.,

2001;Montville et al., 2001; Satter et al., 2001) also suggests that *Listeria* can be transferred during common food handling tasks.

1.7.1. Stainless steel

Based on compositional differences, stainless steel can be classified as ferritic, martinistic, austenitic, or precipitation hardened. Austenitic stainless steel is most commonly used for food processing equipment, whereas ferritic and martinistic stainless steels have seen limited use in the food industry due to their high cost and inferior physical/chemical properties. High tensile strength, yield stress, and hardness are desirable in food-grade stainless steel (Table 1.6.). The high chromium (Cr) and nickel (Ni) (16 to 25 wt.% and 7 to 20 wt.%, respectively) content of austenitic stainless steel results in excellent formability at room temperature with relatively good resistance to oxidation (Table 1.7). American Iron and Steel Institute (AISI) stainless grades 304, and 316 along with their low carbon counter parts 304L and 316L are the most commonly used alloys in the food industry (Smith, 1993) with the 400 series also used for knives. The standard mill surface finishes of stainless steel are designated by AISI and range from basic hot rolled (No. 0 finish) - a very rough finish that is not fully corrosion resistance, to cold rolled (No. 2, including 2B, 2D, and 2BA), mechanically produced polished finishes (No. 3 – 8) and electropolished finishes (Table 1.8).

Corrosion of stainless steel after repeated cleaning and sanitizing likely enhances bacterial attachment and transfer (Barkley, 1979; Bohner and Bradley, 1991). Mechanical and/or physical abuse during cleaning can produce additional

attachment sites as a result of surface marring and scratching. Bacterial attachment and transfer can reportedly be decreased by 'passivation' (i.e., treatment with a mild oxidant to remove surface iron and iron compounds) and by choosing 316 rather than 304 grade stainless with the latter being more corrosion resistant due to the addition of 2.5 wt.% Mo (Arnold and Bailey, 2000). In limited work by Percival (1999), the Mo concentration used in grade 316 stainless steel decreased bacteria viability and reduced biofilm formation. In addition to corrosion resistance and potential biocidal activity of Mo, grade 316 stainless steel has a smoother finish after manufacture and polishing which decreases the number of bacterial attachment sites. (Leclercq-Pelat and Lalande, 1994). Various alloys and polishes including electropolished, 2BA, and 4 finishes are available for food industry applications. However, other alloys and polishes are often too costly for such use (Table 1.9).

Table 1.6. Physical properties of stainless steel (AISI, 2005).

Type	Tensile (ksi) min	Yield (ksi) min	Elongation min	Hardness (Brinell) max	Hardness (Rockwell B) max
300 Series Austinetic					
304	75	30	40% in 2"	183	88
304L	70	30	40% in 2"	183	88
316	75	30	40% in 2"	217	95
316L	70	25	35% in 2"	217	95
400 Series Martinistic					
410	65	30	20% in 2"	217	95
400 Series Ferritic					
430	65	30	22% in 2"	183	88

1.7.2. Attachment and release of *Listeria* from stainless steel

Food interactions with various grades of stainless steel used in the food industry can result in oxidation, pitting, and scoring over time due to both food acidity and cleaning regimens. While many studies have addressed surface wear and oxidation during processing (Arnold and Bailey, 2000; Bohner and Bradley, 1991; Beresford et al., 2001; Bremer et al., 2001), only limited work has been done with respect to retail food contact surfaces such as delicatessen slicers, kitchen knives, and countertops. Studies have shown the influence of stainless steel grade and structure on wear and attachment of bacteria (Akier et al, 1990; Arnold and Bailey, 2000; Arnold and Silvers, 2000; Bohner and Bradley, 1991). Each of these studies indicated that surface finish, grade, and conditioning had a major impact on bacterial attachment and transfer. These studies also emphasized the need for knowledge of stainless grades with the physical and chemical attributes least conducive to bacterial attachment during mechanical and oxidative abuse commonly seen in processing and retail food establishments.

Processing equipment increases the risk of widespread dissemination of foodborne pathogens. Bacterial attachment to surfaces is influenced by surface profile and composition. Previous studies have examined the impact of different surface material compositions and surface finishes on bacterial attachment and biofilm formation (Arnold and Silvers, 2000, Briandet et al., 1999, Jeong and Frank, 1994; Norwood and Gilmour, 1999). According to Briandet et al., (1999) greater bacterial attachment was seen for stainless steel than for rubber when semi-quantitative absorbance values were obtained from recovered bacterial suspensions.

When surface morphologies of different stainless steels of various finishes were examined, differences in bacterial attachment were observed. Relative differences in stainless steel surface morphology based on the type of surface finish: 2B finish, sandblasted, sanded, or electropolished, were also seen using scanning electron microscopy (SEM) and atomic force microscopy (AFM) (Akier et al., 1990; Arnold and Bailey, 2000). Significant differences in attachment to grade 304 stainless steel that was inoculated with a bacterial chicken rinse suspension (2×10^6 CFU/ml) and incubated for 18 h at 37° C were observed using various surface finishes. Electropolished stainless steel had significantly fewer attached bacterial cells (10^2 cells) compared to other surface finishes (10^3 cells). No clumps were observed on electropolished surfaces, whereas more than 12 clumps were seen for the other surface finishes. Evidence from SEM also suggests that bacterial attachment is more prevalent at grain boundaries in stainless steel with corrosion and pitting at these sites further enhancing bacterial attachment (Arnold and Bailey, 2000). Hence, the reduced grain boundary corrosion of low carbon alloys (304L and 316L) would be expected to reduce the number of attachment sites and decrease bacterial transfer.

Table 1.7. Chemical properties of stainless steel (AISI, 2005)

Type	C	Mn	P	S	Si	Cr	Ni	Mb
300 Series Austinetic								
304	0.08	2	0.045	0.03	1	18.00/20.00	8.00/10.50	-
304L	0.03	2	0.045	0.03	1	18.00/20.00	8.00/12.00	-
316	0.08	2	0.045	0.03	1	16.00/18.00	10.00/14.00	2.00/3.00
316L	0.03	2	0.045	0.03	1	16.00/18.00	10.00/14.00	2.00/3.00
400 Series Martinistic								
410	0.15	1	0.04	0.03	-	1	11.5 / 13.5	-
400 Series Ferritic								
430	0.15	1	0.04	0.03	-	1	16.00 / 18.00	-

Table 1.8. Finish grades of stainless steel (AISI, 2005).

Finish	Description	Application
Standard Mill Finish	Broad definition of manufactured steel finish to be used for further processing. Hot rolled resulting in scaling which is subsequently removed by nitric acid	Further processing needed for food applications
No. 0	Referred to as Hot Rolled Annealed (HRA) resulting in scaled black finish. Does not develop fully corrosion resistant film on stainless steel.	Tool and dye applications. Not for food applications.
No. 1	Hot rolled annealed, pickled and passivated. Dull slightly rough finish. Starting finish for cold rolled steel with bright finishes.	Further processing needed food applications
No. 2D	A No. 1 finish after being cold rolled, annealed and passivated. Slightly dull finish improved corrosion resistance.	Industry equipment applications. Not used for food contact surfaces where bright finish is needed
No. 2B	Given a skin pass between cold rolling operations between polishing rolls. Brighter than 2D and precursor to further finishing polishes	Sheet metal applications and industry equipment.
No. 2BA	Commonly referred to as Bright Annealed finish (BA). Cold rolled using high polished rolls for bright finish. Mirror finish similar to 7 or 8.	Sheet metal, construction, and equipment applications where bright surface is needed.
No. 3	Ground unidirectional finish using 80-100 grit abrasive sanding. Starting finish for further polishing	Starting finish for further polishing. Not generally used in food or industry applications.
No.4	Ground unidirectional finish using 150 grit abrasive sanding. Good general-purpose finish subject to rough handling.	Food service applications such as countertops used in restaurants and delicatessens.

Table 1.8. (Con't)

No. 6	Finish produced using rotating cloth mops (Tampico, fibre, muslin, or linen). Non-directional texture with varying reflectiveness. Referred to as satin blend.	Specialty decoration or construction. Not a typical food application finish.
No. 7	Buffed finish with high degree of reflectiveness. Produced using successively finer and finer buffing compounds and abrasives. Minimal fine scratches remaining.	Used where bright highly polished surface is needed (delicatessen displays, equipment fascias).
No. 8	Produced similarly to No. 7 finish with even higher degree of buffing. Final surface is blemish free and true mirror-like appearance.	Used where bright highly polished surface is needed (medical industry). Costly for food applications.
Electropolish	Electrochemical process where phosphoric and sulphuric acids are used in conjunction with high current density to clean and smooth the surface. Raises the proportion of chromium at the surface. Very bright mirror-like finish.	Commonly used in food industry applications for food contact surfaces where high corrosion resistance and mirror like finish is needed

Table 1.9. Typical stainless grades and applications in the food industry (AISI, 2005).

Grade	Cost	Application
304	**Moderate**	Most widely used of all stainless steel grades. Used for numerous food equipment applications and contact surfaces. Moderate corrosion/oxidation resistance and good weldability and physical characteristics. Considered a versatile and reasonably priced stainless grade for many applications.
304 L	**Moderate**	A low carbon derivative of 304 as noted by the "L". Enhanced corrosion/oxidation resistance with excellent weldability due to lowered carbon content. Slightly lower physical strength at high temperatures. Used where corrosion and acid resistance is necessary and high temperature strength not a factor (i.e. food processing).
316	**Moderate**	Improved version of 304 with added molybdenum and slightly higher nickel content. Improved corrosion resistance over 304 with increased physical strength at low temperatures. Lower rate of general corrosion with increased low temperature strength when compared to 304. Many food application uses in low temperature corrosive environments such as brines.
316 L	**High**	A low-carbon derivative of 316 as noted by the letter "L". Excellent weldability and corrosion resistance at high temperatures. Limited use in food applications.
410	**Low**	Lowest alloy content of all basic stainless grades (304, 304L, 316, 316L, 430). A Low cost and general-purpose stainless steel grade. Typical uses secondary handling of food products (packaging, transfer, conveyance).
430	**Low**	A low carbon plain chromium stainless steel. Good corrosion/oxidation resistance in mild environments. Brittle at low temperatures. Limited use in food processing as a low cost alternative to mild food processing environments where strong organic acids are not used.

1.7.3 Other food contact surfaces

Many studies have demonstrated the ability of *L. monocytogenes* to attach to various materials and transfer to subsequent food products (Ak et al., 1994; Arnold and Bailey, 2000; Beresford et al., 2001; Midelet and Carpentier, 2002, Schaffner et al., 2004). Results from these studies suggest a need for increased scrutiny of food contact surfaces and develop of new and innovative materials to reduce bacterial attachment and subsequent transfer. In another study, the microflora on plastic and wooden cutting boards was assessed by either soaking 5-cm square blocks in nutrient broth or by direct plating on nutrient agar (Ak et al., 1994). *Escherichia coli, Listeria innocua, L. monocytogenes,* and *Salmonella* Typhimurium were readily recovered from the plastic boards up to 12 h after inoculation. Recovery was less from wooden boards with all pathogen populations decreasing 98% after 12 h. The absorption and bactericidal activity of wooden cutting boards represented a significant improvement over plastic cutting boards. This study clearly demonstrates the impact of material composition, surface conditioning, and surface structure on bacterial attachment.

Work done by Satter et al. (2001) assessed transfer of *Staphylococcus aureus* from fabrics (100% cotton and 50% cotton/polyester) to hands and other fabrics such as bed linens and garments. Pre-moistened poly cotton had the highest transfer rate (>20%) with the amount of friction significantly impacting transfer. Beresford et al., (2001) also evaluated various polymers and rubber for attachment and subsequent release of *Listeria* after 2 h incubation. Lexan and polypropylene were found to shed 33% and 27% of the *Listeria* population, respectively, demonstrating the impact of

material composition, surface conditioning, and surface structure on bacterial attachment.

1.8. Risk Assessment

Risk assessment is one of the many tools used by both national and international governing organizations to identify chemical, biological, or physical hazards to humans, plants, animals and environments alike. A food-safety risk assessment is compiled to provide a framework for evaluating scientific data to identify the potential for risk of illness and death to a population from exposure to a foodborne pathogen. Several risk assessments developed over the last few years may useful in instituting regulatory policies for control of foodborne pathogens with a possible move away from the "zero tolerance" policy still being enforced in the United States for *L. monocytogenes* in cooked and/or otherwise processed RTE foods.

1.8.1. FDA/CFSAN, USDA, and FAO/WHO

Since 2001, FDA/CFSAN and USDA have developed comprehensive risk assessments for consumer exposure to *L. monocytogenes* through selected categories of RTE foods with the latest revision made available in 2003. The aforementioned risk assessments have ranked delicatessen meats as the leading product for the establishment of listeriosis in pregnant women, neonates, and immunocompromised adults.

Internationally, the Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (WHO) has compiled a risk assessment of *L. monocytogenes* in RTE foods (FAO/WHO, 2004). Various factors including raw ingredients, processing, distribution, and consumption were included as part of the risk analysis for listeriosis (Table 1.10)

Table 1.10. Variables affecting dose for risk of listeriosis (FAO/WHO, 2004)

Point in Food Continuum	Variables Affecting Dose		
	Consumption	Concentration in Contaminated units	Prevalence of Contaminated Units
Consumption	Frequency and amount consumed affected by: season, wealth, ages, sex, culture/region	heating; mixing with other components (e.g.; vinegar in salads); breakdown to smaller units	breakdown to smaller units/serving portions
Home/Food Service		time, temperature, product composition	cross-contamination with other foods
Retail Sale		time, temperature, product composition, breakdown to smaller units	packaging and cross contamination, portioning, breakdown to smaller units
Transport and Storage		time, temperature, product composition	
Processing		Volumetric changes: mixing with other ingredients changes due to dilution or concentration (evaporation, removal of whey) *Growth inactivation changes* brining, heating steps, holding times and temperatures,	cross-contamination, mixing with other bulk ingredients, splitting into smaller units for retail/food service
Raw Ingredients		Environmental sources affecting concentration in ingredients	Season, harvest area fodder and feeding regimes, irrigation water, etc.

Based on current estimates, consumers purchase 24.4 and 75.6% of their luncheon meats prepackaged and delicatessen-sliced, respectively (USDA/FSIS, 2003). USDA/CFSAN data for Class I recalls of *Listeria*-contaminated sliced and unsliced delicatessen meats clearly validates previous estimates for increased sales of delicatessen-sliced as opposed to prepackaged product. Since 1994, a total of 81,623,410 pounds of RTE meat products have been recalled (USDA/FSIS, 2005). From this total, 242,000 pounds were identified as manufacture-sliced or unsliced (e.g. to be sliced at retail delicatessens) luncheon meats. From this total, approximately 186,000 pounds or 77% was destined for retail slicing at delicatessens, which further supports the current consumer preference for delicatessen-sliced meats (Figure 1.2).

Revenue from the sale of delicatessen RTE luncheon meats has grown 4.6% over the last 5 years topping more than 16 billion dollars (Uetz, 2005) (Figure 1.3). In 2004, consumer spending on delicatessen-sliced lunch meats topped 3.1 billion dollars compared to 255 million for unsliced products (Uetz, 2005) (Figures1.4-1.5). Sales projections for RTE luncheon meats are expected to exceed 22 billion dollars by the year 2009 (Uetz, 2005).

Figure 1.2. USDA/FSIS recalls of RTE delicatessen meats identified as sliced or unsliced from January 1994-April 2005 (USDA/FSIS, 2003)

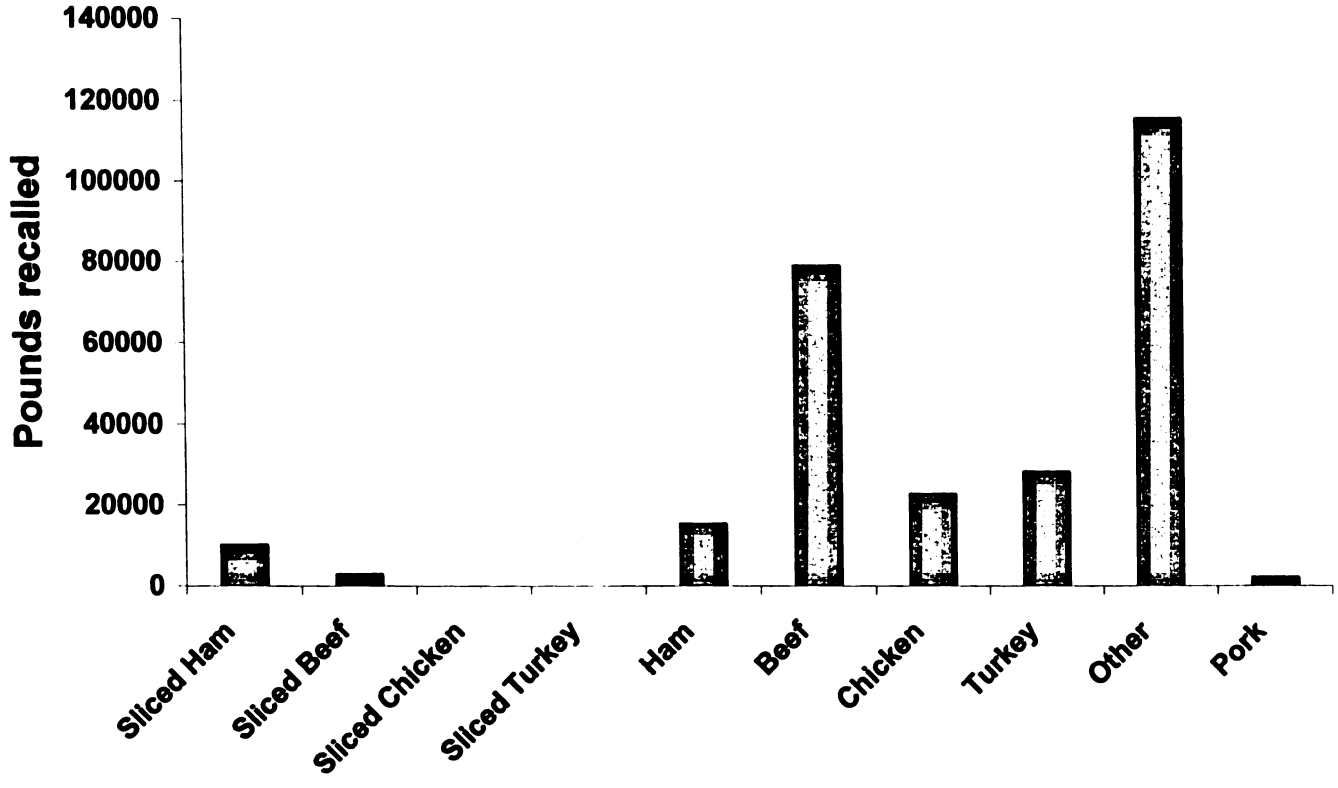


Figure 1.3. Refrigerated RTE luncheon meat sales (Uetz, 2005)

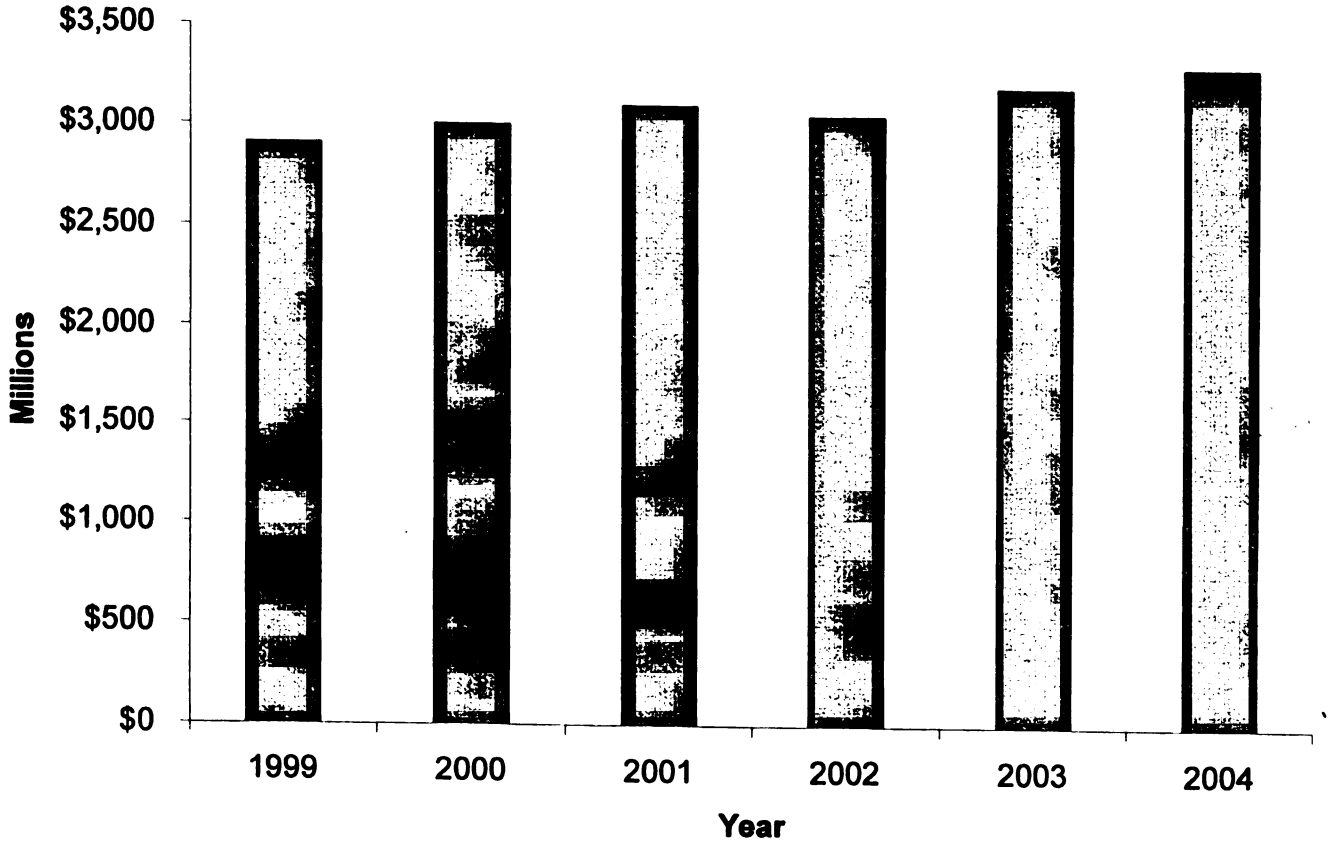


Figure 1.4. Sliced RTE luncheon meat sales (Uetz, 2005)

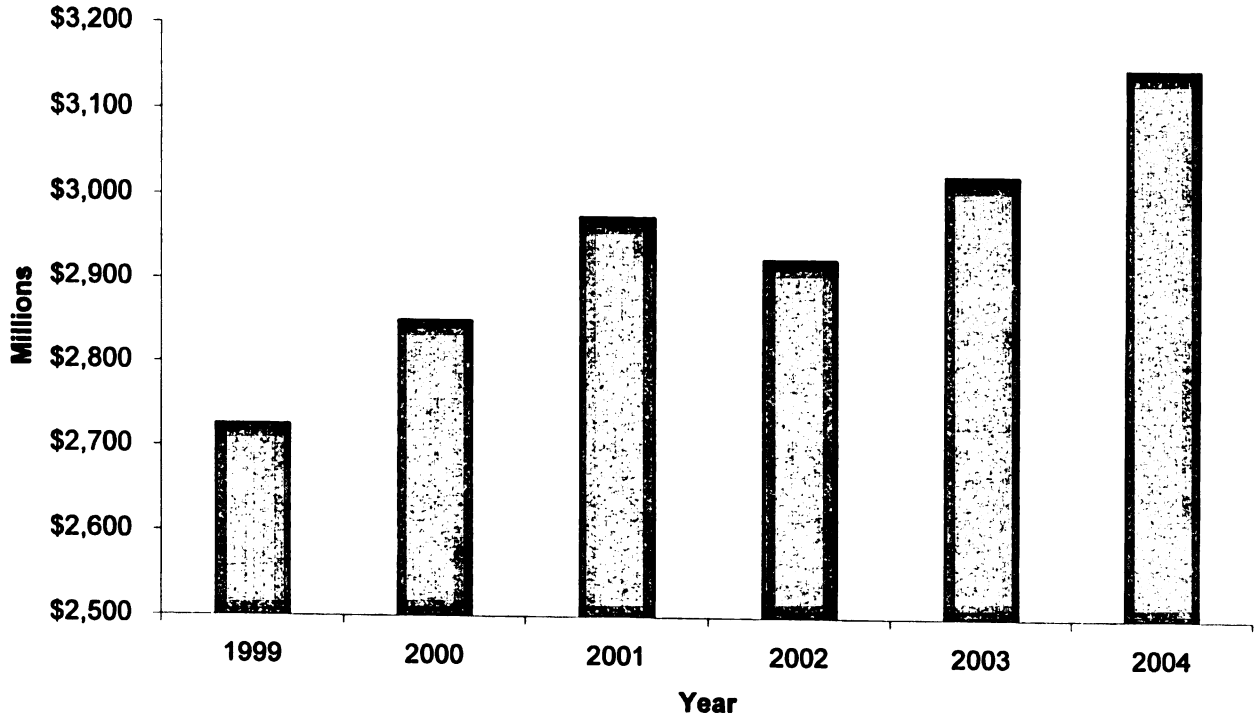
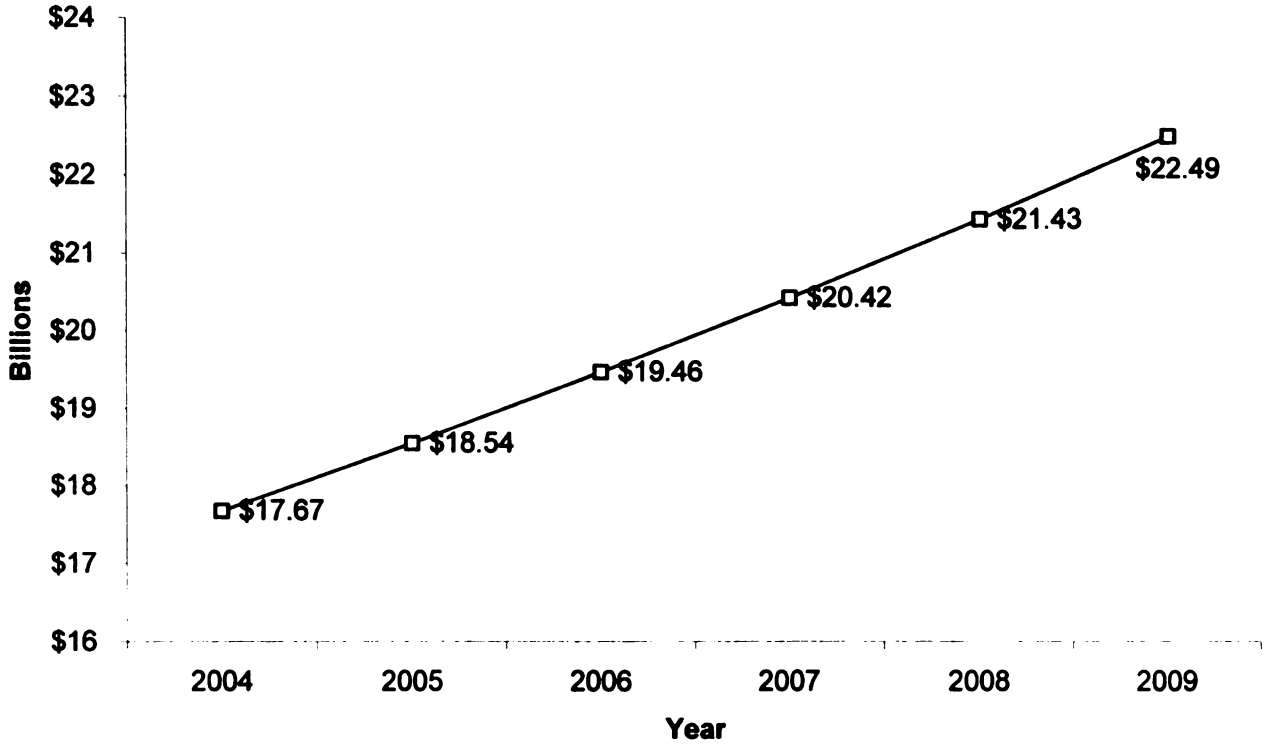


Figure 1.5. Projected RTE luncheon meat sales (Uetz, 2005)



Given the previously reported market trends, post-process contamination of RTE luncheon meats will be a serious concern for many years to come. Work done by Uyttendaele et al. (1999) in which 4.9% of cooked meat products sampled at retail markets tested positive for *L. monocytogenes* emphasizes the risk of listeriosis to the consuming public.

With an estimated 320 fatal listeriosis cases each year from RTE meat product, 242 deaths of which are associated with delicatessen-sliced luncheon meats, minimizing contamination at delicatessens will clearly have a major impact on meeting the goals of Healthy People 2010 (2004), the Presidential directive for reducing the number of listeriosis cases (and other foodborne illnesses) by 50%. The current estimate of 2500 cases annually in the United States has seen a significant reduction over the past 5 years. Laboratory confirmed listeriosis cases have declined from 0.47 cases per 100,000 in 2000 to 0.26 cases per 100,000 in 2005, approaching the target of 0.25 cases per 100,000 to be achieved by 2010.

1.9. Predictive modeling of microbial growth and transfer

Within the last decade risk assessments have promoted the development of more dynamic models that include changes in bacterial survival during distribution and subsequent storage. Reviews done by Buchanan et al., (1997) and Davies (1993) describe the many approaches to experimental design, techniques and approaches to modeling growth, transfer, and dissemination of pathogens in the microbiology, mathematical, engineering and regulatory disciplines.

1.9.1. History and development of microbial modeling

The early years of predictive microbiology centered on growth studies and thermal inactivation at high temperatures, both of which were defined by log-linear relationships (Beck and Arnold, 1977; Bernaerts et al., 2004; Schaffner et al., 1998; Zhoa and Schaffner, 2001) which were developed for food safety and quality assurance programs. Recent cross-disciplinary collaborations have prompted the development of a broad range of dynamic models using microbial, mathematical, and environmental parameters to predict population outcomes at various growth and transfer conditions. (Bernaerts et al., 2004).

1.9.2. Techniques for predictive modeling of microbial growth and transfer

Many growth models, beginning with the USDA Pathogen Modeling Program Version 2.1 (Buchanan and Phillips, 1990) have been developed to predict the growth of *Listeria* and other foodborne pathogens in foods based on pH, storage temperature, and levels of salt and sodium nitrite (Houtsma et al., Conner et al., 1986; Le Marc et al., 2002; and Tamplin, 2002). The most recent USDA model developed by Tamplin comes with a pre-programmed graphical user interface and generates graphs and tabular output for various growth parameters (Tamplin, 2002). This model is freely available to both the public and private sector for estimation of contamination and risk of exposure under various environmental conditions.

For empirical modeling it is desirable to solve for the minimum number of parameters necessary to adequately fit the data. Using a minimum number of parameters increases the degree of stability in the parameter estimation procedure and

the greatest degree of confidence in the calculated parameters. The method of least squares is chosen because it is a simple method and the results are the same as those obtained by maximum likelihood and Gauss-Markov, assuming that the following statistical assumptions are valid, as given by (Beck and Arnold, 1977).

1. The measurement errors are additive in nature to the true (but unknown) bacterial count.
2. The measurement errors, considered over the duration of the experiment, have mean value of zero.
3. The measurement errors have a constant variance over the duration of the experiment.
4. The magnitude of each measurement error is unrelated to its predecessor or successor. In simple terms, the errors are not related.
5. The measurement errors, considered over the duration of the experiment, fall in a normal, or Gaussian, distribution pattern.

Although in most experiments it is difficult to obtain detailed information about experimental errors, the assumptions listed above are not unreasonable for most types of measurements.

1.9.3. Bacterial growth and thermal inactivation models

Mathematical models were first developed to predict bacterial growth and thermal inactivation of foodborne pathogens in various substrates with little regard to the bacterial contamination and transfer that occurs up to the time of consumption

(Bernaerts et al., 2004). Schaffner (2004) recently discussed the framework for developing models that can predict the extent to which pathogens can be transferred from the food processing environment to the final product. Schaffner defined the relationship between the raw product, environment, and finished product as shown in Figure 1.6.

Figure 1.6. Mathematical framework for relationship of raw product, environment and finished products

Raw product CFU x Cross-Contamination Rate = Environmental CFU

Environmental CFU x Persistence Rate = Environmental Reservoir CFU

Environmental Reservoir CFU x Cross-Contamination Rate = Product Contact Surface CFU

Product Contact Surface CFU x Persistence Rate = Product Contact Surface Reservoir CFU

Product Contact Reservoir CFU x Cross-Contamination Rate = **Finished Product CFU**

This framework helps clarify the experimental design and mathematical manipulations for predictive modeling of cross-contamination and subsequent transfer of foodborne pathogens. This model also illustrates the additive effect of each parameter to the framework of the model where each fraction of transfer "f_x" is an additive function of the previous fraction or $f_x = f_a * f_b$ where

"f_a" = raw product and

"f_b" = cross contamination rate

This simplistic approach to predicted distribution of pathogens from raw to finished product is a precursor to more dynamic models including the use of empirical data with parameter estimation and mathematical manipulation (Bernaerts et al., 2004).

In most models, empirical data is fitted to mathematical equations using parameter estimation techniques. Hybrid models that use a combination of empirical data fitting techniques and mathematical manipulations or mechanistic mathematical translations have been described by Bernaerts et al, 2004. These models which can be used to develop manufacturing and mathematical parameters build on parameters described by empirical data while applying mathematical manipulation to each of the parameters as a function of total transfer.

Predictive modeling of microbial pathogens during food production and storage has been approached using previously published models and methods (Bernaerts et al., 2004; Buchanan and Philips, 1990; Houtsma et al., 1996). Each of these approaches has many advantages and disadvantages and has been the subject of much debate. While predictive models based on mathematical translation of

biological functions can be rapid and less costly than empirical models that require little or no laboratory experimentation, they can be greatly influenced by environmental factors not realized in a laboratory setting. In contrast to mathematical translation, curve fitting models that predict population outcomes based on previously obtained experimental data offer an arguably more accurate interpretation of predicted environmental populations. However, these models are costly and may not account for underlying biological parameters and in some cases may be dependent on specific environmental or laboratory conditions. In limited work by Schaffner et al. (2004), modeling of bacterial transfer to and from food contact surfaces was done using a Monte Carlo simulation for plastic cutting boards used in a food service kitchen for raw meats and vegetables over a 2-week period. Results from this study and subsequent simulations predicted a contamination level greater than 20 CFU/4cm^2 after 15 min and greater than 40 CFU/4cm^2 after 45 min. While this study provides some insight into modeling total bacterial transfer during slicing of deli meats, other important parameters including surface scoring, surface roughness, cutting force, and physiological differences in bacterial attachment were not addressed.

In conclusion, empirical data obtained from three years of laboratory work was used to mathematically model the relationship between cutting and slicing of RTE delicatessen meats and transfer of *L. monocytogenes*. This research was performed using a commercially available slicing machine, specially fabricated stainless steel kitchen knives and retail delicatessen meats. The previously reported incidence of *Listeria* contamination in RTE foods is considered a main factor

impacting further contamination of *Listeria*-free foods during subsequent handling at retail. This research was conducted after *Listeria* transfer rates were identified as a key informational gap in the *Draft Listeria Risk Assessment* that was published by the federal government in September 2003 (FDA/FSIS/CDC, 2003).

CHAPTER 2

IMPROVED QUANTITATVE RECOVERY OF *LISTERIA MONOCYTOGENES* FROM STAINLESS STEEL SURFACES USING A 1-PLY COMPOSITE TISSUE

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2.1. ABSTRACT

Four sampling devices a sterile environmental sponge (ES), a sterile cotton-tipped swab (CS), a sterile calcium alginate fiber-tipped swab (CAS), and a 1-ply composite tissue (CT), were evaluated for quantitative recovery of *Listeria monocytogenes* from a food-grade stainless steel surface. Sterile 304 grade stainless steel plates (6 x 6 cm) were inoculated with approximately ~ 10^6 CFU/cm^2 *L. monocytogenes* strain Scott A and dried for 1 h. The ES and CT sampling devices were rehydrated in phosphate buffer solution (PBS). After plate swabbing, ES and CT were placed in 40 ml of PBS, stomached for 1 min and hand-massaged for 30 seconds. Each CS and CAS device was rehydrated in 0.1% peptone before swabbing. After swabbing, CS and CAS were vortexed in 0.1% peptone for 1 min. Samples were spiral-plated on Modified Oxford Agar (MOX) with MOX Rodac Contact® plates used to recover any remaining cells from the stainless steel surface. Potential inhibition from CT was examined in both PBS and in a modified disc diffusion assay. Recovery was 2.70, 1.34, and 0.62 log greater using CT compared to ES, CS, and CAS, respectively, with these differences statistically significant ($P<0.001$) for ES and CT and for CAS, CS and CT ($P<0.05$). Rodac® plates were typically overgrown following ES, positive after CS and CAS, and negative after CT sampling. CT was non-inhibitory in both PBS and the modified disc diffusion assay. Using scanning electron micrscopy, *Listeria* cells were observed on stainless steel plates sampled with each sampling device except CT. The CT device, which is inexpensive and easy to use, represents a major improvement over other methods in quantifying *L. monocytogenes* on stainless steel surfaces and is likely applicable to enrichment of environmental samples.

2.2. INTRODUCTION

The public health significance surrounding *Listeria monocytogenes* has led to a regulatory policy of "zero tolerance" in the United States for this organism in certain ready-to-eat (RTE) foods. *Listeria monocytogenes* is a foodborne pathogen of major concern due to its high fatality rate (20-30%) (Ryser and Marth, 1999) its persistence (Tompkin, 2001) and spread in food-processing environments (Lunden et al., 2002; Vogel et al., 2001), and ability to grow to populations of 10^4 to 10^6 CFU/g on many refrigerated RTE foods such as smoked salmon and luncheon meats (Gombas et al., 2003; Ryser and Marth, 1999). Post-process contamination of cooked/RTE delicatessen products with *L. monocytogenes* has resulted in at least two major outbreaks (MMWR, 2000) and over 80 recalls involving more than 130 million pounds of product, making this pathogen the leading cause of Class I microbiologically related recalls (Levine et al., 2001). However, consumption of low levels of *L. monocytogenes* in food is not uncommon and is not considered a significant risk to most people (Chen et al., 2000).

Environmental sampling within food processing facilities has been plagued by poor repeatability and efficacy when compared to newer methods used in the medical and pharmaceutical industries (Richard and Piton, 1986; Tominaga et al., 2001; Yamaguchi et al., 2003). Several of these newer direct plating methods that utilize adhesive sheets (Yamiguchi et al., 2003) and pads (Tominaga et al., 2001) are reportedly superior to traditional swabbing in terms of both recovery and reproducibility for bacteria on medical devices and in pharmaceutical products. However, recovery of pathogens from equipment used in foodservice and processing environments poses a major hurdle for these newer methods because of the presence of numerous food particulates.

Existing strategies that have been developed to assess presence or absence of *Listeria* in food processing environments lack the needed sensitivity to quantify *Listeria* on solid surfaces (Gombas et al., 2003; Midelet and Carpentier, 2002). The efficiency and reliability of traditional environmental sampling devices such as the sponge and cotton swab have been debated since their introduction in the 1970's (Ware et al., 1999). Studies on meat surfaces also have shown that traditional destructive surface sampling methods such as excision yield higher bacterial recovery ($\geq$ 50%) than non-destructive swab and sponge methods (Gill et al., 2001).

Quantitative transfer to and from slicing machines, knives, and cutting boards in delicatessens was identified as a key informational gap in the 2003 *Draft FSIS risk assessment for Listeria in RTE meat and poultry products* (FDA/FSIS, 2003). In limited work, Humphrey (1990) evaluated retail delicatessen meat slicers in the UK and found *L. monocytogenes* on 10 of 32 slicer blades, thus suggesting ample opportunity for *Listeria* transfer.

In other work, the microflora on plastic and wooden cutting boards was assessed by either soaking 5-cm square blocks in nutrient broth or by direct plating on nutrient agar (Ak et al., 1994). *Escherichia coli, Listeria innocua, L. monocytogenes,* and *Salmonella* Typhimurium (10^6 CFU) were readily recovered from plastic boards up to 12 h after inoculation. Recovery was less from wood blocks with bacterial populations decreasing 98% after 12 h. This study indicates the potential for pathogen transfer from soiled surfaces after extended holding times. Transfer of *Staphylococcus aureus* from fabrics (100% cotton and 50% cotton/polyester) to hands and other fabrics such as bed linens and garments also has been assessed (Satter et al., 2001). The highest transfer rate

(>20%) was seen with moist poly cotton, with friction from moist or re-moistened fabrics significantly impacting the transfer rate.

Foodborne pathogens are also easily transferred in domestic kitchens during common food handling practices (Chen et al., 2000; Satter et al., 2001). One study showed that 40 and 60% of samples from knife handles, chopping boards, wash clothes tested positive for *Salmonella* and *Camplyobactor,* respectively, after contacting contaminated chicken during normal kitchen usage. A correlation between frequency and level of exposure and dose-response was identified as a key element in predicting the relative risk of foodborne pathogens (Tamplin, 2002).

Several studies have used scanning electron microscopy (SEM) to evaluate biofilm formation and bacterial attachment to 304 grade stainless steel of different surface finishes (Arnold and Bailey, 2000; Arnold and Silvers, 2000; Kalmokoff et al., 2001; Mafu et al., 1991). When Arnold and Bailey (2000) used a mixed culture, attachment to stainless steel was 1 log greater on 304 stainless with a 2B or rough finish when compared to 304 electropolished stainless with a mirror-like finish. Thus, SEM can serve as another means to assess recovery of bacteria from stainless steel surfaces.

Given the importance of risk assessments in determining the most vulnerable steps for contamination and growth in a food processing operation, quantification of microbial contaminants on food contact surfaces has become an integral component in establishing the degree of risk to the public. Hence, the objective of this study was to compare the 1-ply composite tissue (CT) to the environmental sponge (ES), cotton-tipped swab (CS) and calcium alginate swab (CAS) for quantitative recovery of *L. monocytogenes* from stainless steel surfaces.

2.3. MATERIALS AND METHODS

2.3.1. Preparation of strains

Listeria monocytogenes strain Scott A (GT 3864) was obtained from Dr. Joseph Madden (Neogen Corp., Lansing, MI) and maintained at -80°C in trypticase soy broth (TSB) (Difco/Becton Dickinson, Sparks, MD) containing 10% (v/v) glycerol. TSB containing 0.6% yeast extract (TSB-YE) (Difco) was inoculated from the frozen stock culture and incubated for 22-24 hours at 37°C. After a second transfer in TSB-YE, the culture was pelleted by centrifugation at 9700 x g / 10 min / 4°C (Sorvall Super T21; Sorvall Products, L.P. Newton, CT) and resuspended in 9 ml of 0.1% peptone (Difco). Cell concentration was determined by spiral plating (Autoplate® 4000 Spiral Plater; Spiral Biotech Inc., Norwood, MA) on trypticase soy agar containing 0.6% yeast extract (TSA-YE) followed by 48 h of incubation.

2.3.2. Stainless steel preparation and inoculation

Unpolished scratch-free grade 304 sanitary stainless steel plates measuring 6 cm x 6 cm x 0.145 cm were obtained from ProAxis, Inc. (Lafayette, IN). Plates were autoclaved at 121°C for 15 min and then inoculated. After use, the plates were treated with mineral oil to prevent surface oxidation. To remove mineral oil before the next use, the plates were rinsed in sterile deionized water, flamed with 95% ethyl alcohol, and then autoclaved for inoculation.

2.3.3. Sampling devices

Four *Listeria* recovery devices were assessed - a sterile environmental sponge (ES) (Nasco Speci-Sponges®; NASCO, Fort Atkinson, WI), a sterile cotton-tipped swab (CS) (Pur-Wraps® Cotton Tipped Applicator; Harwood Products Co. LLC, Guilford, MA), a sterile calcium alginate fiber-tipped swab (CAS) (Fisherbrand® Sterile Swabs; Curtin Matheson Scientific, Houston, TX), and a 1-ply white 11.4 x 21.5 cm tissue (CT) with a basis weight of 11.2 lbs and 3 point thickness (Kim-wipe® Ex-L 1- ply white tissue; Kimberley-Clarke Corp., Roswell, GA). The culture suspension (100 µl) was spotted on stainless steel plates, uniformly spread with a sterile inoculating needle to obtain an inoculum level of ~10^6 *L. monocytogenes* CFU/cm^2 and then allowed to dry for 1 h at ~23°C in a laminar flow hood.

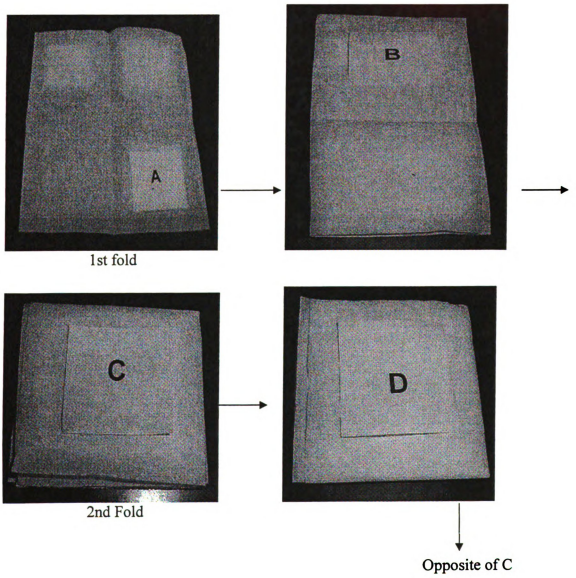
2.3.4. CT and CAS

All CS and CAS devices were rehydrated in PBS with CS and CAS absorbing 0.1 and 0.2 ml of PBS, respectively. Thereafter, the stainless steel plates were swabbed 10 times vertically and horizontally while rotating the swab between movements at a 30° angle as stated in the *Compendium of Methods for the Microbiological Examination of Foods* (Sveum et al., 1992). Thereafter, swab applicators were vortexed (Genie 2; Scientific Industries Inc., Bohemia, NY) in 10 ml of PBS for 60 sec to release *Listeria.* Samples (50 µl) were then spiral-plated on duplicate plates of Modified Oxford Agar (MOX) which were incubated 48 h at 35°C to determine numbers of *L. monocytogenes* recovered.

2.3.5. ES and CT

The CT was folded twice from the side and top edges so as to measure 5.5 cm x 5.5 cm, producing a clean interior and exterior surface which eliminated contact between gloves and the area sampled (Figure 2.1).

Figure 2.1 Folding patter of CT



Using disposable gloves, the ES and CT devices were rehydrated with 10 mL of PBS in a 24 oz Whirl-Pak® bag (NASCO; Fort Atkinson, WI) with ES and CT absorbing 9.8 mL and 0.7 ml of PBS, respectively. After squeezing the opened CT inside the sterile Whirl-Pak® bag to remove excess diluent, the stainless steel plate was swabbed 10 times vertically and horizontally with ES and with CT using the folded exterior surface for CT. After sampling, each ES and CT was returned to the same Whirl-Pak® bag with the CT partially unfolded (Figure 2.2).

Figure 2.2. CT before (A) homogenization

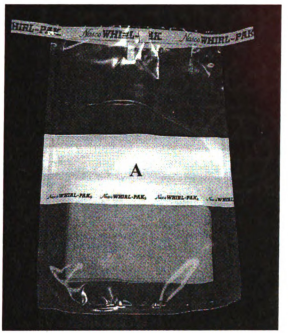
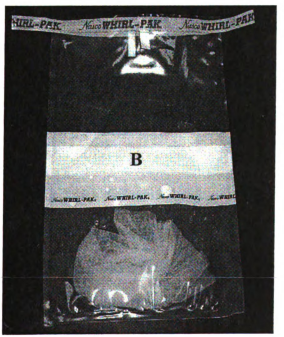


Figure 2.3. CT after (B) homogenization



PBS (40 mL) was added after which the device was homogenized in a Stomacher 400 (Seward, London, U.K.) for 60 sec and then hand-massaged for 30 sec. After homogenization, the CT was unfolded inside the Whirl-Pak® bag (Figure 2.3) and a 50 µL aliquot was spiral-plated in duplicate on MOX as previously described for enumeration of *L. monocytogenes*.

Each ES, CS, and CT recovery test was replicated 5 times using 12 plates (n = 60) with CAS being replicated 3 times (n = 36). After sampling the stainless steel plates with ES, CS, CAS, and CT, Rodac® plates containing MOX were used to quantify any remaining *Listeria* with these plates incubated 48 h at 35°C. Rodac® contact plating was replicated 3 times (n=36).

2.3.6. Potential inhibition in PBS

Sterile Whirl-Pak® bags containing 50 ml of PBS at 21-23°C were inoculated to contain 10^8 *L. monocytogenes* CFU/ml. After homogenizing in a Stomacher for 60 sec, a 0.1 ml aliquot of the homogenate was spiral-plated on TSAYE to determine the initial population. One CT device was then added to PBS with PBS similarly examined for numbers of *Listeria* after 5, 15, and 30 min of exposure. Inhibition studies for each time interval were conducted in triplicate using 10 sample bags (n=30).

2.3.7. Modified disc diffusion assay

TSAYE (20 ml) was inoculated with 100µl of an overnight *L. monocytogenes* culture (10^8 CFU/ml) and poured into standard 100-mm diameter Petri dishes. A 25-mm diameter disc was aseptically cut from the CT and placed in direct contact with the agar

surface after solidification. Plates were visually examined for inhibition zones after 24 h of incubation at 35°C. This assay was replicated twice using 10 inoculated Petri dishes with CT (n=20).

2.3.8. Evaluation of recovery methods using SEM

A field emission scanning electron microscope (CamScan 44FE; CamScan USA Inc., Cranberry Twp., PA) was used to visually evaluate recovery of *Listeria* from duplicate stainless steel plates after sampling with the four different devices. After sampling, each stainless steel plate was treated with 4% formaldehyde (5 min) to fix any remaining cells to the plate and then dehydrated using a series of 25%, 50%, 75% and 95% ethanol for 20 min. After the final dehydration in 95% ethanol, plates sampled using each of the four sampling devices were air-dried for 60 min., placed in the SEM chamber and scanned from end to end.

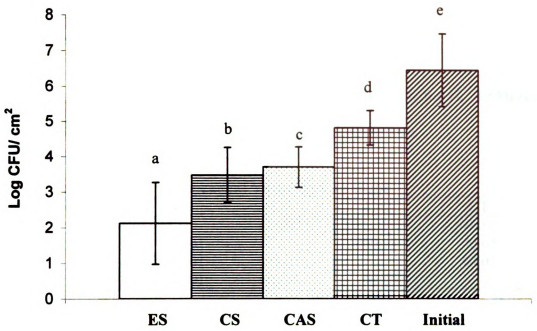
2.3.9. Statistical analysis

Data were analyzed using a general linear model (GLM) with a general randomized complete block design used to compare ES, CS, CAS, and CT devices for quantitative recovery of *L. monocytogenes* (SAS, 1996).

2.4. RESULTS

CT yielded the best recovery with populations 1.11 to 2.70 log/cm^2 higher when compared to the other methods (Figure 2.4).

Figure 2.4. Recovery of *L monocytogenes* from stainless steel.



Means with different letters are significantly different ($P < 0.05$).

Least squares means by average log count were compared for effect of the method and subjected to the least significant difference test. Differences between CT and the other devices were statistically significant *(P*<0.05*)* (Table 2.1).

Table 2.1. Least squares means for effect of method Pr > |t| for H_o: LSMean (i) = LSMean (j) with log count as the dependent variable.

Dependent Variable: Log count				
Method (i/j)	**ES**	**CS**	**CAS**	**CT**
ES		<0.001	<0.001	<0.001
CS	<0.001		>0.05	<0.05
CAS	<0.001	>0.05		<0.05
CT	<0.001	<0.05	<0.05	

ES, which is recommended in most environmental testing protocols, was least effective in quantitatively recovering *Listeria* from stainless steel. Differences between these devices were statistically significant *(P*<0.001*)* with these responses closely following the observed sampling errors for ES.

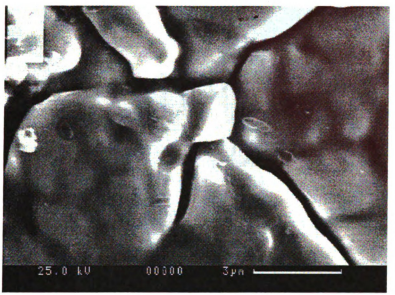
Rodac® plates were used to confirm the presence of *L. monocytogenes* on stainless steel surfaces after swabbing. Confluent growth of *Listeria* was seen for stainless steel plates previously sampled with ES with these Rodac® plates being overgrown. Following CS and CAS, Rodac® counts averaged 2.0 log *L. monocytogenes* CFU/cm^2, whereas no *Listeria* were detected after CT.

Subsequent CT testing showed no inhibition of *L monocytogenes* in PBS with counts of 8.36 and 8.20 log *L. monocytogenes* CFU/ml after 30 min of exposure. The

modified disc-diffusion assay was also negative with no inhibition zone evident after 24 h of incubation.

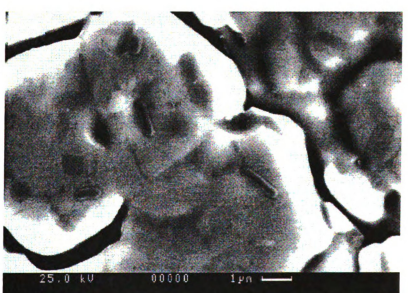
As seen by SEM, *Listeria* cells were present on the stainless steel plates after recovery using each of the devices except CT (Figures 2.5-2.8).

Figure 2.5. Scanning electron micrograph of *Listeria* attached to stainless steel plates after recovery using ES device.



3µm

Figure 2.6. Scanning electron micrograph of *Listeria* attached to stainless steel plates after recovery using CS device.



1µm

Figure 2.7. Scanning electron micrograph of *Listeria* attached to stainless steel plates after recovery using CAS device.

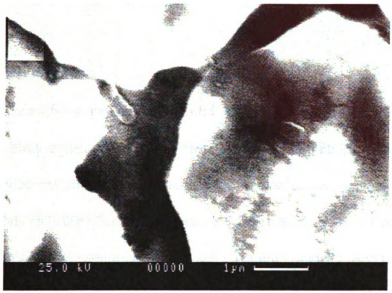
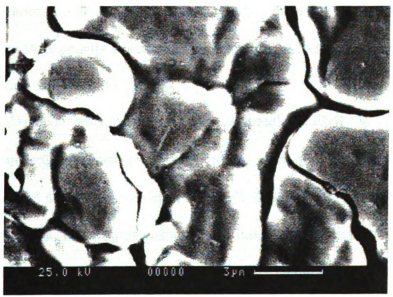


Figure 2.8. Scanning electron micrograph of *Listeria* attached to stainless steel plates after recovery using CT device.



Scanning was performed uniformly after inoculation and recovery from stainless steel plates using each device. Additional scans were performed after CT to confirm absence of *Listeria* cells on the stainless steel plates.

2.5. Discussion

Quantifying pathogens on solid surfaces provides valuable risk assessment data for modeling consumer exposure from cross-contamination in food manufacturing and foodservice environments (International Commission on Microbiological Specifications For Foods (ICMSF) Working Group on Microbial Risk Assessment, 1998). Approved for environmental sampling, ES, CS and CAS are primarily used for bacterial enrichment rather than quantification. Numerous studies have shown that these sampling devices are often awkward to use and inefficient (Pinner et.al. 1992; Richard and Piton, 1986; Salo and Laine, 2000; Scott et al., 1984; Ware et al.,1999;Yamaguchi et al., 2003). Furthermore, CS and CAS as well as Rodac® plates are impractical for quantitatively sampling large heavily soiled areas and typically yield results that are difficult to interpret (Miettinen, 2001).

Listeria monocytogenes can reportedly attach to stainless steel, glass, polypropylene, and Buna-N rubber in as little as 20 min at 4 and 20°C (Akier et al., 1990; Beresford et al.,2001; Briandet et al., 1999; Djordjevic et al., 2002; Herald and Zottola, 1988). Characterization of stainless steel has played an important role in understanding how bacteria attach to these surfaces. Studies using contact angle measurements have shown little difference between flat and penicylinders in terms of the total surface energy for stainless steel (Herald and Zottola, 1988; Hood and Zottola, 1997). The effect of

various cleaners on removal of *L. monocytogenes* from food contact surfaces also has been assessed with the pathogen rapidly attaching to stainless steel and becoming resistant to chemical sanitizers (Krysinski et al., 1992). Hence, proper cleaning and sanitizing is necessary to minimize biofilm formation and subsequent transfer of *Listeria* in delicatessens and food service environments.

The ES device is well suited for qualitative sampling of large heavily soiled areas and offers numerous advantages over CS and CAS. However, the porous cellulose matrix of ES is well known for entrapment of bacteria (Gill et al., 2001; Moore and Griffith, 2002; Salo and Laine, 2000; Ware et al., 1999), which greatly hinders any type of quantitative analysis.

Based on size limitations, CS and CAS are typically used to assess bacterial contamination in cracks, narrow tubing, crevices, joints, and any other difficult-to-sample areas on equipment. While superior to ES, the small size of CS and CAS makes these devices poorly suited for sampling large flat areas such as floors and countertops. While CAS afforded better release of *Listeria* when compared to CS ($P<0.05$), the size of the device was limiting.

Using fiber-tipped swabs, Moore and Griffith (2002) reported that the amount of mechanical energy was more important than the type of wetting solution [1/4 strength Ringer's solution, 2-N-Morpholino-ethanesulfonic acid, Tris buffer, 3% Tween, Spraycult® (a disintegrating reagent)] for recovering *Salmonella* from stainless steel surfaces. Although *Salmonella* recovery increased 16% using ¼ strength Ringer's solution compared to the other wetting solutions, the rate of *Salmonella* release from swabs pre-moistened with 1/4 Ringer's solution was lower (85.2%) compared to dry

swabs (88.2%). The amount of mechanical energy and the type of swab both played pivotal roles with the coarse foam swab having greater recovery (70.4%) when compared to cotton (69.6%), dacron (38.2%), or alginate (55.8%). These findings closely follow our CT data, suggesting that the coarse CT composition enhances scouring of the surface to remove attached cells at a lower application force. Increased recovery using CT may also be due to the inherent antistatic coating, which would aide in the release of bacteria by reducing the electrostatic discharge commonly seen in fiber-tipped swabs.

When CT was used similarly to the other devices, Rodac® plates were typically negative after CT and positive after ES and CS. Following ES, CS, and CAS sampling, *Listeria* cells were readily detected using SEM (Figures 2.5-2.8). However, no *Listeria* cells were seen on stainless steel plates in repeated scans after CT sampling (Figure 2.8).

Differences seen between initial inoculum and final recovery can be attributed to entrapment of *Listeria* cells within the CT device. The problem of entrapment within sampling devices has been of great concern when evaluating bacterial recovery. CT does not have the large porous structure of ES, thus allowing for greater release of *Listeria*. However, CT still likely entraps some cells, as suggested by our data.

CT was advantageous over the other three sampling devices in terms of repeatability and recovery. On a per test basis, the cost of the CT device was also advantageous at $0.02 per device compared to $0.73, 0.19, and 0.10 for each ES, CAS, and CS, respectively (Fisher Scientific; Pittsburgh, PA). Like other currently used environmental sampling devices, CT is amenable for enrichment of environmental samples. The >100-fold increase in recovery of *L. monocytogenes* from stainless steel, combined with ease of use and low cost, makes CT an ideal sampling device for

quantitative (and potentially qualitative) assessment of contamination on hard-to-clean surfaces such as delicatessen slicing blades.

2.6 Summary

Overall findings in the study demonstrate the improved efficacy of CT for *Listeria* recovery from stainless steel when compared to traditional ES, CS, and CAS devices. The CT device was chosen to quantify transfer of *L. monocytogenes* from RTE meats to knife and slicer blades and vice versa in Chapters 3-5. The enhanced ability of CT to detect low levels of bacterial contamination on food contact surfaces and processing equipment will aide in the development of more accurate risk assessments that directly address post-processing contamination in food processing and foodservice establishments. Given the inadequacy of current sampling devices to recover bacteria from large and heavily soiled areas, the CT device represents a significant improvement in quantification of bacteria on food contact surfaces.

CHAPTER 3

TRANSFER OF *LISTERIA MONOCYTOGENES* DURING MECHANICAL SLICING OF TURKEY BREAST, BOLOGNA, AND SALAMI

Vorst K.L., Todd, E.C.D., Ryser, E.T.

3.1. ABSTRACT

A commercial delicatessen slicer was used as the vector for sequential quantitative transfer of *Listeria monocytogenes* from (a) an inoculated slicer blade (~10^8, 10^5, 10^3 CFU/blade) to 30 slices of uninoculated delicatessen turkey, bologna, and salami, (b) inoculated product (~10^8cm^2) to the slicer and (c) inoculated product (10^8, 10^5, 10^3 CFU/cm^2) to 30 slices of uninoculated product via the slicer blade with cutting force and product composition also assessed for their impact on *Listeria* transfer. Five product contact areas on the slicer identified using product bathed in Glow Germ™ were also sampled after slicing inoculated product using a 1-ply composite tissue technique. After slicing with inoculated blades, each slice was surface- or pour-plated using Modified Oxford Agar and/or enriched in University of Vermont Medium. Greater transfer (P<0.05) was seen from inoculated turkey (10^8 CFU/cm^2) to the five slicer contact areas using a cutting force of 10 as opposed to 0 lbs. Using slicer blades inoculated at 10^8 CFU/blade *Listeria* populations decreased logarithmically to 10^2 CFU/slice after 30 slices. Findings for inoculated slicer blade and product (10^5 CFU/blade or cm^2) were similar with *Listeria* counts of 10^2 CFU/slice after 5 slices and enriched samples generally negative after 27 slices. Using 10^3 CFU/blade, the first 5 slices typically contained ~10^1 CFU/slice by direct plating with enrichments negative after 15 slices. The higher fat and lower moisture content of salami compared to turkey and bologna produced a fat layer on the blade that prolonged *Listeria* transfer. When cross-contaminated, delicatessen-sliced meats allowing growth of *Listeria* in home refrigerators may pose an increased public health risk for certain consumers.

3.2. INTRODUCTION

Listeria monocytogenes has long been viewed as a serious post-processing contaminant with this pathogen residing in some food processing facilities for many years (Beresford et al., 2001; Tominaga et al., 2001). Endemic strains that persist in food manufacturing environments possess greater ability to adhere to food contact surfaces (Beresford et al., 2001; Chmielewski and Frank, 2003; Lunden et al., 2000) with some strains attaching to stainless steel in as little as 20 minutes (Mafu, 1991). In one study, Lunden et al. (2002) demonstrated plant-to-plant transfer of *L. monocytogenes* via a dicing machine with the same *Listeria* strain identified at three different facilities. Thus, processing equipment and other food contact surfaces can serve as vectors for the spread of *Listeria* during food manufacture.

Transfer of pathogens through slicing machines was recognized over 40 years ago. An outbreak in Aberdeen, Scotland led to 469 cases of typhoid fever (International Commission on Microbiological Specifications For Foods (ICMSF) Working Group on Microbial Risk Assessment, 1998). The contaminated corned beef was delicatessen sliced resulting in transfer to other meat products via the contaminated slicer over several days. In 1990, Humphrey (1990) recovered *L. monocytogenes* from 10 of 32 retail delicatessen slicers surveyed in the United Kingdom. Three years later, Hudson and Mott (1993) reportedly isolated *L. monocytogenes* from a delicatessen knife and slicing machines in Amsterdam supermarkets with the pathogen also found at most sites near a display case of processed meats.

Listeria monocytogenes is now a well-recognized contaminant of delicatessen products with Sauders et al. (2004) having identified this pathogen in smoked salmon, deli meats and cheeses, hot dogs, and seafood from 20 of 47 retail food establishments surveyed in New York State. In a large-scale survey by Gombas et al. (2003), the incidence of *L. monocytogenes* was approximately seven times greater in delicatessen-sliced (0.4%) as opposed to manufacture-sliced luncheon meats (2.7%) with difficult-to-clean delicatessen slicers and other food contact surfaces presumably being responsible for the higher contamination rate. These findings, along with a report indicating that 75% of consumers purchase delicatessen-sliced rather than pre-packaged luncheon meats (Gombas, 2003), suggest substantial consumer exposure to *Listeria.*

Four major listeriosis outbreaks have been documented in the United States since May of 2000, three of which were traced to consumption of delicatessen-sliced turkey breast, (MMWR 2000, MMWR 2002). These three outbreaks were responsible for a combined total of 91 listeriosis cases, including 11 deaths and 6 miscarriages, in 22 states and the recall of 44.3 million pounds of product. These outbreaks prompted the development of three USDA-mandated alternatives for controlling *Listeria* in delicatessen meats – (a) post-package pasteurization, (b) product reformulation to prevent *Listeria* growth and/or (c) increased product and environmental testing (FSIS, 2003) and also raised concerns regarding current food handling practices at the retail level as specified in the Food Code (FDA/CFSAN, 2001). Based on a FSIS risk assessment for *Listeria* in ready-to-eat meat and poultry products (FSIS, 2003), 242 of the estimated 500 listeriosis fatalities each year are thought to be traceable to delicatessen meats. Thus,

minimizing contamination at delicatessens will clearly have a major impact on reducing the incidence of listeriosis and in meeting the goals of *Healthy People* 2010 (2004)

Given that the numbers of *Listeria* transferred between commercial slicing machines and delicatessen meats was cited as both a major public health concern and a key data gap in several *Listeria* risk assessments (FSIS, 2003; USDA/FSIS, 2003), the objectives of this study were to assess: (a) impact of cutting force on transfer of *L. monocytogenes* from contaminated RTE luncheon meats to a delicatessen slicer, (b) transfer of *L. monocytogenes* from an inoculated delicatessen slicer blade to uninoculated roast turkey, salami and bologna, (c) transfer of *L. monocytogenes* from inoculated product to a delicatessen slicer and then to uninoculated product, and (d) slicer blade wear over a 2-year duration.

3.3. MATERIALS AND METHODS

3.3.1. *Listeria monocytogenes* strains

The following six strains of *Listeria monocytogenes* (obtained from Dr. Catherine W. Donnelly, University of Vermont, Burlington, Vermont): CWD 205 (source unknown), CWD 578 (dairy plant), CWD 701 (Azore cheese), CWD 730 (dairy plant), CWD 845 (dairy plant), and CWD 1002 (pork sausage) were chosen from a set of more than 190 strains based on their ability to form weak (CWD 205, CWD 578), medium (CWD 701, CWD 1002) or strong (CWD 730, CWD 845) biofilms in a microtiter plate assay (Keskinen et al., 2003). All strains were maintained at -80°C in trypticase soy broth (TSB) (Difco/Becton Dickinson, Sparks, MD) containing 10% (v/v) glycerol. TSB

containing 0.6% yeast extract (TSB-YE) (Difco) was inoculated from the frozen stock cultures and incubated for at 37°C for 24 h. After a second transfer in TSB-YE, each culture was pelleted by centrifugation at 9700 x g / 10 min / 4°C (Sorvall Super T21; Sorvall Products, L.P. Newton, CT), resuspended in 9 ml of 0.1% peptone (Difco) and combined in equal volumes to produce one 6-strain cocktail containing approximately 10^8 CFU/ml. Cell concentration was determined by optical density at 600 nm and spiral plating (Autoplate® 4000 Spiral Plater; Spiral Biotech Inc., Norwood, MA) an appropriate dilution on Modified Oxford (MOX) agar followed by 48 h of incubation at 35°C.

3.3.2. Delicatessen meats

One retail brand each of restructured roast turkey breast, Genoa hard salami and bologna (5.5 to 6.5 lbs each) was purchased in chub-form from a local retailer (Gordon Food Service, Lansing, MI), held at 4°C and used within 20 d. Based on the package label, product compositions were as follows: turkey breast (composed of each turkey breast, turkey broth, < 2% each of salt, dextrose, and sodium phosphates); salami (composed of pork, beef, salt, < 2% each of dextrose, water, natural spices, sodium ascorbate, lactic acid starter culture, garlic powder, sodium nitrite, BHA, BHT, and citric acid); and bologna (composed of beef, pork, water, salt, and < 2% each of dextrose, potassium lactate, sodium diacetate, sodium erythorbate, sodium nitrite, and oleoresin of paprika).

Fat, moisture, and crude protein contents were determined in triplicate for two lots of each product according to the Association of Official Analytical Chemists (AOAC) methods 991.36, 950.46, and 992.15, respectively (AOAC, 2003).

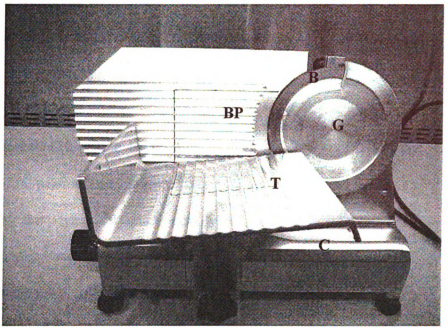
3.3.3. Delicatessen slicer

A commercial gravity fed delicatessen slicer (Model 220F, Omcan Manufacturing; Niagara, Falls, NY) manufactured with an electropolished 304 stainless steel blade and other non-electropolished components was used for slicing. In order better quantify numbers of *Listeria* recovered from the various slicer components, the slicer blade was milled from a diameter of 22 cm to 15.5 cm while maintaining the original surface profile, which had a beveled cutting edge 2.5 cm wide. The guard and back plate were scaled down to conform to the milled blade.

3.3.4. Identification of delicatessen slicer product contact areas

A chub of turkey breast was bathed in Glow-Germ™ powder (Glo-Germ™; Moab, UT) and immediately sliced (5 slices) using the delicatessen slicer. The entire slicer was then viewed under UV light (260 nm) to identify the most likely parts to be contaminated. From this, the following product contact surfaces and areas for later sampling: table (T) - 160 cm^2, back plate (BP) - 192 cm^2, guard (G) - 161 cm^2, blade (B) - 181 cm^2, and collection area (C) - 176 cm^2 (Figure 3.1).

Figure 3.1. Contact areas of gravity feed delicatessen slicer



(T) = table, (BP) = back plate, (B) = blade, (G) = guard, (C) = collection area

3.3.5. Surface profiling of delicatessen slicer blade

Blade roughness values and overall surface profiles were obtained at the University of Illinois - Center for Microanalysis of Materials (Urbana, IL) using a Sloan Dektak^3 ST stylus surface profilometer (Veeco Instruments Inc., Woodbury, NY). Surface profilometer measurements were taken along three radial 10-mm lines marked at approximately 120-degree intervals on the front and backside of new and used blades after 1 and 2 years of use. Surface roughness data points were collected by recording the height of the stylus 40 times per second while traveling along the 10-mm line. Measurements were made along these lines with the stylus movement, ending approximately 0.5 mm from the blade edge. The data was then short-pass filtered to remove the effects of blade surface curvature during the milling process from the manufacturer and provide a base line for pitting and scoring from slicing and cleaning regimens.

3.3.6. Evaluation of slicer blade wear using SEM

A field emission scanning electron microscope (CamScan 44FE; CamScan USA Inc., Cranberry Twp., PA) was used to visually assess new and used stainless steel slicer blades for pitting and oxidation. Three 4 x 4 cm pieces were cut from new and 2-year old slicer blades using a computerized numeric control laser cutter (ProAxis Inc.; West Lafayette, IN). Each slicer blade piece was cleaned with 95% ethanol, placed in the SEM chamber and scanned from end to end.

3.3.7. Impact of force on *L. monocytogenes* transfer from turkey to a delicatessen slicer.

A replicated study (n = 3) involving inoculated roast turkey breast (~10^5 CFU/cm^2) was conducted using the 6-strain cocktail. Each turkey chub (22 cm in length x 8 cm in diameter) was surface inoculated with the 6-strain cocktail (100 µl) lengthwise along a 1-cm wide strip and held for 1 h at 4°C to allow the inoculum to absorb into the product. Forces of 0 and 10 ± 2 lbs were applied to the product against the back plate while slicing and were continuously monitored using a Chatillion® force gauge (Amtek, Largo, FL) equipped with 10 x 10 cm product contact platform. After each slice, the five previously identified contact areas were swabbed using the 1-ply composite tissue (CT) recovery method developed by Vorst et al. (2004) and added to stomacher bags containing 50 ml of phosphate buffered saline (PBS). Samples were homogenized in a Stomacher 400 (Seward; Norfolk, England) for 1 min and spiral plated on MOX followed by 48 h of incubation at 35°C. For each replication, the numbers of *Listeria* transferred as impacted by force were analyzed using multiple linear regression and analysis of variance (SAS, 1996).

3.3.8. Slicer blade inoculation

A turkey slurry was prepared for inoculating the delicatessen slicer blade by diluting 25 g of turkey breast 1:10 in sterile deionized water and homogenizing in a model DIFP2 blender (General Electric; Bridgeport, CT) at high speed for 1 min. Thereafter, the slurry was filtered through five layers of cheesecloth, heated in an 80°C water bath for 20 min, cooled and stored in 50 ml aliquots at -20°C. For use, 50 ml of the

turkey slurry was thawed overnight at 4°C, poured into a sterile 15-cm diameter glass bowl to a depth of 1.5 cm and inoculated with 0.35 ± .05 ml of the 6-strain cocktail so as to contain 10^9, 10^6, or 10^4 CFU/ml. The product-blade contact area of the alcohol-flamed and cooled slicer blade, as previously identified by Glo-Germ™ (161 cm^2), was inoculated by rotating the blade through 5 revolutions in the bowl so as to contain 10^8, 10^5, or 10^3 CFU/blade and then dried for 1 h in a laminar flow cabinet. Although unrealistically high, these inoculations level were deemed necessary to quantify *Listeria* transfer during sequential slicing.

3.3.9. Transfer of *L. monocytogenes* from an inoculated delicatessen slicer blade to uninoculated product

The previously inoculated slicer blade was used to obtain thirty 2 to 3 mm-thick slices of turkey, salami or bologna weighing approximately 25 g each with each experiment replicated 3 times. For slicer blades containing 10^8 CFU/blade, all 30 slices were diluted 1:5 (w/v) in PBS, homogenized in a Stomacher for 2 minutes and spiral plated (50 µl) on MOX. For inoculum levels of 10^5 and 10^3 CFU/blade, all slices were diluted 1:5 in UVM and homogenized in a Stomacher for 1 minute. Duplicate 5 ml aliquots of the homogenized sample were pour-plated in 25 ml of MOX using 150-mm diameter disposable Petri dishes (Fisher Scientific; Chicago, IL) and incubated at 35°C for 48 h with populations determined as the number of listeriae per slice. When *Listeria* was not detected by direct plating, MOX plates from the previously enriched samples were examined for presence/absence of *Listeria* after 48 h of incubation at 35°C.

3.3.10. Transfer of *L. monocytogenes* from inoculated product via the slicer to uninoculated product

Transfer of *L. monocytogenes* from inoculated turkey, salami, and bologna to the delicatessen slicer and then to uninoculated product was replicated 3 times for each of the three products. The turkey, salami, and bologna chubs were surface-inoculated with the aforementioned 6-strain *L. monocytogenes* cocktail to obtain approximately 10^8 and 10^5 CFU/cm^2 as determined from spiral plating. These inoculation levels were again necessary to quantify numbers of *Listeria* in consecutive slices. After 1 h at 4°C to allow the inoculum to absorb, three to five slices were generated from each chub to artificially contaminate the blade with this same blade then immediately used to obtain 30 slices of uninoculated product of the same or different type.

When product containing 10^8 CFU/cm^2 was cut to contaminate the blade and followed by uninoculated product, the first 20 slices were diluted 1:5 in PBS and spiral-plated on MOX. The 10 remaining slices were diluted 1:5 in UVM, incubated 48 h at 35°C and streaked to MOX. For products containing 10^5 CFU/cm^2, *L. monocytogenes* was recovered by homogenizing a 1:5 dilution in UVM and then pour plating duplicate 5 ml aliquots in 25 ml of MOX using 150-mm dia. Petri plates. After 48 h of incubation at 35°C, all *Listeria*-like colonies on the MOX plates were counted to determine the number of listeriae per slice. When *Listeria* was not detected by direct plating, the MOX plates streaked after enrichment were examined for presence/absence of *Listeria* after 48 h of incubation at 35°C.

3.3.11. Quantification of injured *Listeria* on slicer blades

Five 4 x 4 cm pieces cut from the cutting edge of a new stainless steel slicer were inoculated by spreading 100 µl of the aforementioned six-strain cocktail on the surface and then drying in a laminar flow cabinet for 1 h. The five slicer blade pieces were sampled using the previously described CT method with 1 ml of PBS added to CT before swabbing. After adding the CT to 9 ml of PBS and homogenizing in a Stomacher for 1 min, aliquots (50 µl) were spiral-plated in duplicate on tryptose phosphate agar (DIFCO) containing ferric ammonium citrate (0.5 g/l) and esculin (1 g/l) (mTPA) for recovery of healthy and injured cells, and on mTPA with sodium chloride (40 g/l) (NaCl) (mTPAN) and MOX for recovery of healthy cells as previously described (Matthew and Ryser, 2002). All plates were counted after 48 h at 35° C. Percent injury was determined by the following equation:

% injury = [(non-selective count – selective count)/ non-selective count]* 100.

3.3.12. Cleaning and decontaminating the slicer

After use and complete disassembly, the slicer table, guard, and blade were wiped with a 1-ply composite tissue and soaked for 30 min in a pan containing an activated 32% alkaline glutaraldehyde solution (CIDEX®; Advanced Sterilization Products, Irvine, CA). Non-removable components of the slicer (back plate and collection area) were disinfected with the same 32% alkaline glutaraldehyde solution and allowed to air dry for 30 min. Thereafter, a 1-ply composite tissue was soaked in 70% ethanol (v/v) was used to clean all removable and non-removable parts of the slicer after which all components were

rinsed with deionized water and dried. Follow-up sampling using the CT method showed that the slicer was free of *Listeria*. To prevent surface oxidation during storage, the slicer blade was coated with a thin layer of mineral oil, which was removed by flaming with 95% ethanol, rinsing with sterile deionized water and drying with KimWipe® immediately before use.

3.3.13. Statistical analysis

All *Listeria* transfer experiments were replicated three times. Impact of cutting force on transfer of *Listeria* to the five slicer contact areas and direct/sequential transfer from the inoculated slicer blade to uninoculated product and from inoculated product to uninoculated product via the slicer blade were analyzed using a general linear model and analysis of variance (ANOVA) for least significant differences in mean recovery (Scott et al., 1984). Mean differences in surface topography were analyzed using a general liner model at each time point (n=3) (Scott et al., 1984).

3.4. RESULTS

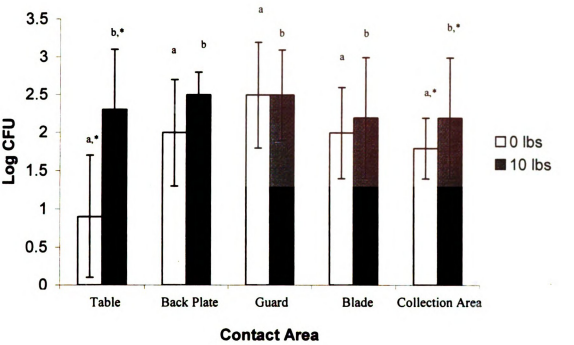
3.4.1. Proximate analysis

Based on analyses of duplicate lots, roast turkey breast, bologna and salami contained an average of 78, 60 and 43 % moisture, <1, 27 and 36% fat, and 19, 10 and 17% protein, respectively.

3.4.2. Impact of force on *L. monocytogenes* transfer from turkey to a delicatessen slicer

A force of 4.5 kg applied against the product while slicing yielded significantly greater *Listeria* transfer than 0 kg. ($P<0.05$). Less transfer was seen to the table than other slicer contact areas at 0 kg ($P<0.05$) with transfer of *Listeria* to the back plate, guard, and blade not significantly different ($P>0.05$) (Figure 3.2). However, significantly greater ($P<0.05$) numbers of listeriae were recovered from the collection area using an application force of 4.5 kg. No significant difference ($P<0.05$) was seen between the table, back plate, guard and blade at a force of 4.5 kg, suggesting uniform contamination (Figure 3.2).

Figure 3.2. Number of *Listeria monocytogenes* recovered at an application force of 0 and 10 lbs.

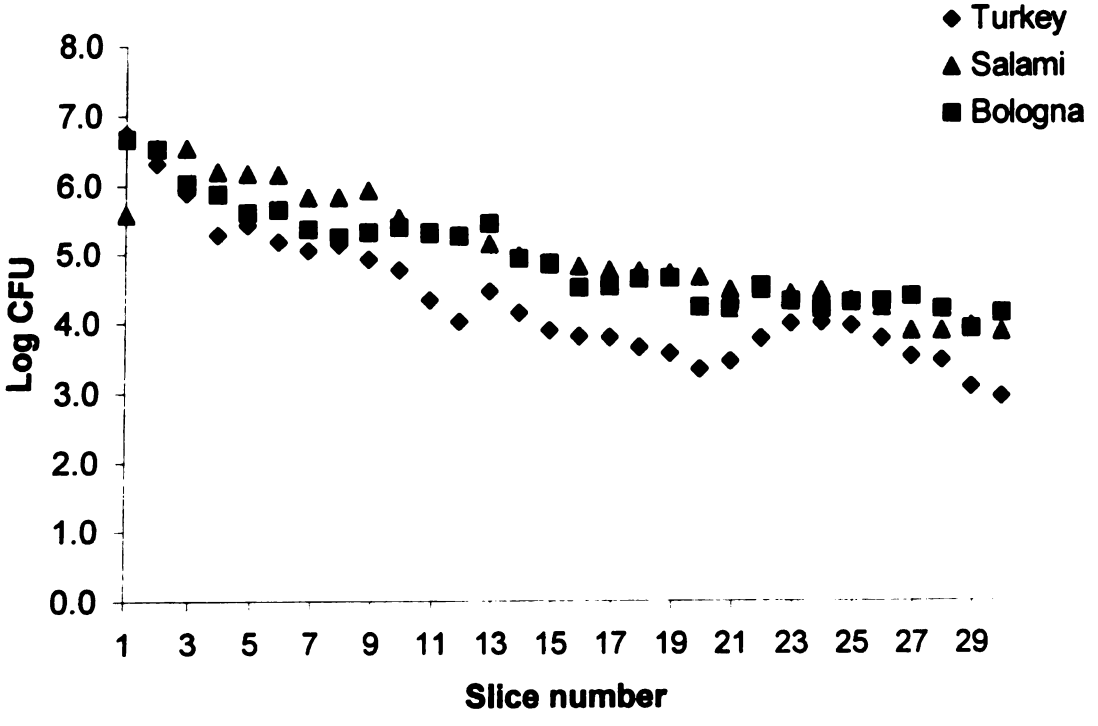


Means with different superscripts are significantly different for total transfer ($P < 0.05$)
Means with (*) are significantly different within each contact area between force treatments ($P < 0.05$)

3.4.3. Transfer of *L. monocytogenes* from an inoculated delicatessen slicer blade to uninoculated product

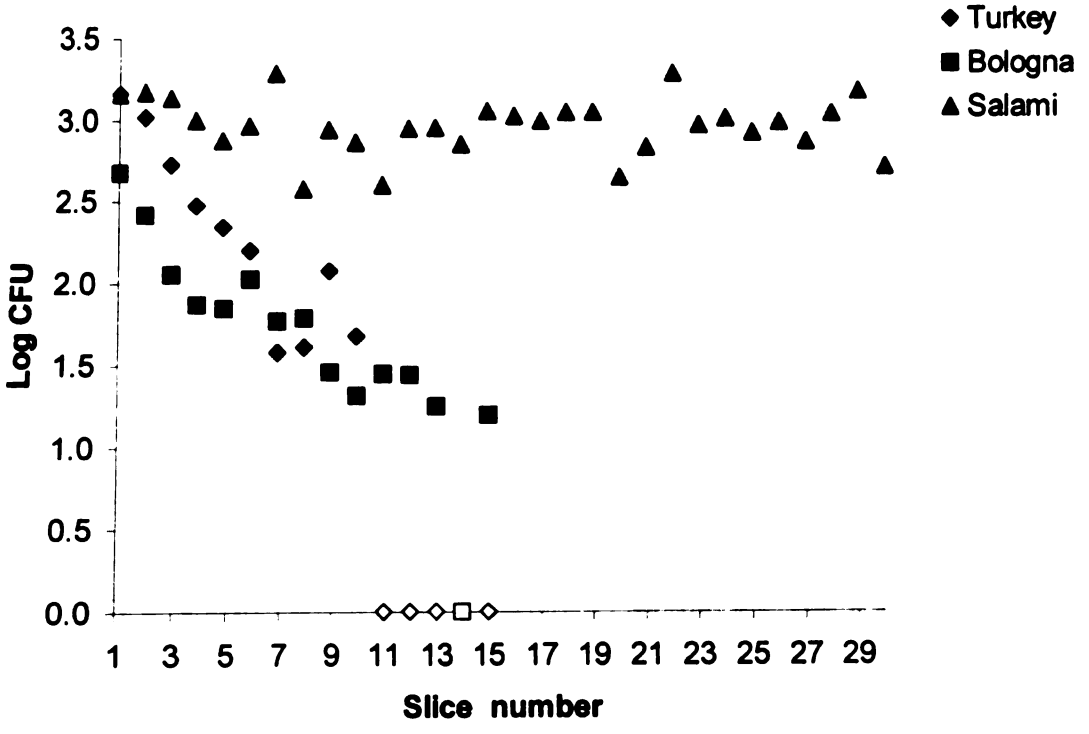
Listeria monocytogenes transfer from an inoculated slicer blade containing 10^8 CFU/blade to uninoculated roast turkey and bologna was generally logarithmic (R^2 >0.92) and linear for salami (R^2 = 0.93) with no significant differences (P<0.05) in average recovery seen between the three products (Figure 3.3).

Figure 3.3. Transfer of *L. monocytogenes* from inoculated slicer blade 10^8 (CFU/blade) to uninoculated turkey, salami and bologna



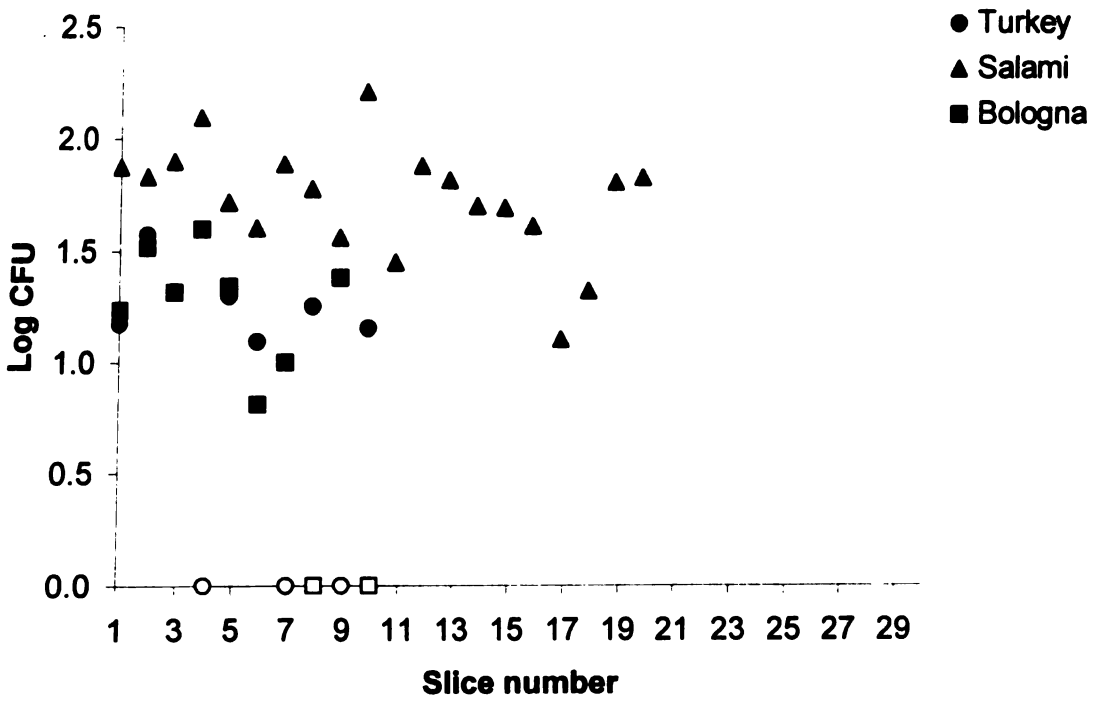
All three products yielded direct counts for each of the 30 slices with a 2-log reduction seen after the first 20 slices. Except for salami, similar results were obtained at an inoculation level of 10^5 CFU/blade (Figure 3.4).

Figure 3.4. Transfer of *L. monocytogenes* from an inoculated slicer blade 10^5 (CFU/blade) to uninoculated turkey, salami and bologna. Open symbols not quantifiable by direct plating.



While a linear and logarithmic decrease in numbers of *Listeria* transferred was not seen for salami, enrichment results were positive out to 30 slices at an inoculation level of 10^3 CFU/blade (Figure 3.5).

Figure 3.5. Transfer of *L. monocytogenes* from an inoculated slicer blade 10^3 (CFU/blade) to uninoculated turkey, salami and bologna. Open symbols not quantifiable by direct plating.



Salami was significantly different (P <0.05) from turkey and bologna at 10^5 CFU/blade. Unlike turkey and bologna, a decrease in *Listeria* transfer was not evident during slicing of salami. *Listeria* populations transferred to turkey and bologna were not significantly different (P >0.05) at an inoculation level of 10^3 CFU/blade. Similarly to 10^5 CFU/blade, salami was significantly different (P <0.05) from both turkey and bologna. Using 10^5 CFU/blade, enrichments for turkey and bologna were typically positive out to 22 slices with all 30 slices of salami positive by direct plating. At 10^3 CFU/blade, enrichments were typically positive out to 23 slices for turkey and salami and 20 slices for bologna (Table 3.1).

Table 3.1. Number of samples yielding *Listeria* by direct count and/or enrichment (N=3) for delicatessen slicer-product (DS-P) and product-delicatessen slicer-product (P-DS-P) transfer for turkey (T), bologna (B), and salami (S).

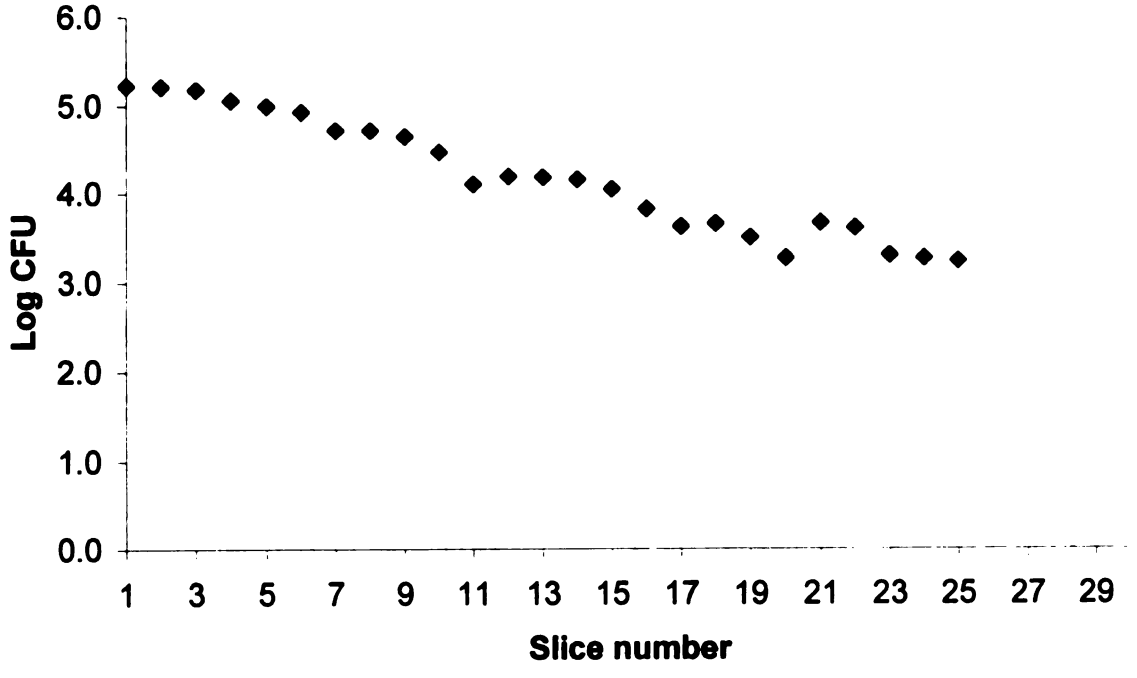
	10^3 CFU/blade (DS-P)			10^6 CFU/blade (DS-P)			10^6 CFU/cm^2 (P-DS-P)		
Slice	T	B	S	T	B	S	S-DS-S	S-DS-T	T-DS-S
1	3/3[a]	3/3	3/3	3/3	3/3	3/NT[b]	3/3	3/3	3/3
2	3/3	3/3	3/3	3/3	3/3	3/NT	3/3	1/3	1/3
3	3/3	2/3	3/3	3/3	3/3	3/NT	3/3	3/3	3/3
4	0/3	1/3	3/3	3/3	3/3	3/NT	3/3	1/3	3/3
5	2/3	2/3	3/3	3/3	3/3	3/NT	3/3	1/3	3/3
6	1/3	1/3	3/3	2/3	3/3	3/NT	3/3	1/3	3/3
7	0/3	1/3	3/3	1/3	3/3	3/NT	3/3	1/2	3/3
8	2/3	2/3	3/3	1/3	3/3	3/NT	2/3	2/2	3/3
9	0/3	2/3	3/3	2/3	3/3	3/NT	3/3	0/0	2/3
10	1/3	0/3	3/3	1/3	3/3	3/NT	0/3	0/1	2/3
11	0/3	0/2	3/3	0/3	2/3	3/NT	3/3	0/0	2/3
12	0/3	0/2	3/3	0/3	2/3	3/NT	2/3	0/0	1/3
13	0/3	0/2	3/3	0/3	2/3	3/NT	0/3	0/0	1/3
14	0/3	0/2	3/3	0/3	0/3	3/NT	0/3	0/0	0/2
15	0/3	0/2	3/3	0/3	2/3	3/NT	0/3	0/0	0/2
16	NT/3	NT/1	2/3	0/3	0/3	3/NT	NT/3	NT	NT/0
17	NT/3	NT/0	3/3	0/3	0/3	3/NT	NT/3	NT	NT/1
18	NT/2	NT/1	2/3	0/3	0/2	3/NT	NT/3	NT	NT/0
19	NT/2	NT/2	2/2	0/3	0/2	3/NT	NT/3	NT	NT/0
20	NT/3	NT/1	½	0/1	0/2	3/NT	NT/3	NT	NT/0
21	NT/3	NT/0	NT/3	NT/1	NT/2	3/NT	NT/3	NT	NT
22	NT/3	NT/0	NT/3	NT/2	NT/2	3/NT	NT/3	NT	NT
23	NT/2	NT/0	NT/2	NT/0	NT/2	3/NT	NT/3	NT	NT
24	NT/0	NT/0	NT/0	NT/1	NT/1	3/NT	NT/3	NT	NT
25	NT/2	NT/0	NT/0	NT/0	NT/1	3/NT	NT/3	NT	NT
26	NT/0	NT/0	NT/0	NT/0	NT/1	3/NT	NT/2	NT	NT
27	NT/0	NT/0	NT/0	NT/0	NT/1	3/NT	NT/2	NT	NT
28	NT/1	NT/0	NT/0	NT/0	NT/0	3/NT	NT/2	NT	NT
29	NT/0	NT/0	NT/0	NT/0	NT/1	3/NT	NT/2	NT	NT
30	NT/0	NT/0	NT/0	NT/0	NT/0	3/NT	NT/2	NT	NT

[a] Number of samples positive by direct plating / number of samples positive by enrichment,
[b] NT- Not Tested

3.4.4. Sequential transfer of *L. monocytogenes* from inoculated product to a delicatessen slicer and then to uninoculated product

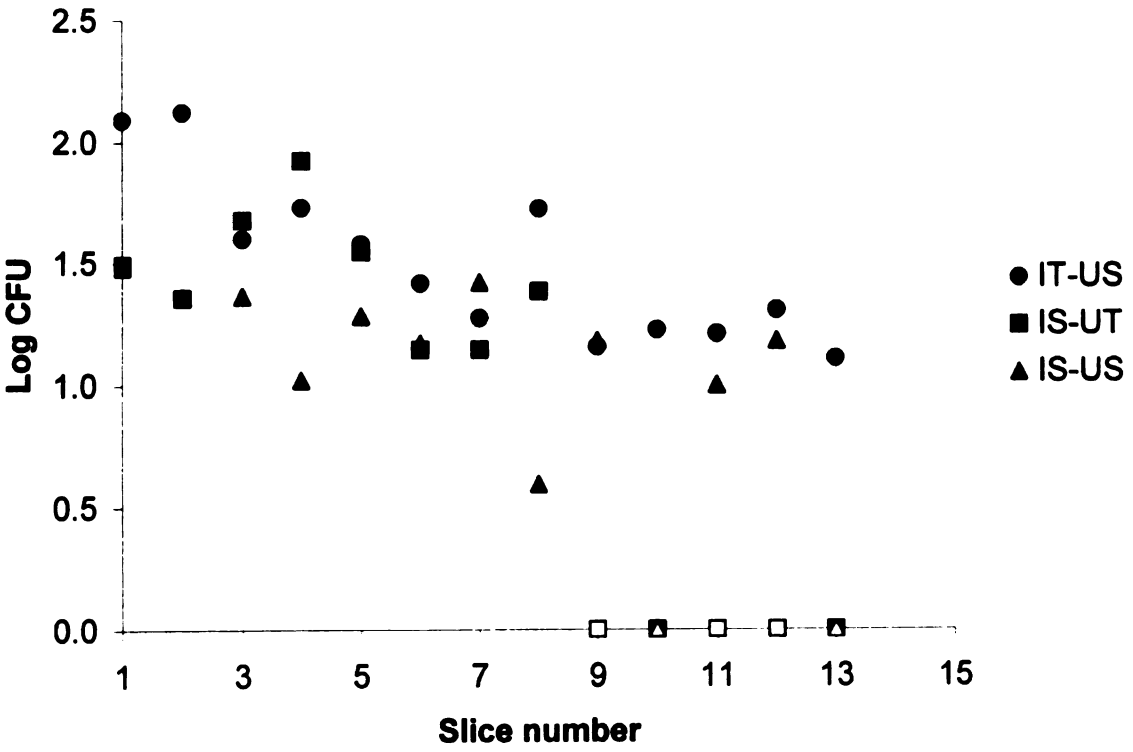
Numbers of *Listeria* transferred from surface-inoculated turkey containing 10^5 CFU/cm^2 to uninoculated turkey during slicing were not quantifiable by direct plating. At the higher inoculum level (10^8 CFU/cm^2), *L. monocytogenes* populations decreased ~2 logs after 15 slices with enriched samples positive out to 30 slices (Figure 3.6).

Figure 3.6. Transfer of *L. monocytogenes* from inoculated turkey 10^8 (CFU/cm^2) via the slicer blade to uninoculated turkey.



Using product inoculated to contain 10^5 CFU/cm^2, *Listeria* was quantifiable when inoculated turkey was sliced before uninoculated salami, when inoculated salami was sliced before uninoculated turkey, and when inoculated salami was sliced before uninoculated salami. After slicing inoculated turkey, the first 14 slices of uninoculated salami yielded *Listeria* by direct plating. After slicing inoculated salami, *Listeria* was also quantifiable in uninoculated turkey with a high degree of variability seen between slices. Slicing inoculated salami followed by uninoculated turkey yielded the greatest variability in direct counts for the first ten slices (Figure 3.7).

Figure 3.7. Transfer of *L. monocytogenes* from inoculated turkey (IT) and inoculated salami (IS) 10^5(CFU/cm^2) to uninoculated turkey (UT) and uninoculated salami (US) during slicing. Open symbols not quantifiable by direct plating.

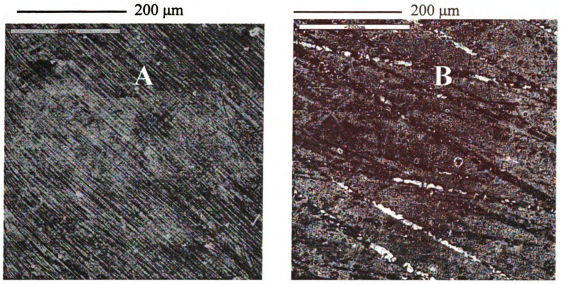


A product inoculation level of 10^5 CFU/cm^2 yielded positive enrichment results out to 30, 8 and 15 slices when inoculated salami was followed by uninoculated salami or turkey or when inoculated turkey was followed by uninoculated salami, respectively (Table 3.1). At an inoculation level of 10^5 CFU/cm^2, a comparison of means showed significant differences (P <0.05) for average recovery between inoculated turkey followed by uninoculated salami when compared to inoculated salami followed by uninoculated turkey and inoculated salami followed by uninoculated salami.

3.4.5. Slicer blade surface profiling

A significant difference (P<0.001) in surface topography was seen for both the front and back surfaces of the grade 304 stainless steel electropolished slicer blades over time. Initial average roughness values for the front and back sides increased from 653 and 752 μm to 935 and 836 μm after year 1 and to 3251 and 5045 μm after year 2. Electron micrographs taken with the SEM showed substantial wear and pitting on used as compared to new slicer blade chips (Figure 3.8).

Figure 3.8. SEM micrographs of new (A) and used (B) slicer blades after 1 year of use



3.4.6. Quantification of injured *Listeria* on slicer blades

The non-selective medium (mTPA) afforded greater recovery of healthy and injured *Listeria* cells from stainless steel slicer blade pieces blade compared to selective media. Using mTPAN and MOX, 61% and 73% of the *Listeria* inoculum was injured, respectively, after 1 h of drying in a laminar flow cabinet.

3.5. DISCUSSION

The three products for slicing were chosen based on differences in fat and moisture content with turkey having the lowest fat (<1%) and highest moisture (78%) and salami having the highest fat (36%) and lowest moisture (43%). These variations in product composition resulted in visual differences in soiling after slicing. The higher fat and lower moisture content of salami compared to turkey and bologna produced a

pronounced fat layer on the blade, which provided an excellent menstrum for *Listeria* dispersion as well as protection from the normal frictional forces during slicing. Unlike salami, the higher moisture and lower fat content of turkey had a washing affect on the slicer blade with fewer visible meat particles remaining on the blade after consecutive slicing. Lin et al. (2004a) also reported that a layer of fat developed on slicer blades and conveyor belts after slicing salami but not after slicing bologna or turkey. Salami does not support growth of *L. monocytogenes* and has not been implicated as a vehicle for listeriosis. However, the fat layer that develops on these blades after slicing salami provides an ideal mechanism for prolonged *Listeria* transfer and cross-contamination of other *Listeria*-free products. The amount of mechanical energy applied to a stainless steel food contact surface during microbiological sampling also has a significant impact on bacterial recovery. Findings by Moore and Griffith (2002) demonstrated greater bacterial recovery from stainless steel with increased mechanical energy. Our findings also suggest a direct relationship between mechanical energy, application force and *Listeria* transfer. Significantly greater ($P<0.05$) transfer of *L. monocytogenes* was seen from inoculated turkey breast to the slicer using a cutting force of 10 lbs as opposed to 0 lbs. Applying a 10 lb force to turkey breast during slicing led to more uniform contamination of the slicer with the amount of exudate increasing as more force was applied to the product during slicing.

Further differences in the numbers of *Listeria* transferred can be attributed to the fat and moisture content of these products. The presence of a fat layer on a conveyor belt during slicing of salami yielded the greatest numbers of *Listeria* on the blade, slicer housing and conveyor belt. Further work done by Lin et al. (2004a) showed increased

Listeria transfer from contaminated slicer parts to turkey, salami, and bologna during slicing. In their study, a commercial delicatessen slicer blade was inoculated with a five-strain cocktail of *L. monocytogenes* (10^2 CFU) and then used to slice turkey, bologna, and salami. Packages containing five slices of each product were vacuum-sealed and assessed for *Listeria* growth after 1, 30, 60, and 90 days of storage at 4°C. While *Listeria* populations increased in roast turkey breast, numbers gradually declined in salami and bologna and fell below detectable limits after 60 and 90 days of storage. In most cases, our results demonstrated *Listeria* transfer out to 30 slices. Thus, the potential exists for growth of *Listeria* to potentially hazardous levels in roast turkey breast during refrigerated storage as demonstrated by Lin et al. (2004b).

Previous research has helped identify surfaces such as stainless steel and polyethylene that more conducive to bacterial transfer than rubber (Arnold and Silvers, 2000; Beresford, 2001; Midelet and Carpentier, 2002). Stainless steel is prone to scratching, pitting and corrosion with chlorine- and acid-based sanitizers hastening this process (Barkley, 1979; Bohner and Bradley, 1991; Stone and Zottola, 1985). When present on slicer blades, such pits provide preferential sites for bacterial attachment and biofilm formation (Arnold and Bailey, 2000; Chmielewski and Frank, 2003), which impact the bacterial transfer rate during slicing. Recent studies have also demonstrated areas of a table-top bowl chopper most susceptible to contamination during processing of beef contaminated with *Escherichia coli* O157:H7 (Flores, 2004). Areas of the bowl chopper most likely to be contaminated were the top of the comb/knife guard and the knife. Cleanability of stainless steel is a problem in the dairy industry. Similar studies have evaluated cracks in stainless steel surfaces after cleaning with solvent-based

cleaners. Hairline cracks were found in 6 of 9 stainless steel milk holding tanks and all 13 cheese vats in one dairy processing facility (Barkley, 1979), suggesting environmental niches for bacterial pathogens.

When SEM and atomic force microscopy (AFM) were used to assess stainless steel surfaces of different finishes Arnold and Bailey (2000) saw relative differences in surface morphology for different surface finishes with fewer bacterial cells attaching to electropolished stainless steel (10^2 cells/SEM area) compared to 2B finished, sandblasted, sanded, and electropolished stainless steel (10^3 cells/SEM area). In addition, no bacterial cell clumps were observed on electropolished surfaces, whereas greater than 12 clumps were seen on all other surfaces.

In our study, significant changes in slicer blade surface topography were observed during two years of continuous use with repeated, mildly abrasive cleaning and sanitizing producing pits or areas of high oxidation from the disintegration of the electropolished finish. These topographical changes likely impacted *Listeria* transfer with the rougher blades allowing for increased attachment. Electron micrographs of new and used slicer blades (Figure 3.8) illustrate differences in surface finish topography as a result of use and cleaning cycles. Numerous pits and scores on worn slicer blades can serve as harborage sites for bacterial attachment and thus lead to more extended transfer during slicing.

Transfer of the low-level inoculum (10^3 CFU/blade) was difficult to quantify with a tailing effect observed followed by sporadic recovery after 5 slices for all three products. Enrichment data provided some insight on likelihood of transfer after extended slicing (>10 slices). Differences seen between initial inoculum levels and total numbers

of *Listeria* cells recovered can be accounted for in part by injury. Using selective and non-selective media, an average of 67% of the *Listeria* inoculum became injured on our stainless steel blades after 1 h of drying with this injury accounting for differences in recovery using selective media for direct plating and enrichment. Salami continued to be an outlier when compared to the other two products with 22% of the initial inoculum (10^3 CFU/blade) recovered by direct plating during sequential slicing. Listeriae not accounted for by direct plating and injury results were likely transferred to other unsampled surfaces of the delicatessen slicer or lost as aerosols.

Based on guidelines established in the 2001 Food Code (FDA/CFSAN, 2001), equipment used for food preparation with food contact services must be cleaned every 24 h if held at < 5° C or every 10 h when held at 10-12.8° C with cleanliness being defined as "clean to sight and touch". These recommendations clearly allow ample time for bacterial attachment, growth, and subsequent transfer to previously uncontaminated products between cleanings. Food preparation equipment such as mechanical slicers have numerous components including the slicer blade and guard that are difficult to clean with soiling not always visually apparent. Findings presented in this study suggest ample opportunity for transfer of *Listeria* in delicatessens via mechanical slicers with the highest risk of consumer exposure coming from the first 10 slices. Thereafter, sporadic transfer was seen out to 28-30 slices for all inoculation levels and transfer scenarios (inoculated slicer to uninoculated product and inoculated product to uninoculated product via the slicer). Based on one recent report (Draughon, 2005), delicatessen sliced luncheon meats were more frequently contaminated with *Listeria monocytogenes* when

sliced in succession thus suggesting repeated cross contamination from delicatessen slicer.

3.6 SUMMARY

Overall findings presented in this study suggest that the greatest risk of exposure to *Listeria* during slicing of delicatessen meats occurs within the first 10 slices. Given that an estimated 75% of all luncheon meats sold are being sliced at delicatessens, ample opportunity exists for the contamination of delicatessen-sliced meats. Depending on product formulation, certain delicatessen meats that permit growth of *Listeria* may pose a public health risk to consumers when stored in home refrigerators for long periods of time. These findings identified improved equipment design, stainless steel grade, and finish as future research areas of importance for food manufacturers and retail establishmets.

CHAPTER 4

TRANSFER OF *LISTERIA MONOCYTOGENES* DURING SLICING OF TURKEY BREAST, BOLOGNA, AND SALAMI USING KITCHEN KNIVES

Vorst, K.L., Todd, E.C.D., Ryser, E.T.

4.1. ABSTRACT

In response to continued concerns regarding *Listeria* cross-contamination of ready-to-eat meat and poultry products in both retail and home kitchens, a series of specially prepared grade 304 and 316 stainless steel knife blades were inoculated with a 6-strain *L. monocytogenes* cocktail comprised of two weak, two medium, and two strong biofilm forming strains so as to contain ~10^8, 10^5, 10^3 CFU/blade. Thereafter, whole chubs of delicatessen turkey breast, bologna, and salami (3 replicates) were sliced to entirety (30 slices) at a cutting speed of 20 mm/min using an Instron 5565 electromechanical compression analyzer. Slices were diluted 1:5, homogenized and then surface- or pour-plated using Modified Oxford Agar and enriched in University of Vermont Medium, *Listeria* transfer from knife blades inoculated at 10^8 CFU/blade was logarithmic with a 2-log decrease after 12 slices and direct counts obtained thereafter out to 30 slices. However, blades containing 10^5 and 10^3 CFU/blade typically yielded direct counts out to only 20 and 5 slices, respectively. Normalizing data on a log scale for the first 10 slices resulted in significantly greater *Listeria* transfer and "tailing" from grade 304 as opposed to grade 316 stainless ($P<0.05$) for all three products. After one year of use, knife blade roughness values as determined by surface profilometry were significantly greater ($P<0.001$) for grades 304 than 316 stainless. Force and knife sharpness were not significantly different ($P>0.05$) within stainless steel grade ($P<0.05$) for each product. However, significant differences in force were seen between salami and turkey ($P<0.05$) for grades 304 and 316 stainless steel respectively. Compositional differences of deli meats and knife blades, knife blade wear and scoring will also likely increase *Listeria* transfer during slicing.

4.2. INTRODUCTION

Cross-contamination of cooked and ready-to-eat (RTE) foods with *Listeria monocytogenes* has been identified as a serious public health concern with delicatessen meats ranking fourth for predicted relative risk to the North American population (FDA, 2003). Four major listeriosis outbreaks have been documented in the United States since May of 2000, three of which were traced to consumption of delicatessen-sliced turkey breast, (MMWR, 2000; MMWR 2002). These three outbreaks were responsible for a combined total of 91 listeriosis cases, including 11 deaths and 6 miscarriages, in 22 states and the recall of 44.3 million pounds of product. These outbreaks prompted the development of three USDA-mandated alternatives for controlling *Listeria* in delicatessen meats – (a) post-package pasteurization, (b) product reformulation to prevent *Listeria* growth and/or (c) increased product and environmental testing (FSIS, 2003). While processing environments are still major sources of contamination, very little research has been done with respect to *Listeria* cross-contamination from food contact surfaces and utensils in delicatessens (Chen et al., 2000; Lunden et al., 2002, Uttendaele, 1999). Studies have shown a high incidence of microbial contamination associated with retail delicatessens. In one UK survey, 10 of 32 slicer blades yielded *L. monocytogenes* (Humphrey, 1990) with Uyttendaele et al. (2001) reporting that 4.9% of cooked meat products sampled at retail markets tested positive for *Listeria monocytogenes*. This is higher than for commercially processed meat [data such as Gombas et al., 2003] and indicates that there is a likelihood of cross contamination in the retail operations.

Routes of cross–contamination in kitchen environments have been well documented with studies assessing bacterial survival on common kitchen items including

cutting boards (Ak et al., 1994; Akier et al., 1990; Sattar et al., 2001), sponges (Michaels et al., 2002), oven mitts (Michaels et al., 2002), pot holders (Michaels et al., 2002) and cloth towels (Satter et al., 2001) as well as food contact surfaces comprised of stainless steel (Arnold and Bailey, 2000; Arnold and Silvers, 2000; Herald and Zottola, 1988; Norwood and Gilmour, 1999) or other materials (Beresford et al., 2001). Using plastic and wood cutting boards, Ak et al. (1994) showed increased transfer of a bacterial suspension including *Listeria, Escherichia coli*, and *Salmonella* species with scored or scratched plastic boards compared to wooden boards. Montville et al. (2001) reported bacterial transfer rates of 0.01 and 10% when food workers handled chicken meat with and without vinyl gloves, respectively. Due to sampling difficulties and wide variations in both design and use, knife blades have received inadequate attention in regards to bacterial transfer. *Listeria* has been shown to attach to stainless steel in as little as 20 minutes (Mafu et al., 1991) with the extent of attachment dependent on both the grade of stainless steel and the type of surface finish. Using scanning electron microscopy (SEM) and atomic force microscopy, Arnold and Bailey (2000) measured biofilm formation and surface morphology of grade 304 stainless steel of different surface finishes including 2B, sandblasted, sanded, and electropolished. When all four surfaces were exposed to a mixed bacterial culture obtained from a poultry carcass rinse, bacterial attachment was at least 1 log lower on electropolished stainless steel compared to the other three surfaces. These findings have important ramifications in the manufacture of stainless steel knife blades, delicatessen slicer blades and other food contact surfaces found on processing equipment as well as in retail delis.

In limited work by King (1999) using an Instron electromechanical compression analyzer, a light weight high speed knife sustained less damage during slicing of lamb rib bones compared to traditional knife blades used for processing with fewer meat particulates being generated. This work suggests that cross-contamination and subsequent contamination of processing environment can be lessened by improving knife blade designs with reduced aerosols and meat particulates as a result of the cutting and sawing process.

The three primary objectives of the present study were to quantify transfer of *L. monocytogenes* from (a) contaminated knife blades to turkey, salami, and bologna, (b) contaminated roast turkey, salami and bologna to knife blades and (c) inoculated product to a knife and then to uninoculated product. As a secondary objective, stainless steel grade, surface roughness, knife sharpness, and cutting speed were also assessed for their impact on *Listeria* transfer during slicing of deli meats. .

4.3. MATERIALS AND METHODS

4.3.1. *Listeria monocytogenes* strains

Six strains of *Listeria monocytogenes* (obtained from Dr. Catherine W. Donnelly at the University of Vermont, Burlington, Vermont): CWD 205 (source unknown), CWD 578 (dairy plant environment), CWD 701 (Azore cheese), CWD 730 (dairy plant environment), CWD 845 (dairy plant environment), and CWD 1002 (pork sausage) were chosen from more than 190 strains based on their ability to form weak (CWD 205, CWD 578), medium (CWD 701, CWD 1002) or strong (CWD 730, CWD845) biofilms in a

microtiter plate assay (Keskinen et al., 2003). All strains were maintained at -80°C in trypticase soy broth (TSB) (Difco/Becton Dickinson, Sparks, MD) containing 10% (v/v) glycerol. TSB containing 0.6% yeast extract (TSB-YE) (Difco) was inoculated from the frozen stock cultures and incubated at 37°C for 24 h. After a second transfer in TSB-YE, each culture was pelleted by centrifugation at 9700 x g / 10 min / 4°C (Sorvall Super T21; Sorvall Products, L.P. Newton, CT), resuspended in 9 ml of 0.1% peptone (Difco) and combined in equal volumes to produce one 6-strain cocktail containing approximately 10^8 CFU/ml. Cell concentration was verified by optical density at 600 nm and spiral plating (Autoplate® 4000 Spiral Plater; Spiral Biotech Inc., Norwood, MA) an appropriate dilution on Modified Oxford Agar (MOX) followed by 48 h of incubation at 35°C.

4.3.2. Delicatessen meats

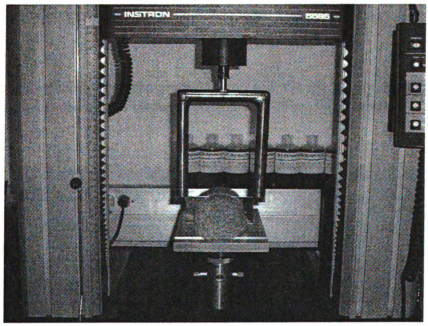
One retail brand each of restructured roast turkey breast, Genoa hard salami and bologna (5.5 to 6.5 lbs each) was purchased in chub-form from a local retailer (Gordon Food Service, Lansing, MI), held at 4°C and used within 20 d. Based on the package label, each product contained the following ingredients: turkey breast (turkey breast, turkey broth, < 2% each of salt, dextrose, and sodium phosphates); salami (pork, beef, salt, < 2% each of dextrose, water, natural spices, sodium ascorbate, lactic acid starter culture, garlic powder, sodium nitrite, BHA, BHT, and citric acid); and bologna (beef, pork, water, salt, and < 2% each of dextrose, potassium lactate, sodium diacetate, sodium erythorbate, sodium nitrite, and oleoresin of paprika). Fat, moisture, and crude protein content were determined in triplicate for two lots of each product according to the

Association Official of Analytical Chemists International (AOAC Int.) methods 991.36, 950.46, and 992.15, respectively (AOAC, 2003).

4.3.3. Knife blades

A series of sharp and medium sharp electropolished grade 304 and 316 stainless steel knife blades measuring 12 cm x 5 cm (product contact area of 60 cm^2 for each side of the blade) with a thickness of 1.4mm were manufactured by ProAxis, Inc. (Lafayette, IN) (Figure 4.1).

Figure 4.1. Instron 5565 electromechanical compression analyzer

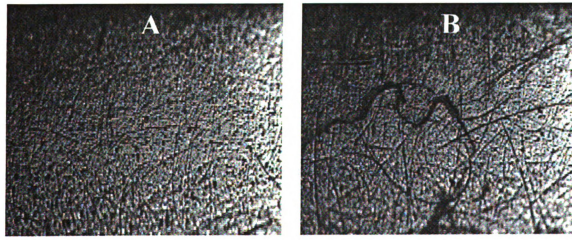


Each knife blade had a built-in 1 cm x 2 cm flange at each end so that the blade could be screwed to the support bracket and then secured to an Instron electromechanical compression analyzer. Sharp knives were machined to allow for sharp point by milling at a 45° angle 10 mm from the end of the blade. Medium sharp blades were machined with a blunt end (0.5 mm from tip) to simulate a broken knife blade.

4.3.4. Quantification of cutting force and speed

An Instron 5565 electromechanical compression analyzer (Instron; Canton, MA) was used to quantify force at a cutting speed of 20 mm/min. A custom-made knife support bracket to which all knife blades were attached was secured to the upper load cell (1124 lb) for complete cutting of all deli meats (Figure 4.2).

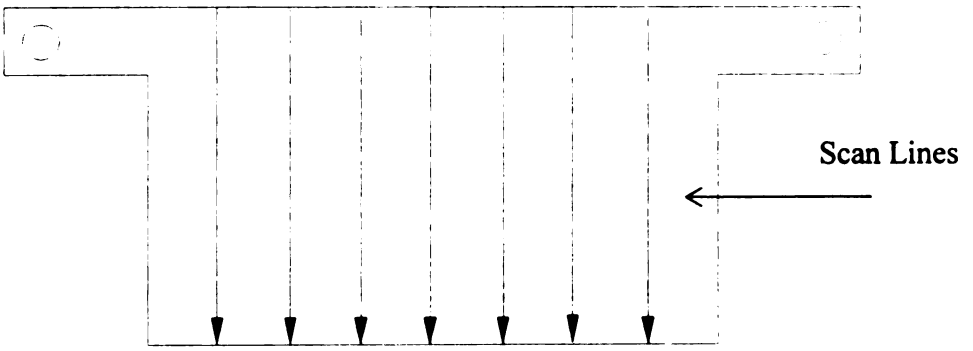
Figure 4.2. Surface scoring of used grade 316 (A) and 304 (B) electropolished stainless steel knife blades after 6 months of use (approximately 500 slices).



4.3.5. Surface profiling of knife blades.

Knife blade roughness values and overall surface profiles were obtained from the University of Illinois Center for Microanalysis of Materials (Urbana, IL) using a Sloan Dektak^3 ST stylus surface profilometer (Veeco Instruments Inc., Woodbury, NY). Initially and after one year of use, surface profilometer measurements were taken along six defined 10-mm lines on the front and backside of the blade (Figure 4.3).

Figure 4.3. Stylus locations on blade for surface profile measurements



Surface roughness values were obtained by recording the stylus height 40 times per second as the stylus traveled towards the edge of the blade along each 10-mm line at predetermined intervals. Measurements were stopped when the stylus came within 0.5 mm of the blade edge.

4.3.6. Knife blade inoculation

A turkey slurry was prepared for inoculating the knife blade by diluting 25 g of turkey breast 1:10 in sterile deionized water followed by homogenization in a model DIFP2 blender (General Electric; Bridgeport, CT) at high speed for 1 min. The slurry was then filtered through five layers of cheesecloth, heated in an 80°C water bath for 20 min, and stored in 50 ml aliquots at -20°C. For use, 50 ml of the turkey slurry was thawed overnight at 4°C after which 1 ml of the 6-strain cocktail was inoculated into 9 ml of turkey slurry. One face of the 75% (v/v) ethanol flame sterililzed knife blade was then inoculated with 100 µl of this turkey slurry. After uniformly spreading the inoculum on the 60 cm^2 surface of the blade with a inoculating needle so as to contain 10^8, 10^5, and 10^3 CFU/blade, the blades were dried for 1 h in a laminar flow cabinet at 23°C and 30 - 40% relative humidity recorded with a hygrometer (Fisher Scientific; Hampton, NH) and then immediately used to obtain 30 slices of turkey, salami or bologna.

4.3.7. Transfer of *L. monocytogenes* from inoculated grade 304 stainless steelknife blades to uninoculated product

In a replicated study (n=3), 30 slices each of previously uninoculated turkey breast, salami, and bologna were obtained using knife blades inoculated to contain 10^8, 10^5, and 10^3 *L. monocytogenes* CFU/blade. These unrealistically high inoculation levels were necessary to quantify numbers of *Listeria* in consecutive slices for subsequent modeling of *Listeria* transfer. For knife blades containing 10^8 CFU/blade, all slices were diluted 1:5 (w/v) in PBS, homogenized in a Stomacher 4000 (Seward; Norfolk, England) for 1 min and spiral-plated (50µl) on MOX. For blade inoculum levels of 10^5 and 10^3

CFU/blade, all slices were diluted 1:5 (w/v) in University of Vermont Medium (UVM) (Difco-Becton Dickenson; Detroit, MI) and homogenized in a Stomacher for 1 min. Duplicate 5 ml aliquots of the homogenized sample were pour-plated in 25 ml of MOX using 150-mm diameter disposable Petri dishes (Fisher Scientific; Chicago, IL) and incubated at 35°C for 48 h with populations determined as the number of listeriae per slice. When *Listeria* was not detected by direct plating, MOX plates streaked from the previously enriched sample at 30°C were examined for presence/absences of *Listeria* after 48 h at 35°C.

4.3.8. Transfer of *L. monocytogenes* from inoculated grade 304 and 316 stainless steel knife blades to uninoculated product.

Listeria transfer studies from inoculated grade 304 and 316 stainless steel knife blades to turkey, salami and bologna were replicated three times for each of the three products. Sterile grade 304 and 316 stainless steel knife blades were inoculated to contain 10^3 CFU/blade as previously described and used to obtain 20 slices of each product. All slices were diluted 1:5 (w/v) in UVM and homogenized in a Stomacher for 1 min. Duplicate 5 ml aliquots of the homogenized sample were pour-plated in 25 ml of MOX using 150-mm diameter disposable Petri dishes (Fisher Scientific; Chicago, IL) and incubated at 35°C for 48 h with populations determined as the number of listeriae per slice. When *Listeria* was not detected by direct plating, MOX plates streaked from the previously enriched samples were examined for presence/absences of *Listeria* after 48 h at 35°C.

4.3.9. Transfer of *L. monocytogenes* from inoculated product to grade 304 stainless steel knife blades and then to uninoculated product

Replicated studies (n=3) assessing transfer of *L. monocytogenes* from inoculated turkey, salami, and bologna to knife blades and then to uninoculated product were conducted using each of the three products. The turkey, salami, and bologna chubs were surface-inoculated with the aforementioned 6-strain cocktail along a 1 x 1 cm strip to obtain approximately 10^5 CFU/cm^2 as determined from subsequent spiral plating of diluted samples on MOX. This high inoculation level was again necessary for quantification of *Listeria* in consecutive slices. After a 1-h hold at 4°C, each product was sliced 3-5 times to contaminate the grade 304 stainless steel knife blade with this same blade then immediately used to obtain 30 slices of uninoculated product of the same or different type.

Listeria was recovered by homogenizing a 1:5 dilution in UVM followed by pour plating duplicate 5 ml aliquots in 25 ml of MOX using 150-mm dia. Petri plates. After 48 h of incubation at 35°C, all MOX plates were counted to determine numbers of listeriae per slice. When *Listeria* was not detected by direct plating, MOX plates streaked after enrichment were examined for presence/absence of *Listeria* after 48 h at 35°C.

4.3.10. Cleaning/decontaminating knife blades

The knife blades were removed from the support bracket after use, soaked in 75% ethanol (v/v), wiped with a 1-ply composite tissue (CT) and rinsed with deionized water. Adequacy of this cleaning/sanitizing regimen was confirmed using the CT developed by Vorst et al. (2004). Before use, the knife blades were rinsed with sterile deionized water

and dried with a 1-ply composite tissue. To prevent surface oxidation during storage, the knife blades were coated with a thin layer of mineral oil, which was removed before use by flaming with 95% ethanol and a final rinse in sterile deionized water.

4.3.11. Quantification of injured *Listeria* on knife blades

Five unused grade 304 and 316 knife blades were inoculated at 10^5 CFU/blade as previously described. All knife blades were sampled using the CT method of Vorst et al. (2004) with 1 ml of PBS added to CT before swabbing. After adding the CT to 9 ml of PBS and homogenizing in a Stomacher for 1 min, aliquots (50 µl) were spiral-plated in duplicate on tryptose phosphate agar (DIFCO) containing ferric ammonium citrate (0.5 g/l) and esculin (1 g/l) (mTPA) for recovery of healthy and injured cells, and on mTPA with sodium chloride (40 g/l) (mTPAN) and MOX for recovery of healthy cells as previously described (Mathew and Ryser, 2002). All plates were counted after 48 h at 35° C. Percent injury was determined by the following equation:

% injury = [(non-selective count – selective count)/ non-selective count]* 100

4.3.12. Statistical Analysis

All *Listeria* transfer experiments were replicated three times. *Listeria* transfer to/from knife blades and direct/sequential transfer from inoculated blades to product and inoculated product to uninoculated product via the knife blade were analyzed using a general linear model and analysis of variance (ANOVA) for least significant differences in mean recovery (SAS, 1996). Mean differences in surface topography for 304 and 316

grade stainless were replicated five times and analyzed using a general liner model at each of 5000 points for 6 defined areas across the front and back of the knife blade (Figure 4.3) (SAS, 1996).

4.4. RESULTS

4.4.1. Knife blade surface profiling

Initial roughness values for new knife blades prepared from grades 304 and 316 stainless steel were 105 and 70 μm, respectively. After one year of use, a significant difference ($P<0.0001$) in surface topography was observed for both the front and back of grade 316 blades with average roughness values of 2083 and 3079 μm, respectively, whereas no such difference ($P>0.05$) was seen for grade 304 blades. The total mean roughness values of 7409 and 2581 μm (front and back) for 304 and 316 blades respectively were significantly different ($P<0.001$).

4.4.2. Proximate Analysis

Based on proximate analyses, the turkey breast, salami, and bologna sliced in this study contained 78, 43 and 60% moisture, <1, 36, and 27% fat, and 19, 17, and 10 protein, respectively.

4.4.3. Effect of stainless steel grade, product, and sharpness on transfer

The average force needed to cut salami was the highest for grades 304 and 316 stainless steel blades at 50 ± 7 and 48 ± 5 lbs., respectively for sharp blades. Significant

differences in cutting force were seen between salami, bologna and turkey ($P<0.05$) for grades 304 and 316 stainless steel (Table 4.1).

Table 4.1. Average slicing force (lbs) for turkey, salami, and bologna using medium sharp (MS) and sharp (S) knife blades manufactured from 304 and 316 grade stainless steel

	Grade 304		Grade 316	
Product	**MS**	**S**	**MS**	**S**
Turkey	34 ± 4^{a}	21 ± 3^{b}	30 ± 3^{a}	22 ± 3^{b}
Bologna	11 ± 3^{c}	8 ± 1^{c}	8 ± 1^{c}	8 ± 1^{c}
Salami	57 ± 5^{d}	50 ± 7^{d}	59 ± 6^{d}	48 ± 5^{d}

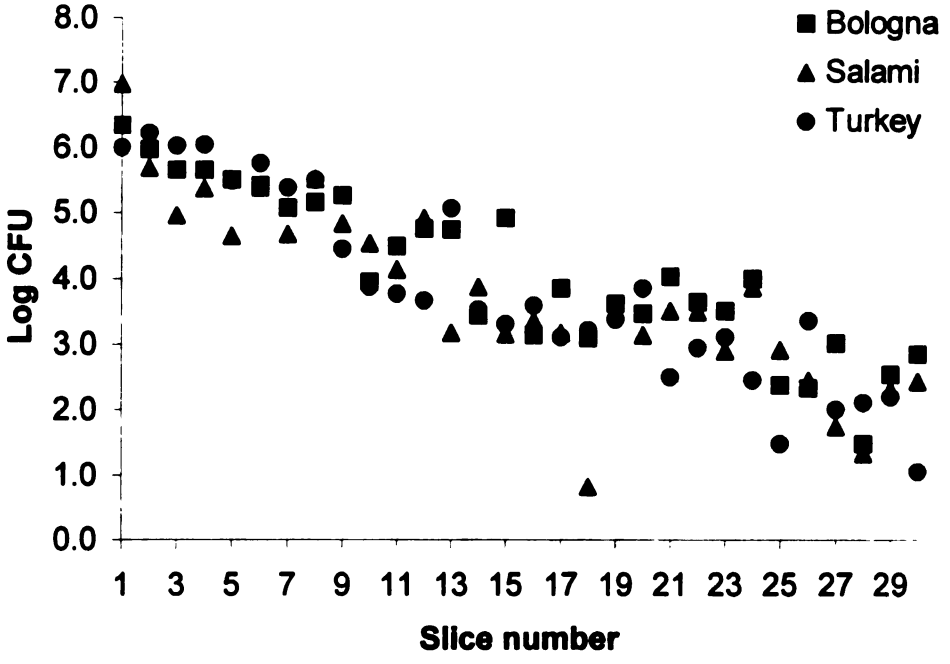
Means with different superscripts significantly different ($P<0.05$)

Average cutting force for turkey and salami was 22 ± 3 and 48 ± 5 lbs for grade 316 and 21 ± 3 and 50 ± 7 lbs. for grade 304 stainless steel blades, respectively. Bologna had the lowest average cutting force at 8 ± 1 lbs. Preliminary data showed no significant differences ($P>0.05$) in transfer using inoculated sharp (S) and medium sharp (MS) 304 grade stainless knife blades (10^8 CFU/blade) with transfer at 5.02 and 5.07 CFU/slice for MS and S respectively.

4.4.4. Transfer of *L. monocytogenes* from inoculated grade 304 and 316 stainless steel knife blades to uninoculated product

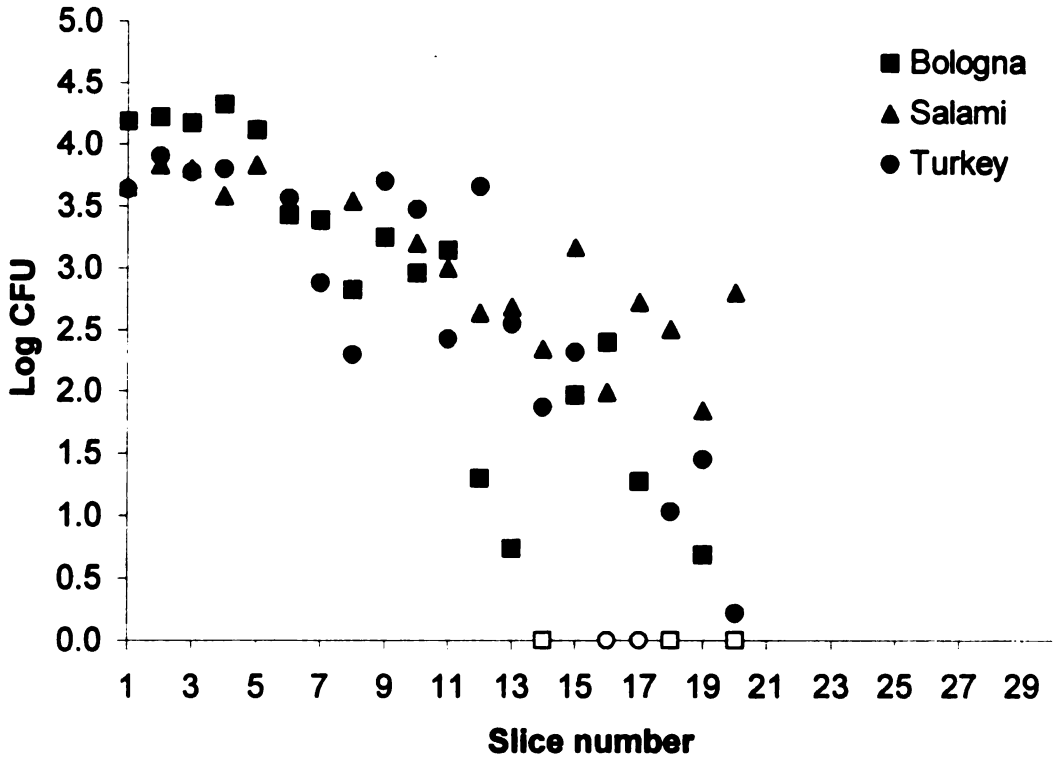
Transfer of *Listeria* from inoculated knife blades (10^8 CFU/blade) was generally logarithmic for all three products with populations decreasing 2 logs on the blade after the first 8-12 slices (Figure 4.4).

Figure 4.4. Transfer of *L. monocytogenes* from an inoculated knife blade (10^8 CFU/blade) to uninoculated turkey, salami and bologna



The total number of *Listeria* CFUs transferred was not significantly different ($P<0.05$) between products. At an inoculation level of 10^5 CFU/blade, *Listeria* was quantified in slices 13 to 20 by direct plating for all three products (Figure 4.5).

Figure 4.5. Transfer of *L. monocytogenes* from an inoculated knife blade (10^5 CFU/blade) to uninoculated turkey, salami and bologna. Open symbols not quantifiable by direct plating.



Enrichment results were typically negative for turkey and bologna after 26 slices and positive for salami out to 30 slices (Table 4.2).

Table 4.2. Number of direct counts and positive enrichments for blade -product (B-P) and product-blade-product (P-B-P) transfer for turkey (T), bologna (B), and salami (S)

	10^3 CFU/Blade (BP)			10^5 CFU/Blade (BP)			10^5 CFU/cm^2 (PBP)		
Slice	T	B	S	T	B	S	TBT[b]	SBT[c]	TBS[d]
1	3/3[a]	3/3	2/3	3/3	3/3	3/3	3/3	3/3	2/3
2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3
4	3/3	2/3	3/3	3/3	3/3	3/3	3/3	3/3	1/3
5	3/3	1/3	3/3	3/3	3/3	3/3	3/3	2/3	1/3
6	2/3	1/3	0/3	3/3	3/3	3/3	3/3	1/3	1/3
7	1/3	1/3	2/3	3/3	3/3	3/3	3/3	2/3	1/3
8	0/3	1/3	2/3	3/3	3/3	3/3	3/3	3/3	1/3
9	0/3	2/3	1/3	3/3	3/3	3/3	3/3	3/3	1/3
10	0/3	2/3	3/3	3/3	3/3	3/3	3/3	1/3	3/3
11	0/1	1/3	3/3	3/3	3/3	3/3	3/3	0/3	1/3
12	0/2	0/2	2/3	3/3	2/3	3/3	3/3	0/3	0/2
13	0/1	1/3	0/1	3/3	2/3	3/3	3/3	0/3	1/2
14	0/1	0/3	1/1	2/3	0/3	3/3	3/3	0/3	0/1
15	0/1	0/3	0/2	2/3	1/3	3/3	3/3	1/3	2/2
16	0/1	0/2	0/2	1/2	1/3	3/3	3/3	1/3	0/3
17	0/1	0/2	0/1	0/3	1/3	3/3	3/3	0/3	0/1
18	0/0	0/2	0/0	1/2	0/3	3/3	3/3	1/1	0/1
19	0/0	0/3	0/0	2/3	1/3	3/3	2/3	0/1	0/2
20	0/0	0/1	0/0	1/3	0/3	3/3	3/3	0/3	0/2
21	NT[e]	NT	NT	1/2	0/2	NT/3	3/3	0/2	0/1
22	NT	NT	NT	1/2	0/1	NT/3	2/3	0/2	0/1
23	NT	NT	NT	0/2	0/1	NT/3	2/3	0/1	0/0
24	NT	NT	NT	0/2	0/2	NT/3	3/3	0/2	0/2
25	NT	NT	NT	NT/2	NT/1	NT/3	3/3	0/1	0/1
26	NT	NT	NT	NT/2	NT/1	NT/3	3/3	0/1	0/0
27	NT	NT	NT	NT/0	NT/0	NT/3	1/1	0/0	0/2
28	NT	NT	NT	NT/0	NT/0	NT/3	1/3	0/1	0/2
29	NT	NT	NT	NT/0	NT/0	NT/3	1/1	0/0	0/0
30	NT	NT	NT	NT/0	NT/1	NT/3	1/1	0/0	0/0

[a] Direct counts separated by slash following enrichment results for 3 replicates

[b] T-B-T – inoculated turkey to blade to turkey

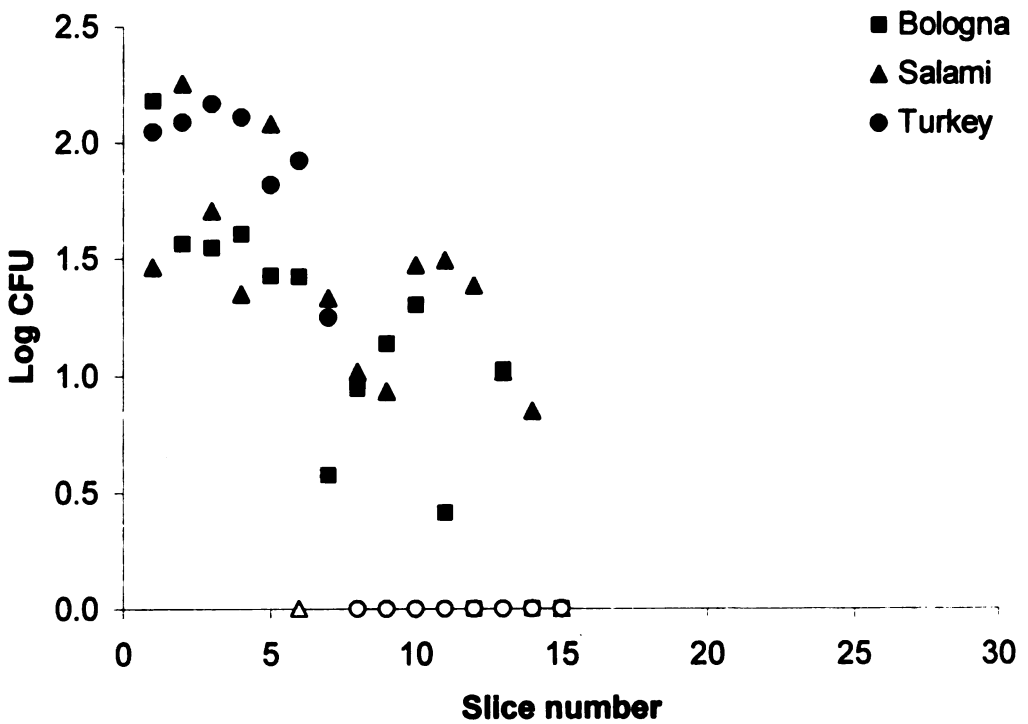
[c] S-B-T – inoculated salami to blade to turkey

[d] T-B-S – inoculated turkey to blade to salami

[e] NT-Not Tested

Low-level inoculation (10^3 CFU/blade) identified a weak logarithmic association for bologna and no association for turkey and salami (Figure 4.6).

Figure 4.6. Transfer of *L. monocytogenes* from an inoculated knife blade (10^3 CFU/blade) to uninoculated turkey, salami and bologna. Open symbols not quantifiable by direct plating.



Differences in transfer between stainless steel grade (304 vs. 316) were compared for each turkey, bologna, and salami product. Using 10^3 CFU/blade, stainless steel type did not significantly impact ($P>0.05$) numbers of listeriae transferred during the first 20 slices. *Listeria* was quantifiable in the first 5 slices by direct plating, regardless of stainless steel grade or product type. Direct counts were obtained out to 5 slices for all products and both grades of stainless. Normalizing data on a log scale for the first 10 slices resulted in significantly greater ($P<0.05$) transfer for grade 316 as opposed to grade 304 stainless steel. For slices 10 through 15, more direct counts were obtained from grade 304 than grade 316 stainless steel (Figures 4.7-4.9).

Figure 4.7. Transfer of *L. monocytogenes* from inoculated knife blades (grade 304, grade 316, 10^3 CFU/blade) to uninoculated turkey

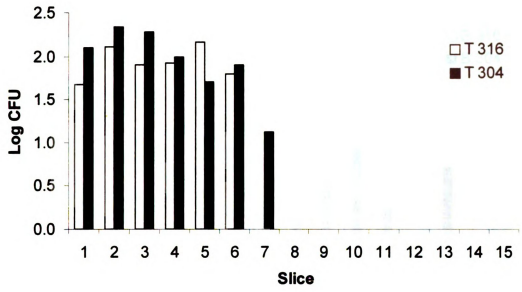


Figure 4.8. Transfer of *L. monocytogenes* from inoculated knife blades (grade 304, grade 316, 10^3 CFU/blade) to uninoculated salami

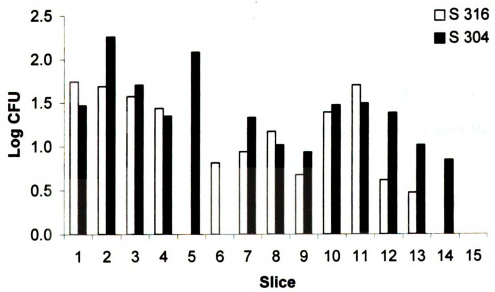
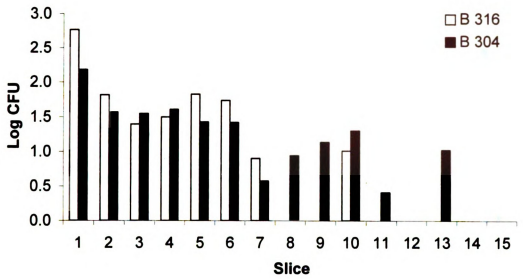


Figure 4.9. Transfer of *L. monocytogenes* from inoculated knife blades (grade 304, grade 316, 10^3 CFU/blade) to uninoculated bologna

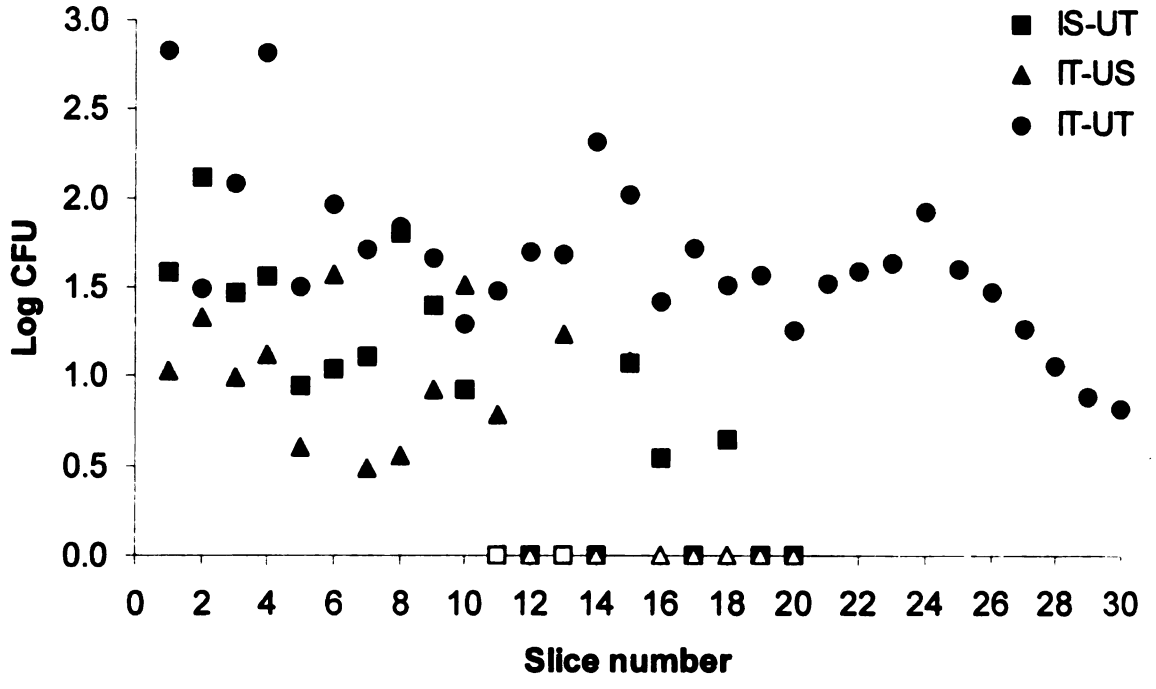


For slices 10-15, total numbers of *Listeria* transferred were greater using grade 304 as opposed to grade 316 stainless steel blades, resulting in significant differences ($P<0.05$) for mean recovery as a function of total CFUs transferred.

4.4.5. Sequential transfer of *L. monocytogenes* from inoculated product to a knife blade and then to uninoculated product using grade 304 stainless steel knife blades

Numbers of *Listeria* transferred from surface-inoculated turkey and to uninoculated salami (10^5 CFU/cm^2), inoculated salami to uninoculated turkey (10^5 CFU/cm^2) and inoculated turkey (10^5 CFU/cm^2) to uninoculated turkey were quantifiable out to 11th,16th, and 30th slices using direct plating (Figure 4.8).

Figure 4.10. Transfer of *L. monocytogenes* from inoculated turkey (IT) (10^5 CFU/cm^2) and inoculated salami (IS) (10^5 CFU/cm^2) to uninoculated turkey (UT) and uninoculated salami (US) during slicing. Open symbols not quantifiable by direct plating.



Mean recovery was significantly greater ($P<0.05$) for inoculated turkey sliced before uninoculated turkey when compared to inoculated salami sliced before uninoculated turkey and inoculated turkey sliced before uninoculated salami. A 2-log reduction was seen within the first 15 slices for all transfer scenarios with transfer out to more slices with inoculated turkey sliced before uninoculated turkey. Enrichment results were typically negative after 28 slices for inoculated turkey sliced before uninoculated salami and inoculated salami sliced before uninoculated turkey. Inoculated turkey sliced before uninoculated turkey resulted in positive enrichments to 30 slices.

4.4.6. Quantification of injured *Listeria* on 304 and 316 knife blades

The non-selective medium (mTPA) afforded greater recovery of healthy and injured *Listeria* cells from stainless steel blades compared to selective media. When mTPAN and MOX was used for recovery of *L. monocytogenes* after 1 h of drying in a laminar flow cabinet on grade 304 and 316 stainless steel, 46% and 72% of the *Listeria* CFU were injured, respectively,.

4.5. DISCUSSION

Products chosen for this study were based on fat and moisture content with turkey having the lowest fat (<1%) and highest moisture (78%). Salami had the highest fat (36%) and lowest moisture (43%) content. The knife blades used in this study were manufactured to specific specifications to minimize variability seen in commercially available knife blades. Use of the Instron eliminated operator variations in cutting speed, force, and cutting action (sawing versus chopping). Cutting force and knife sharpness were not significantly different within stainless steel grades and product ($P<0.05$). Force required to slice the product was significantly higher ($P<0.05$) for salami when compared to turkey and bologna.

Compositional differences in products were not observed to have an impact on transfer with the exception of inoculated turkey subsequently sliced with uninoculated turkey. While Lin et al. (2004a) reported the development of a visible fat layer during slicing of salami with a delicatessen slicer, this same fat layer was not pronounced after slicing the same product with a knife blade. Hence, the chopping action did not support formation of a fat layer when compared to the high-speed rotation and centrifugal force

of a delicatessen slicer. In our study, *Listeria* transfer was similar when turkey, salami, and bologna were sliced with an inoculated knife blade. However, when inoculated turkey was sliced and followed with uninoculated turkey, greater transfer was seen to more slices compared to when inoculated salami or turkey was followed by uninoculated salami.

Stainless steels of different grades are used in all segments of the food industry due to their superior mechanical properties (good ductility, toughness, strength, and workability) and corrosion resistance. In food processing environments, AISI (American Iron and Steel Institute) grades 304, 304L, 316, and 316L stainless steel are the most common alloys (Smith, 1993; Stone and Zottola, 1985; Suzuki, 2000) with the 400 series also commonly used for knives. Stainless steel knives are universally used in food preparation with a seemingly endless variety of industrial and kitchen knives being marketed. In addition to obvious differences based on intended use, knife blades also differ in surface finish/polish type, stainless steel grade (e.g., carbon, nickel and molybdenum content), cutting edge styles (serrated and straight) and overall surface area. Knife blade sharpness was not shown to have an impact on *Listeria* transfer but did result in a lower cutting force for sharp blades. Although likely having a limited impact on transfer, the variability between within products sliced was too high to identify any discernable differences. Furthermore, large changes in surface topography most likely masked any subtle difference seen in knife force or sharpness. Current methods for quantifying *Listeria* on heavily soiled food contact surfaces are too imprecise to allow the use of more realistic contamination levels (10^1 - 10^2 CFU/cm^2) (Gombas, 2003; Moore and Griffith, 2002; Satter, 2001). Once improved recovery methods have been

developed, further research needs to be conducted at very low-level inoculation to fully understand the impact of knife sharpness on bacterial transfer during slicing of RTE foods.

Stainless steel grade did not significantly impact ($P>0.05$) the total number of *Listeria* cells transferred during slicing. However, differences in stainless steel grade were significantly different by slice ($P<0.05$) for the first ten slices. Most notably, a far more pronounced tailing affect was seen when grade 304 rather than 316 knife blades were used for slicing. In contrast, greater transfer of *Listeria* to fewer slices was evident using grade 316 stainless steel knife blades which may be related to the smoother finish, greater durability and easier cleanability of grade 316 as opposed to 304 stainless steel (Arnold and Bailey 2000, Leclercq-Pelat and Lalande, 1994). Our surface topography results support these findings with overall surface roughness values being significantly lower and less variable for 316 when compared to 304 electropolished stainless steel. Surface scoring was also less pronounced on grade 316 stainless steel blades with obvious score marks on the same areas of 304 grade steel blades after 6 months of repeated use and cleaning. According to Percival, the molybdenum concentration used in grade 316 stainless steel may be factor in terms of decreasing viability of bacteria and reducing biofilm formation (Percival, 1999). While our findings cannot confirm or deny these biocidal claims of molybdenum, use of grade 316 stainless steel resulted in greater injury of *Listeria* (72%) compared to grade 304 grade stainless (46%).

Based on guidelines established in the 2001 Food Code (FDA/CFSAN, 2001), all food contact surfaces on equipment must be cleaned every 24 h if held at < 5° C or every 10 h when held at 10-12.8° C with cleanliness being defined as "clean to sight and

touch". These recommendations clearly allow sufficient time for bacterial attachment, growth, and subsequent transfer to previously uncontaminated products between cleanings. Food preparation equipment such as knives will score over time as demonstrated in this study and have the potential to harbor pathogens even after cleaning.

4.6. SUMMARY

Based on our findings, ample opportunity exists for transfer of *Listeria* using kitchen knives in both commercial and home settings with the highest risk of consumer exposure coming from the first 5-15 slices, depending on the grade of stainless steel used. While the numbers of listeriae transferred in such settings would be admittedly very low, even these few cells may pose a public health risk to consumers if the product formulation permits growth of *Listeria* in home refrigerators during extended storage. Further recommendations to equipment manufacturers and food processors would include use of electropolished knife blades and grade 316 stainless steel for reduced surface scoring and transfer of bacteria to fewer slices.

CHAPTER 5

TRANSFER COEFFICIENTS AND PREDICTIVE MODELS FOR *LISTERIA MONOCYTOGENES* DURING SLICING OF READY-TO-EAT, TURKEY BOLOGNA, AND SALAMI

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5.1. ABSTRACT

Previous data was used to develop a series of *Listeria* transfer coefficients during slicing of deli meats with a mechanical delicatessen slicer and kitchen knives prepared from grades 304 and 316 stainless steel. Transfer coefficients were calculated for two different *Listeria* transfer scenarios – (a) inoculated blade to uninoculated product and (b) inoculated product to uninoculated product via an uninoculated blade. A mathematical model was then developed to predict the least favorable conditions for *Listeria* transfer during slicing of ready-to-eat turkey breast, salami, and bologna. The model and subsequent program is based on the following three assumptions: 1) the expected number of *Listeria* cells transferred during slicing is the fraction "f_1" that describes the number of *Listeria* cells on the blade just before each sequential slice, 2) the expected number of *Listeria* cells transferred to the surrounding areas is the fraction "f_2", and 3) the number of *Listeria* cells on the blade available for transfer before any slicing begins is N_0. Based on these assumptions, a predictive model was developed that may prove to be beneficial in refining the current risk assessments for RTE meat and poultry products purchased at retail delicatessens.

5.2. INTRODUCTION

Since May of 2000, four major outbreaks of foodborne listeriosis have been documented in the United States; three were linked to consumption of delicatessen-sliced turkey breast. These later three outbreaks were responsible for 91 cases of listeriosis, including 11 fatalities and 6 miscarriages, and resulted in the recall of at least 44.3 million pounds of product. In all three outbreaks, post-processing contamination of the product was to blame.

Listeria monocytogenes can reside in food processing facilities for many years (Tompkin, 2001) those strains that are most persistent in factory environments possessing greater capability to adhere to food contact surfaces (Lundun et al., 2000, 2002; Norwood and Gilmour, 1999). Attachment of *Listeria monocytogenes* to stainless steel surfaces in as little as 20 min has been reported after which this pathogen can be transferred to previously uncontaminated food and food contact surfaces during food preparation and subsequent handling (Mafu et al., 1990). In one study, Lunden et al. (2002) demonstrated plant-to-plant transfer of *L. monocytogenes* via a dicing machine with the same strain of *Listeria* identified at three different facilities. At the retail level, Hudson and Mott (1993) collected various environmental swab samples from a supermarket delicatessen and isolated *L. monocytogenes* from a knife and slicing machine with the pathogen also found at most sites near a display case of processed meats.

In response to the aforementioned outbreaks, quantitative transfer to and from commercial slicing machines, knives, and cutting boards in delicatessens was identified as both a major public health concern and a key informational gap in the 2003 FDA *Listeria Risk Assessment* (FDA/FSIS/CDC, 2003). In one study of 32 retail meat slicers,

10 machines were positive for *L. monocytogenes* (Humphrey, 1990). This information, combined with a recent report by Gombas et al. (2003) indicating *L. monocytogenes* populations as high as 10^4 CFU/g on delicatessen luncheon meats, suggests ample opportunity for transfer of *L. monocytogenes* via slicing machines to previous uncontaminated delicatessen meats sold at retail with such contamination putting susceptible individuals at greatest risk of infection.

Many growth models, beginning with the US Department of Agriculture (USDA) Pathogen Modeling Program (Buchanan and Phillips, 1990) have been developed to predict the growth of *Listeria* and other foodborne pathogens in foods based on pH, storage temperature, and levels of salt and sodium nitrite (Houtsma et al., 1996; 1986; Le Marc et al., 2002; and Tamplin, 2002). The most recent USDA model developed by Tamplin (2002) comes with a pre-programmed graphical user interface and generates graphs and tabular output for various growth parameters. In most of these models, empirical data is fitted to mathematical equations using parameter estimation techniques. The method proposed herein uses a combination of emipirical data fitting techniques and mathematical manipulations as described by Bernaerts et al, (2004). Techniques proposed in this study build on parameters described by empirical data while applying mathematical manipulation to each of the parameters as a function of total transfer.

The objective of this study was to develop a predictive model describing the relationship between numbers of *Listeria* cells transferred based on previous data obtained from the slicing of turkey, salami, and bologna with inoculated delicatessen slicer and kitchen knife blades. a) the expected number of *Listeria* cells transferred during slicing is the fraction "f_1" that describes the number of *Listeria* cells on the blade

just before each sequential slice, b) the expected number of *Listeria* cells transferred to the surrounding areas is the fraction "f_2" that describes how many cells are on the blade just before slicing, and c) the number of *Listeria* cells on the blade available for transfer before any slicing begins is N_0. Based on these assumptions, a model was generated that describes transfer of *L. monocytogenes* during slicing of RTE deli meats. The transfer coefficients and mathematical model developed can be used to refine the current risk assessments for RTE meat and poultry products.

5.3. MATERIALS AND METHODS

5.3.1. Transfer coefficients for *Listeria monocyctogenes* during slicing of turkey, bologna and salami.

Previous data obtained from the slicing of roast turkey breast, salami and bologna with a mechanical delicatessen slicer (Vorst et al., 2005a) and kitchen knives (Vorst et al., 2005b) prepared from grades 304 and 316 stainless steel. *Listeria* transfer coefficients were developed for two different scenarios – (a) inoculated blade to uninoculated product and (b) inoculated product to uninoculated product via an uninoculated blade. The following equation was used for calculating transfer coefficients where: "N_o" is the original inoculum on the blade, "f_1" is the fraction of bacteria left on the blade just before each sequential slice and "f_2" is the expected number of bacteria transferred to surrounding areas. The equation for the transfer coefficient presented as a cumulative percentage of total transfer is as follows:

$$\% \text{ transfer} = 100 * \frac{\left(\frac{f_1 N_o}{f_1 + f_2}\right)}{N_o} \quad (1a)$$

This can be arranged as

$$\% \text{ transfer} = \frac{100 * f_1}{f_1 + f_2} \quad (1b)$$

The cumulative transfer coefficient was found by summing sequential transfer coefficients for each slice.

5.3.2. Predictive modeling of *L. monocytogenes* transfer during slicing of turkey breast, bologna, and salami.

A model based on the following three assumptions was developed to predict the previously calculated transfer coefficients: a) the expected number of colony forming units (CFU) transferred during slicing is the fraction "f_1" that describes what is left on the blade just before each sequential slice b) the expected amount of transfer to the surrounding areas is a different fraction "f_2" that describes what is left on the blade just before slicing, and c) the number of CFU's on the blade available for transfer before any slicing begins is N_0.

The following consequences of these assumptions are as follows:

1st Slice

CFU on Meat = $f_1 N_0$ (2a)

CFU to Surroundings = $f_2 N_0$ (2b)

CFU left on Blade = $N_0 - f_1 N_0 - f_2 N_0$

$$= (1 - f_1 - f_2) N_0 \quad (2c)$$

2nd Slice

CFU on Meat = $f_1(1 - f_1 - f_2) N_0$ (3a)

CFU to Surroundings = $f_2(1 - f_1 - f_2) N_0$ (3b)

CFU left on Blade = $(1 - f_1 - f_2) N_0 - f_1(1 - f_1 - f_2) N_0 - f_2(1 - f_1 - f_2) N_0$

$$= (1 - f_1 - f_2)^2 N_0 \quad (3c)$$

3rd Slice

CFU on Meat = $f_1(1 - f_1 - f_2)^2 N_0$ (4a)

CFU to Surroundings = $f_2(1 - f_1 - f_2)^2 N_0$ (4b)

CFU left on Blade = $(1 - f_1 - f_2)^2 N_0 - f_1(1 - f_1 - f_2)^2 N_0 - f_2(1 - f_1 - f_2)^2 N_0$

$$= (1 - f_1 - f_2)^3 N_0 \quad (4c)$$

Xth Slice

CFU on Meat = $f_1(1 - f_1 - f_2)^{X-1} N_0$ (5a)

CFU to Surroundings = $f_2(1 - f_1 - f_2)^{X-1} N_0$ (5b)

CFU left on Blade = $(1 - f_1 - f_2)^{X-1} N_0 - f_1(1 - f_1 - f_2)^{X-1} N_0 - f_2(1 - f_1 - f_2)^{X-1} N_0$

$$= (1 - f_1 - f_2)^X N_0 \quad (5c)$$

5.3.3. Predicting CFU's on meat as a function of slice number (X).

The model predicts that the number of CFUs transferred to slice X is:

$$\text{CFU (X)} = f_1 (1 - f_1 - f_2)^{X-1} N_0 \qquad (6a)$$

This can be arranged as:

$$\text{CFU (X)} = \frac{f_1 N_0}{1 - f_1 - f_2} \quad (1 - f_1 - f_2)^X \qquad (6b)$$

This can be rewritten as:

$$\text{CFU (X)} = ka^X \qquad (6c)$$

Where "k" and "a" are constants related to the model parameters.

5.3.4. Fitting the equation to data (finding "k" "a"). Taking the natural log of the predictive equation gives the general equation for a straight line.

$$y = b + mx \qquad (7a)$$

This equation can then be fitted to the data to slope (m) and intercept (b)

Where:

$y = \ln(CFU)$
$b = \ln(k)$
$m = \ln(a)$

It then follows that:

$$a = 1 - f_1 - f_2 = e^m \tag{7b}$$

$$k = f_1 N_0 (1 - f_1 - f_2) = e^b \tag{7c}$$

5.3.5. Interpretation of fit results.

The previously identified equation has three unknowns that can be determined if the original inoculum level is known and both the slope and y-intercept are derived. The parameter "a" is the fraction of CFUs remaining on the blade after any slice. The slope "m" from the fit will always be negative, so $(1 - f_1 - f_2) < 1$. The number of CFUs transferred from the blade to the first slice is $f_1 N_0$. From the relationships between the fit parameters "m" and "b" and the model parameters "f_1", "f_2" and "N_0" it follows that:

Fraction remaining on blade = e^m

CFUs transferred to 1st slice = e^{m+b}

Given the inoculation level or original number of CFU's on the blade, " f_1 ", " f_2 " and " N_0 " can be found as follows using these previous equations:

$$f_1 = \frac{e^{m+b}}{N_0} \tag{8a}$$

$$f_2 = 1 - f_1 - e^m$$

5.4. RESULTS

5.4.1. Transfer coefficients for *Listeria monocyctogenes* during slicing of turkey breast, bologna and salami.

Listeria transfer coefficients for each mechanical slicer and knife blade scenario are presented in Figures 5.1-5.8. Except for salami sliced on a mechanical slicer at 10^5 and 10^3 CFU blade, a similar trend was seen for all other mechanical slicer and knife blade scenarios with 99% of the original *Listeria* population being transferred in the first 10-15 slices. When salami was sliced using a mechanical slicer blade containing 10^5 CFU/blade, *Listeria* continually transferred out to 30 slices without a plateau and out to 20 slices before reaching a plateau or becoming stationary for a slicer blade inoculated at 10^3 CFU/blade.

Figure 5.1. Cumulative *L. monocytogenes* transfer (%) from an inoculated slicer blade (10^8 CFU/blade) to turkey, salami and bologna.

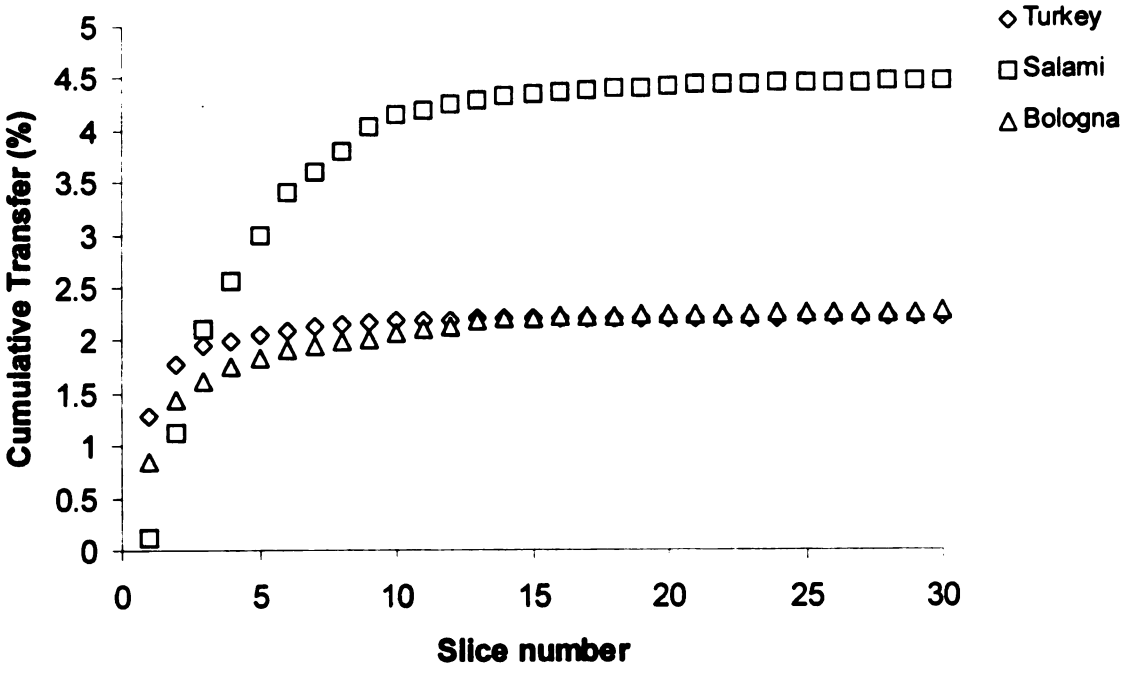


Figure 5.2. Cumulative *L. monocytogenes* transfer (%) from an inoculated slicer blade (10^5 CFU/blade) to turkey, salami and bologna.

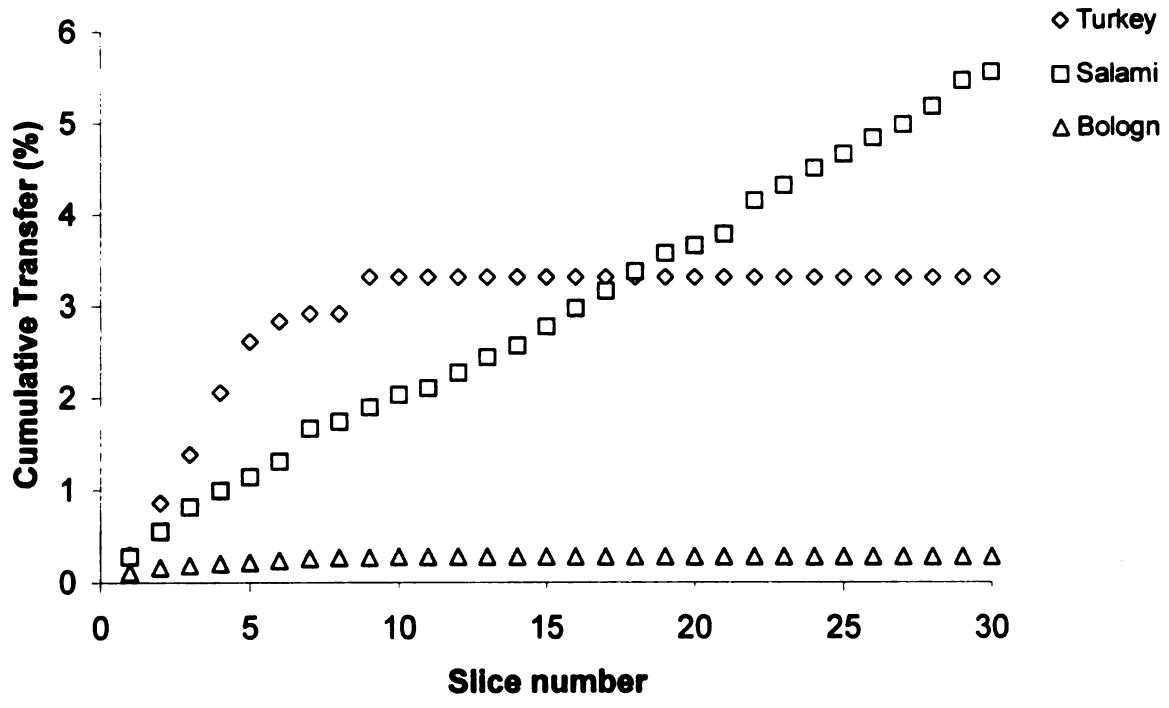


Figure 5.3. Cumulative *L. monocytogenes* transfer (%) from an inoculated slicer blade (10^3 CFU/blade) to turkey, salami and bologna.

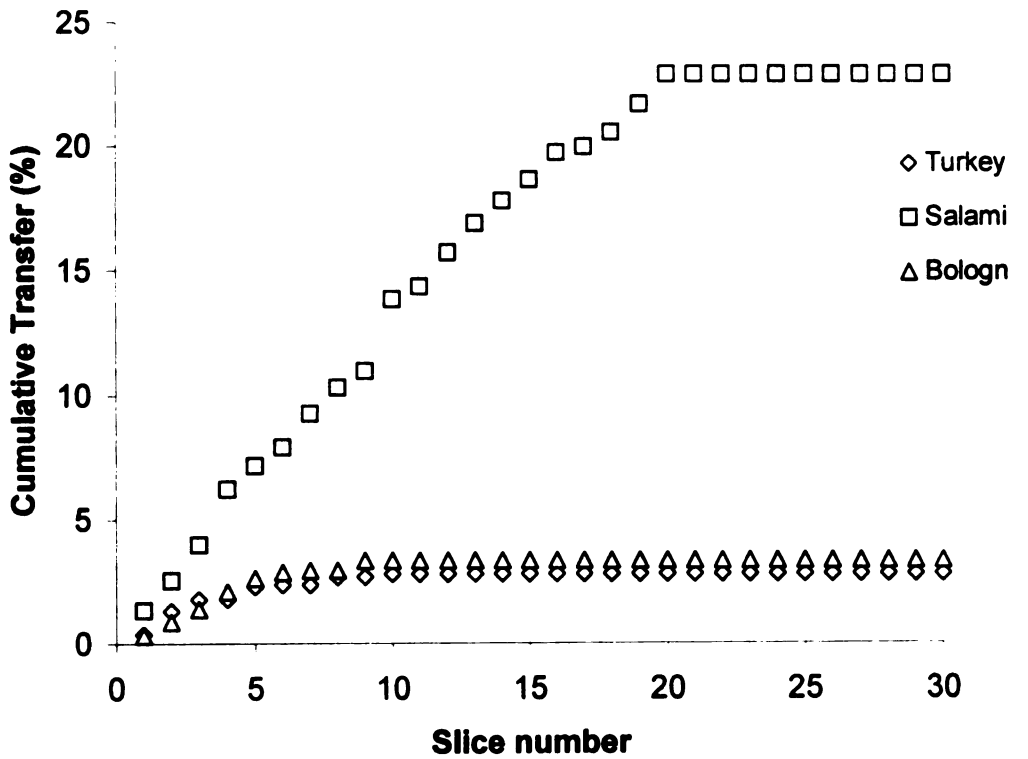


Figure 5.4. Cumulative *L. monocytogenes* transfer (%) from inoculated turkey (IT) and salami (IS)(10^5 CFU/cm^2) to uninoculated turkey (UT) and salami (US) during mechanical slicing.

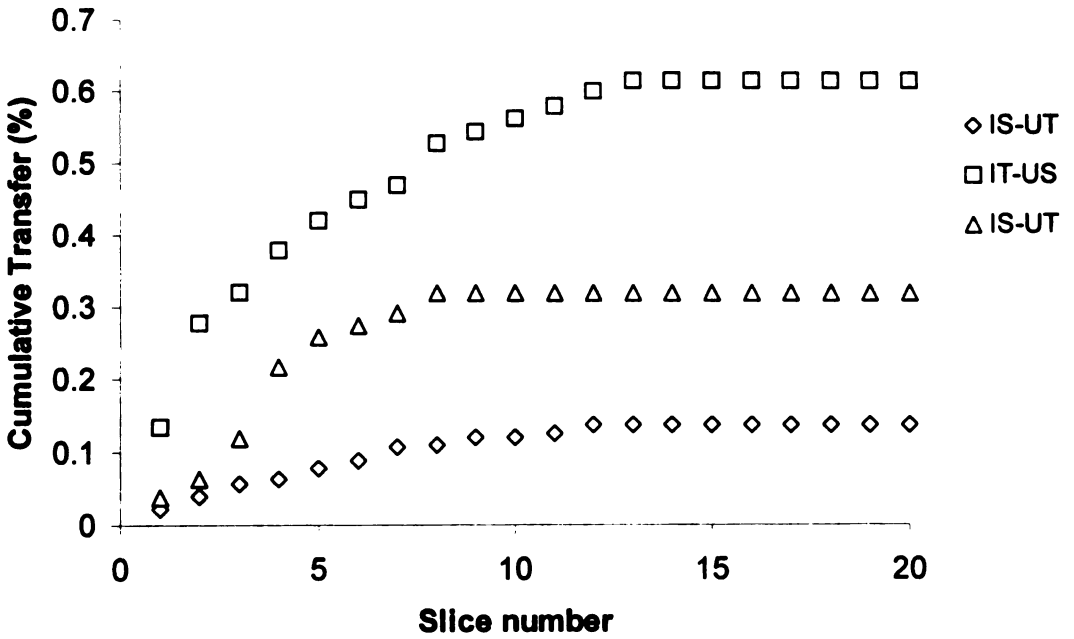


Figure 5.5. Cumulative *L. monocytogenes* transfer (%) from an inoculated) knife blade (10^8 CFU/blade) to turkey, salami and bologna.

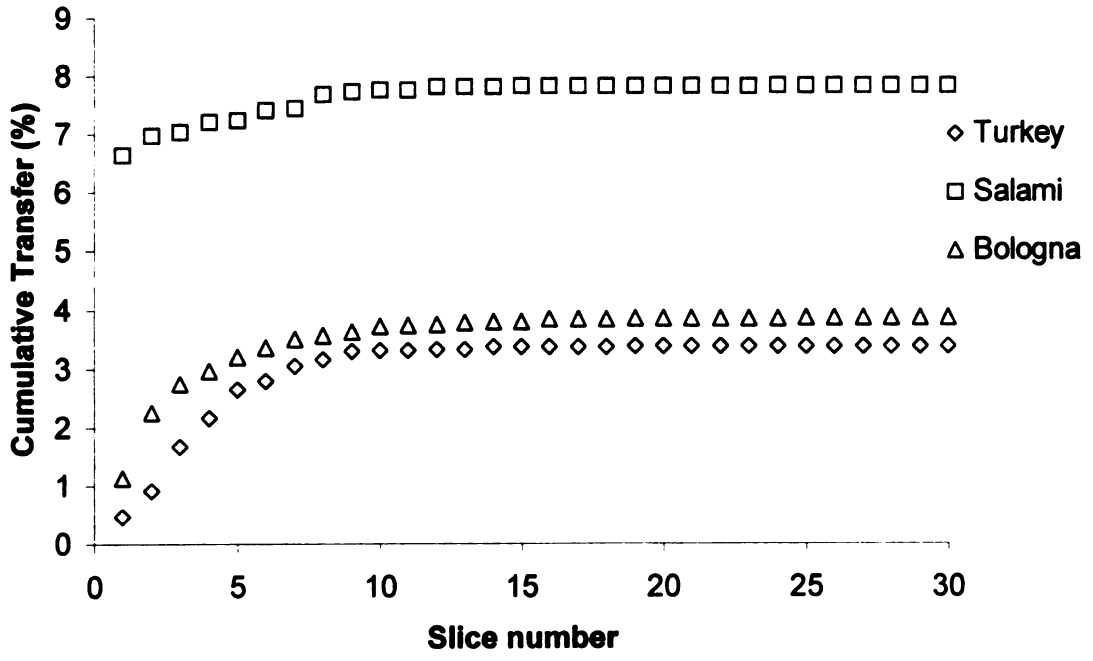


Figure 5.6. Cumulative *L. monocytogenes* transfer (%) from an inoculated knife blade (10^5 CFU/blade) to turkey breast, salami and bologna

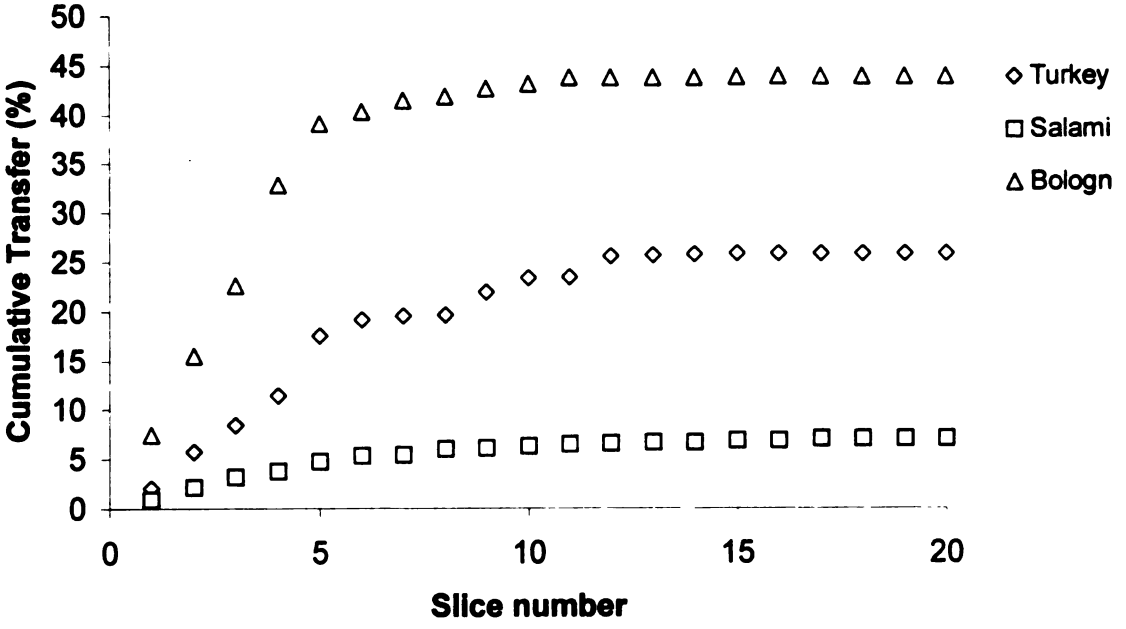


Figure 5.7. Cumulative *L. monocytogenes* transfer (%) from an inoculated knife blade (10^3 CFU/blade) to turkey breast, salami and bologna

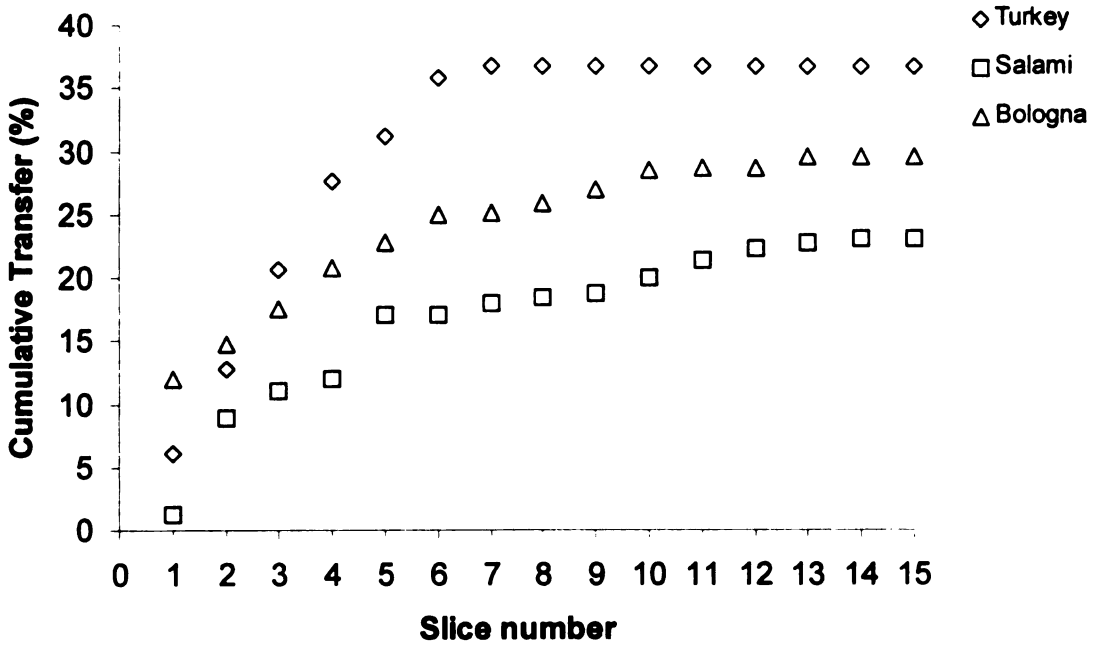
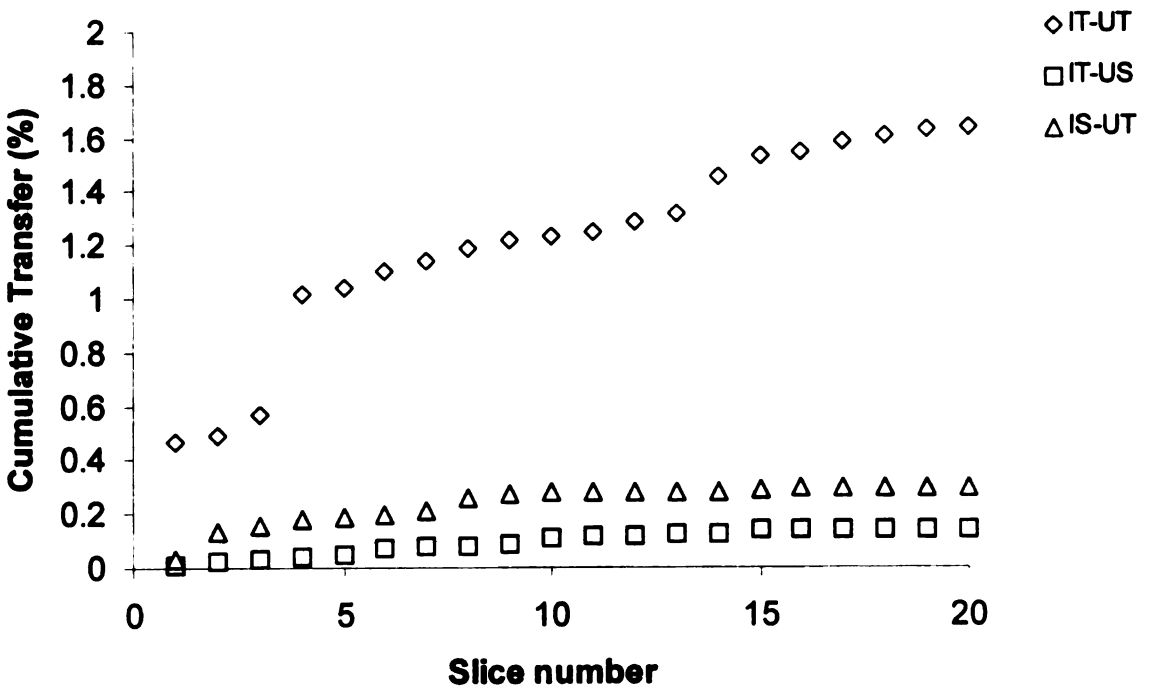


Figure 5.8. Cumulative *L. monocytogenes* transfer (%) from inoculated turkey (IT) and salami (IS)(10^5 CFU/cm^2) to uninoculated turkey (UT) and salami using an uninoculated knife blade.



5.4.2. Predictive model for *L. monocytogenes* transfer during slicing of turkey, bologna, and salami using a mechanical slicer.

Using the previously identified predictive model, a program was developed using GWBasic for a series of correlation coefficients of predicted versus observed values for transfer using slicer and knife blade scenarios with example output illustrated in Figures 11-14. High-level inoculation of products (10^8 CFU) resulted in the lowest variance or fractional differences (R^2>0.90) for observed vs. predicted values for all models tested (Figure 5.9).

Figure 5.9. Example: GWBasic output for salami sliced using an inoculated knife blade (10^8 CFU/blade)

Fraction left on blade during each slice= .8150896

CFUs transferred to 1st slice= 2173269

Above results are independent of N_0

If initial CFUs on the blade= 1E+08, then .

fraction transferred to the product during each slice = 2.173269E-02

fraction transferred to surroundings during each slice = .1631777

fitted equations (all equivalent) are:

1) ln CFUs = -.2044573 *s + 14.7962

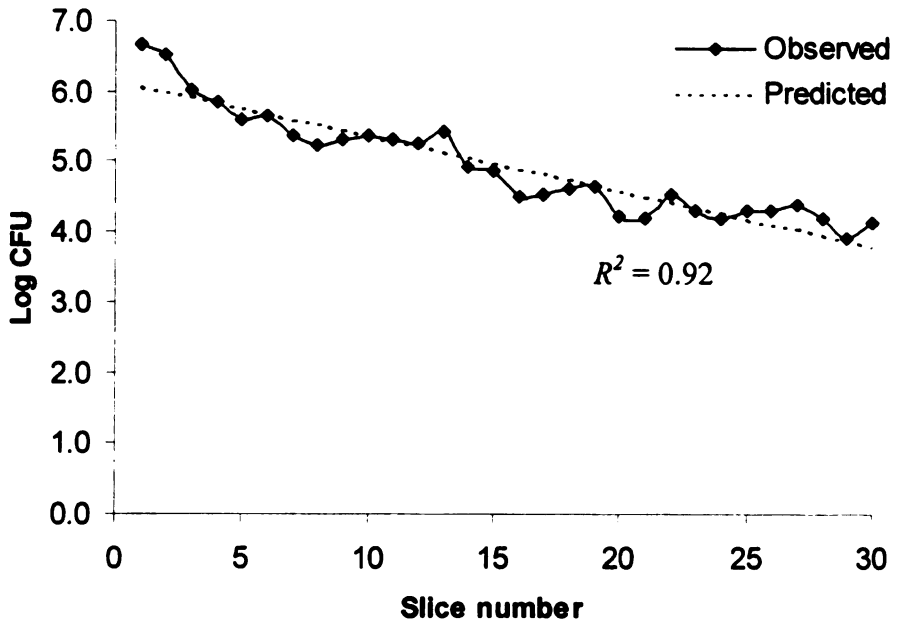
2) CFUs = 2666295 * .8150896 ^s

3) CFUs = 2666295 *e^(-.2044573 *s)

4) CFUs == 2666295 *10^(-8.879466E-02 *s)

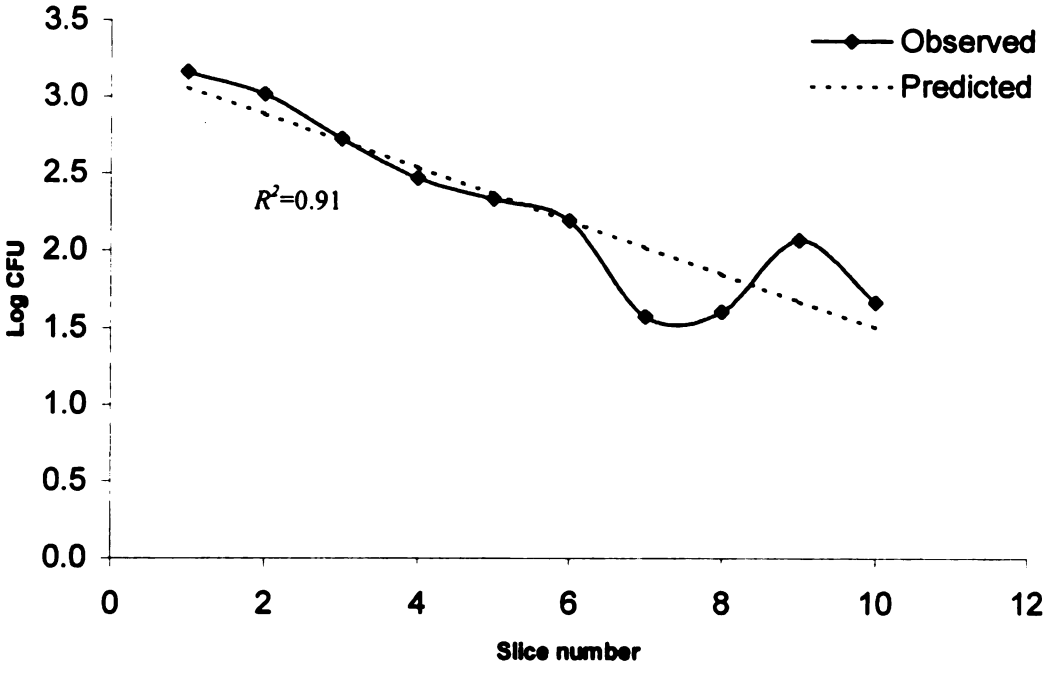
correlation coefficient for fit is R^2 = .964417

Figure 5.10. Plotted output using GWBasic for assessing *L. monocytogenes* transfer from an inoculated slicer blade (10^8 CFU/blade) to salami



Identifying a predictive model for lower level inoculations (10^5 and 10^3 CFU/blade) was much more difficult with different models being selected based on the product sliced. Turkey and bologna were similar ($R^2 > 0.90$) at 10^5 CFU/blade (Figure 5.11). Salami was not modeled at 10^5 CFU/blade for product sliced on the delicatessen slicer due to continued transfer out to 30 slices in the absence of any trend or regression (Figure 5.2).

Figure 5.11. Plotted output using GWBasic for predicting *L. monocytogenes* transfer from an inoculated slicer blade (10^5 CFU/blade) to turkey.



Low-level inoculation (10^3 CFU/blade) resulted in greater variance in all models tested. The model identified for turkey and bologna resulted in large differences between observed and predicted values ($0.52 \leq R^2 \leq 0.65$) when compared to higher inoculation levels (Figure 5.12). Salami showed a weak correlation coefficient ($R^2 < 0.40$) for all models tested (Figure 5.13) with residuals greater than 50% of the measured values..

Figure 5.12. Plotted output using GWBasic for predicting *L. monocytogenes* transfer from aninoculated slicer blade (10^3 CFU/blade) to turkey and bologna

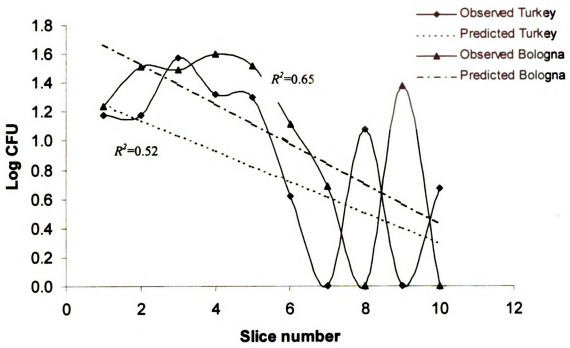
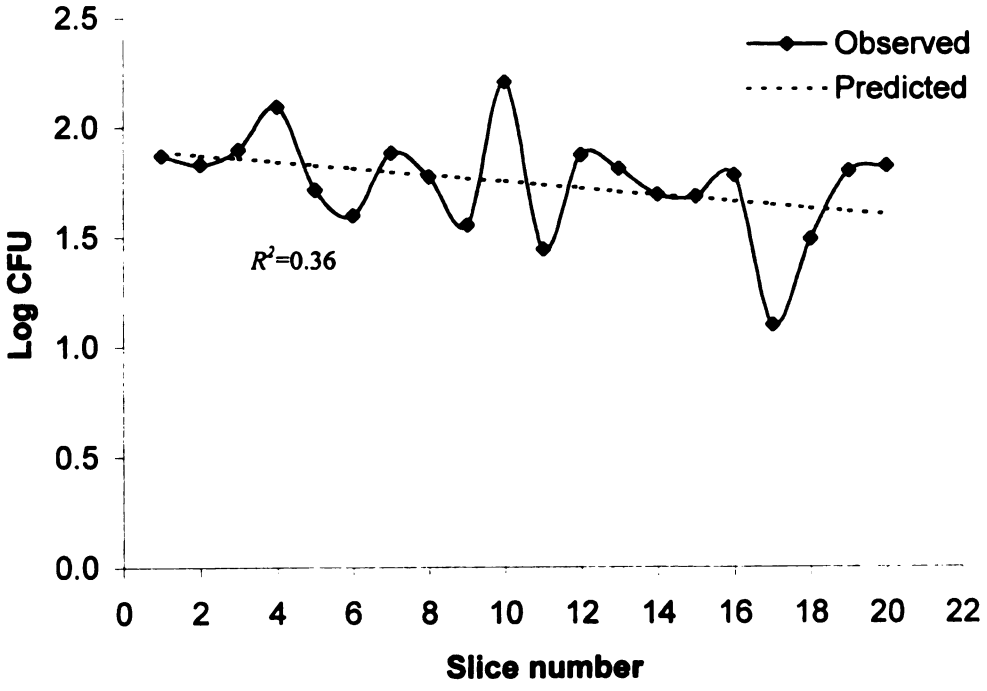


Figure 5.13. Plotted output using GWBasic for predicting *L. monocytogenes* transfer from an inoculated slicer blade (10^3 CFU/blade) to salami



5.5. DISCUSSION

Predictive modeling of microbial pathogens during food production and storage has been approached using various mathematical models and methods. Common approaches have included such empirical modeling or mechanistic mathematical translation of various factors including attachment properties and metabolic functions (Bernaerts et al., 2004). Each of these approaches has many advantages and disadvantages. Predictive models based on mathematical translation of biological functions can be rapid and less costly than empirical models requiring little or no laboratory experimentation. While derived mathematical models use biological mechanisms to predict population outcome, environmental scenarios may strongly influence an outcome that is not readily identified or foreseen with mathematical derivations and manipulation. Curve fitting models predict population outcomes based on previously obtained experimental data offering arguably more accurate interpretation of predicted environmental populations. However, many curve fitting models based on empirical data do not account for underlying biological factors that influence the fitting of results and in some cases may be dependent on specific environmental or laboratory conditions. Limited work has been done modeling bacterial transfer to and from food contact surfaces. A study by Schaffner et al. (2004) used a Monte Carlo simulation for predicting bacterial populations on cutting boards over time. Results from this study and subsequent simulation predicted a contamination level greater than 20 CFU/4cm^2 after 15 min and greater than 40 CFU/4cm^2 after 45 min. While this study provides some insight into modeling total bacterial transfer during slicing of deli meats, other important

parameters including surface scoring, surface roughness, cutting force, and physiological differences in bacterial attachment were not addressed.

Models generated for this research were based on empirical data generated in a laboratory during mechanical and knife slicing of turkey, salami, and bologna with the following 12 product, environmental and blade variables affecting the outcome of these models:

1. Product fat content – high fat (salami) vs. low fat (turkey)
2. Product moisture content – high moisture (turkey) vs. low moisture (salami)
3. Product composition – homogeneous (bologna) vs. heterogeneous (salami)
4. Product temperature – frozen (< 0°C) vs. refrigerated (<0-7°C) or abusive (7-23° C)
5. Environment – low (<50%) versus high (>50%) relative humidity
6. Blade stainless steel grade – 304 vs.316
7. Blade sharpness – sharp vs. dull or broken
8. Blade thickness – thin vs. medium or thick.
9. Blade cutting speed/force – slow vs. fast
10. Blade age – changes in surface roughness, /wear, and scoring, and pitting over time.
11. Blade surface finish – 2B vs. electropolished.
12. Blade/knife edge – serrated vs smooth

Assumptions made for fitted equations in this model were: a) the expected number of *Listeria* transferred (CFU) during slicing is the fraction "f_1" that describes how many *Listeria* are on the blade just before each sequential slice b) the expected number of *Listeria* transferred to the surrounding areas is a different fraction "f_2" that describes how many *Listeria* are on the blade just before slicing, and c) N_0. the number of *Listeria* cells on the blade that are available for transfer before any slicing begins.. These fractional assumptions "f_1" and "f_2" are expected to be constant because of the degree of adhesion between *Listeria* and the blade/meat surface. A surface exhibiting a low-level of adhesion would allow the fraction transferred to the meat to approach 1 and the fraction transferred to the surface to approach 0. This would allow the fraction left on the blade "f_1" to approach 0 while the fraction left on the surface "f_2" would approach 1. The reverse is true for a surface exhibiting a high-level of adhesion where the fraction left on the surface "f_1" approaches 1 and the fraction on the meat "f_2" approaches 0. When using a mechanical slicer, it is assumed that any *Listeria* on the blade are uniformly and randomly distributed due to the exceedingly high rotation rate of 8 rotations per second during slicing. Given the high number of revolutions between slicing (60-80), *Listeria* is uniformly transferred from the blade to the product or from the product to the blade. This assumption was verified when turkey, salami, and bologna were sliced using knife blades. *Listeria* transfer coefficients were dependent on both the type of product being sliced and the method of slicing. Using the delicatessen slicer, transfer coefficients were higher for high fat, low moisture salami when compared to low fat and high moisture turkey. When turkey and salami were sliced using the knife blades, turkey yielded a higher transfer coefficient for blades inoculated at 10^5 and 10^3 CFU/blade. Lin et al.

(2004a) reported similar findings using a mechanical slicer with the presence of a fat layer extending transfer out to more slices. These findings indicate that different *Listeria* transfer scenarios can be expected based on the previously identified 12 parameters.

The four fitted models described herein of the form [CFU (X) = ka^X] along with the program written in GWBasic can be used if any two of the following three values are known: a) initial inoculum level, b) total bacterial transfer, c) fraction of bacteria remaining on blade after consecutive slicing, solving for each model parameter CFU(X), k, or a. Further extrapolations can be done using previously reported data for turkey, salami, and bologna to estimate each of these model parameters using predicted fitted equations having correlation coefficients greater than 85%. While many low inoculum levels exhibited weak correlation coefficients, the last 10 slices and the first 5-10 slices of high-level (10^8 CFU/blade or cm^2) and low-level inoculations (10^3 CFU/blade or cm^2) can be used to estimate the number of *Listeria* transferred during slicing.

5.6. SUMMARY

Based on our model, the greatest number of *Listeria* (>90%) will be found in the first 15 slices of delicatessen meats after mechanical or knife slicing. The model presented in this research has been simplified using limited factors based on fractions transferred to the blade, meat and surroundings. This model can be used as a starting point to identify additional parameters that impact bacterial transfer. Depending on any one or combination of the aforementioned 12 parameters affecting transfer, certain delicatessen meats that permit growth of *Listeria* may pose a public health risk to certain consumers if the product is subjected to extended refrigerated storage. The mathematical

construction of the model presented in this study provides a framework for designing future models with different parameters, environments, and processes.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Post-process contamination of ready-to-eat meats from *Listeria monocytogenes* continues to be a health risk to the public and a safety and financial risk to the processor and retailer alike. Results from this research demonstrate the ability of *L. monocytogenes* to transfer from contaminated product to uncontaminated products using a slicer or knife blade as a vector. Quantitative recovery of *Listeria* from solid surfaces was identified as a hurdle for both accurate and precise assessmen t of transfer from contaminated products to food contact surfaces.

The first objective of this research, optimizing quantitative recovery of *L. monocytogenes* from stainless steel surfaces, was improved (> 0.5 log cfu) using a composite tissue device, which was inexpensive and easy to use when compared to traditional sponge, and swabbing devices. In addition to improved efficacy, larger heavily soiled areas could be sampled without using multiple devices. While this device represents a significant improvement to traditional sampling devices, future research needs to be conducted to further develop new and innovative sampling devices for improved accuracy and precision when sampling solid surfaces.

The second objective of this research was to determine direct and sequential transfer rates for *L. monocytogenes* from artificially contaminated ready-to-eat luncheon meats to a delicatessen slicer and vice versa. Our findings provided valuable insight into distribution of *L. monocytogenes* during slicing of contaminated ready-to-eat meats. Results from this study demonstrated greater transfer ($P<0.05$) from inoculated turkey (10^8 CFU/cm^2) to the five slicer contact areas using a cutting force of 10 as opposed to 0 lbs. When slicer blades were inoculated at 10^8 CFU/blade *Listeria* populations decreased

logarithmically to 10^2 CFU/slice after 30 slices. Findings for inoculated slicer blade and products (10^5 CFU/blade or cm^2) were similar with *Listeria* counts of 10^2 CFU/slice after 5 slices and enriched samples generally negative after 27 slices. Using 10^3 CFU/blade, the first 5 slices typically contained ~10^1 CFU/slice by direct plating with enrichments negative after 15 slices. Product composition had a major impact on transfer during slicing. Higher fat and lower moisture of salami compared to turkey and bologna resulted in prolonged *Listeria* transfer with a fat layer developing on the blade. Our finding also suggest a major impact of product composition on transfer with blade surface roughness changing significantly (>100%) during one year of use. Numerous areas of future research were identified during this study. Material composition of the slicer, surface finish, effects of cleaning regimens on slicer oxidation and pitting, as well as environmental temperature and relative humidity are limited examples of interactions that are poorly understand and in most cases not documented.

Similar to objective two, the third objective, determining the effects of cutting force, stainless steel grade, sharpness, and product composition on transfer of *L. monocytogenes* from artificially contaminated ready-to-eat luncheon meats to knives and vice versa, identified product composition as having a significant impact on transfer. *Listeria* transfer from knife blades inoculated at 10^8 CFU/blade was logarithmic with a 2-log decrease after 12 slices and direct counts obtained thereafter out to 30 slices. However at lower inoculation levels of 10^5 and 10^3 CFU/blade, direct counts were typically only observed out to 20 and 5 slices, respectively. Stainless steel grade had a significant impact on transfer with greater "tailing" or more prolonged transfer from grade 304 as opposed to grade 316 stainless ($P<0.05$) for all three products. After one

year of use, knife blade roughness values were significantly greater ($P<0.001$) for grades 304 than 316 stainless. Surprisingly, force and knife sharpness were not significantly different ($P>0.05$) within stainless steel grade ($P<0.05$) for each product. However, significant differences in force were seen between salami and turkey ($P<0.05$) for grades 304 and 316 stainless steel. This study also identified numerous areas of future research with material composition (chemical and physical properties), surface finish, effects of cleaning regimens on blade oxidation and pitting, as well as environmental temperature and relative humidity being limited examples of interactions that are poorly understand and in most cases not documented.

In the final objective, development of one or more mathematical models based on the transfer coefficients obtained from the previous three objectives that will predict the numbers of *L. monocytogenes* cells transferred during slicing of delicatessen meats, four variations of a model identified as [CFU (X) = ka^X] along with a program in GWBasic. The model variations and subsequent program were based on the following three assumptions: 1) the expected number of *Listeria* cells transferred during slicing is the fraction "f_1" that describes the number of *Listeria* cells on the blade just before each sequential slice, 2) the expected number of *Listeria* cells transferred to the surrounding areas is the fraction "f_2" after each slice, and 3) the number of *Listeria* cells on the blade available for transfer before any slicing begins is N_0. The aforementioned model and variations thereof can be used if any two of the following three values are known: (a) initial inoculum level, (b) total bacterial transfer, (c) fraction of bacteria remaining on blade after consecutive slicing, solving for each model parameter CFU(X), k, or a. The overall finding using transfer coefficients in the models resulted in the greatest number of

Listeria (>90%) being found in the first 15 slices. As a result of this study, numerous areas of future research were identified emphasizing the future research needs presented in objectives two through four. In addition to better understanding of material and product composition, experimental design using a hybrid of mathematical manipulation and empirical data gathering is needed to evaluate parameter effects. Traditional approaches using empirical data gathering to find parameter effects are very costly and may result in the neglect of underlying factors hidden with the parameters being evaluated. Modifications to each of these approaches (mathematical manipulation and empirical data gathering) will minimize cost and time spent finding each of these parameter effects. In addition to model identification and development, future research needs to be done validating such models using independent researchers and laboratories.

Many recommendations to food manufacturers, retailers, and researchers can be taken from these studies. Transfer of bacterial pathogens was found to be sequential with scoring and surface finish changing significantly with usage for both slicer and knife blades. As a result of these studies recommendations to slicer manufactures would include better polishing of food contact areas using electropolishing or high grade buffing. Current slicer configurations use a low-grade stainless (304) and polish (No. 4) on all areas except the slicer blade which is typically electropolished. A higher grade stainless such as 316 would result in greater resistance to oxidation and corrosion and subsequently decrease scoring and areas of attachment for bacteria. Improving surface finish by electropolishing to obtain a mirror-like buffed finish on all food contact areas would decrease food particulate and bacterial attachment as well as enhancing visual soiling. Slicer design has also been identified as an area for improvement by eliminating

unnecessary machining and creating smoother transitions between contact areas to ease in cleaning.

Additional recommendations resulting from this study can be applied to food utensil manufacturers. Food utensils have many variations in design, composition and surface finish. Based on our findings, 316 grade stainless was superior to grade 304 and is recommended for improved initial smoothness and surface wear after continued use. Future research needs include the compositional effects of grade 316 stainless on cell recovery. Addition of molybdenum and other compounds may result in new and innovative alloys with bactericidal properties, thus minimizing biofilm formation and subsequent transfer.

Overall, this work represents a major contribution to the area of bacterial transfer in processing and retail environments and has generated many new research avenues for further investigation of post-process contamination at the retail level. Future research building on this study will contribute greatly to a better understanding of the distribution and dissemination of bacterial pathogens in the food supply along with major improvements in the current risk assessments.

APPENDIX I

Preliminary Analysis of Dr. Robert McMaster

11 October 2003

Background:

This is a preliminary report based on the two sets of data I've received from Keith Vorst on slicing the turkey breast. There were 21 data points taken on 18 June 2003 and 29 points measured on 28 August 2003. Keith forwarded the information to me on 15 September 2003.

Analysis:

I have analyzed the data using seven different functions, allowing them to compete on the basis of closeness of fit. Each function contains either 2, 3 or 4 parameters, which are optimized in order to obtain the closest fit for the respective functional form. Each of these parameters could be considered a type of "transfer coefficient" as we had set forth in our original proposal.

Once the best fit for each function is found, the standard of deviation of the residuals is then compared for each function in order to determine which function provides the best fit. The residuals are simply the differences between each measured data point and the value of the same point as calculated by the model. The model with

the smallest standard deviation in the residuals is generally assumed to be the most appropriate. However, this must be tempered by the number of parameters used in the function.

Functions with more parameters may provide a better fit, not necessarily on the basis of a more appropriate modeling of the physical behavior of the problem, but because of the additional degrees of freedom offered by the higher order model. In order to determine the most appropriate model, then, statistical methods can be employed to discern whether the additional parameters in a higher order model provide improvement in the residuals of statistical significance. For example, using the "F" statistic, the "F" test can be employed in evaluating the reduction in the standard deviation of the residuals in comparing a 2 parameter model to a 3 parameter model. The "F" statistic table is entered with the number of parameters and the number of measurements and the result gives the necessary reduction in the standard deviation of the residuals from the 3 parameter model to justify inclusion of the 3^{rd} parameter beyond the 2 parameter model. For cases where there is no physical reason to justify the inclusion of the 3^{rd} parameter, the "F" test should show that the inclusion of the additional parameter in the model is not warranted.

Results:

Of the two tests on hand to date, seven models were fitted to each as shown in Tables 1 and 2 below. Table 1 summarizes the 21 data points taken on 18 June 2003 and Table 2 summarizes the 29 points measured on 28 August 2003. The accompanying figures show plots of the raw data with the best fit function superimposed. As can be seen in Table 1, the lowest standard deviation in the residuals is achieved by both Models 3 and 4. However, Model 3 has only 2 parameters (or "transfer coefficients") and Model 4 requires 3 parameters. Therefore, the "F" test is not required in this case, since the additional parameter in model 4 offered no improvement in the model performance.

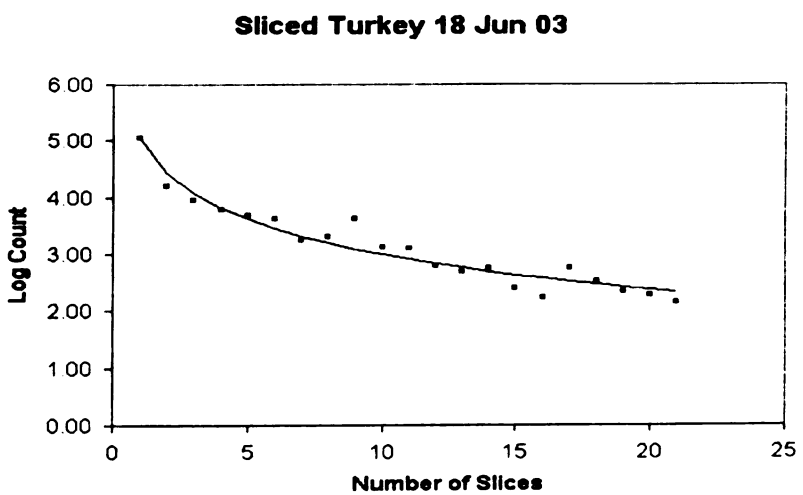


Table 1 21 data points taken on 18 June 2003						
Model	Equation	A	B	C	D	Standard Deviation of Residuals
1	$F(n) = A + Be^{-Cn}$	1.885348	3.070936	0.097862		0.192888
2	$F(n) = Ae^{-Bn}$	4.613156	0.037861			0.216080
3	$F(n) = A - B\ln(n)$	5.051415	0.891713			0.185079
4	$F(n) = A - B\ln(Cn)$	5.023455	0.891714	0.969131		0.185079
5	$F(n) = A - Bn^C$	0.632403	4.596126	-0.29112		0.236264
6	$F(n) = A - Bn + Cn^2$	4.776439	0.217829	0.00472		0.200547
7	$F(n) = A - Bn + Cn^2 + Dn^3$	5.000451	0.328542	0.017063	-0.00037	0.190184

In Table 2, we see that the same seven models are used, since the curve has the same basic shape as the data analyzed in Table 1. In this case, Model 7 emerges as the best fit. However, this model requires 4 parameters and the magnitude of the improvement in the standard deviation of the residuals is very small. Moreover, the "F" test would not show justification to include the additional parameter added in model 7 from model 6. More significant still is the fact that Model 1 is superior to Model 6, even though it has one fewer parameter. Therefore, Model 1 is clearly the most appropriate choice of the functions which were fit to this set of data.

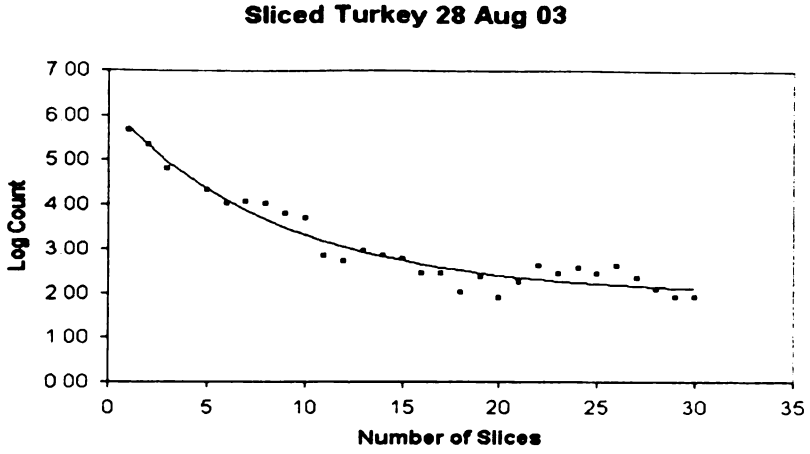
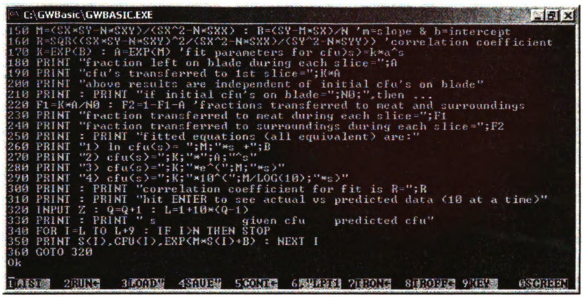


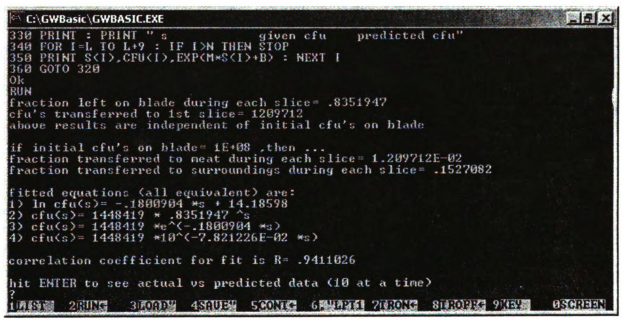
Table 2 29 data points taken on 28 August 2003						
Model	Equation	A	B	C	D	Standard Deviation of Residuals
1	$F(n) = A + Be^{-Cn}$	1.961016	4.219396	0.113016		0.253891
2	$F(n) = Ae^{-Bn}$	5.316928	0.038814			0.373251
3	$F(n) = A - B\ln(n)$	6.068183	1.197134			0.296056
4	$F(n) = A - B\ln(Cn)$	6.059808	1.19713	0.993037		0.296056
5	$F(n) = A - Bn^C$	-58.4602	64.55685	-0.0192		0.298025
6	$F(n) = A - Bn + Cn^2$	5.783087	0.296413	0.00606		0.263702
7	$F(n) = A - Bn + Cn^2 + Dn^3$	6.107309	0.414355	0.01547	-0.0002	0.242961

APPENDIX II

The example program using GWBasic is as follows:



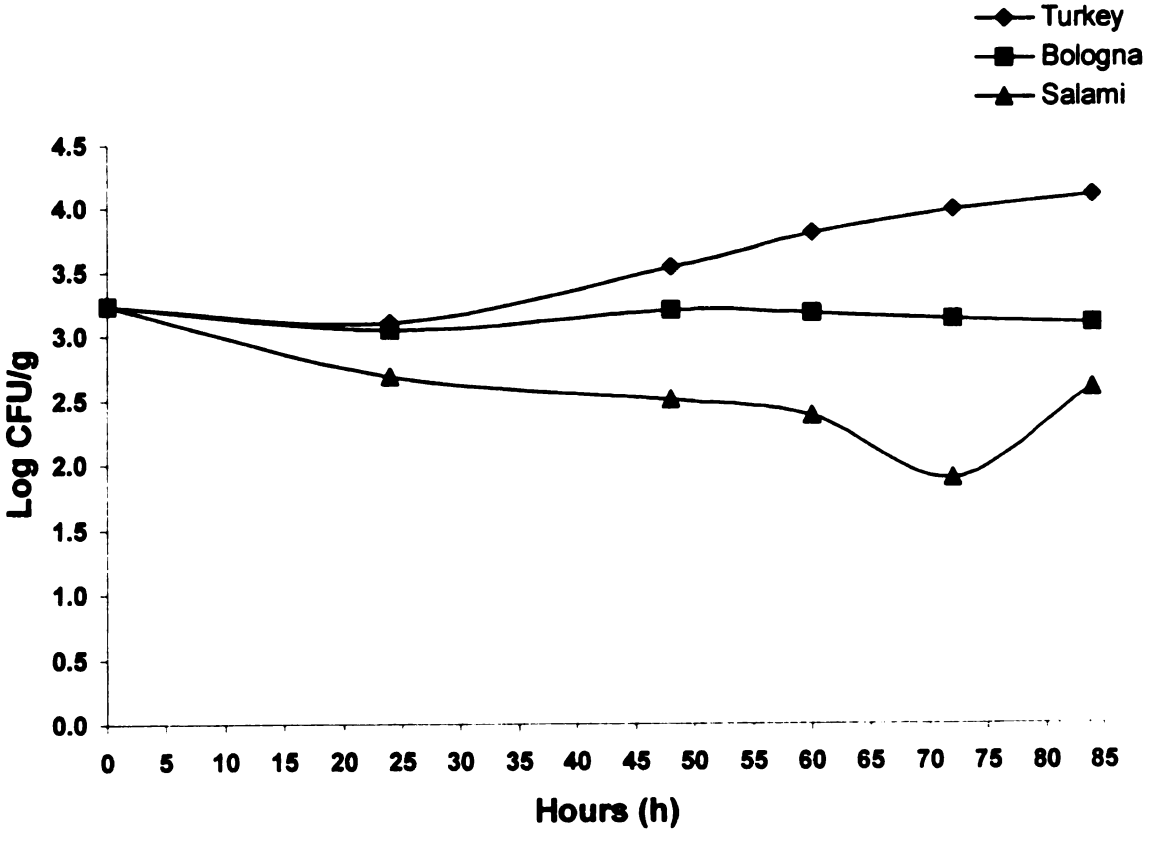
Using data obtained from a delicatessen slicer inoculated at 10^8 CFU/blade the program developed in GW basic is as follows:



APPENDIX III

Lag (LT) and Generation Time (GT) of *Listeria monocytogenes* on turkey, bologna and salami at 4° C.

Food	Temperature (C)	GT (h)	LT (h)
Roast Turkey	4	29	24
Bologna	4	N/G	N/G
Salami	4	N/G	N/G



APPENDIX IV

From: Whiting, Richard C [mailto:Richard.Whiting@cfsan.fda.gov]
Sent: Thursday, January 13, 2005 4:39 PM
To: 'Keith Vorst'
Cc: Ryser, Elliot; Ewen Todd; 'carl.custer@fsis.usda.gov'
Subject: RE: Data for Deli Slicer and knife transfer

Keith,

I've looked at the data and propose you consider an exponential or first-order decay model. This has a constant amount of material removed with each subsequent slice. Log Ns = log No - ks Ns is the counts on a slice, No is the original amount on the knife/blade, s is the slice number and k is the parameter value. On the attached spreadsheet of yours the k appears to be about 0.1, this means that 90% of the material remains on the knife and 10% goes to the slice. The next slice that passes the knife removes another 10% but from a slightly smaller amount remaining on the knife. The spreadsheet has my electronic doodling and graphing of your data.

The difference between the inoculum and first slice appears to be about 2.08 log 10 or about 0.8%.

However, I see this as a mass transfer not really a microbial transfer. It would be useful to know the mass transferred from the original contaminated product to the knife/blade. Then 10% of that product would be transferred with the first slice.

The three products appear to be similar, it would be interesting to see how other products (including cheeses) would transfer. This might be related to the product's friability and adhesion or it might be mostly adhering water. Your pressure, surface texture would also be factors here.

I could see one model for the amount of mass (with bacteria) transferred to a blade and the second model above for the removal from the blade to subsequent clean product with each slice.

Let me know what this sounds like to you.

Richard Whiting

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5100 Paint Branch Parkway
College Park, MD 20740-3835

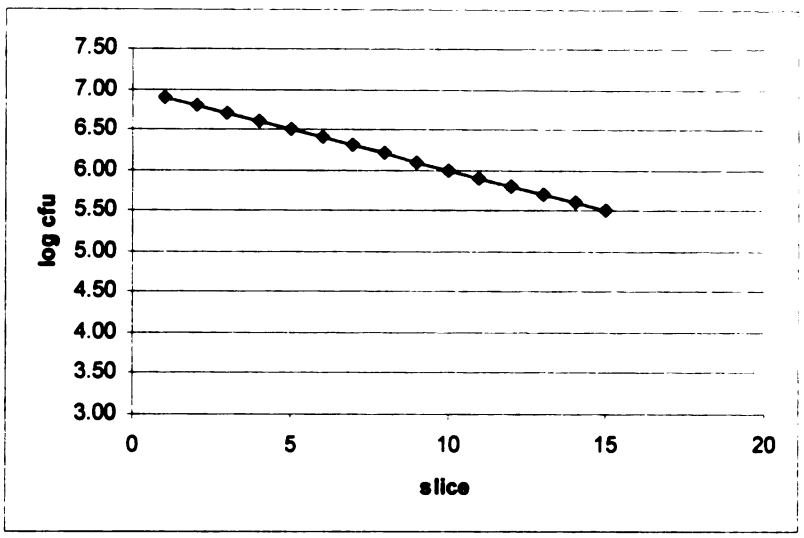
phone 301-436-1925
fax 301-436-2632
e-mail rwhiting@cfsan.fda.gov

Model Generated by Dr. Whiting FDA/CFSAN

Ns = No -
exp(ks)
No = amount on first slice
s =
slice

No	k
7	0.1

slice	Ns
1	6.90
2	6.80
3	6.70
4	6.60
5	6.50
6	6.40
7	6.30
8	6.20
9	6.10
10	6.00
11	5.90
12	5.80
13	5.70
14	5.60
15	5.50



		No	k				
10^8	Bologna	6.843802	0.137483	5.853894	0.073729	5.374298	0.031559
	Salami	6.246282	0.097275	6.363965	0.085096	6.50417	0.086507
	Turkey	6.059119	0.110711	5.846207	0.105168	5.395735	0.075915

10^5	Bologna	
	Salami	
	Turkey	

With exception of 10^5
salami:
If appears about 1% of pathogens were transferred to blade and were removed with first slice

Subsequent slices have a k = 0.10, this means that 90% remains and 10% removed each slice.
If back calculate to slice zero, then original concentration was 2.08 + 0.1 + 2.19

This is a transfer of mass, bacteria are along for the ride.

			Nprod-No
ave		0.08	1.98
	6.02	0.09	1.63
	6.37	0.10	2.23
	5.77		
		0.11	2.56
	2.44	0.01	2.10
	2.90	0.11	1.99
	3.01	0.10	2.08
ave		n=5	

Slicer Blade to Product (10^8 CFU/Blade)

Product	Bolgona = 1, Salami = 2, Turkey =3						
REP							bologna
Slice #					bologna rep 1		rep 2
Count	CFU				No	k	No
Level	10^8				6.843802	0.137483092	5.853894
							k
Rep	Slice	Count	Product	log count	model	diff sq	0.073729
1	1	7.78E+06	1	6.89	6.71	0.034	
1	2	8.35E+06	1	6.92	6.57	0.125	
1	3	2.55E+06	1	6.41	6.43	0.001	
1	4	1.71E+06	1	6.23	6.29	0.004	
1	5	8.54E+05	1	5.93	6.16	0.051	
1	6	1.01E+06	1	6.00	6.02	0.000	
1	7	4.42E+05	1	5.65	5.88	0.056	
1	8	2.94E+05	1	5.47	5.74	0.076	
1	9	3.49E+05	1	5.54	5.61	0.004	
1	10	4.48E+05	1	5.65	5.47	0.033	
1	11	3.98E+05	1	5.60	5.33	0.072	
1	12	3.36E+05	1	5.53	5.19	0.110	
1	13	5.97E+05	1	5.78	5.06	0.518	
1	14	9.25E+04	1	4.97	4.92	0.002	
1	15	5.70E+04	1	4.76	4.78	0.001	
1	16	8.86E+03	1	3.95	4.64	0.485	
1	17	1.22E+04	1	4.09	4.51	0.177	
1	18	8.20E+03	1	3.91	4.37	0.207	
1	19	9.27E+03	1	3.97	4.23	0.070	
1	20	1.11E+04	1	4.05	4.09	0.002	
1	21	6.85E+03	1	3.84	3.96	0.015	
1	22	2.12E+04	1	4.33	3.82	0.258	
1	23	3.84E+03	1	3.58	3.68	0.010	
1	24	2.27E+03	1	3.36	3.54	0.035	
1	25	1.61E+03	1	3.21	3.41	0.040	
1	26	2.69E+03	1	3.43	3.27	0.026	
1	27	5.16E+03	1	3.71	3.13	0.337	
1	28	0.00E+00	1		2.99		
1	29	8.76E+02	1	2.94	2.86	0.007	sum
1	30	0.00E+00	1		2.72		2.756232
2	1	2.89E+06	1	6.46	5.78	0.464	
2	2	5.74E+05	1	5.76	5.71	0.003	
2	3	3.31E+05	1	5.52	5.63	0.013	
2	4	3.23E+05	1	5.51	5.56	0.003	
2	5	2.24E+05	1	5.35	5.49	0.018	
2	6	2.34E+05	1	5.37	5.41	0.002	
2	7	1.76E+05	1	5.25	5.34	0.008	
2	8	8.76E+04	1	4.94	5.26	0.103	

2	9	2.02E+05	1	5.31	5.19	0.013	
2	10	9.63E+04	1	4.98	5.12	0.018	
2	11	8.34E+04	1	4.92	5.04	0.015	
2	12	1.11E+05	1	5.05	4.97	0.006	
2	13	1.30E+05	1	5.11	4.90	0.047	
2	14	9.28E+04	1	4.97	4.82	0.021	
2	15	6.22E+04	1	4.79	4.75	0.002	
2	16	2.36E+04	1	4.37	4.67	0.090	
2	17	2.48E+04	1	4.40	4.60	0.042	
2	18	3.71E+04	1	4.57	4.53	0.002	
2	19	2.23E+04	1	4.35	4.45	0.011	
2	20	1.90E+04	1	4.28	4.38	0.010	
2	21	1.82E+04	1	4.26	4.31	0.002	
2	22	1.59E+04	1	4.20	4.23	0.001	
2	23	8.79E+03	1	3.94	4.16	0.046	
2	24	1.71E+04	1	4.23	4.08	0.022	
2	25	1.03E+04	1	4.01	4.01	0.000	
2	26	1.02E+04	1	4.01	3.94	0.005	
2	27	1.48E+04	1	4.17	3.86	0.094	
2	28	3.95E+03	1	3.60	3.79	0.037	
2	29	5.90E+03	1	3.77	3.72	0.003	sum
2	30	7.57E+03	1	3.88	3.64	0.056	1.158075
3	1	3.17E+06	1	6.50	5.34	1.343	
3	2	8.62E+05	1	5.94	5.31	0.390	
3	3	2.23E+05	1	5.35	5.28	0.005	
3	4	1.60E+05	1	5.20	5.25	0.002	
3	5	1.09E+05	1	5.04	5.22	0.032	
3	6	8.29E+04	1	4.92	5.18	0.071	
3	7	7.54E+04	1	4.88	5.15	0.076	
3	8	1.39E+05	1	5.14	5.12	0.000	
3	9	7.23E+04	1	4.86	5.09	0.053	
3	10	1.86E+05	1	5.27	5.06	0.044	
3	11	1.33E+05	1	5.12	5.03	0.009	
3	12	9.88E+04	1	4.99	5.00	0.000	
3	13	8.91E+04	1	4.95	4.96	0.000	
3	14	7.35E+04	1	4.87	4.93	0.004	
3	15	1.01E+05	1	5.00	4.90	0.011	
3	16	6.32E+04	1	4.80	4.87	0.005	
3	17	6.30E+04	1	4.80	4.84	0.001	
3	18	8.33E+04	1	4.92	4.81	0.013	
3	19	1.02E+05	1	5.01	4.77	0.054	
3	20	2.01E+04	1	4.30	4.74	0.193	
3	21	2.27E+04	1	4.36	4.71	0.127	
3	22	6.77E+04	1	4.83	4.68	0.023	
3	23	4.96E+04	1	4.70	4.65	0.002	
3	24	2.81E+04	1	4.45	4.62	0.028	
3	25	4.76E+04	1	4.68	4.59	0.009	
3	26	4.92E+04	1	4.69	4.55	0.019	
3	27	5.24E+04	1	4.72	4.52	0.039	

3	28	4.38E+04	1	4.64	4.49	0.023	
3	29	1.77E+04	1	4.25	4.46	0.044	sum
3	30	3.44E+04	1	4.54	4.43	0.012	1.289239
1	1	6.59E+05	2	5.82	6.15	0.11	
1	2	1.70E+06	2	6.23	6.05	0.03	
1	3	1.58E+06	2	6.20	5.95	0.06	
1	4	1.15E+06	2	6.06	5.86	0.04	
1	5	8.63E+05	2	5.94	5.76	0.03	
1	6	5.56E+05	2	5.75	5.66	0.01	
1	7	8.57E+05	2	5.93	5.57	0.14	
1	8	5.73E+05	2	5.76	5.47	0.08	
1	9	3.19E+05	2	5.50	5.37	0.02	
1	10	5.11E+04	2	4.71	5.27	0.32	
1	11	7.45E+04	2	4.87	5.18	0.09	
1	12	8.64E+04	2	4.94	5.08	0.02	
1	13	6.40E+04	2	4.81	4.98	0.03	
1	14	7.01E+04	2	4.85	4.88	0.00	
1	15	1.69E+04	2	4.23	4.79	0.31	
1	16	3.91E+04	2	4.59	4.69	0.01	
1	17	2.40E+04	2	4.38	4.59	0.05	
1	18	2.03E+04	2	4.31	4.50	0.04	
1	19	6.70E+04	2	4.83	4.40	0.18	
1	20	3.80E+04	2	4.58	4.30	0.08	
1	21	1.80E+03	2	3.26	4.20	0.90	
1	22	2.16E+04	2	4.33	4.11	0.05	
1	23	1.54E+04	2	4.19	4.01	0.03	
1	24	1.81E+04	2	4.26	3.91	0.12	
1	25	1.91E+04	2	4.28	3.81	0.22	
1	26	1.04E+04	2	4.02	3.72	0.09	
1	27	4.35E+03	2	3.64	3.62	0.00	
1	28	1.18E+03	2	3.07	3.52	0.20	
1	29	2.07E+03	2	3.32	3.43	0.01	sum
1	30	3.38E+03	2	3.53	3.33	0.04	3.31
2	1	2.88E+05	2	5.46	6.28	0.67	
2	2	3.93E+06	2	6.59	6.19	0.16	
2	3	5.10E+06	2	6.71	6.11	0.36	
2	4	1.50E+06	2	6.18	6.02	0.02	
2	5	1.09E+06	2	6.04	5.94	0.01	
2	6	2.49E+06	2	6.40	5.85	0.29	
2	7	4.22E+05	2	5.63	5.77	0.02	
2	8	5.33E+05	2	5.73	5.68	0.00	
2	9	3.81E+05	2	5.58	5.60	0.00	
2	10	4.33E+05	2	5.64	5.51	0.02	
2	11	1.64E+05	2	5.21	5.43	0.05	
2	12	9.29E+04	2	4.97	5.34	0.14	
2	13	1.02E+05	2	5.01	5.26	0.06	
2	14	8.41E+04	2	4.92	5.17	0.06	
2	15	8.86E+04	2	4.95	5.09	0.02	

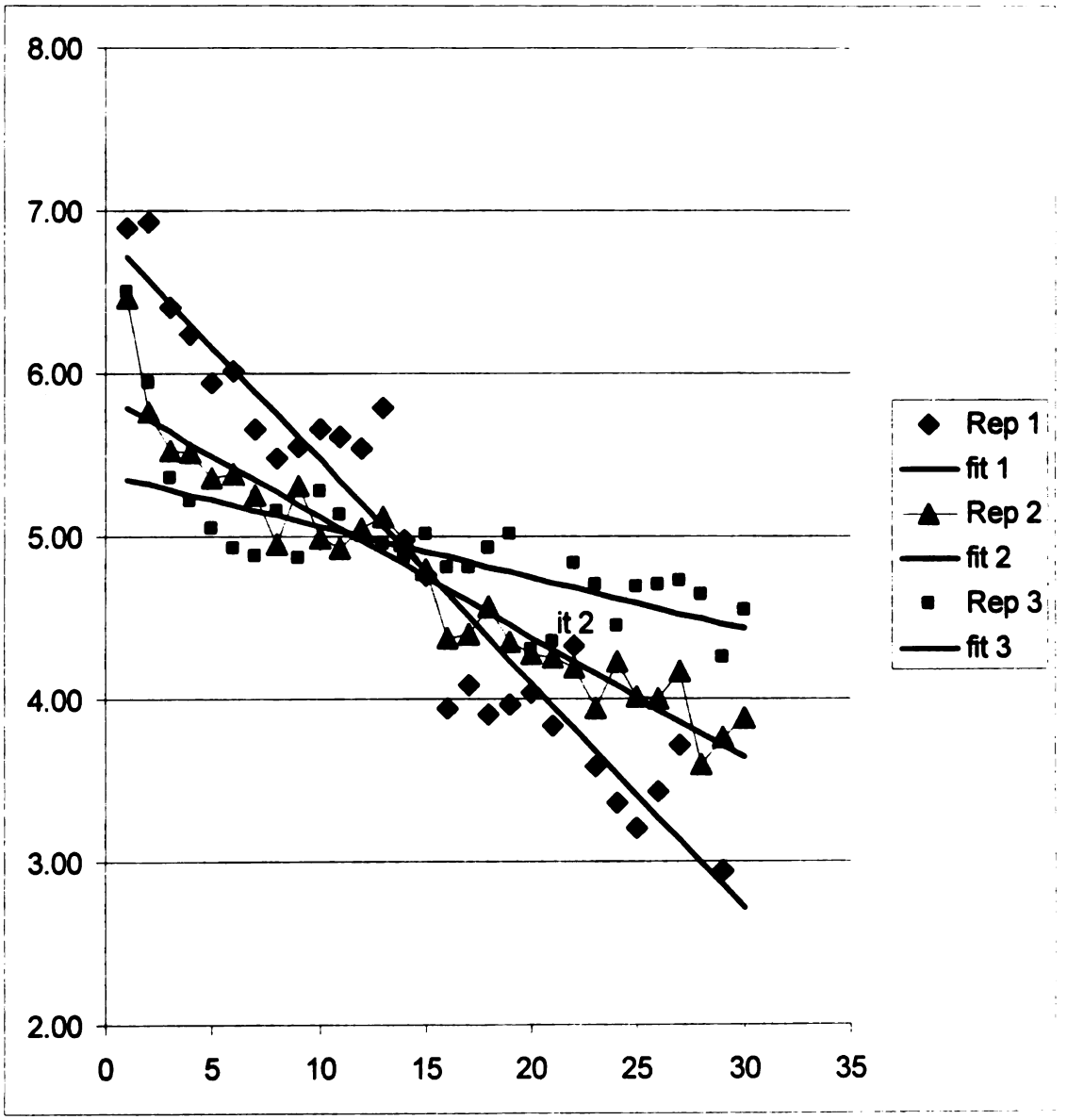
2	16	8.23E+04	2	4.92	5.00	0.01		
2	17	8.36E+04	2	4.92	4.92	0.00		
2	18	7.12E+04	2	4.85	4.83	0.00		
2	19	4.12E+04	2	4.61	4.75	0.02		
2	20	4.05E+04	2	4.61	4.66	0.00		
2	21	3.91E+04	2	4.59	4.58	0.00		
2	22	3.13E+04	2	4.50	4.49	0.00		
2	23	3.69E+04	2	4.57	4.41	0.03		
2	24	3.30E+04	2	4.52	4.32	0.04		
2	25	1.35E+04	2	4.13	4.24	0.01		
2	26	8.48E+03	2	3.93	4.15	0.05		
2	27	8.95E+03	2	3.95	4.07	0.01		
2	28	1.10E+04	2	4.04	3.98	0.00		
2	29	1.64E+04	2	4.21	3.90	0.10	sum	
2	30	9.83E+03	2	3.99	3.81	0.03		2.19
3	1	1.70E+05	2	5.23	6.42	1.41		
3	2	4.55E+06	2	6.66	6.33	0.11		
3	3	3.34E+06	2	6.52	6.24	0.08		
3	4	1.95E+06	2	6.29	6.16	0.02		
3	5	2.42E+06	2	6.38	6.07	0.10		
3	6	1.18E+06	2	6.07	5.99	0.01		
3	7	7.48E+05	2	5.87	5.90	0.00		
3	8	8.82E+05	2	5.95	5.81	0.02		
3	9	1.78E+06	2	6.25	5.73	0.28		
3	10	5.25E+05	2	5.72	5.64	0.01		
3	11	3.54E+05	2	5.55	5.55	0.00		
3	12	3.84E+05	2	5.58	5.47	0.01		
3	13	2.47E+05	2	5.39	5.38	0.00		
3	14	1.31E+05	2	5.12	5.29	0.03		
3	15	1.08E+05	2	5.03	5.21	0.03		
3	16	7.47E+04	2	4.87	5.12	0.06		
3	17	6.56E+04	2	4.82	5.03	0.05		
3	18	7.81E+04	2	4.89	4.95	0.00		
3	19	5.15E+04	2	4.71	4.86	0.02		
3	20	6.09E+04	2	4.78	4.77	0.00		
3	21	4.95E+04	2	4.69	4.69	0.00		
3	22	3.64E+04	2	4.56	4.60	0.00		
3	23	2.74E+04	2	4.44	4.51	0.01		
3	24	3.55E+04	2	4.55	4.43	0.01		
3	25	2.99E+04	2	4.48	4.34	0.02		
3	26	3.23E+04	2	4.51	4.26	0.06		
3	27	1.01E+04	2	4.00	4.17	0.03		
3	28	1.08E+04	2	4.03	4.08	0.00		
3	29	9.07E+03	2	3.96	4.00	0.00	sum	
3	30	9.37E+03	2	3.97	3.91	0.00		7.87
1	1	9.35E+06	3	6.97	5.95	1.05		
1	2	4.01E+06	3	6.60	5.84	0.59		
1	3	1.28E+06	3	6.11	5.73	0.14		

1	4	LOST	3		5.62			
1	5	4.32E+05	3	5.64	5.51	0.02		
1	6	2.12E+05	3	5.33	5.39	0.00		
1	7	2.35E+05	3	5.37	5.28	0.01		
1	8	2.19E+05	3	5.34	5.17	0.03		
1	9	1.11E+05	3	5.05	5.06	0.00		
1	10	9.83E+04	3	4.99	4.95	0.00		
1	11	1.25E+04	3	4.10	4.84	0.55		
1	12	8.07E+03	3	3.91	4.73	0.68		
1	13	2.09E+04	3	4.32	4.62	0.09		
1	14	1.22E+04	3	4.09	4.51	0.18		
1	15	1.24E+04	3	4.09	4.40	0.09		
1	16	5.25E+03	3	3.72	4.29	0.32		
1	17	5.46E+03	3	3.74	4.18	0.19		
1	18	2.05E+03	3	3.31	4.07	0.57		
1	19	4.64E+03	3	3.67	3.96	0.08		
1	20	1.22E+03	3	3.09	3.84	0.58		
1	21	3.03E+03	3	3.48	3.73	0.06		
1	22	7.11E+03	3	3.85	3.62	0.05		
1	23	5.49E+03	3	3.74	3.51	0.05		
1	24	7.37E+03	3	3.87	3.40	0.22		
1	25	5.47E+03	3	3.74	3.29	0.20		
1	26	4.35E+03	3	3.64	3.18	0.21		
1	27	2.61E+03	3	3.42	3.07	0.12		
1	28	1.67E+03	3	3.22	2.96	0.07		
1	29	1.27E+03	3	3.10	2.85	0.07	sum	
1	30	1.58E+03	3	3.20	2.74	0.21		6.43
2	1	1.52E+06	3	6.18	5.74	0.19		
2	2	1.30E+06	3	6.11	5 64	0.23		
2	3	5.63E+05	3	5.75	5.53	0.05		
2	4	1.39E+05	3	5.14	5.43	0.08		
2	5	2.41E+05	3	5.38	5.32	0.00		
2	6	1.75E+05	3	5.24	5.22	0.00		
2	7	6.55E+04	3	4.82	5.11	0.09		
2	8	1.09E+05	3	5.04	5.00	0.00		
2	9	9.09E+04	3	4.96	4.90	0.00		
2	10	5.85E+04	3	4.77	4.79	0.00		
2	11	3.75E+04	3	4.57	4.69	0.01		
2	12	9.03E+03	3	3.96	4.58	0.40		
2	13	5.71E+04	3	4.76	4.48	0.08		
2	14	2.56E+04	3	4.41	4.37	0.00		
2	15	8.85E+03	3	3.95	4.27	0.10		
2	16	9.32E+03	3	3.97	4.16	0.04		
2	17	4.64E+03	3	3.67	4.06	0.15		
2	18	6.06E+03	3	3.78	3.95	0.03		
2	19	2.55E+03	3	3.41	3.85	0.19		
2	20	2.32E+03	3	3.37	3.74	0.14		
2	21	3.17E+03	3	3.50	3.64	0.02		
2	22	2.98E+03	3	3.47	3.53	0.00		

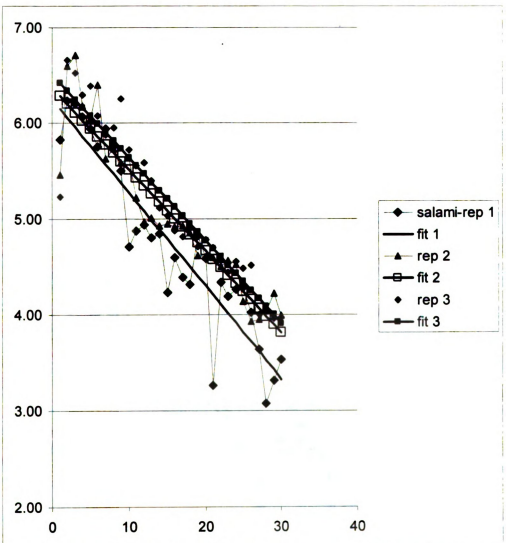
2	23	5.96E+03	3	3.78	3.43	0.12		
2	24	1.07E+04	3	4.03	3.32	0.50		
2	25	3.00E+03	3	3.48	3.22	0.07		
2	26	5.48E+03	3	3.74	3.11	0.39		
2	27	1.33E+03	3	3.12	3.01	0.01		
2	28	2.77E+03	3	3.44	2.90	0.29		
2	29	3.22E+02	3	2.51	2.80	0.08	sum	
2	30	1.54E+02	3	2.19	2.69	0.25		3.54
3	1	5.37E+06	3	6.73	5.32	1.99		
3	2	7.00E+05	3	5.85	5.24	0.36		
3	3	4.45E+05	3	5.65	5.17	0.23		
3	4	2.39E+05	3	5.38	5.09	0.08		
3	5	1.06E+05	3	5.03	5.02	0.00		
3	6	6.38E+04	3	4.80	4.94	0.02		
3	7	3.29E+04	3	4.52	4.86	0.12		
3	8	7.44E+04	3	4.87	4.79	0.01		
3	9	4.50E+04	3	4.65	4.71	0.00		
3	10	1.98E+04	3	4.30	4.64	0.12		
3	11	1.39E+04	3	4.14	4.56	0.17		
3	12	1.36E+04	3	4.13	4.48	0.12		
3	13	5.64E+03	3	3.75	4.41	0.43		
3	14	4.28E+03	3	3.63	4.33	0.49		
3	15	2.15E+03	3	3.33	4.26	0.85		
3	16	4.29E+03	3	3.63	4.18	0.30		
3	17	8.27E+03	3	3.92	4.11	0.04		
3	18	5.17E+03	3	3.71	4.03	0.10		
3	19	3.60E+03	3	3.56	3.95	0.16		
3	20	2.89E+03	3	3.46	3.88	0.17		
3	21	2.19E+03	3	3.34	3.80	0.21		
3	22	7.47E+03	3	3.87	3.73	0.02		
3	23	1.74E+04	3	4.24	3.65	0.35		
3	24	1.16E+04	3	4.06	3.57	0.24		
3	25	1.91E+04	3	4.28	3.50	0.61		
3	26	7.80E+03	3	3.89	3.42	0.22		
3	27	6.06E+03	3	3.78	3.35	0.19		
3	28	4.25E+03	3	3.63	3.27	0.13		
3	29	2.03E+03	3	3.31	3.19	0.01	sum	
3	30	LOST	3		3.12			7.76

bologna rep 3

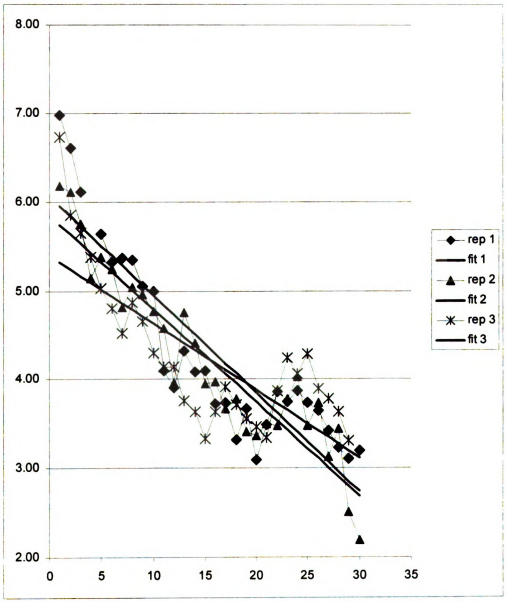
No	k
5.374298	0.031559



Salami	rep 1		rep 2		rep 3	
No	k		No	k	No	k
6.246282	0.097275		6.363965	0.085096	6.50417	0.08651



		turkey rep 2		turkey rep 3	
	k	N0	k	N0	k
turkey rep 1 N0 6.059119	0.110711	5.846207	0.105168	5.395735	0.075915



Slicer blade to product (10^5 CFU/blade)

Product Bolgona = 1, Salami = 2, Turkey =3
REP 1-3
Slice # 1-15
Count CFU
Level 10^5
* zero indicates negative direct plate but positive enrichement

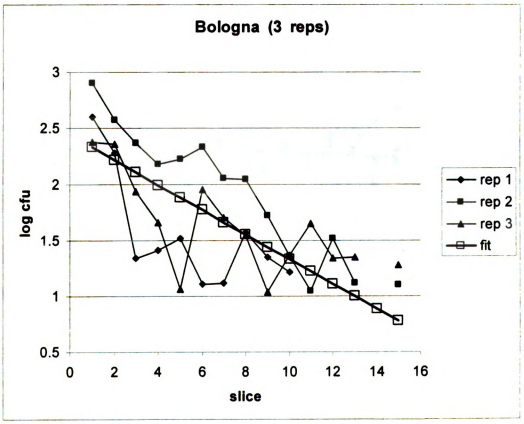
Rep	Slice	Count	Product	log count
1	1	4.0E+02	1	2.60
1	2	1.9E+02	1	2.27
1	3	2.2E+01	1	1.34
1	4	2.6E+01	1	1.41
1	5	3.3E+01	1	1.52
1	6	1.3E+01	1	1.11
1	7	1.3E+01	1	1.11
1	8	3.7E+01	1	1.57
1	9	2.2E+01	1	1.35
1	10	1.6E+01	1	1.21
1	11	0.0E+00	1	
1	12	0.0E+00	1	
1	13	0.0E+00	1	
1	14	0.0E+00	1	
1	15	0.0E+00	1	
2	1	8.0E+02	1	2.90
2	2	3.7E+02	1	2.57
2	3	2.3E+02	1	2.37
2	4	1.5E+02	1	2.18
2	5	1.7E+02	1	2.22
2	6	2.2E+02	1	2.33
2	7	1.1E+02	1	2.05
2	8	1.1E+02	1	2.05
2	9	5.3E+01	1	1.72
2	10	2.3E+01	1	1.35
2	11	1.1E+01	1	1.04
2	12	3.3E+01	1	1.52
2	13	1.3E+01	1	1.11
2	14	0.0E+00	1	
2	15	1.2E+01	1	1.09
3	1	2.4E+02	1	2.37
3	2	2.3E+02	1	2.36
3	3	8.6E+01	1	1.94
3	4	4.6E+01	1	1.66

3	5	1.2E+01	1	1.06
3	6	9.1E+01	1	1.96
3	7	5.0E+01	1	1.70
3	8	3.5E+01	1	1.55
3	9	1.1E+01	1	1.04
3	10	2.3E+01	1	1.36
3	11	4.5E+01	1	1.65
3	12	2.2E+01	1	1.34
3	13	2.2E+01	1	1.35
3	14	0.0E+00	1	
3	15	1.9E+01	1	1.28
1	1	3.4E+03	2	3.53
1	2	3.4E+03	2	3.53
1	3	2.4E+03	2	3.38
1	4	2.7E+03	2	3.43
1	5	1.7E+03	2	3.22
1	6	1.7E+03	2	3.23
1	7	8.4E+02	2	2.92
1	8	0.0E+00	2	
1	9	0.0E+00	2	
1	10	0.0E+00	2	
1	11	3.3E+02	2	2.52
1	12	1.0E+03	2	3.02
1	13	6.4E+02	2	2.81
1	14	1.5E+03	2	3.17
1	15	2.3E+03	2	3.37
2	1	3.7E+02	2	2.57
2	2	7.8E+02	2	2.89
2	3	3.5E+02	2	2.55
2	4	2.4E+02	2	2.38
2	5	4.8E+02	2	2.68
2	6	1.4E+02	2	2.13
2	7	4.1E+03	2	3.62
2	8	3.1E+02	2	2.50
2	9	3.2E+02	2	2.51
2	10	7.5E+02	2	2.87
2	11	4.8E+02	2	2.68
2	12	8.4E+02	2	2.93
2	13	2.0E+03	2	3.30
2	14	2.0E+02	2	2.31
2	15	4.1E+02	2	2.61
3	1	5.9E+02	2	2.77
3	2	2.7E+02	2	2.43
3	3	1.4E+03	2	3.14
3	4	7.7E+01	2	1.89
3	5	9.8E+01	2	1.99
3	6	0.0E+00	2	

3	7	7.9E+02	2	2.90
3	8	7.5E+02	2	2.87
3	9	1.4E+03	2	3.15
3	10	7.0E+02	2	2.84
3	11	3.8E+02	2	2.58
3	12	7.3E+02	2	2.86
3	13	6.0E+00	2	0.78
3	14	4.4E+02	2	2.64
3	15	6.4E+02	2	2.81
1	1	1.3E+03	3	3.12
1	2	2.0E+03	3	3.30
1	3	1.1E+03	3	3.05
1	4	3.4E+02	3	2.53
1	5	7.4E+01	3	1.87
1	6	0.0E+00	3	
1	7	1.1E+02	3	2.06
1	8	0.0E+00	3	
1	9	1.8E+02	3	2.25
1	10	1.4E+02	3	2.15
1	11	0.0E+00	3	
1	12	0.0E+00	3	
1	13	0.0E+00	3	
1	14	0.0E+00	3	
1	15	0.0E+00	3	
2	1	1.1E+03	3	3.06
2	2	6.5E+02	3	2.81
2	3	2.4E+02	3	2.39
2	4	2.9E+02	3	2.47
2	5	3.0E+02	3	2.48
2	6	3.6E+02	3	2.56
2	7	0.0E+00	3	
2	8	0.0E+00	3	
2	9	1.8E+02	3	2.25
2	10	0.0E+00	3	
2	11	0.0E+00	3	
2	12	0.0E+00	3	
2	13	0.0E+00	3	
2	14	0.0E+00	3	
2	15	0.0E+00	3	
3	1	1.9E+03	3	3.27
3	2	4.9E+02	3	2.69
3	3	2.3E+02	3	2.36
3	4	2.6E+02	3	2.41
3	5	2.8E+02	3	2.45
3	6	1.1E+02	3	2.03
3	7	0.0E+00	3	
3	8	1.2E+02	3	2.09

3	9	0.0E+00	3
3	10	0.0E+00	3
3	11	0.0E+00	3
3	12	0.0E+00	3
3	13	0.0E+00	3
3	14	0.0E+00	3
3	15	0.0E+00	3

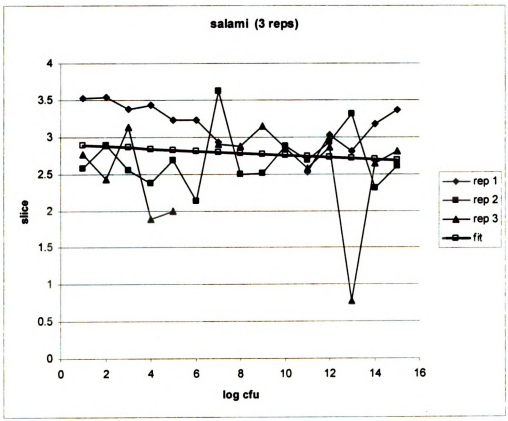
bologna					No 2.43755	k 0.110726
Slice	Rep 1	Rep 2	Rep 3	Ave	fit	(ave-fit)^2
1	2.598572	2.900695	2.374382	2.62	2.33	0.089
2	2.273418	2.573336	2.360972	2.40	2.22	0.035
3	1.342423	2.366983	1.936514	1.88	2.11	0.050
4	1.407561	2.180928	1.658965	1.75	1.99	0.060
5	1.518514	2.219899	1.061452	1.60	1.88	0.081
6	1.108565	2.334212	1.958468	1.80	1.77	0.001
7	1.112605	2.052309	1.702431	1.62	1.66	0.002
8	1.567732	2.047664	1.546049	1.72	1.55	0.028
9	1.347135	1.724276	1.038223	1.37	1.44	0.005
10	1.21272	1.353339	1.362482	1.31	1.33	0.000
11		1.04454	1.651278	1.35	1.22	0.016
12		1.516932	1.339253	1.43	1.11	0.102
13		1.113943	1.351796	1.23	1.00	0.055
14					0.89	0.787
15		1.09482	1.277838	1.19	0.78	0.168
					sum	1.479



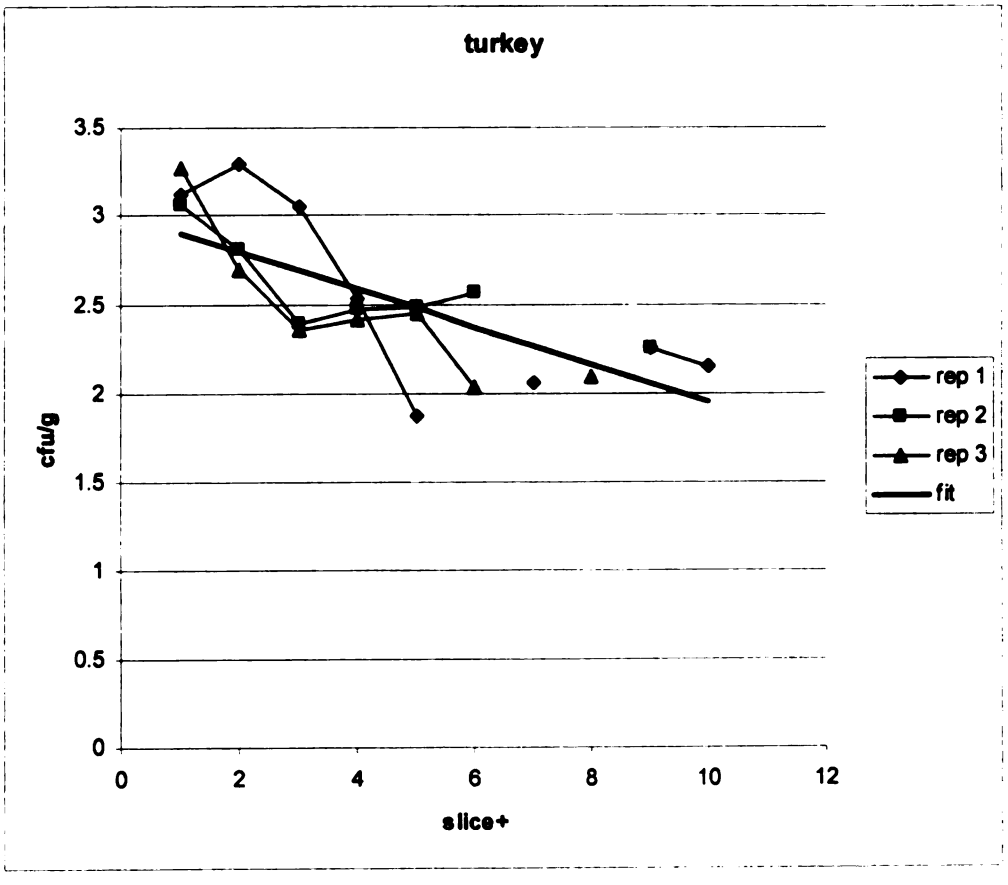
					No	k
					2.895622	0.013779

salami

slice	1	2	3	Ave	fit	(ave-fit)^2
1	3.528039	2.572058	2.771914	2.96	2.88	0.006
2	3.534517	2.8927	2.425906	2.95	2.87	0.007
3	3.37541	2.547701	3.135324	3.02	2.85	0.027
4	3.425899	2.376526	1.886626	2.56	2.84	0.077
5	3.220788	2.678518	1.990836	2.63	2.83	0.039
6	3.227914	2.134993		2.68	2.81	0.017
7	2.922195	3.617631	2.895644	3.15	2.80	0.120
8		2.495822	2.874366	2.69	2.79	0.010
9		2.507316	3.148997	2.83	2.77	0.003
10		2.872785	2.844104	2.86	2.76	0.010
11	2.520237	2.679064	2.576756	2.59	2.74	0.023
12	3.02111	2.926748	2.863085	2.94	2.73	0.043
13	2.808929	3.302902	0.778151	2.30	2.72	0.176
14	3.171538	2.306245	2.643528	2.71	2.70	0.000
15	3.369162	2.60892	2.808621	2.93	2.69	0.058
					sum	0.616



					No	k
					3.010	0.106
turkey						
slice	1	2	3	Ave	fit	(ave-fit)^2
1	3.11883	3.058046	3.272306	3.15	2.90	0.060
2	3.296054	2.80956	2.686636	2.93	2.80	0.018
3	3.050622	2.38739	2.361728	2.60	2.69	0.008
4	2.525796	2.468347	2.41162	2.47	2.58	0.014
5	1.870989	2.480007	2.450249	2.27	2.48	0.045
6		2.559907	2.033424	2.30	2.37	0.006
7	2.055799			2.06	2.27	0.044
8			2.08636	2.09	2.16	0.005
9	2.253411	2.247973		2.25	2.05	0.039
10	2.148726			2.15	1.95	0.041
11						
12					sum	0.280



APPENDIX V

Example calculations and modeling of turkey sliced on a delicatessen slicer (inoculated blade to uninoculated product)

GW Basic Output: turkey (10^8 CFU/blade)

fraction left on blade during each slice= .7995544

cfu's transferred to 1st slice= 568016.4

above results are independent of initial cfu's on blade

if initial cfu's on blade= 1E+08 ,then ...

fraction transferred to meat during each slice= 5.680164E-03

fraction transferred to surroundings during each slice= .1947655

fitted equations (all equivalent) are:

1) ln cfu(s)= -.2237008 *s + 13.47361

2) cfu(s)= 710416.2 * .7995544 ^s

3) cfu(s)= 710416.2 *e^(-.2237008 *s)

4) cfu(s)= 710416.2 *10^(-.097152 *s)

correlation coefficient for fit is R= .8978895

Example calculations of output for turkey (10^8 CFU/blade)

$$\% \text{ transfer} = \frac{100 * f_1}{f_1 + f_2}$$

f_1=5.680164E-3 = 0.005680164

f_2 = 0.1947655

$$\% \text{ transfer} = \frac{100 * 0.005680164}{0.005680164 + 0.1947655}$$

% transfer = 2.83

GW Basic Output: turkey (10^5 CFU/blade)

fraction left on blade during each slice= .672946

cfu's transferred to 1st slice= 1144.353

above results are independent of initial cfu's on blade

if initial cfu's on blade= 100000 ,then ...

fraction transferred to meat during each slice= 1.144353E-02

fraction transferred to surroundings during each slice= .3156105

fitted equations (all equivalent) are:

1) ln cfu(s)= -.3960902 *s + 7.438685

2) cfu(s)= 1700.512 * .672946 ^s

3) cfu(s)= 1700.512 *e^(-.3960902 *s)

4) cfu(s)= 1700.512 *10^(-.1720198 *s)

correlation coefficient for fit is R= .9150966

Example calculations of output for turkey (10^5 CFU/blade)

$$\% \text{ transfer} = \frac{100 * f_1}{f_1 + f_2}$$

f_1= 1.14435E-2 = 0.0114435

f_2 = 0.315610

$$\% \text{ transfer} = \frac{100 * 0.0114435}{0.0114435 + 0.315610}$$

% transfer = 3.49

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