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I am submitting herewith a thesis written by Jessica Anne Clemons entitled “Novel Approaches for the Efficient Sampling and Detection of *Listeria monocytogenes* and *Brochothrix thermosphacta* on Food Contact Surfaces.” I have examined the final electronic copy of this thesis for form and content and recommended that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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**Novel Approaches for the Efficient Sampling and Detection
of *Listeria monocytogenes* and *Brochothrix thermosphacta* on
Food Contact Surfaces**

A Thesis Presented
For the Master of Science Degree
The University of Tennessee, Knoxville

Jessica Anne Clemons
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Dedication

I want to dedicate this thesis to my grandparents: **Jean and Wayne Perryman.**

For though their life on earth has passed, their legacy remains alive and flourishing within my soul. The sweet memories of my childhood serve as fuel for my future, igniting a pursuit for the road less traveled and burning brighter with every passing day.

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Romans 8:29

Abstract

The primary step in the microbiological assessment of highly dynamic and complex food processing conditions is environmental sampling. The objectives of this study were to: (1) compare the efficacy of four sampling devices including Microbial-Vac system (MV), cellulose sponge (SP), polyester swab (SW) and composite tissue (CT), for the recovery of *Listeria monocytogenes* and *Brochothrix thermosphacta* on five surfaces and (2) to determine if there was a significant difference between the recovery of low (10 CFU/900cm²) and high (100 CFU/900cm²) *L. monocytogenes* inoculum levels using the sampling devices in a simulated food processing environment. Surfaces used for this study were stainless steel (SS), polyethylene cutting board (CB), polyurethane conveyor belt (PB), open hinge flat top belt conveyor (FT) and mesh conveyor belt (MB). Environmental surfaces were inoculated with *L. monocytogenes* to obtain a final cell population of 10 (low) or 100 (high) CFU/900 cm². An average cell density of 10,000 CFU/25 cm² was used for inoculating *B. thermosphacta* on each of the surfaces. Inoculated surfaces were dried and held for two hours at 4°C then sampled and processed for detection. Because *L. monocytogenes* is a zero tolerance pathogen in ready-to-eat foods, the qualitative analysis included an enrichment step to detect the presence/absence of *L. monocytogenes* in the sample. In comparison, *B. thermosphacta* was directly plated in order to quantify the recovery capability of each device. Results indicated for recovery of 100 CFU/900 cm² *L. monocytogenes*, there was no significant difference among devices on SS, CB or PB surfaces ($p>0.05$). However, a significant difference was detected at 10 CFU/900 cm² on SS between MV and CT, 62.97 and 17.34%, respectively ($p=0.0086$). Results for FT indicated MV was superior over SP and SW ($p=0.0004$) for detection of high and low *L. monocytogenes*. There

was no difference between the quantitative recovery of *B. thermosphacta* on PB and SS; however, there was a difference ($p=0.0371$) among devices on CB indicating MV was superior over SP and CT. The swab recovered $3.25 \log \text{CFU}/25\text{cm}^2$ from flat top belts and was significantly lower ($p=0.0259$) than MV and SP devices, 4.29 and $4.12 \log \text{CFU}/25\text{cm}^2$, respectively.

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Chapter 1 Introduction

1.1 Environmental Sampling

Environmental sampling is the first step in the microbiological assessment of highly dynamic and complex food processing environments. The purpose of environmental sampling is to monitor and assess industry sanitation programs, detect spoilage and pathogenic microorganisms and ensure the safety of our food supply. Thus, it is essential the devices used in environmental sampling have the ability to provide accurate and reliable assessments across a variety of food contact surfaces. Also imperative, these devices need the capability to detect pathogenic organisms at low levels due to the low infectious doses of some foodborne pathogens such as *Listeria monocytogenes* as well as quantitatively assess microbial load on a given surface (Kathariou, 2002 and Moore and Griffith, 2002). *L. monocytogenes* is the leading cause of death among foodborne pathogens with a reported 25-30% fatality rate (Kushwaha *et al.*, 2009 and Norton, 2002) and has the ability to survive long-term within adverse growth conditions increasing its competitive advantage to contaminate the final product making it a pathogen of particular concern in ready-to-eat (RTE) foods (Kalpana *et al.*, 2009, Kushwaha *et al.*, 2009, Norton, 2002 and Thimothe *et al.*, 2004). U.S. regulatory agencies have established a zero tolerance policy in which RTE food products contaminated with *L. monocytogenes* at ≥ 1 CFU/25 grams are considered adulterated (Gasanov *et al.*, 2005 and Thimothe *et al.*, 2004). The Food and Drug Administration has proposed an acceptable defect level of 100 CFU/g for *Listeria* spp. in non-RTE foods (Kushwaha and Muriana, 2009). Currently, the Food Safety and Inspection Service (FSIS) states that even the presence of undifferentiated *Listeria* spp. could represent the presence of *L. monocytogenes* since it is likely both share the same contamination sources.

The conditions necessary for microbial growth are consistently present within food processing environment (Gabis and Faust, 1988) and food contact surfaces such as sinks, tables, conveyor belts and workbench surfaces, are points frequently involved in cross-contamination (Mariott, 1994; Sveum, Moberg, Rude and Frank, 1992 and Taylor and Holah, 1996). Microbial contamination can develop and persist on both porous and non-porous surfaces and it is essential to develop effective sampling methods to assess the efficacy of decontamination efforts (Buttner *et al.*, 2007). Hazard Analysis Critical Control Points (HACCP) have increased the awareness and understanding of the risks associated with microbiological contamination. The Food Safety and Inspection Service (FSIS) published a final rule in July 1996 mandating that HACCP be implemented as the system of process control in all inspected meat and poultry plants. HACCP requires that regular hygiene monitoring of production facilities provides results in a timely manner for remedial action to be taken and regain product control (Moore and Griffith, 2002). Sampling devices are a central tool in following industry HACCP plans and specifications as well as abiding government standards.

Sampling devices used to assess microbial surface contamination are various types of hygiene swabs, cellulose sponges and contact plates. Since the 1970's, environmental sampling has been plagued by evidence of low recovery rates and poor detection capability (Favero *et al.*, 1968, Gill and Jones, 1999, Kang *et al.*, 2007, Moore and Griffith, 2002, Pepperell *et al.*, 2005, Rose *et al.*, 2004 and Vorst *et al.*, 2004). Nevertheless, these devices are the primary option for assessing the safety of the food environment because of their familiarity, ease of use and affordability. Gaining notable attention in recent studies are composite tissues for their inherent antistatic coating and abrasive structure which increases this device's ability to remove microorganisms from food contact surfaces (Vorst *et al.*, 2004). In 1990, Microbial-Vac Systems, Inc. introduced a more aggressive and automated approach that utilizes wet-vacuum technology

to simultaneously collect and extract the sample from various types of surfaces. This device has the capability to penetrate and sample tiny cracks and crevices and completely eliminates the extraction step associated with sponges, swabs and tissues (Microbial-Vac Systems, Inc).

All of these previously mentioned sampling devices lack universally adopted and government regulated sampling protocols (Kovacevic *et al.*, 2008). Given the numerous shortcomings of sponges and swabs, there is a serious need to adopt novel, aggressive, automated and accurate sampling devices to more reliably assess, both qualitatively and quantitatively, complex food contact surfaces for pathogenic and spoilage microorganisms from highly dynamic environments. The objectives of this study were to: (1) compare the efficacy of four sampling devices including Microbial-Vac system (MV), cellulose sponge (SP), polyester swab (SW) and composite tissue (CT), for the recovery of *Listeria monocytogenes* and *Brochothrix thermosphacta* on five surfaces and (2) to determine if there was a significant difference between recovery of low (10 CFU/surface) and high (100 CFU/surface) *L. monocytogenes* inoculum levels using the sampling devices in a simulated food processing environment.

Chapter 2 Literature Review

2.1 Traditional Hygiene Swabs, Sponges and Swab-Rinse Methods

Hygiene swabs and sponges have been widely used throughout the food and healthcare industries to assess the efficacy of the cleaning and disinfection procedures within facilities and on food contact surfaces. The first environmental sampling technique, swab-rinse method, was developed in 1917 to assess bacterial contamination on food utensils (Rose *et al.*, 2004). The swab-rinse method is a rapid, simple and inexpensive way to assess microbial contamination of food surfaces (Jay *et al.*, 2005). This technique has since evolved into a more specific and sensitive method of targeting surface level microorganisms (Eblen *et al.*, 2005). By 1960, NASA had modified this method for use in spacecraft equipment and recommended swabs not be used for greater than 4 in² and include a two-minute sonication during the extraction step. (Favero *et al.*, 1968, Jay *et al.*, 2005 and Rose *et al.*, 2004)

Swabs have the advantage of being simple, affordable and rapid methods of retrieving environmental samples from hard to reach areas. Commonly used swabs have a wood or plastic shaft with various types of buds, such as polyester, rayon, cotton-alginate fibers and Dacron. In addition, there is a wide variety of diluents available to aid in the recovery of injured bacteria and/or neutralize any residual disinfectants present within the sample. Common procedures for collecting swab samples involve rubbing the designated area thoroughly with a moistened swab and returning the device to the test tube containing diluent. When calcium alginate swabs are used, the organisms are released into the diluent upon dissolution of the alginate by sodium hexametaphosphate (Jay *et al.*, 2005). Typically, sponges are used in the same manner following similar swab methodology and are advantageous for sampling larger surface areas which

increase the likelihood of capturing pathogenic microorganisms that are of concern in low numbers such as *L. monocytogenes* and *E. coli* O157:H7 (Lindblad, 2007).

The accuracy of swab and sponge devices and methodologies are dependent upon a multitude of variables including but not limited to: the skill of the technician, device type, size and material, surface type and size, moisture content on the surface, amount of mechanical energy between surface and device, presence of biofilms, ability of device to affectively remove contaminants from the surface and subsequent release of these microorganisms from the device head for detection. Surface hygiene swabbing is a non-automated process and difficult to standardize due to the extreme variation in processing conditions and degrees of pressure applied by the technician during sample collection (Buttner *et al.*, 2007, Capita *et al.*, 2004, Dorsa *et al.*, 1996, Gill and Jones, 2000, Moore and Griffith, 2007 and Vorst *et al.*, 2004). This is reflected statistically by the large standard deviations in reported data which supports the theory that swab data is often difficult or near impossible to repeat and reproduce under identical experimental conditions much less in variable processing environments. Further adding to the weaknesses of hygiene swabs and sponges is the evidence that these devices harbor a significant amount of the microorganisms within the bud which inhibit release into the detection devices and result in reduced recovery rates (Moore and Griffith, 2007, Rose *et al.*, 2004 and Vorst *et al.*, 2004). These shortcomings can be attributed to the wide range of variables including: the complex nature of the food particulates on contact surfaces, vast array of surface types within testing conditions, variation of collection methodology, skill of the technician, type of contaminant to be recovered and the lack of universally accepted protocols (Buttner *et al.*, 2007, Foschino *et al.*, 2003, Kang *et al.*, 2007, Moore and Griffith, 2002 and Zottola and Sasahara, 1994). Nonetheless, for the food industry, swabs and sponges remain the primary device choice for detection because

of their simplicity, affordability and ability to access areas within the food processing facility such as cracks, crevices and geometrically abnormal spaces (Foschino *et al.*, 2003).

Rose *et al.*, (2004) compared four different swab materials, using dry and pre-moistened, for their ability to recovery artificially, and directly inoculated *Bacillus anthracis* spores from nonporous stainless steel coupons. Additionally, vortexing, sonication and minimal agitation were compared during processing to potentially increase extraction of spores. Results indicate that directly inoculated swabs, post-vortexing, released significantly ($p<0.01$) higher percentages (84%-94%) of spores than swabs used for sampling inoculated stainless steel coupons which obtained <50% of the original inoculum level. No significant difference was found between polyester, macrofoam and rayon in their abilities to release spores ($p>0.05$). However, the polyester swab released significantly less than the other three materials ($p<0.01$). Pre-moistened swabs were more efficient than dry swabs ($p<0.05$). Regarding recovery efficiency, results indicate that macrofoam and cotton had the highest recovery capability of the four materials, with only 30.7% and 27.7% recovery of spores, respectively, with no significant difference ($p=1.0$) between these two materials. Polyester and rayon swabs (10.6% and 10.0%, respectively) were significantly less efficient ($p<0.01$) than the cotton and macrofoam devices. Use of sonication did not increase spore recovery. However, recovery rates did significantly increase by vortexing cotton and macro-foam pre-moistened swabs ($p<0.01$). This study and others concluded that swabs should be pre-moistened with a surfactant, such as Tween 80, for a maximum retrieval of microorganisms (Buttner *et al.*, 2007, Davidson *et al.*, 1999, Moore and Griffith, 2002, Moore and Griffith, 2007 and Rose *et al.*, 2004).

Moore and Griffith (2007) conducted a study to assess the factors affecting the recovery of *Escherichia coli* and *Staphylococcus aureus* from stainless steel using a variety of swab/solution combinations. Results indicate cotton-tipped and brush-textured nylon flock absorbed significantly larger volumes of microbial suspension than rayon and Dacron-tipped swabs. The larger absorption volume can be attributed to the hydroxyl groups within the cellulose fibers of the cotton that attract water molecules (Hearle, 1963). Despite the cotton swabs superior ability to absorb moisture ($p < 0.05$), this device has a significantly lower recovery rate of *E. coli* and *S. aureus* compared to the brush spatula, brush-textured and Dacron-tipped swabs. Therefore, the very nature of the cotton swab that enables it to absorb and remove microorganisms from a surface may be the very same characteristics that aid in the entrapment of cells within the device resulting in poor recovery rates. Thus, the devices ability to release organisms from the device into the diluent is at least equally, if not of greater importance than its ability to recover bacteria from surfaces (Buttner *et al.*, 2007 and Moore and Griffith, 2007). In addition, as with prior mentioned studies (Rose et al. 2004), a higher percentage of recovery was related to the directly inoculated swabs when compared to the swabs used for sampling which highlights the shortcomings associated with swab recovery and subsequent release of mechanisms.

Previous studies (Dorsa *et al.*, 1997, Moore and Griffith, 2002, Moore and Griffith, 2007 and Vorst *et al.*, 2004) have shown that increasing mechanical energy between sampling device and surface improves detachment of bacteria. Therefore, use of more rigid swab stems with abrasive bud materials has the ability to increase recovery rates by penetrating biofilms and firmly attached bacteria by utilizing mechanical energy (Moore and Griffith, 2007). However, choice of swabbing materials can only marginally improve efficiency of detecting

microorganisms. Given the poor detection capability, swabs and sponges should not be the ‘gold standard’ to determine surface contamination because of the numerous weaknesses associated with these devices which prevent accurate and trustworthy microbial assessments of food processing environments.

2.1a Comparison of Swab and Sponge

The detection of indicator organisms, such as coliforms is widely used in the food industry to measure the effectiveness of sanitation programs (Buchanan, 2000 and Moore and Griffith, 2002). The presence of indicator organisms substantially increases risk of microbial contamination and possible pathogenic foodborne related illnesses (Frank *et al.*, 1990 and Moore and Griffith, 2002). In a study completed by Moore and Griffith (2002), the sponge device was 100 times less sensitive than the cotton swab for extraction of low level coliforms from a wet stainless steel surface. This could be explained by ineffective bacterial release from the device head. Sponges are very absorbent compared to swabs which prove advantageous for sampling a larger volume and surface area. However, any bacteria that are removed from the surface during sampling are likely to become entrapped within the matrix of the sponge (Daley *et al.*, 1995 and Moore and Griffith, 2002). Further exacerbating inhibition of bacterial release is the repeated compressions of the sponge within the diluent which make the microorganisms likely to be reabsorbed into the device.

A study by Buttner *et al.* (2007) compared the sponge and macrofoam swab for the recovery of *Erwinia herbicola* on a variety of surface types including: wood, glass, metal, plastic, nylon, concrete and vinyl. Quantitative polymerase chain reaction (QPCR) amplification assay was used for detection purposes. The greatest concentrations of *E. herbicola* were found on smooth, non-porous surfaces such as glass and metal. Overall recoveries ranged from 0.7 to

52.2%. This data correlates with previous findings (Rose *et al.*, 2004) that concluded pre-moistened (with surfactant Tween 80) macrofoam and cotton swabs had the highest detection rates whereas sponge had the lowest recovery and detection rates.

2.2 Replicate Organism Direct Agar Contact Plates (RODAC)

Replicate organism direct agar contact (RODAC) plates have the advantage of being a non-destructive, simple, possibly selective and quick type of environmental sampling procedure for non-porous, flat surfaces when microbial contamination levels are low (<100 CFU). This method, originated in 1945, uses a special Petri plate which has a raised agar surface. After proper incubation and further enumeration, the direct contact of the plate to the sample surface provides a mirror image of the bacterial load on the designated area (Capita *et al.*, 2004 and Jay *et al.*, 2005). Foschino *et al.* (2003) have indicated that the accuracy of the RODAC plate results is directly influenced by contact time and the adherence capability of the target microorganism to the sampling surface. A disadvantage of RODAC methods are the possibility of overgrown plates due to spreading colonies if bacterial load exceeds 100 CFU/cm² and subsequent ineffectiveness for heavily contaminated areas. Furthermore, the RODAC plate samples are only as large as the surface area the device comes into contact with which leads to multiple sample sites to yield representative data. Microbial counts are often <1% of those obtained with destructive methods because of the contact plates lack of ability to penetrate the cracks and crevices and recover surface microbes in carcass samples (Capita *et al.*, 2004).

2.3 Composite Tissue

Recently, use of composite tissues (CT) has gained notoriety in published research as a device that is a suitable and cheap alternative to sponges and swabs for surface sampling. In addition, the tissue is abrasive which increases the device's ability to scour the designated

surface and potential to remove microorganisms. Studies report detection rates for CT are equally, if not better, than that of hygiene swabs (Kovacevic *et al.*, 2008). Nonetheless, it takes a substantial amount of time to prepare the composite tissue (approximately 65 minutes for 40 samples) and has a greater chance of contamination due to device handling during the preparation steps which make it undesirable for daily and routine sampling in food processing environments (Kovacevic *et al.*, 2008). The accuracy of each of these previously mentioned devices is dependent upon the ability to adequately remove the microorganism from the surface followed by completely dislodging the microorganisms from the sampling head into the diluent and respective detection device (Buttner *et al.*, 2007 and Moore and Griffith, 2007). Whether the purpose is qualitative or quantitative assessment, the extraction methodology for each of these devices is fundamental to obtaining accurate microbial detection.

2.4 Microbial-Vac Systems

Environmental conditions within food processing facilities are highly dynamic and traditional methodology and devices are not able to accurately represent, collect or model true microbial contamination. It is not only unrealistic, but costly and dangerous for consumers to continue relying on familiar sampling habits to ensure food safety. Instead, more efficient, affordable and widely applicable sampling options are needed for food processors and health officials to better assess daily sanitation programs and reduce the opportunity for foodborne illness outbreaks. Recent Microbial-Vac validation studies provide evidence this device offers an effective and accurate alternative at potentially lower costs to the industry compared to traditional devices (Vorst *et al.*, 2004). The Microbial-Vac (MV) System is a robust surface sampling device that approaches sample collection with an aggressive perspective. The system consists of a Support Equipment Case (Figure 2-1) that provides the necessary vacuum action

and delivers pressurized (15 PSI) sterile solution. The Support Equipment Case (SEC) is calibrated to provide over 2,000 hours of maintenance free service, assuming all maintenance recommendations are followed. In addition, the MV Kit is required which consists of a sampling head (Figure 2-2) connected to extruded tubing to an air/liquid separator with the attached plastic collection reservoir. The sampling head has a solution control switch that regulates flow of the sterile solution and vacuum port around the circumference of the head. Additionally, there is a flexible surface contact ring that allows for maximum interface with the designated surface. The tubing connecting the sampling head to the air/liquid separator has two attached lines. One which serves as a sup line delivering sterile liquid and the other necessary to carry the vacuumed sample from the surface to the collection bottle.

The MV is a wet vacuum device that simultaneously sprays a sterile buffer onto the surface while collecting the solution and microbes into a collection bottle. The solution is able to penetrate hard to reach areas such as cracks and crevices commonly seen in production facilities (Figure 2-3). The MV is breakthrough technology that eliminates the need for a separate extraction step commonly associated with traditional sponge and swab devices. In addition, the MV takes advantage of the abrasive action and mechanical energy that is needed to penetrate biofilms and remove firmly attached bacteria. Practical applications of the MV include a wide variety of surfaces such as stainless steel, carcass, beef trimmings, tile, vegetables, ultra high molecular weight polyethelene (UHMWP) and conveyor belts. After completion of sampling, the bottle is easily removed and sealed with a cap readily available for further detection analyses. Upon completion of the use of MV, the device is rinsed, cleaned and sanitized with a semi-automated 180°F hot water rinse system using a peristaltic pump (Figure 2-4).



Figure 2-1 MV Standard Equipment Casing (SEC)

Photo provided by Microbial-Vac Systems, Inc

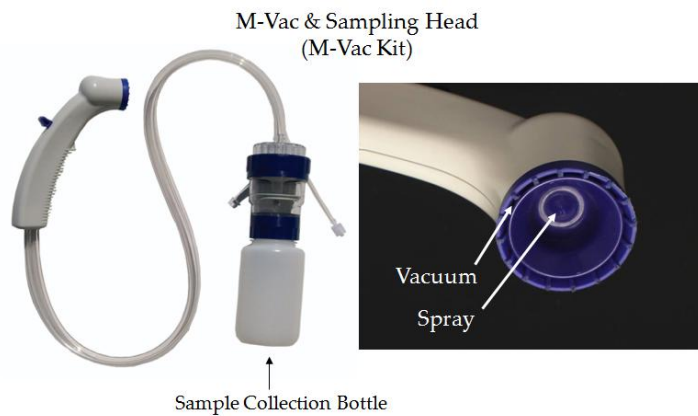


Figure 2-2 MV Sampling Head

Photo provided by Microbial-Vac Systems, Inc

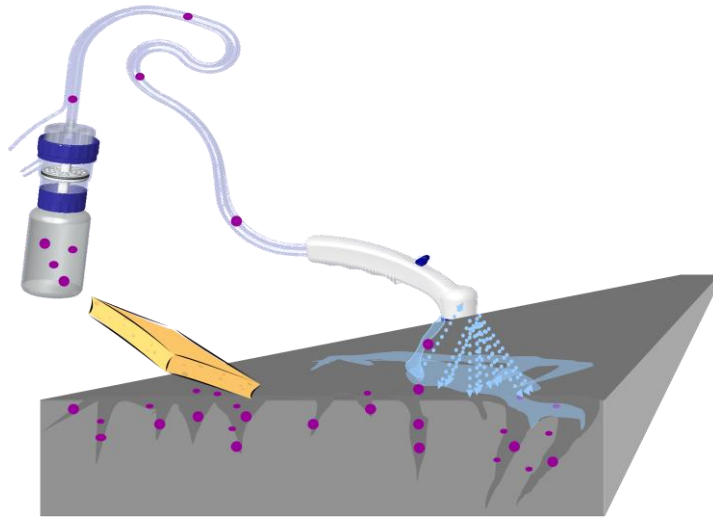


Figure 2-3 MV Sampling

Photo provided by Microbial- Vac Systems, Inc.

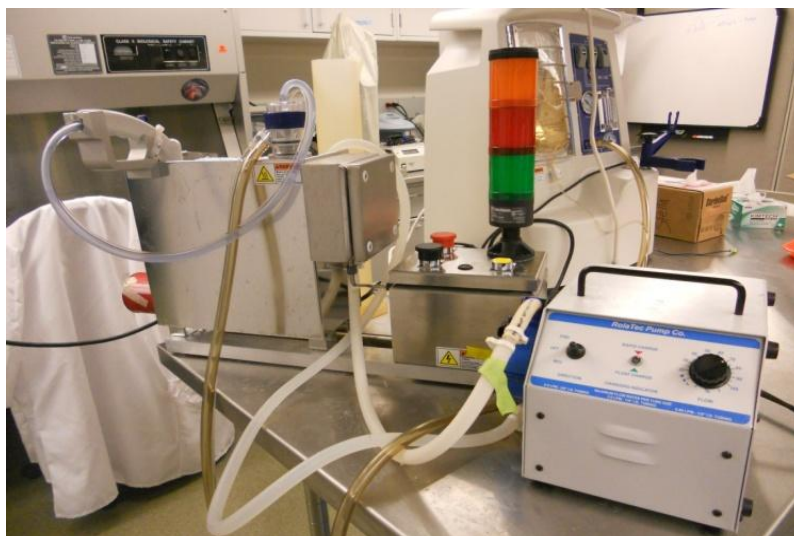


Figure 2-4 Hot-water rinse system and peristaltic pump for MV

2.5 Comparison of Sampling Devices

To further investigate and discuss available environmental sampling devices, a summary of relevant published research is presented. However, it is vital to understand that any direct comparison among studies mentioned would result in biased conclusions due to lack of uniform experimental design and sampling conditions from study to study. Thus, comparisons between chosen methodologies will only be discussed and compared within each of the research articles.

2.5a RODAC Contact Plate and Swab

Scott *et al.* (1983) investigated 64 homes for domestic microbial contamination by sampling 9 environmental sites with calcium alginate swabs and contact plates. Spearman correlation was used for analyzing data. Higher correlation was found at wet sites (both hard and soft) than at dry ($r=0.71$, $p<0.0005$). The swab device was superior to the contact plate for sampling surfaces suggesting that the mechanical break-up of clumps during swabbing gives rise to more colony forming units. However, at lower contamination (less than 100 CFU) the difference between contact plate and swab recovery was less significant, especially for flat hard surfaces such as board, work top benches and floors. In conclusion, when high levels of contamination are expected, the swab device is more effective at capturing true microbial load. Yet, when contamination is less than 450 colonies/25 cm² and the surface is flat, the contact plate can be suitable for regular environmental assessment.

Foschino *et al.* (2003) conducted a study with two purposes. One objective was to compare RODAC agar contact plate and swabbing for the recapture of *E. coli* cells or *Aspergillus niger* spores inoculated in low numbers onto metal surfaces. The second objective compared the cleanability of stainless steel with different finishes, specifically whether or not shot peening was used. Shot peening is a surface treatment useful to homogenize the roughness

of a metal surface at points in which it is variable or at welded points. The results indicated the accuracy of the RODAC plate is influenced by contact time. In the early stages of growth, *E. coli* cells are regular straight rods; *A. niger* spores are spheres with irregularities that support the attachment to the surface. The adhesion of *A. niger* on stainless steel surfaces is higher in these experimental conditions than that of *E. coli* since it appears to be more resistant to rinsing action and cleaning procedures. RODAC plate technique plus a prolonged contact time was able to significantly ($p < 0.05$) recover more of the microbial population present on the surface, whereas swabbing was unsuitable for recovering low level cells or spores. The type of finishing related to the stainless steel did not have any effect on the cleanability of surfaces and the shot peening treatments does not involve any change in recovery of *E. coli* cells or *A. niger* spores.

2.5b Sponge, Swab, and Composite Tissue

Vorst *et al.* (2004) compared traditional sampling devices for the quantitative recovery of *Listeria monocytogenes* from stainless steel surfaces. The devices include: composite tissue (CT), environmental sponge (ES), cotton-tipped swab (CS) and calcium alginate swab (CAS). CT yielded the most efficient recovery, with a 1.1 to 2.7 log CFU/cm² higher recovery when compared to the other devices.

As indicated by the scanning electron microscope data, *Listeria* cells were detected on stainless steel after recovery using each of the devices except CT. Following CS and CAS, RODAC plates averaged 2.0 log CFU/cm². The superiority of the CT could be explained by the coarse composition which enhances scouring of the surface and the inherent antistatic coating which aides in the release of the contaminant by reducing the electrostatic discharge commonly associated with fiber-tipped swabs. Yet, CT still entraps some cells within the device as suggested by the significant differences between device and inoculum level (Figure 2-5). The

low cost, abrasive material and enhanced ability to detect microorganism make the composite tissue an ideal sampling device for the quantitative (and potentially qualitative) assessment of contamination on hard-to-clean surfaces.

Over a period of five months, Kovacevic *et al.* (2009) collected 720 environmental samples from meat processing facilities to detect presence of *Listeria* spp. using cotton swab (CS), sterile sponge (SS) and composite tissue (CT). The SS and CT methods were similar ($p>0.05$) in their ability to recover *Listeria* spp. and significantly more efficient ($p<0.01$) than the CS. Although these results follow closely Vorst *et al.* (2004), the major difference was Kovacevic study found the sponge is equivalent to the CT, not inferior, as concluded by Vorst. However, these two studies were performed under different experimental conditions. Vorst *et al.* (2004) inoculated coupons with a pure culture and simulated bacterial behavior in food processing conditions and compared the devices based upon quantitative recovery. Kovacevic *et al.* (2009) compared the devices in real processing environments for qualitative recovery since the current U.S. regulatory policies are based upon presence or absence of *L. monocytogenes* in ready-to-eat foods. The CT and SS were similar in their recovery capability; however, the time and labor associated with CT preparation and additional difficulties in handling of this device make it an undesirable option for routine sampling.

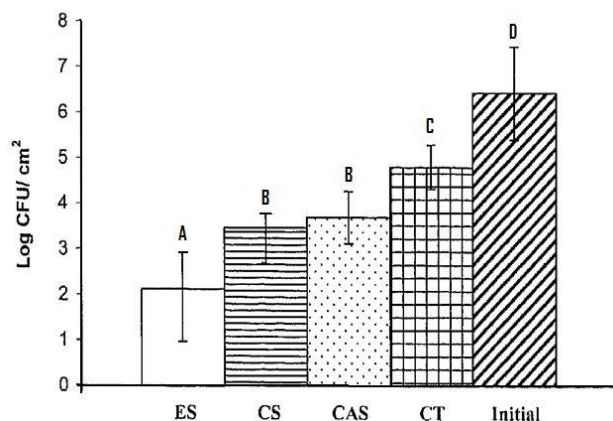


Figure 2-5 Mean quantitative recovery (log CFU/cm²) of *L. monocytogenes* from stainless steel using composite tissue (CT), calcium alginate swab (CAS), cotton swab (CS) and environmental sponge (ES)

Means with different letter are significantly different ($p < 0.05$) (Vorst *et al*, 2004)

2.5c Swab, Rinse, Direct Contact and Sonication

Quantifying microorganisms on surfaces provides valuable modeling data for consumer exposure due to cross-contamination and gives a basis for risk management efforts domestically and industrially. Kang *et al.* (2007) examined the ability of a sonic toothbrush to enhance quantitative recovery of *Listeria monocytogenes* from the surface of stainless steel and compared these results to traditional environmental surface devices: swab, rinse and direct contact plates. The mean recovery rates were significantly different ($p < 0.05$) from the inoculum (Table 1). Results regarding traditional devices found, after a 1 hour drying time, the rinse method yielded the highest mean detection compared to agar contact plate and swab. Overall, swab and contact were the least efficient and yielded 20 and 21% total recovery, respectively. Regarding sonicating devices, brush non-contact and contact methods yielded the highest detection rates of 61 and 59% total recovery, respectively (Figure 2-6).

Table 1 Mean recovery of *L. monocytogenes* dried for 1h at 20°C with 50-60% relative humidity

Sampling Method	Mean recovery per coupon ($\log_{10}$)*	Standard Deviation
Inoculum	5.32 ^A	0.14
Brush noncontact	5.10 ^B	0.12
Brush contact	5.09 ^B	0.15
Sonic Bath	4.98 ^{BC}	0.17
Rinse	4.90 ^C	0.13
Agar Contact	4.64 ^D	0.16
Swab	4.62 ^D	0.14

*Means not followed by same superscript letter are significantly different. $P < 0.05$. $N = 15$ (per sampling method). (Kang *et al.*, 2007)

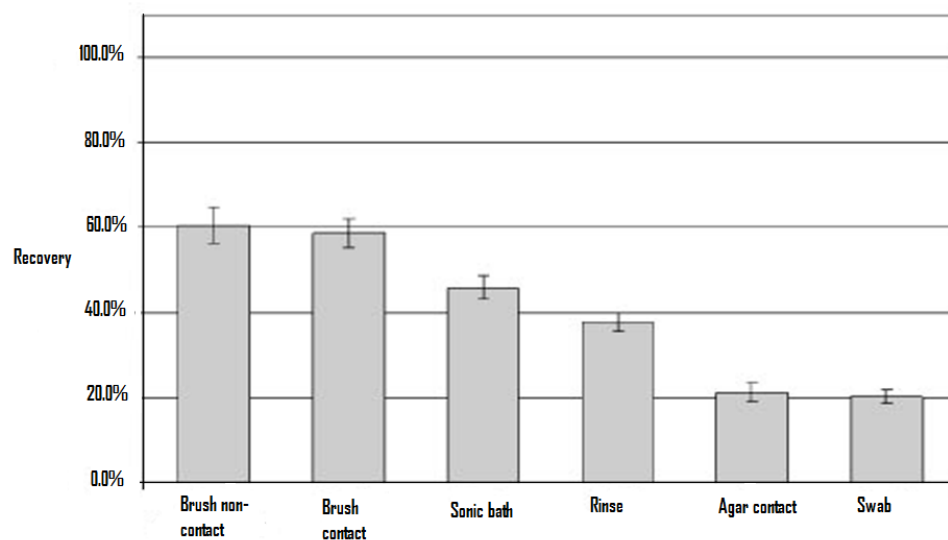


Figure 2-6 Mean (%) recovery of *L. monocytogenes* by various sampling methods dried for 1h at 20°C with 50-60% relative humidity

(Kang *et al.*, 2007)

After an extended drying time of 12 hours, there was a range of 0.77-1.74 log₁₀ loss (Table 2) from the mean recovery of the initial inoculum (p<0.05). Results followed closely the pattern from the 1h drying time; indicating swab had the lowest recovery (2%) and brush contact method had the highest recovery (17.3%, Figure 2-7). The consistent poor performance of swab devices led the authors to conduct an auxiliary test to enumerate viable cells entrapped within the device. After 1h drying followed by sampling, the swab was exposed to ultrasonication in a water bath and recovery increased approximately 20-25%. Using the same methods, this effect was only a 0.2% increase for the 12h drying time using the swab device. This technique was applied to the sonicating device head yet recovery increased only 1-2% and did not significantly add to total recovery.

Table 2 Mean recovery of *L. monocytogenes* dried for 12h at 20°C with 50-60% relative humidity

Sampling Method	Mean recovery per coupon (log ₁₀)*	Standard Deviation
Inoculum	5.39 ^A	0.12
Brush noncontact	4.62 ^B	0.17
Brush contact	4.57 ^{BC}	0.23
Sonic Bath	4.31 ^{CD}	0.27
Rinse	4.21 ^{CD}	0.26
Agar Contact	4.02 ^D	0.15
Swab	3.65 ^E	0.27

*Means not followed by the same superscript letter are significantly different. P< 0.05. N=10 (per method). (Kang *et al.*, 2007)

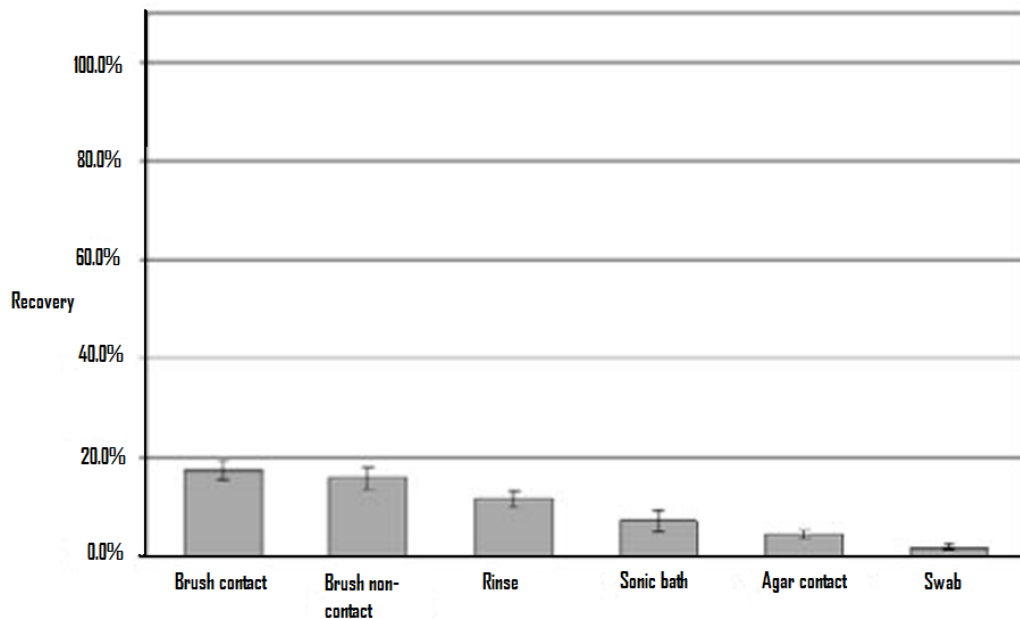


Figure 2-7 Mean (%) recovery of *L. monocytogenes* by various sampling methods dried for 12h at 20°C with 50-60% relative humidity

(Kang *et al.*, 2007)

In conclusion, Kang *et al.*, revealed a correlation between efficiency and the distance of the bristles to the surface; the closer the oscillating brush head was to the surface, the higher the recovery rate. The key to aping this acoustic energy through cavitating action is the necessary fluid medium which is likely impractical in an industrial setting. In addition, a sonicating head has the potential to disperse microorganisms and possibly aerosolize them. However, considering the availability, affordability and promising results of increased recovery, these types of devices provide useful methodology for future application of more accurately enumerating surface contaminants.

2.5d MV Validation Studies

Recently, studies have been conducted using the Microbial-Vac to assess the accuracy of microbial recovery in comparison to widely adopted sampling devices. In an environmental study, the MV was compared to sponging and swabbing for the quantitative recovery of *Salmonella enterica* and *Listeria innocua* from four different environmental surfaces. Results indicate the MV recovered significantly higher ($p < 0.05$) levels of *Listeria* and *Salmonella* from each surface type in a simulated setting when compared to cellulose sponge and cotton swab (Table 3). This device has a multitude of advantages over sponges, swabs and tissues: the ability to assess a wide variety of environmental surfaces (including carcasses), eliminates extraction step, automated methodology, aggressive wet-vacuum sampling approach and the ability to penetrate cracks and crevices by simultaneously wetting and sampling the surface over areas as large as 1800 cm² (Microbial-Vac Systems, Inc).

Table 3 Quantitative recovery (log CFU) from environmental surfaces using Microbial-Vac, cellulose sponge and cotton swab

Organism	Device	Stainless Steel*	Cutting Board*	Ceramic Tile*	Conveyor Belt*
<i>Listeria</i>	MV	7.49a	7.07a	7.31a	7.51a
	Sponge	5.65b	5.77b	5.52b	5.55b
<i>Salmonella</i>	MV	7.29a	7.37a	7.05a	7.30a
	Sponge	6.06b	6.57b	5.92b	6.03b
	Swab	6.02b	5.48c	5.62c	5.75b

*Means not followed by the same letter are significantly different ($p < 0.05$) $n = 15$ (for each mean)

(Microbial-Vac Systems, Inc)

Chapter 3 Materials and Methods

3.1 Preparation of Inoculum

3.1a *Listeria monocytogenes* Strain Scott A

Listeria monocytogenes strain Scott A was obtained from the Department of Food Science and Technology at the University of Tennessee, Knoxville. Cultures were revived in sterile 10 mL trypticase soy broth (Difco; Becton, Dickinson and Company, Sparks, MD) supplemented with 0.6% (wt/vol) yeast extract (TSBYE) and incubated for 24h at 35°C. *L. monocytogenes* cultures were maintained using sterile trypticase soy agar (Difco) containing 0.6% yeast extract (TSAYE) slants at 4°C. The culture was cultivated in 10 mL TSBYE for 24h at 35°C from stock slants. At 24h intervals, the culture was transferred by a single loop inoculation into 10 mL TSBYE. It was sub-cultured in this manner at least twice, consecutively, before use. A standard plate count method determined the average concentration was about 1.0×10^9 CFU/mL after 24h of incubation at 35°C.

3.1b *Brochothrix thermosphacta*

Brochothrix thermosphacta (ATCC 11509TM) was obtained from Fisher Scientific (Pittsburgh, PA) and maintained at 4°C. Cultures were revived in sterile 10 mL trypticase soy broth (Difco) supplemented with 0.6% yeast extract (TSBYE) and incubated for 24h at 25°C. *B. thermosphacta* cultures were maintained on sterile trypticase soy agar (Difco) containing 0.6% (wt/vol) yeast extract (TSAYE) slants at 4°C. The culture was cultivated in 10 mL TSBYE for 24h at 25°C from stock slants. At 24h intervals, the culture was transferred by a single loop inoculation into 10 mL TSBYE. It was sub-cultured in this manner at least twice, consecutively,

before use. A standard plate count method determined the average concentration was about 2.67×10^8 CFU/ml after 24h of incubation at 25°C.

3.2 Preparation of Food Contact Surfaces

Five surfaces commonly found in food processing environments were used for this study: unpolished, grade 304 stainless steel plates (SS), polyethylene cutting boards (CB), polyurethane conveyor belt (PB), open hinge flat-top conveyor belt (FT) and mesh conveyor belt (MB). SS and CB surfaces for this study were new, whereas PB, FT and MB had been thoroughly used in a food processing environment prior to sample collection (Appendix I).

For *Listeria monocytogenes* sampling, the materials were cut or divided into squares measuring 30cm x 30 cm² (900cm²). For *Brochothrix thermosphacta* sampling, a 5cm x 5 cm (25cm²) area was measured and marked on each surface. All surfaces were immersed in 10% bleach for 20 minutes, rinsed with tap water and dried with ultra-violet light in a biosafety level II cabinet for approximately 30 minutes prior to inoculation.

3.3 Inoculation of Food Contact Surfaces

Stock cultures were serially diluted in sterile 0.1% peptone water. Low level inoculum, 10 CFU/surface, and high level inoculum, 100 CFU/surface, were used for inoculation of *L. monocytogenes* on each of the 5 surfaces (900 cm²) in a randomized application. An inoculum level of 10,000 CFU/surface was used for inoculation of *B. thermosphacta* on each of the five surfaces (25 cm²) in a randomized application. The culture suspension was blindly inoculated with 0.01ml in 10 randomized spots (0.1 ml total volume) on each of the five surfaces. Blind inoculation means that a technician different from the technician conducting sampling recovery performed the inoculation to reduce bias in sample recovery. Inoculated surfaces were dried in a

biosafety level II hood for approximately 20 minutes. After drying, the inoculated surfaces were placed in a 4°C refrigerator for two hours to simulate processing facility conditions. Surfaces inoculated with sterile diluent (sterile 0.1% peptone water) were used as negative controls.

3.4 Sampling Devices

Four sampling devices (Appendix II) were compared for their efficacy in recovering *L. monocytogenes* and *B. thermosphacta* from SS, CB, PB, FT and MB. The devices include: polyester-tipped swab (SW; Copan Diagnostics, Inc.), cellulose sponge (ES; 3M™), composite tissue (CT; KimWipe™, Kimberly-Clark) and Microbial-Vac system (MV; Microbial-Vac Systems, Inc., Jerome, ID).

3.4a Polyester-Tipped Swab (SW)

For the SW method, (*Compendium of Methods for Micro. Exam Food*, 4th ed, Chpt 3, Section 3.52), Neutralizing Buffer (Difco) plus 0.05% polysorbate 80 (Tween) was used as the sterile rinse solution (SRS). The swab was rehydrated with 10 ml SRS and surfaces were swabbed by holding the device at a 30° angles in contact with the surface using 10 vertical strokes followed by 10 horizontal strokes rubbing the head thoroughly and vigorously over the entirety of the designated surface. The swab was returned to the tube and broken/cut with a sterile device leaving only the swab head in the tube. The screw cap of the test tube was replaced containing 10 ml SRS and vortexed for 15 seconds. Because of the surface area and size of the swab head, swabs were used only for evaluating recovery on mesh and flat top conveyor belts.

3.4b Cellulose Sponge (SP)

Neutralizing Buffer (Difco) plus 0.05% polysorbate 80 (Tween) was used as the sterile rinse solution (SRS). The SP was rehydrated with 10 ml of SRS and sampled by passing the

sponge over the surface 10 times in the horizontal direction and 10 times in the vertical direction. The sponge was aseptically placed back into the Whirl-Pak bag (Nasco) with 40 ml SRS and stomached for two minutes at 260 rates per minutes (RPM). (*Compendium of Methods for Micro. Exam Food*, 4th ed, Chpt 3, Section 3.52)

3.4c Composite Tissue (CT)

The CT was folded twice from the side and top edges (measured 5.5 cm by 5.5 cm) producing a clean interior and exterior surface. This eliminated contact between gloves and the sampled area. Neutralizing Buffer (Difco) plus 0.05% polysorbate 80 (Tween) was used as the sterile rinse solution (SRS). The CT was rehydrated with 10 ml of SRS in a sterile Whirl-Pak bag. Excess diluent was removed from the tissue by squeezing the CT inside the Whirl-Pak bag. Then, the surface area was sampled with 10 vertical and 10 horizontal strokes using the folded exterior surface. The CT was returned to the Whirl-Pak bag and partially unfolded. Then, 40 ml of SRS was added to the bag, homogenized in a stomacher for 60 seconds at 260 RPM and hand massaged for 30 seconds (Vorst *et al.* 2004).

3.4d Microbial-Vac Systems (MV)

The MV incorporates liquid (SRS) and air assisted microbiological detachment and capture technology to collect microorganisms from a surface (Microbial-Vac Systems, 2007). A sterile MV sampling head kit was used for sampling by passing the head over the entire surface slowly using vertical and horizontal strokes with the vacuum and SRS solution in the “on” position at approximately 18 psi. The SRS was turned “off” and the MV sampling head was passed back over the surface to collect residual solution. This procedure was repeated until collection of 25-100 ml, depending on the surface being sampled. The collection bottle was

removed, capped and processed using the following recovery and detection techniques. Prior to and between each sample, the MV sampling head was sterilized using a hot water rinse system with peristaltic pump at 82°C to ensure no microorganisms were carried over between each sample.

3.5 Enrichment of *Listeria monocytogenes*

The USDA and Association of Analytical Chemists (AOAC/IDF) method 993.12 for the enrichment of *Listeria* from food or environmental samples were used as the reference method for this study. This two-stage enrichment method uses a modification of University of Vermont medium (UVM; Difco) containing acriflavin and naladixic acid for primary enrichment, followed by secondary enrichment in Fraser broth (FB; Difco) and plating onto modified Oxford (MOX; Difco) agar containing the selective agents moxalactam and colistin sulphate (Gasanov *et al.*, 2005).

Primary enrichment was conducted immediately after completion of sampling. A predetermined volume (10, 50 or 100 ml) of double strength UVM was added to each SW, SP, CT and MV sample inside a whirl-pak bag (Figure 3-1). Each whirl-pak bag was homogenized in a stomacher at 260 RPM for two minutes. Then, the bagged sample was incubated for 24h at 30°C. Following incubation, secondary enrichment was completed by transferring 0.1 ml of each primary enrichment sample from the whirl-pak bag into 10 ml of sterile FB. The inoculated FB tubes were incubated at 35°C for 24-48h.

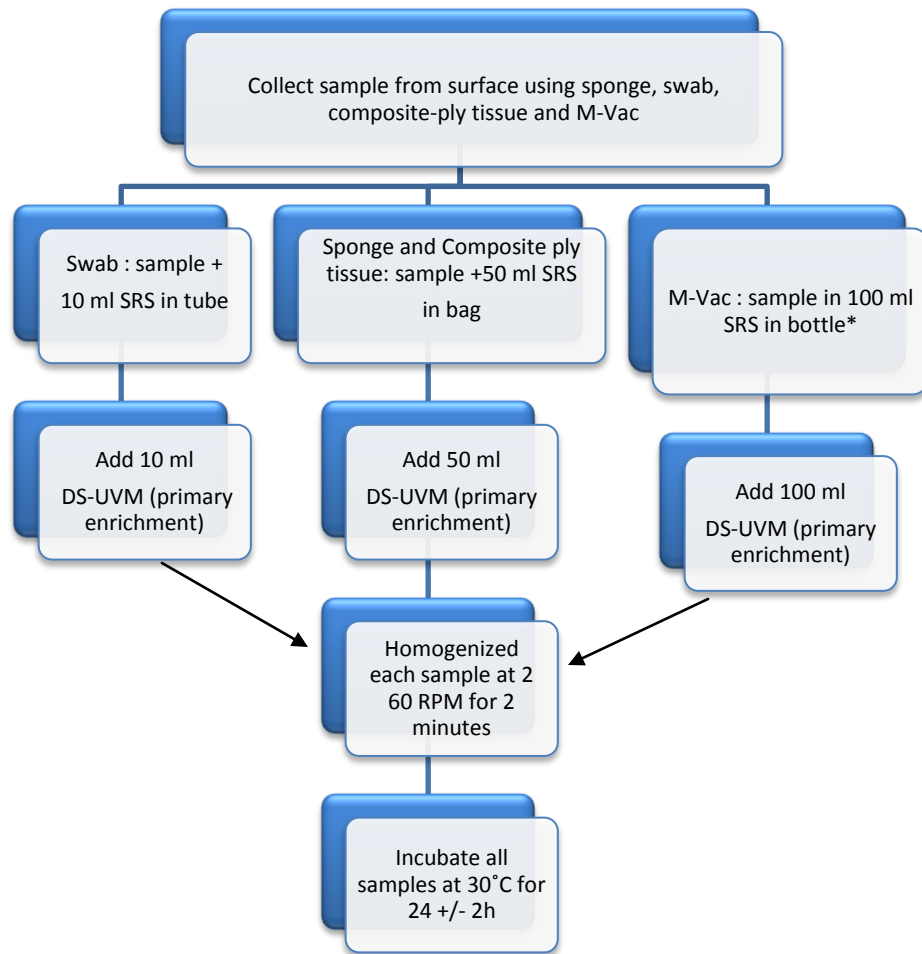


Figure 3-1 Recovery and primary enrichment of *L. monocytogenes* from food contact surfaces.

3.6 Detection of *Listeria monocytogenes*

After incubation for 24h, the FB tubes were visually examined for potential presence of *L. monocytogenes* indicated by darkening (black test tubes) due to esculin hydrolysis. If darkening was indicated, FB culture was streaked onto a MOX plate. The inoculated MOX plates were incubated at 35°C for 24h which were examined for colonies present with a dark zone (black colonies). If black colonies were present, the sample was considered positive and detection was completed (Figure 3-2).

After incubation for 24h, the FB tubes that were considered negative (no black/darkening present) were incubated for an additional 24h at 35°C. If any darkening was indicated, a MOX plate was streaked for isolation (as described above). If no darkening was indicated after 48h of incubation, the FB tubes were considered negative for presence of *L. monocytogenes*. A positive reaction on MOX plates was considered positive for *L. monocytogenes* without further confirmation.

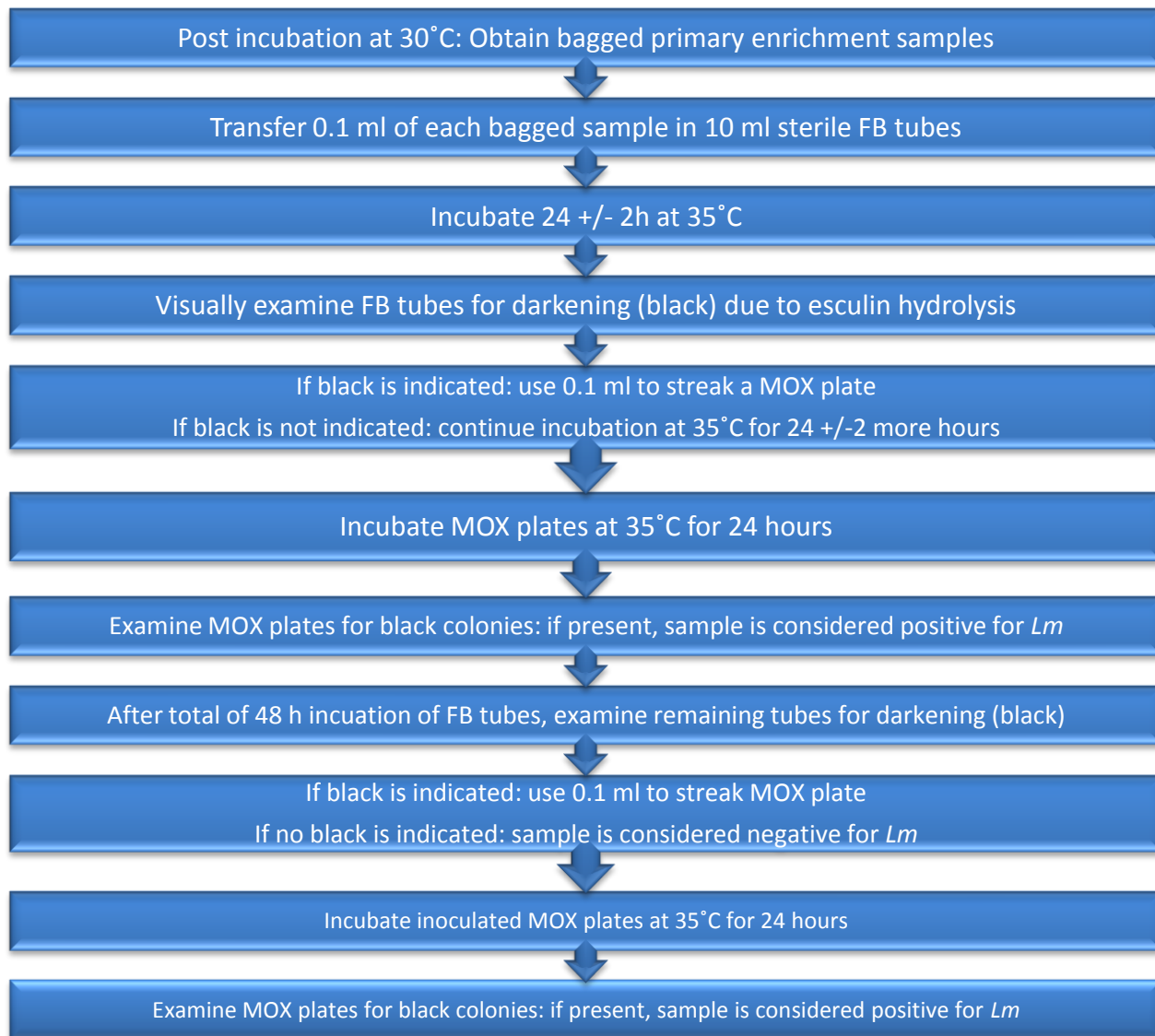


Figure 3-2 Secondary enrichment of *L. monocytogenes* from food contact surfaces

3.7 Recovery and Detection of *Brochothrix thermosphacta*

Following sampling, 0.1 ml was transferred directly from each sample and plated onto TSAYE in duplicates. The inoculated TSAYE plates were incubated at 25°C for 24-48h. After incubation the colonies were counted and an average quantitative recovery was determined per sampling method for each surface.

3.8 Statistical Analysis

3.8a *Listeria monocytogenes*

Data were analyzed using SAS (version 9.1.3; SAS Inst., Cary, N.C., U.S.A.). The generalized linear mixed models procedure, PROC GLIMMIX, was used to analyze the binomial response variable, presence or absence of *L. monocytogenes* on stainless steel, cutting board and polyurethane conveyor belt. However, due to variability in data and model convergence criteria, flat top conveyor belts were analyzed using the normal approximation to the binomial distribution. The experimental design for all surfaces was a randomized block design (RBD) blocked on day with a factorial treatment design and replication. Results were expressed as mean percent recovery (% samples where *Listeria* was detected) +/- standard deviation for each method. The statistical model included the following factors: block (day), food contact surface (SS, CB, PB and FT), treatment (sampling devices: MV, CT, SP and SW) and inoculum level (High and Low). A significance level of 0.05 was used for all surfaces. When significant effects were found, Fisher's least significant difference method was used for mean separation (Appendix III).

3.8b *Brochothrix thermosphacta*

Data were analyzed using SAS (version 9.1.3; SAS Inst., Cary, N.C., U.S.A.). The %mmaov macro (http://dawg.utk.edu/glossary/whatis_mmaov_macro.htm) was used to perform

mixed model analysis of variance (ANOVA) testing for differences among the means. The experimental design was a randomized block (day) design with sampling method as treatment, with separate analysis for each surface. Normality of model residuals was evaluated using the Shapiro-Wilk test. Levene's F-test was used to test equality of group variances and a Tukey single degree of freedom test was used to test for interaction of block and treatments. A log transformation was used to normalize the residuals. Results with $p < 0.05$ were considered significant. Fisher's LSD method was used for mean separation of any significant ANOVA effects. Least squares means were back-transformed to the original data scale (Appendix IV).

Chapter 4 Results and Discussion

4.1 *Listeria monocytogenes*

Foodborne transmission of *Listeria* spp. was first established in 1981 and has been linked with numerous outbreaks of foodborne illness mostly associated with ready-to-eat (RTE) foods. *L. monocytogenes* is the only species among the genus *Listeria* that is considered a human pathogen (Gasnov *et al.*, 2005, Grady *et al.*, 2007, Kathariou, 2002 and Thimothe *et al.*, 2003). Post-processing contamination of RTE foods with *L. monocytogenes* has caused over 80 recalls of more than 130 million pounds of product, which makes this pathogen of major concern to public health organizations and food manufacturers (Grady *et al.*, 2008 and Vorst *et al.*, 2004). *L. monocytogenes* has been implicated in at least 11 human foodborne epidemics worldwide and causes approximately 2500 cases of foodborne illnesses in the United States annually. *L. monocytogenes* can survive and grow at temperatures from 2 to 45°C, is resistant to freezing (as low as -20°C) and high salt concentrations (10-12% NaCl) and can form a biofilm on a variety of food contact surfaces (USDA; http://fsrio.nal.usda.gov/fsheet_pf.php). Research indicates this pathogen can form a biofilm on stainless steel in as little as 20 minutes at 4 and 20°C (Kushwaha and Muriana, 2009 and Taormina and Beuchat, 2002).

4.1a Detection of *L. monocytogenes* on stainless steel and cutting board using Microbial-Vac, composite tissue and cellulose sponge

Over a period of 6 months, 420 samples were collected and analyzed for the mean (%) detection of *L. monocytogenes* Scott A using four sampling devices on food contact surfaces. Of the 420 samples, 252 samples were collected from stainless steel (SS) and cutting board (CB) using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP) devices and analyzed for the presence of *L. monocytogenes* using a high (100 CFU/900 cm²) and low (10

CFU/900 cm²) inoculum. Results showed that at 100 CFU/900 cm² there was no significant difference ($p>0.05$) among detection using MV, CT or SP on stainless steel (Table 4). In addition, there was no significant difference ($p>0.05$) among MV, CT and SP detection on cutting board at 100 CFU/900 cm² (Table 5). However, on stainless steel at a low inoculum level (10 CFU/900cm²) there was significant difference among sampling devices ($p=0.0324$). The Microbial-Vac yielded the highest recovery rate of 63% in comparison to CT and SP, 17 and 37%, respectively (Table 6). The MV detected *L. monocytogenes* at low inoculum level better than CT ($p=0.0086$). Although not different from SP ($p=0.1211$), the MV did detect an average of 24.21% more than SP. Overall, on cutting board and stainless steel, the cellulose sponge detects *L. monocytogenes* less often (ranging from 5% to 25% less) as compared to Microbial-Vac.

Results of the detection of 10 CFU/900cm² *L. monocytogenes* on cutting board showed no difference ($p>0.05$) was detectable between MV, CT or SP devices (Table 7). Cutting boards have an inherent hydrophobic surface, so liquid droplets tend to be repelled on top of the boards. This may have caused significant loss of inoculation during the surface preparation, drying and sampling steps resulting in low, poor and/or inaccurate detection results.

Table 4 Detection of *L. monocytogenes* at 100 CFU/900 cm² on stainless steel using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Treatment	Mean (%)*	Standard Deviation (%)**
MV	47.42 A	26.22
CT	62.97 A	29.99
SP	42.23 A	37.09

*Values represent the mean percentage of positive samples (number of positive samples/total samples). Means with a common letter do not differ (p>0.05). N=21 (per treatment).

**Std. deviation of percent detection across blocks

Table 5 Detection of *L. monocytogenes* at 100 CFU/900 cm² on cutting board using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Treatment	Mean (%)*	Standard Deviation (%)
MV	57.18 A	31.70
CT	47.02 A	26.23
SP	41.99 A	31.70

*Values represent the mean percentage of positive samples (number of positive samples/total samples). Means with a common letter do not differ (p>0.05). N=21 (per treatment).

Table 6 Detection of *L. monocytogenes* at 10 CFU/900 cm² on stainless steel using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Treatment	Mean (%)*	Standard Deviation (%)**
MV	62.97 A	23.00
CT	17.34 B	26.23
SP	37.07 AB	35.63

*Values represent the mean percentage of positive samples (number of positive samples/total samples). Means with a common letter do not differ (p>0.05). N=21 (per treatment)

**Std. deviation of percent detection across blocks

Table 7 Detection of *L. monocytogenes* at 10 CFU/900 cm² on cutting board using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Treatment	Mean (%)*	Standard Deviation (%)**
MV	41.99 A	25.20
CT	47.02 A	26.23
SP	27.26 A	23.00

*Values represent the mean percentage of positive samples (number of positive samples/total samples). Means with a common letter do not differ (p>0.05). N=21 (per treatment).

** Std. deviation of percent detection across blocks

Because *L. monocytogenes* is a “zero tolerance” microorganism in RTE foods and has a low infectious dose, it is vital for the safety of consumers and the security of the food industry that sampling devices are able to detect *L. monocytogenes* at low levels of contamination. These results indicate that the Microbial-Vac system compared to composite tissue and sponge has the highest rate for detecting pathogenic microorganisms at low levels of contamination from stainless steel food contact surfaces in a simulated food processing environment. In agreement with Kovacevic *et al.* (2008), the sponge and composite tissue were equivalent for the recovery of *L. monocytogenes*. The advantage of the sponge over the tissue is the ease of sample preparation and collection. The CT is undesirable for routine sampling due to the labor intensive steps and greater potential for contamination involved with preparation of these devices. Because *L. monocytogenes* can readily form a biofilm (Vorst *et al.*, 2004) it is vital that sampling devices have the ability to penetrate, recover and detect pathogens. The MV overcomes weaknesses associated with traditional sampling devices by introducing an aggressive wet-vacuum technique that increases the likelihood of penetrating biofilms and recovering pathogens. In addition, MV eliminates the need for an extraction step that devices such as swabs, sponge and tissues require for detection purposes.

4.1b Detection of *L. monocytogenes* on flat top conveyor belts using Microbial-Vac, composite tissue, cellulose sponge and polyester-tipped swabs

Frequently used surfaces in processing facilities, such as flat top belts, contain narrow cracks and crevices that are near impossible to access with sponges and swabs. Nevertheless, these hard to reach areas are ideal growth environments for microorganisms harboring areas for long term survival and potential food borne contamination outbreaks that are devastating to food companies and processors alike. Past studies, highlighting the inefficiencies of swabs and

sponges, have indicated a crucial need for altering outdated sampling methodology and to develop a more reliable process of recovering pathogenic microorganisms (Kang *et al.*, 2007, Kovacevic *et al.*, 2008, Moore and Griffith, 2005 and Vorst *et al.*, 2004). This study, along with the others mentioned, provides results that further promote the use of more aggressive and automated methodology such as the Microbial-Vac. Four sampling devices were compared for mean (%) detection of low and high inoculum *L. monocytogenes* on flat top conveyor belts. At 10 CFU/900cm², the MV detected an average of 83.33% and was significantly better ($p=0.0004$) than SP and SW devices. Although not different ($p>0.05$), the MV did detect an average of 16.67% more than CT (Table 8). At 100 CFU/900cm², again the MV was the superior device and detected an average of 100%, significantly different ($p<0.05$) than SP and SW (Table 9). There was no difference between MT and CT for the detection of *L. monocytogenes* at high inoculum levels.

Overall, regarding CT, SP and SW, these findings are in agreement with the results from Kovacevic *et al.* (2009) following the same general trend of mean detection per device. The current study, along with the prior mentioned, indicate the swab is the least efficient qualitative microbial assessment technique, most likely due to the inability to sample large surface areas, difficulty in release of microorganism from swab head and oversaturation of sampling head. The sponge and composite tissue have a variety of shortcomings including the entrapment of bacteria, variation of application pressure and size limitations which prevent these devices from sampling cracks and crevices commonly found in the processing environment. However, it is important to note the superiority of the CT over SP and SW which could be explained by the coarse composition and the inherent antistatic coating aiding in the recovery and release of bacteria by reducing the electrostatic discharge commonly seen in cotton-tipped swabs (Vorst *et al.*, 2004).

Table 8 Detection of *L. monocytogenes* at 10 CFU/900 cm² on flat top conveyor belts using Microbial-Vac (MV), composite tissue (CT), cellulose sponge (SP) and polyester-tipped swab (SW)

Treatment	Mean (%)*	Standard Deviation (%)**
MV	83.33 A	28.87
CT	66.67 AB	28.87
SP	33.33 BC	28.87
SW	0 C	0

*Values represent the mean percentage of positive samples (number of positive samples/total samples). Means with a common letter do not differ (p>0.05). N=12 (per treatment).

** Std. deviation of percent detection across blocks

Table 9 Detection of *L. monocytogenes* at 100 CFU/900cm² on flat top conveyor belts using Microbial-Vac (MV), composite tissue (CT), cellulose sponge (SP) and polyester-tipped swabs (SW)

Treatment	Mean (%)*	Standard Deviation (%)**
MV	100 A	0
CT	83.33 AB	28.87
SP	50.0 BC	0
SW	33.33 C	28.87

Values represent the mean percentage of positive samples (number of positive samples/total samples). Means with a common letter do not differ (p>0.05). N=12 (per treatment).

** Std. deviation of percent detection across blocks

4.1c Detection of *L. monocytogenes* on polyurethane conveyor belts using Microbial-Vac, composite tissue and cellulose sponge

There was no difference found between detection methods when recovering *L. monocytogenes* on polyurethane belts ($p=0.7047$) at high or low inoculum levels (Table 10). At 100 CFU/900cm², all sampling devices were equally capable of detecting presence of *L. monocytogenes* with 100% success rate. Therefore, there was no variability in the data so further analysis was needed. Given the high detection rates for this surface compared to the previously mentioned, it appears *L. Monocytogenes* was unable to initiate development of a biofilm on PB. Research indicates food contact surfaces play a key role in the foodborne illness outbreaks. Moore and Griffith (2002) found as porosity and surface roughness decreases, few bacteria become attached within the material and remain accessible to recovery processes. Additionally, this study concluded that recovery rates of *Salmonella* were higher from flat, non-porous surfaces than from polyethylene or wood. Previous studies agree that microorganisms are more efficiently transferred from nonporous then from porous surfaces (Rusin *et al.*, 2002). Thus, the same inherent characteristics that enable this surface to be easily sampled and *L. monocytogenes* to be detected, are the same characteristics that increase the likelihood that *Listeria* could contaminate the final product if industry sanitation protocols are not properly developed, regulated and practiced (Moore and Griffith, 2007).

Table 10 Detection of *L. monocytogenes* at 10 CFU/900 cm² on polyurethane conveyor belts using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Treatment	Mean (%)*	Standard Deviation (%)**
MV	96.25 A	19.24
CT	88.74 A	38.49
SP	96.25 A	19.24

*Values represent the mean percentage of positive samples (number of positive samples/total samples). Means with a common letter do not differ ($p>0.05$). N=18 (per treatment)

** Std. deviation of percent detection across blocks

4.1d Mesh Conveyor Belts

Data collection was abandoned due to the mechanics of the Microbial-Vac in combination with the porosity of the mesh conveyor belt. Initially, a tray was used to contain the mesh-conveyor belt during sampling and to prevent potentially hazardous spread of *L. monocytogenes* and loss of SRS fluid (sample) in the lab environment. With use of the tray, the MV was collecting residual solution that would otherwise be lost or uncollected during routine environmental sampling in a food processing facility. The tray was removed from the sampling procedure to increase the likelihood of collecting unbiased data among the 4 sampling methods and to more accurately mimic practical sampling procedures. However, problems still occurred due to the spread and more importantly the loss of MV SRS fluid (sample) along with pathogenic microorganisms during routine data collection. For future research on these types of surfaces, an unbiased and practical sampling protocol needs to be developed to address these difficulties.

4.2 *Brochothrix thermosphacta*

B. thermosphacta is a Gram-positive, rod shaped, non-motile, non-spore forming, psychrotrophic microorganism commonly associated with spoilage of pre-packaged meats. This organism favors growth at 4°C with limited oxygen sup and elevated CO₂ concentrations. *B. thermosphacta* has a close phylogenetic relationship to the genus *Listeria* and is included in the Listeriaceae family (Kilcher *et al.*, 2010). Over a period of 5 weeks, 264 samples were collected and analyzed for the quantitative recovery of *B. thermosphacta* on SS, CB, PB and FT. The inoculum level was approximately 10,000 CFU/25 cm². The dependent variable of this study was log difference (Inoculated log CFU/25cm²- recovered log CFU/25cm²) per method on each surface calculated for each day of sampling.

4.2a Detection of *B. thermosphacta* on polyurethane conveyor belt, stainless steel and cutting board using Microbial-Vac, composite tissue and cellulose sponge

There was no significant differences between the devices ability to quantitatively assess *B. thermosphacta* on polyurethane belts (p=0.527) and stainless steel (p=0.9217), which are the flat, non-porous, non-waxy surfaces used in this study (Table 11 and 12, respectively). However, on cutting board, MV was significantly different (p=0.0371) than CT and SP (Table 13) and collected the highest amount of the original inoculum.

Table 11 Mean quantitative detection (log CFU/25cm²) of *B. thermosphacta* on polyurethane conveyor belts using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Method	Mean (log CFU)*	Standard Deviation
Inoculum	4.66 A	0.61
MV	3.74 A	1.29
CT	4.13 A	0.45
SP	3.53 A	1.68

*Means with a common letter do not differ (p>0.05). N=20 per method.

Table 12 Mean quantitative detection (log CFU/25cm²) of *B. thermosphacta* on stainless steel using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Method	Mean (log CFU)*	Standard Deviation
Inoculum	4.53 A	0.31
MV	3.91 A	1.10
CT	3.78 A	1.23
SP	3.95 A	0.74

*Means with a common letter do not differ (p>0.05). N=16 per method.

Table 13 Mean quantitative detection (log CFU/25cm²) of *B. thermosphacta* on cutting board using Microbial-Vac (MV), composite tissue (CT) and cellulose sponge (SP)

Method	Mean (log CFU)*	Standard Deviation
Inoculum	4.53 A	0.31
MV	4.35 A	0.35
CT	3.87 B	1.15
SP	3.62 B	1.55

*Means with a common letter do no differ (p>0.05). N=16 per method.

4.2b Detection of *B. thermosphacta* on flat top conveyor belts using Microbial-Vac, composite tissue, cellulose sponge and polyester tipped swab

The swab recovered the least amount of *B. thermosphacta* inoculum, 3.25 log CFU/25cm², from flat top belts and was lower ($p=0.0259$) than MV and SP devices, 4.29 and 4.12 log CFU/25cm², respectively. Furthermore, SP was significantly better at quantifying *B. thermosphacta* than SW but still less than MV. CT did not differ significantly from SW or SP ($p>0.05$). Overall, MV had the highest recovery capability, on average capturing all of the original inoculum (Table 14).

Overall, the swab yielded the lowest bacterial recovery of all the devices used in this study followed closely by cellulose sponge. Swabs and sponges have been plagued by studies highlighting the number of weaknesses associated with these devices (Foschino *et al.*, 2003, Kang *et al.*, 2007, Kovacevic *et al.*, 2009, Moore and Griffith, 2002, Vorst *et al.*, 2004). Besides the limitation to sample large surface areas, results indicate these devices harbor collected bacteria in the sampling head decreasing the overall ability to accurately quantify or even qualitatively assess food processing environments. Consequently, the characteristics that enable a swab or sponge to scour surfaces and entrap bacteria in the sampling head may then be the same characteristics that prevent the microorganisms from being then released for detection. Furthermore, these are non-automated devices and the standard deviations are reportedly larger compared to more novel techniques. This could be explained by the variability in application pressure, methodology and the manual skill of the technician.

Numerous studies report that increasing mechanical energy between sampling device and surface increases the number of bacteria removed from the surface (Gill and Jones, 1998, Moore and Griffith, 2005 and Vorst *et al.*, 2004). These results indicate the superiority of MV to

quantitatively assess complex surfaces more efficiently than sponges and swabs. Using the Microbial-Vac overcomes a variety of weaknesses previously discussed by introducing a robust wet-vacuum device that simultaneously moistens and samples the surface in an aggressive and automated manner. In addition, use of the MV eliminates the need for an extraction step which is one of the causes of decreased quantitative accuracy related to sponges, swabs and tissues.

Table 14 Mean quantitative detection (log CFU/25cm²) of *B. thermosphacta* on open hinge flat top conveyor belts using Microbial-Vac (MV), composite tissue (CT), cellulose sponge (SP) and polyester-tipped swab (SW)

Sampling Method	Mean (log CFU)*	Standard Deviation
Inoculum	4.29 A	0.12
MV	4.29 A	0.38
SP	4.12 A	0.47
CT	3.85 AB	1.37
SW	3.25 B	0.29

*Means with a common letter do not differ (p>0.05). N=16 per method.

Chapter 5 Conclusions

L. monocytogenes is responsible for over 2,500 cases of foodborne listeriosis and about 500 fatalities in the United States each year costing approximately \$2.33 billion (Kornacki, 2010). The efficiency of detecting and quantifying pathogenic microorganisms from food processing environments is primarily dependent upon the use of sensitive, accurate and rapid sampling devices (Kovacevic *et al.*, 2008). This study determined the Microbial-Vac was better at detecting both high and low level *Listeria monocytogenes* from flat top conveyor belts as compared to commonly used cellulose sponges and polyester swabs ($p < 0.05$) and superior to composite tissue at recovering low level, 10 CFU/900cm², contamination on stainless steel. In addition, Microbial-Vac quantitatively assesses bacterial contamination on flat top conveyor belts at greater levels ($p < 0.05$) as compared to polyester swabs. In general, there was no difference between composite tissue and cellulose sponge ability to detect or recover microorganisms among all surfaces. Overall, the polyester swab was the least efficient device for the quantitative or qualitative assessment of either pathogenic or spoilage microorganisms regardless of surface type, surface area or level of contamination. In conclusion, the Microbial-Vac overcomes the weaknesses commonly associated with widely adopted sampling devices, sponges and swabs, by introducing automated, robust and aggressive sampling device that increases the likelihood of detecting foodborne pathogens and quantitatively assessing microbial contamination on a variety of food contact surfaces.

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Appendices

Appendix I: Environmental Surfaces



Figure I-0-1 Grade 304 stainless steel

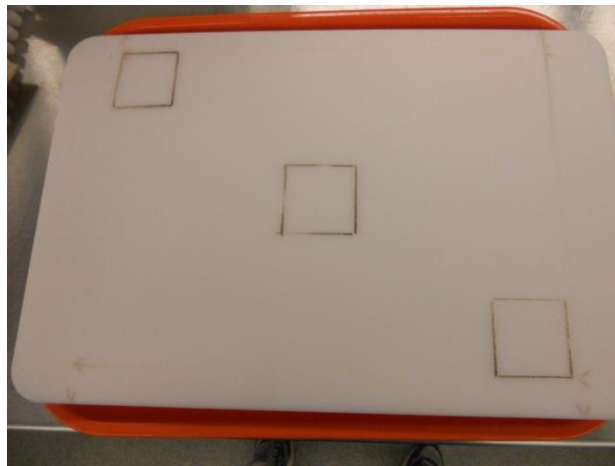


Figure I-0-2 Polyethylene cutting board

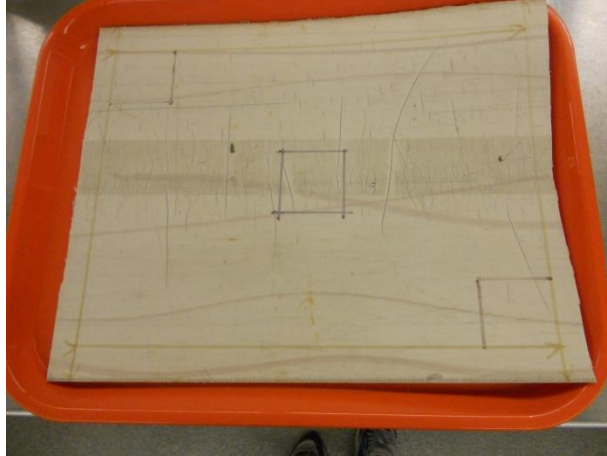


Figure I-0-3 Polyurethane conveyor belt



Figure I-0-4 Open hinge flat top conveyor belt



Figure I-0-5 Mesh conveyor belt

Appendix II: Sampling Devices



Figure II-0-6 Copan polyester tipped swab

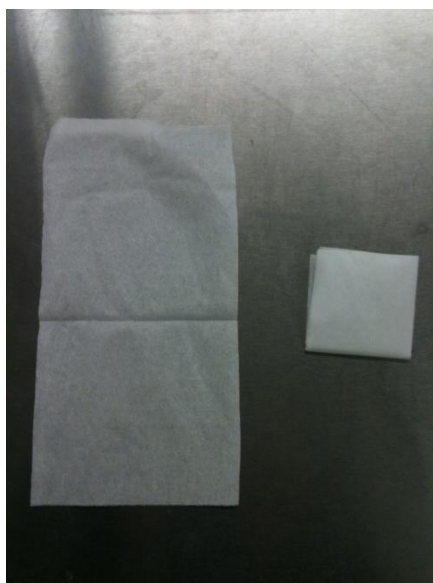


Figure II-0-7 Kim-wipe™ composite tissue



Figure II-0-8 3M cellulose sponge

Appendix III: *Listeria monocytogenes* SAS code

CB and SS only:

```
options ls=72 nonumber nodate;
%include 'c:\AS572\DandA.sas';

PROC IMPORT OUT= WORK.listeria
    DATAFILE= "C:\Users\Jessica Anne\Desktop\Listeria7Blks.xls"
    DBMS=EXCEL REPLACE;
    SHEET="Listeria";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;

*using the imported data, sum the 0,1 values across the reps to get the
counts of the number of times
    listeria was detected in each block by surface by treatment by highlow
combination;

proc means data=listeria mean std n;
class block surface treatment hilow;
var outcome;
output out=sums sum=outcome;
run;
data usesums; set sums;
if _type_=15;
num=3;
run;
proc means data=usesums mean std n ;
class surface treatment hilow;
var outcome;
run;

* run glimmix using the events/trials syntax or outcome/num;
* results show a surface by treatment by hilow interaction if you use
alpha=0.05;
* mean separation that follows slices by hilow and shows which treatments
differ
    by which surface for each category of hilow;
proc sort;
by surface;
proc glimmix data=usesums;
by surface;
class block surface treatment hilow;
model outcome/num=treatment|hilow/ link=logit dist=binomial;
random block;
lsmeans treatment*hilow/pdiff ilink ;
ods output lsmeans=mmm diffs=ppp;
title ' ';
run;

%pdmix800(ppp,mmm,slice=hilow);
```

FT, MB and PB only:

```
options ls=72 nonumber nodate;

PROC IMPORT OUT= WORK.listeria
            DATAFILE= "C:\Users\Jessica Anne\Desktop\Listeria7Blks.xls"
            DBMS=EXCEL REPLACE;
    SHEET="Listeria All";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;

%include 'c:\AS572\DandA.sas';

options ls=72 nonumber nodate;

proc means data=listeria std n sum;
class block surface treatment hilow;
var outcome;
output out=sums sum=outcome n=number;
run;

*drop some of the summary records in the sums dataset;
data usesums; set sums;
if _type_=15;
run;

proc means data=listeria std n sum ;
class surface treatment hilow;
var outcome;
run;

* For surfaces 4 and 5, run glimmix using the events/trials syntax or
outcome/num;
* The three-way interaction will not converge so I've reduced the model
effects;
* I found that the surface by treatment interaction was sig. as was the
simple
  effect of hilow;
* mean separation that follows slices by surface and shows which treatments
differ
  by surface, the lsmeans for hilow is included but there is no mean
separation
  since there are only two levels;
proc sort data=listeria;
by surface ;
proc mixed data=listeria;
by surface ;
  where surface in(4,5);
  class block surface treatment hilow;
  model outcome= treatment|hilow/ outp=rrr;
  random block;
  lsmeans treatment|hilow /pdiff ;
```

```

ods output lsmeans=mmm diffs=ppp;
title ' ';
run;
proc univariate plot normal data=rrr;
var resid;
run;

%pdmix800(ppp,mmm, slice=hilow);

* For surface 3, I could not get convergence with hilow in the model so I ran
  the treatment model separately for the levels of hilow;
*But hilow=1 will not converge;

proc glimmix data=usesums;
  where hilow=1 and surface=3;
  class block treatment;
  model outcome/number=treatment/ link=logit dist=binomial;
  random block;
  lsmeans treatment/ pdiff ilink;
  title ' Surface 3, Hilow=1 ';
run;
*Here is why it does not converge,
  recovery is complete, it does not vary by treatment for hilow=1,
  surface=3;
*You can also see this in the descriptive stats for surface 3 below;
proc freq data=usesums;
  where surface=3;
  tables hilow*treatment;
  weight outcome; run;

*Hilow=2;
proc glimmix data=usesums;
  where hilow=2 and surface=3;
  class block treatment;
  model outcome/number=treatment/ link=logit dist=binomial;
  random block;
  title ' Surface 3, Hilow=2 ';
  lsmeans treatment/pdiff ilink;
  ods output lsmeans=mmm diffs=ppp;
run;
%pdmix800(ppp,mmm, alpha=0.05);

```

Appendix IV: Brochothrix thermosphacta SAS code

```
options ls=72 nonumber nodate;

PROC IMPORT OUT= WORK.brox
            DATAFILE= "C:\Users\Jessica Anne\Documents\BrAll.xls"
            DBMS=EXCEL REPLACE;
    SHEET="BrAll";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;

%include 'c:\AS572\DandA.sas';

*add labels for surface;
proc format;
    value trt 1='PolyBelt'
              2='StSteel'
              3='CtBoard'
              4='MshBoard'
              5='FltTop';

data use; set brox;
format surface trt.;
run;

data day1to5;
set use;
if day<=5;
if method='Swab' then delete;
if surface='4' then delete;
run;

data surf1;
set use;
if day<=5;
if surface=1;
run;

data surf2;
set use;
if day<=5;
if surface=2;
run;

data surf3;
set use;
if day <=5;
if surface=3;
run;
```

```

data surf5;
set use;
if surface=5;
if day<=5;
run;

title 'RDB ANOVA on logof Difference: Days 1 to 5, Surf1';
%mmaov(surf1,diff,class=day surface method,fixed=surface|method,
    random=day day*surface*method,transtype=log,transvalue=.5);

    proc means data=surf1 n mean std maxdec=2;
    class surface method;
    var diff;
    types surface method surface*method;
    title2 'Descriptive Stats'; run;

title 'RDB ANOVA on logof Difference: Days 1 to 5, Surf2';
%mmaov(surf2,diff,class=day surface method,fixed=surface|method,
    random=day day*surface*method,transtype=log,transvalue=.5);

    proc means data=surf2 n mean std maxdec=2;
    class surface method;
    var diff;
    types surface method surface*method;
    title2 'Descriptive Stats'; run;

    title 'RDB ANOVA on logof Difference: Days 1 to 5, Surf3';
%mmaov(surf3,diff,class=day surface method,fixed=surface|method,
    random=day day*surface*method,transtype=log,transvalue=.5);

    proc means data=surf3 n mean std maxdec=2;
    class surface method;
    var diff;
    types surface method surface*method;
    title2 'Descriptive Stats'; run;

title 'RDB ANOVA on logof Difference: Days 1 to 5, Surfaces 5, Swab method
included';
%mmaov(surf5,diff,class=day surface method,fixed=surface|method,
    random=day day*surface*method,transtype=log,transvalue=.5);

    proc means data=surf5 n mean std maxdec=2;
    class surface method;
    var diff;
    types surface method surface*method;
    title 'RDB ANOVA on logof Difference: Days 1 to 5, Surfaces 5,
Swab method included';
    title2 'Descriptive Stats'; run;

```

Vita

Jessica Clemons was born in Knoxville, Tennessee on September 12, 1983. She was raised in Clinton, TN by her father and mother, Landy and Cindy Clemons along with her grandparents, Wayne and Jean Perryman. She has one younger brother, Jordan Clemons. She graduated from Clinton High School in 2001 and received her Bachelor of Science in Agriculture from the University of Tennessee in 2005. She began graduate school at UT in the spring of 2009 where she worked under the supervision of Dr. David Golden, Dr. Bill Morris and Dr. Majorie Penfield as a graduate research assistant. She graduated with a Master of Food Science and Technology/Minor in Statistics in December 2010. Jessica plans to move to Arequipa, Peru in January 2011 to begin short-term mission work helping to spreading the gospel to the unreached people in the world. There she will work on an oregano farm assisting with the production, implementing good agricultural practices and establishing international trade to benefit local farmers in Peru while serving God in a daily pursuit of the road less traveled.