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To the Window, To the Wall: Improving Environmental Monitoring Protocols

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Food Science

by

Sarah L. Jones
Kansas State University
Bachelor of Science in Food Science and Industry, 2017

May 2022
University of Arkansas

This dissertation is approved for the recommendation to the Graduate Council.

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Abstract

Detecting microorganisms on environmental surfaces via an environmental monitoring (EM) program is part of a preventive food safety culture. Environmental monitoring should 1) verify that food safety plans are reducing cross-contamination risk from surfaces to food, 2) pinpoint microbial niches, and 3) prevent the transmission of pathogens. Environmental monitoring programs utilize EM tools, such as sponges, to sample food contact and non-food contact surfaces. However, EM tool selection is determined by the individual food firm. This dissertation evaluated and characterized factors influencing EM program effectiveness in the food industry. Specifically, this dissertation focuses on the release of microorganisms from EM tools and the recovery of microorganisms from environmental surfaces. First, the release of microorganisms from EM tools was investigated. This study characterized polyurethane foam (PUF) and cellulose (CELL) EM tools for their ability to release foodborne pathogens (*Listeria monocytogenes*, *Salmonella* Typhimurium, Tulane virus [TV; human norovirus surrogate]) from their sponge matrices. This study aimed to 1) compare the ability of EM tools to release microorganisms, 2) characterize EM tool performance at decreasing inoculum concentrations, and 3) assess the impact of elution method during EM sample processing. Data indicated that EM tool type impacted microbial release (p=0.0001), whereas the PUF EM tool released microorganisms more readily than the CELL EM tool. Conversely, no significant differences were observed across inoculum levels or elution method (stomacher versus manual). Next, the influence of environmental factors on the recovery of microorganisms using the PUF EM tool was determined. The specific objective of this study was to determine if environmental conditions and surface composition impact the recovery of select microorganisms found in food processing environments. These data were compared across 1) microorganism type (*L. monocytogenes*, *S.* Typhimurium, TV), 2) surface type (polypropylene, stainless steel, neoprene), 3) environmental temperature and relative humidity (30°C/30%, 6°C/85%, 30°C/85%), and 4) exposure time (24 h or 72 h). Data indicate that microbial recovery from

environmental surfaces significantly ($p \leq 0.05$) varies by microorganism type, environmental conditions, and exposure time. However, overall, surface type did not significantly impact the recovery of microorganisms. Then, research focused on pathogen-food associated pairs and the impact of food residues on microbial recovery. Data generated from studies on *L. monocytogenes* and ready-to-eat food residues (lettuce rinsate, blended lettuce, whole milk, lowfat milk) suggest little variability in recovery amongst food residue types overall with greater differences apparent at the 24 h sampling period. Conversely, studies on *S.* Typhimurium and low water activity foods (all-purpose flour, whole milk dairy powder, infant formula) show significant differences in the mean log loss of *S*. Typhimurium ($p < 0.05$) between all food residue types at 1 h and 24 h sampling times. Moreover, significant differences were found within each food residue type over time. Overall, the findings within this dissertation provide insights on the factors that influence microbial release and recovery from EM tools utilized in the food industry. This information will assist the food industry in making informed decisions about EM programs and enhance EM protocols.

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Dedication

This dissertation is dedicated to my parents, Michael and Debra Jones.

To Mom and Dad,

You have made me who I am today and have supported every adventure I have pursued. I am forever grateful for your love, support, and kindness. I carry my love for you both wherever I go. I am so proud to be your daughter.

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Chapter 3. Jones, S. L. & Gibson, K. E. (2021). Factors impacting microbial release from environmental monitoring tools. *International Journal of Food Microbiology*, *347*, 109198. doi: https://doi.org/10.1016/j.ijfoodmicro.2021.109198. Published.

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Chapter 1: Introduction: Environmental Monitoring Programs in the Food Industry

1. Environmental Monitoring

Foodborne illness is an important public health issue, with an estimated 48 million illnesses annually in the U.S. (Scallan et al., 2011a; 2011b). Food can become contaminated with pathogens at any point in the food chain; therefore, food manufacturers must mitigate public health risks by routinely preventing and controlling microbiological hazards. One of the avenues that foodborne pathogens are introduced into the food supply is via cross-contamination, where one contaminated product, or surface, transfers the pathogen to non-contaminated products, thus spreading the microbiological hazard (Pérez-Rodríguez et al., 2008). For example, cross-contamination can be caused by insufficient hygiene practices, contaminated equipment, food handlers, or further product processing (Carrasco et al., 2012).

In 2011, the FDA's Food Safety Modernization Act (FSMA) was signed into law. Over nearly a decade, FSMA's rules and regulations have been promulgated across the food industry. Within the FSMA Final Rule for Preventive Controls for Human Food (PCHF), the requirement for environmental monitoring (EM) programs was outlined. Environmental monitoring is an established environmental surface swabbing program unique to each food processing facility. Environmental monitoring sampling is conducted by utilizing a tool (e.g., a swab or sponge), swiping the tool across the surface, and then sending the sample to be tested for select microorganisms (e.g., *Listeria monocytogenes*). The purpose of EM programs is multifaceted. For instance, to seek-and-destroy microorganisms within a food processing facility, an EM program attempts to find the pathogen or non-pathogenic microbial indicators of concern so that corrective actions can be made before the product is compromised.

Additionally, EM programs are frequently employed within food processing facilities to evaluate the efficacy of preventive controls and verify if surface cleaning and sanitation programs are effective (Ismaïl et al., 2013). Finally, environmental surfaces are sampled during other investigative surface sampling events, such as foodborne disease outbreaks (FBDO) and

regulatory inspections. However, while EM is required under FSMA and regulatory rules and guidance documents have been developed to help food manufacturers create EM programs using best practices, there is no standardization for the EM tools themselves.

2. Microorganisms of Concern

Many foodborne pathogens are of concern in the food supply (Scallan et al., 2011b). However, some pathogens are associated with certain foods and/or their ingredients, while others are associated with the manufacturing environment. For instance, *L. monocytogenes* is most often introduced via the contaminated food processing environment and not through the raw contaminated product (Lin et al., 2006). *L. monocytogenes* is a foodborne bacterial pathogen of significant concern to the food industry (Teixeira et al., 2007; Poimenidou et al., 2009). Listeriosis in humans is uncommon but serious, with a fatality rate of 20 to 30% (Swaminathan and Gerner-Smidt, 2007). *L. monocytogenes* can grow and persist in adverse conditions where many pathogens would not, such as growth in temperatures as low as 2°C (Rocourt and Bille, 1997) and salt concentrations up to 10% (te Giffel and Zwietering, 1999). Thus, *L. monocytogenes* can persist in the food manufacturing environment for extended periods of time.

Salmonella spp. are a group of pathogenic bacteria responsible for one of the most frequent foodborne diseases in the United States (Teixeira et al., 2007). While there are many serotypes of *Salmonella*, *Salmonella enterica* serovars Enteritidis and Typhimurium are the *Salmonella* types most frequently associated with foodborne illness (Liu et al., 2011). *Salmonella* can reside on various food-contact surfaces with different degrees of adhesion (Teixeira et al. 2007). During a study of bacteria adhesion to food contact surfaces by Teixeira et al. (2007), *Salmonella* strains strongly adhered to stainless steel, a surface found extensively in food processing. Environmental monitoring of *Salmonella* within a facility is important for hygienic monitoring due to its prevalence in raw product and food processing environments.

While bacteria such as *Salmonella* and *L. monocytogenes* are of the utmost concern in food processing facilities, there are pathogenic microorganisms of concern that are not routinely monitored. Despite causing approximately 58% of all foodborne illnesses in the U.S. annually, human norovirus is often not monitored on food contact surfaces and the adjacent, non-food contact surfaces. While cleaning and sanitation programs, such as standard sanitary operating procedures (SSOPs) and current good manufacturing practices (cGMPs), aim to monitor and control microbiological activity in the food processing environment, these regulations are based on bacteria (Bosch et al, 2018; Fortin et al., 2021). Overall, in the U.S., there is no standardized environmental swabbing method available for human enteric viruses which are known for notable transmission via contaminated environmental surfaces (Rzeżutka and Cook, 2004; Boone and Gerba, 2007; Hall, 2012).

3. Influential Factors in Environmental Monitoring

Environmental monitoring is a broad-brushed program promulgated by the FSMA PCHF and some portions of the FSMA Final Rule on Produce Safety (FDA, 2015a; 2015b). However, despite EM being a required activity for nearly all food manufacturers, little consideration has gone into the factors that can influence the efficacy of EM programs. Naturally, due to the diverse nature of the food supply, many factors can influence EM programs. Extrinsic factors that can influence the microbial recovery in EM programs include, but are not limited to, the surface material type, types of microorganisms, the number of microorganisms present on the surface, moisture on the surface, surface sampling area, organic load on the surface, and the EM tool type and material. For example, FSMA does not designate a specific EM sampling tool material or type for EM, but sponges and swabs are recommended (Feng et al., 1998, Andrews et al., 2002; FDA, 2015c). In the past, cellulose swabs and sponges have been the most common sampling material used in EM (personal communication). Today, EM sampling devices are made from alternative polymers, such as polyurethane, rayon, and polyester, which are in addition to cellulose. Ultimately, the decision is left up to the food firm to decide what EM tool to

select. Thus, due to all of these factors, the appropriateness and efficacy of EM programs may be difficult to determine.

4. Improving Environmental Monitoring Programs

In this dissertation, the research has focused on the characterization of EM tools and identified the external factors influencing EM program efficacy. This research aims to enhance EM programs in the food industry. An in-depth look at the current state of EM in the food industry was conducted to identify the factors that impact EM programs (Chapter 1).

This led to exploring and evaluating factors impacting microbial release from environmental monitoring tools (Chapter 2). This focused on the release of microorganisms by 1) comparing the ability of EM tools to release *L. monocytogenes*, *S.* Typhimurium, and Tulane virus (TV), a human norovirus surrogate, into a recovery eluent, 2) characterizing EM tool performance at decreasing inoculum concentrations, and 3) assessing the impact of various operators during the processing of EM samples before ever introducing the environmental surface into the equation. These data can assist in determining the appropriateness of the EM tool selected for use within the food industry. To continue characterizing other aspects of EM programs, this dissertation explores the environmental factors of exposure time, temperature and relative humidity, and the surface type and their impact on the microbial recovery of *L. monocytogenes*, *S.* Typhimurium, and Tulane virus (TV) from surfaces (Chapter 3). These data focused on the recovery of microorganisms from clean surfaces and are critical to understanding environmental sampling after cleaning and sanitation. However, not all EM sampling is conducted on clean surfaces.

In order to understand the influence of soiled surfaces on EM programs, organic matter (i.e., food residues) were added to the surfaces (Chapters 4 and 5). Foodborne microorganisms are frequently attributed to contamination of certain foods. Historically, *L. monocytogenes* is often associated with ready-to-eat food products, including fresh produce and dairy products (FDA, 2020). On the other hand, low water activity food products, such as flour and tree nuts,

are becoming more frequently associated with pathogens such as *Salmonella*. Lastly, to better determine the influence of organic matter on the microbial recovery from surfaces, the recovery of *L. monocytogenes* from surfaces with whole milk, lowfat milk, lettuce rinsate, and blended lettuce were evaluated (Chapter 4). Similar research was completed evaluating the influence of all-purpose flour, whole milk dairy powder, and infant formula on the recovery of *S.* Typhimurium from surfaces (Chapter 5).

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Chapter 2: Swabbing the surface: Critical factors in environmental monitoring and a path towards standardization and improvement

Abstract

Cross-contamination can be broadly defined as the transfer, direct or indirect, of microorganisms from a contaminated product to a non-contaminated product. Events that may result in cross-contamination include inadequate hygiene practices, contaminated equipment surfaces, contamination via food handling personnel, further product processing, or storage abuse. All of these niches require consistent environmental surveillance systems to monitor microbial harborage sites to prevent foodborne illnesses via cross-contamination. Environmental surveillance is achieved through routine surface sampling of the food contact surfaces and surrounding areas. To better understand cross-contamination, the role of environmental surface transmission during outbreaks due to the presence and persistence of pathogenic microorganisms on various food contact surfaces must be investigated. However, studies on environmental sampling techniques are rarely performed in an actual food processing environment but rather under controlled variables within a laboratory-setting. Moreover, results and conclusions of studies differ because of the considerable variability across surface sampling tools due to individual operator dependency, low recovery rates, and low reproducibility. Information is also often lacking on environmental sampling tools used within a processing facility, the characterization of these tools, and the optimization of recovery of microorganisms for surface sampling. Thus, this review aims to: (1) discuss and compare factors impacting the recovery of microorganisms and the standardization of surface sampling methods for optimal recovery of microorganisms and (2) examine how research strategies could focus more towards the development of standard methodologies for surface sampling.

1. Introduction

In the United States, an estimated 47.8 million people acquire foodborne illnesses from contaminated food and beverages—a common and impactful problem resulting in over 1,000

fatalities annually (Centers for Disease Control and Prevention 2018a, Scallan et al. 2011). Bacteria, viruses, and parasites can all cause foodborne disease, and the most prominent infectious agents implicated in foodborne disease outbreaks are viruses with bacteria following a close second (Li et al. 2012). An estimated 9.4 million illnesses are caused by 31 major pathogens, and of those, human norovirus (hNoV) is the leading causative agent resulting in 58% of the foodborne illnesses domestically (Scallan et al. 2011) and an additional 125 million cases worldwide (Kirk et al. 2015). The next most prevalent causative agent is nontyphoidal *Salmonella* contributing 11% of the estimated foodborne illnesses in the U.S. (Scallan et al. 2011).

While food can become contaminated with pathogens at any point in the food chain, foods associated with most of the outbreak-associated illnesses are those consumed with little processing (Centers for Disease Control and Prevention 2017a, Bhunia 2008). For instance, a food product not requiring any further inactivation steps (e.g., thermal treatment) prior to consumption leaves the consumer more vulnerable to foodborne infection or intoxication (Painter et al. 2013; Gould et al. 2011). To mitigate this public health risk, food processing facilities must routinely prevent and control any microbiological threats. However, monitoring and controlling microorganisms within the food-manufacturing environment has proven to be a constant challenge due to the diversity of reservoirs and growth environments of pathogens. Often animal reservoirs can be a source of bacteriological pathogens, such as *Escherichia coli* and *Salmonella*, by shedding the bacteria through their feces (Swartz 2002; Himathongkham et al. 2000; Callaway et al. 2006; Van Donkersgoed, Graham, and Gannon 1999; Edrington et al. 2006; Munns et al. 2015). Meanwhile, pathogens such as *Campylobacter* are present in the microbiota in the lower gastrointestinal tract of animals and may contaminate via evisceration processes (Horrocks et al. 2009). Other pathogens may be ubiquitous in the environment (e.g. *Listeria monocytogenes*) (Farber and Peterkin 1991) or humans may be the primary reservoir (e.g. *Staphylococcus aureus*) (Argudín, Mendoza, and Rodicio 2010). This diversity amongst

reservoirs requires a multifaceted system to prevent entry of the microorganisms into the food processing facility. However, the direct transmission of the microorganism from a particular reservoir is not the only vehicle for foodborne pathogens. Cross-contamination tends to exacerbate the spread of microorganisms throughout a food processing facility (Carrasco, Morales-Rueda, and García-Gimeno 2012).

Cross-contamination can be broadly defined as the "term which refers to the transfer, direct or indirect, of bacteria or virus from a contaminated product to a non-contaminated product" (Pérez-Rodríguez et al. 2008). Cross-contamination can result from events involving inadequate hygiene practices, contaminated equipment surfaces, contamination via food handling personnel, further product processing, or storage abuse (Carrasco, Morales-Rueda, and García-Gimeno 2012). All of these niches require consistent environmental surveillance systems to monitor microbial harborage sites to prevent foodborne illnesses via cross-contamination (Tompkin 2002). Environmental surveillance is achieved through routine surface sampling of the food contact surfaces and surrounding areas. To better understand cross-contamination, the role of environmental surface transmission during outbreaks due to the presence and persistence of pathogenic microorganisms on various food contact surfaces must be investigated. However, despite the demand for routine surveillance within a food processing facility, studies on environmental sampling techniques are rarely performed in an actual food processing environment and are normally aimed at sampling method optimization during foodborne outbreaks. Results and conclusions of studies differ because of the considerable variability across surface sampling tools due to individual operator dependency, low recovery rates, and low reproducibility (Favero et al., 1968; Ismaïl et al., 2013; Moore and Griffith, 2007). In addition, information is often lacking on environmental sampling tools used within a processing facility, the characterization of environmental monitoring tools, and the optimization of recovery of viruses and bacteria for surface sampling. Thus, this review aims to: (1) discuss and compare factors impacting the recovery of microorganisms and the standardization of

surface sampling methods for optimal recovery of microorganisms and (2) examine how research strategies could focus more on the development of standard methodologies for surface sampling.

2. Background

2.1. Microbial transmission due to environmental surface contamination

Microbiological contamination of environmental surfaces in the food industry is a well-established transmission pathway for numerous pathogenic microorganisms (Lahou and Uyttendaele 2014; Otter, Yezli, and French 2011). Two factors should be accounted for when assessing the risk of foodborne pathogenic infections associated with cross-contamination: the level of microbial contamination on the surfaces and the probability of its transfer to the food itself (Bloomfield and Scott 1997). For instance, *L. monocytogenes* is most often introduced via the food processing environment, not raw contaminated product (Lin et al. 2006). Thus, once microorganisms are unintentionally introduced into the food system, the presence of pathogenic microorganisms on food contact surfaces can result in a cross-contaminated food product. For example, a study of *L. monocytogenes* contamination in a cold-smoked rainbow trout processing plant revealed that the predominant *L. monocytogenes* isolates from finished food products were associated with brining and slicing operations in further processing, not the raw materials (Autio et al. 1999).

2.2. The impact of microbial sampling in a historical and economic context

Historically, the past standard procedure for recalls has been confirmation of a contaminant on the actual finished product—a detected threat. Thus, in the past, rigorous microbial sampling has been the general reaction to foodborne illnesses, such as the 1990's response to *E. coli* O157:H7 involved in a chain outbreak that served undercooked hamburger patties resulting in 731 confirmed cases, 170 hospitalizations, 56 cases of hemolytic uremic syndrome (HUS) and the death of four children (Bell et al. 1994). This devastating *E. coli* outbreak garnered the attention of the general public and lawmakers alike. As reviewed by

authors Baker et al. (2016a), noted public outcry pushed policymakers to improving food safety monitoring procedures within the meat industry as well as motivated the U.S. Department of Agriculture to initially recognize *E. coli* O157:H7 followed by six other Shiga toxin (Stx)-producing *E. coli* (STEC) and their Stx toxins, as adulterants in raw, non-intact ground beef products.

Once a pathogen becomes recognized as an adulterant in the food supply, there is an increased demand for development of rapid and sensitive detection methods for application in foods associated with low infectious dose pathogens (e.g., STEC) (Baker et al. 2016b). Moreover, as pathogens evolve and diversity increases, new adulterants will inevitably be identified, and sufficient methods will need to be continually modified to meet the demands of the public. However, this adaptation has proven challenging in the past due to the "physiological elusiveness" of pathogens such as STECs and their uncanny ability to adapt to their environment (Baker et al. 2016b). Due to the complex nature of the food supply, the efficacy of an assay can be dependent on factors such as food type, ingredients, and quality so there is a validation and verification procedure required for untried food systems (Hoorfar 2011; Fu, Rogelj, and Kieft 2005; Baker et al. 2016a). For instance, the age and concentration of organic molecules can vary in meat products and thus alter the function of an assay potentially leading to false negatives or even false positives (Fu, Rogelj, and Kieft 2005; Baker et al. 2016b). Although the adaptation to the ever-changing genetic lineages and phenotypic transitions of microorganisms has proven to be challenging, the subsequent methodology success and failures are critical to the food industry and public health as a whole.

More recently, the Peanut Corporation of America's (PCA) deadly *Salmonella* Typhimurium outbreak resulted in 714 persons ill with salmonellosis in 2009 (Centers for Disease Control and Prevention 2009). This outbreak was attributed to insanitary processing and widespread environmental contamination within multiple PCA food processing facilities (Leighton 2016). The lack of routine environmental monitoring and other criminal negligence led

to the widespread *Salmonella* outbreak (Leighton 2016). This multi-state *Salmonella* outbreak, and others, caught the attention of policymakers. Soon thereafter an overhaul of the United States' food safety regulations was brought into law.

Enacted in 2011, the U.S. Food and Drug Administration Food Safety Modernization Act (FSMA) aims to ensure the safety of the U.S. food supply by moving the focus from reacting to pathogenic contamination to preventing its occurrence within the food supply (U.S. Food and Drug Administration 2018a). Within FSMA, the Preventive Controls for Human Food (PCHF) Rule highlights the importance of an effective environmental monitoring program. This environmental monitoring program exhaustively tries to find the pathogen or non-pathogenic microbial indicators of concern so that corrective action can be made before the product is compromised (Food Safety Preventive Controls Alliance 2016, U.S. Food and Drug Administration 2018c). For example, in 2015, there were 626 recalls due to all causes (Mayberry 2016). Comparatively, in 2016, there were over 700 recalls due to all causes with 196 recalls attributed to potential *Listeria* contamination alone (Mayberry 2017). It should be noted that while an increase in recalls seems unfavorable to producers of food products, recalls are an important tool for preventing additional illnesses caused by the specific pathogen detected. A more recent analysis of the cost of a food product recall by Food Safety Tech (2017) considered the following as direct areas of cost: assembling a crisis team, removing the product from the market, shipping of product, investigating and addressing the cause, and managing the public relations fallout. Meanwhile, indirect areas of cost include litigation, stock value decline, fines, loss in sales, and overall impact on brand reputation (Food Safety Tech Staff 2017). The minimum direct costs of a recall can be demonstrated through the following example provided by Food Safety Tech. In 2016, 10 million pounds (>4,000 metric tons) of flour were recalled after it was linked to an outbreak of *E. coli* O121, and the estimated minimum direct cost of this recall was $5.7 million USD (Food Safety Tech Staff 2017). Overall, food product recalls resulting from a recognized foodborne disease outbreak can be costly. In a recent analysis of USDA-FSIS

recalls by Seys et al. (2017), *Salmonella* illness-related recalls were associated with 1) larger amounts of recalled product, 2) smaller percentages of recalled product recovered, and 3) greater days between production date and recall dates when compared to non-illness related recalls (e.g., detection of *Salmonella* in the food processing environment) . Moreover, based on data from 2000 to 2012, it is estimated that *Salmonella* illness-related recalls prevented 19,000 illnesses while non-illness related recalls prevented an additional 8,300 illnesses, or 27,300 total illnesses. Thus, pathogen environmental monitoring programs implemented under FSMA are likely going to play a greater role in removing potentially contaminated product from the market prior to an outbreak. To satisfy these environmental monitoring programs, routine surface sampling is carried out throughout food processing facilities.

2.3. Current requirements and criteria for surface sampling and analysis

As cited in Section 2.2., the FDA's FSMA aims to ensure the safety of the U.S. food supply by shifting the focus to a preventive approach rather than reactive. An aspect of both the Produce Safety Rule (PSR) and the PCHF includes the requirement for environmental monitoring where a food processing facility is kept in a sanitary condition to prevent hazards such as environmental pathogens (U.S. Food and Drug Administration 2018c). Potential biological hazards include pathogens such as *L. monocytogenes* (Gaul et al. 2013; Pouillot et al. 2016; Samadpour et al. 2006; Aureli et al. 2000; Centers for Disease Control and Prevention 2011), nontyphoidal *Salmonella* serovars (Harvey et al. 2017; Angelo et al. 2015; Centers for Disease Control and Prevention 2018b; 2017b), and human enteric viruses (Centers for Disease Control and Prevention 2013; 2016; Sarvikivi et al. 2012). Overall, these pathogens are capable of persisting within the manufacturing, processing, packing, or holding environments that may contaminate food and result in illness if that food is consumed without a proper kill-step treatment.

To prevent cross-contamination from surfaces to food, environmental monitoring systems play a significant role within food processing facilities to assess the efficacy of

preventive controls and verify if surface cleaning and sanitation programs are effective (Ismaïl et al. 2013). Food processing facilities are not alone when it comes to environmental sampling. The United States' regulatory agencies similarly complete routine microbiological testing of environmental surfaces during inspections of food processing facilities as well as during foodborne disease outbreak investigations (U.S. Food and Drug Administration 2018b).

Since the FDA expansion of the criteria for recalls, including the presence of contaminants in the general environment where the food is processed, more environmental monitoring is being conducted as a result of FSMA and its related rules (U.S. Food and Drug Administration 2018c). With the implementation of PCHF, more products are being recalled due to finding *Listeria* in the food processing environment, where it is often found, indicating the shift from reactive to preventive approaches within the food industry. For instance, a hummus manufacturer recalled several products due to concerns over *L. monocytogenes*, which was identified at the processing facility but not in tested finished product (U.S. Food and Drug Administration 2016). Considering this expansion of recalls, it is important to understand the multifaceted factors impacting the recovery of microorganisms from the environmental surfaces.

3. Factors impacting the recovery of microorganisms from surfaces

3.1. Surface type

During environmental monitoring, there are many different factors impacting the recoverability of microorganisms from surfaces. One of those factors is the surface type being sampled. Each surface is defined by its own characteristics such as configuration, porosity, surface roughness, material composition, and hydrophobicity (Briandet et al. 1999; Cunliffe et al. 1999; Flint, Brooks, and Bremer 2000; Hood and Zottola 1997; Jullien et al. 2003). While not limited to the aforementioned characteristics, these surfaces must be thoroughly understood to select the most appropriate sampling devices. Food contact surfaces are typically chosen for their ability to be cleaned during the sanitation cycles at the food processing facility (Schmidt and Erickson 2009). If the surface is not easily washed and sanitized, it would not be chosen for

a food processing facility whether it is a direct food contact surfaces or in the surrounding areas (Jullien et al. 2003). Ideally, materials would be smooth, non-porous, and abrasion resistant. Often, stainless steel, plastics, rubber, and concrete components are all commonly found within a processing facility (Speers et al. 1984; Ayebah and Hung 2005; Beresford, Andrew, and Shama 2001; Holah and Thorpe 1990).

Studies assessing the impact of food contact surfaces on the recovery of enteric viruses have been thoroughly reviewed by Turnage and Gibson (2017). The authors concluded that surface type has been shown to influence surface sampling recovery efficiencies of enteric viruses (Turnage and Gibson 2017). For example, stainless steel, which is perhaps the most common surface in a processing facility, is a hydrophilic and negatively charged surface. There has been a noted irreversible attachment between the stainless steel and select microorganisms within one minute leading to a more challenging surface recovery . Moreover, studies by Silva et al. (2008) and Lahou and Uyttendaele (2014) both demonstrated a decreased viability on stainless steel surfaces in comparison with polypropylene and high-density polyethylene surfaces, respectively . From these studies it was concluded that recovery is also affected by the composition of the surface. Surface types can also impact the ability to sanitize the surface if a biofilm is present – this is addressed further in Section 3.2. Additionally, Taku et al. (2002) reported that a greater recovery efficiency of microorganisms could be achieved with a given sampling device if the collection buffer was allowed to cover the surface for 15 minutes . This concept, however, is limited to surfaces that are horizontal and thus not applicable for an all-encompassing environmental monitoring program involving routine sampling of the numerous vertical surfaces (e.g., walls, sides of machinery) and other small niches (e.g., wheels on equipment) routinely sampled.

3.2. Types of microorganisms and density

In addition to surface characteristics, the types of microorganisms and their density on the surface impact the recovery ability. As addressed previously, human enteric viruses cause

the most foodborne related illnesses worldwide. While viruses are not currently considered during mandated environmental monitoring programs, enteric viruses, such as hNoV, can survive on fomite surfaces for an extended period of time, and the case for virus-targeted standardized environmental sampling is growing (Escudero et al. 2012; Wikswo et al. 2015). Turnage and Gibson (2017) acknowledge that the U.S. does not have an official methodology for enteric virus environmental monitoring, but it is noted that the Centers for Disease Control and Prevention (CDC) does recommend the use of swabs for collecting hNoV from environmental surfaces. However, the CDC has also reported that swabbing is highly variable and that the interpretation of results could be unreliable (Centers for Disease Control and Prevention 2012).

Another factor to consider when completing environmental monitoring is what type of bacteria are likely to be present on the food contact surface. Gram-positive bacteria, such as *Listeria* and *Clostridium*, contain a thick peptidoglycan layer comprised of proteins on the outer layer of the bacterium (Bhimji and Unakal 2018). In contrast, Gram-negative bacteria, such as *Salmonella, E. coli* and *Campylobacter*, present a much thinner peptidoglycan layer between their outer membrane and the inner cytosolic membrane (Bhimji and Unakal 2018). This morphology impacts the way bacteria present themselves on the surfaces. More recently, Keeratipibul et al. (2017) noted that bacterial Gram-type impacted the efficiency of bacterial recovery on dry surfaces. This is important to consider because *Listeria* (Gram-positive) and *Salmonella* (Gram-negative) are top priorities in the food industry in relation to environmental contaminants . Another important variable is the level of inoculation in laboratory-based studies. These laboratory-based studies are being used to identify recovery potential of environmental sampling tools. Studies often inoculate at high concentrations (> 10^4 colony forming units [CFU] per unit area) and yield promising results (as shown in Table 1), but then find when applied at a lower inoculation level, little to no recovery of microorganisms. More research is needed

following evaluation at high inoculation levels on the limit of detection of the sampling device through progressively lower inoculation levels.

An issue that often presents itself within the food industry is the formation of biofilms on surfaces (Van Houdt and Michiels 2010; Blackman and Frank 1996; Chmielewski and Frank 2003; Stepanović, Ćirković, and Ranin 2004). Biofilms are an aggregated group of bacteria on a surface that adhere to each other and the surface (Chmielewski and Frank 2003). During this aggregation, the bacterial cells begin producing extracellular polymeric substances (EPS) which help form and protect the biofilm itself thus enhancing its adherence (Carpentier and Cerf 1993). Multispecies biofilms can form on packaging and equipment surfaces, including stainless steel, plastic packaging, rubber, and glass found within a food processing facility (Krysinski, Brown, and Marchisello 1992). Biofilms have increasingly become recognized as a hazard within the food industry due to their increased, resident-like adherence to the surface compared to transient bacteria (Chmielewski and Frank 2003; Bredholt et al. 1999; Carpentier and Cerf 1993; Bridier et al. 2011; 2015)

For example, Pan, Breidt Jr., and Kathariou (2006) concluded that the resistance of bacterial cells in biofilms to a sanitizer was greater on the Teflon surfaces than on the stainless steel surfaces, demonstrating that microbial attachment is a multifaceted link not limited to one singular aspect. Bremer et al. (2002) reported that there was a significant difference in the effectiveness of the sanitizers against cells attached to the stainless steel surfaces than to the conveyer belt surfaces . Comparable results were found by Krysinski et al. (1992), who determined that the resistance of *L. monocytogenes* biofilms on stainless steel was less than that on polyester or polyester-blend surfaces. As illustrated, biofilm formation is a prominent issue within the food industry (Brooks and Flint 2008; Chmielewski and Frank 2003). Biofilms present an increased surface-adhesion and structural complexity compared to weakly associated microorganisms (Donlan 2002, Garrett, Bhakoo, and Zhang 2008). Consequently, the presence of biofilms may result in lower microorganism recovery using current

environmental monitoring tools (Branck et al. 2017). Further research is needed to address the recovery capabilities of environmental monitoring tools and biofilm formation (Cappitelli, Polo, and Villa 2014). Overall, the great variety of environmental pathogens and density of microorganisms on surfaces result in many types of environmental monitoring tools selected for use in microbial evaluations.

3.3. Sampling device options

For the FSMA related rules and regulations, there is not a mandated sampling tool required for environmental monitoring, but sponges and swabs are recommended (Feng et al. 1998; Andrews et al. 2002; U.S. Food and Drug Administration 2015). In the past, cellulose swabs and sponges have been the standard sampling material used in environmental monitoring. As sampling methods have advanced, different materials have been applied to the same swab or sponge shape. Now, there are sampling devices made from alternative polymers, such as polyurethane, rayon, and polyester. In addition to using the same sampling framework with new compositional materials, other sampling devices have incorporated new technologies. Additional approaches include wipes, sponges, and minirollers, all made of various materials (Moore and Griffith 2002; Keeratipibul et al. 2017; Gómez et al. 2012). These technologies as well as others are discussed in more detail in Section 4.

3.4. Drying time

Another key point to acknowledge is drying time, as in, identifying if the surface is wet during sampling, and if not, how long has the surface been dry. It has been established that microorganisms on dry surfaces have a lower survivability than if the surface has moisture present (Moore and Griffith 2002; Davidson et al. 1999). Although it is known that viability is limited on dry surfaces, it is important to understand to what degree drying time impacts surface sampling and microbial survivability (Davidson et al. 1999; Nocker et al. 2012). Drying conditions are not typically defined within an industrial processing environment due to the nature of food processing, and these conditions could underrepresent surface contamination (Verran et

al. 2010). In fact, Moore, Blair, and McDowell (2007) observed an average 2.47 log_{10}-reduction of viable *Salmonella* Typhimurium cells recovered from four surface types (stainless steel, Formica, polypropylene, and wood) within the first 1-hour period of drying. When this study was continued for an additional 5 hours a 0.95 average log_{10}-reduction occurred (Moore, Blair, and McDowell 2007). The slower decline in recovered bacterial cells suggests that the primary loss in viability occurs within the first hour of moisture evaporation (Moore, Blair, and McDowell 2007).

Even though viability is reduced on dry surfaces, it has also been suggested that some microorganisms can retain viability for several weeks (Wilks, Michels, and Keevil 2005) increasing the risk of cross contamination (Verran 2002). Authors Verran et al. (2010) concluded that when the inoculum had dried, recovery was reduced (recovery was approximately 30%, and became more reduced). The authors acknowledge that, outside of cell death, another impacting factor could be increased adherence of bacterial cells to the surface after drying (Verran et al. 2010). Furthermore, a study by Kusumaningrum et al. (2003) highlighted that foodborne pathogens may remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time (e.g., at least 96 hours for *S. aureus* at approximately 10^7 and 10^5 CFU/100 cm^2 initial contamination levels) . The viability of microorganisms in a dry environment can also be impacted by the characteristics and surface structures of the microorganisms which was addressed previously in Section 3.2. Additionally, Park et al. (2015) studied the impact of drying times of hNoV recovered from stainless steel surfaces. Testing multiple types of swabs at different drying times, Park et al. (2015) concluded that when the hNoVs are dried on surfaces there was a significant negative effect on sampling efficiency. For example, a macrofoam swab performed the best out of the various swab types analyzed (Park et al. 2015). With the macrofoam swab, the rates of hNoV recovery ranged from 18.2 to 25.7% when the drying time of the inoculum was less than 24 hours yet, after 48 hours

of drying, the rate of virus recovery was reduced significantly to approximately 10.0% (Park et al. 2015).

3.5. Types of elution buffers

During environmental monitoring, the respective sampling device is placed into an elution buffer for the microorganisms to be released for microbial analysis. To achieve accurate results, an elution buffer must (1) be able to neutralize any sanitizer that may be present on a surface sampled, (2) maintain microbial viability until the sampling device is processed, and (3) not cause any interference with interpreting microbial analysis assays. In addition to the three primary requirements of an elution buffer, some may also include growth promoting nutrients to help resuscitate injured bacteria (McFeters, Cameron, and LeChevallier 1982, Reasoner and Geldreich 1985) and contain properties that assist in the disruption of biofilms on the surface (Moore and Griffith 2007). For example, Tween 80, which is a surfactant, is present in Dey-Engley (D/E) neutralizing broth and may aid in release of cells from a surface (Moore and Griffith 2007). Moore and Griffith (2007) suggested that the presence of Tween 80 within a wetting solution is likely to reduce the surface tension of the liquid on the surface and may reduce the mechanical energy generated by the swabbing action, thus, minimizing bacterial injury.

Furthermore, choosing a buffer based on one's sampling needs can be challenging due to the logistical differences in applied-industry methods and laboratory-based experiments. Occasionally, laboratory-based experiments do not use neutralizing broths since the surface is sterile prior to inoculation thus eliminating the need to use common industry sanitizers unless the study is specifically addressing sanitizers (see Table 1). In fact, the comparison of microbial structures within a laboratory may not accurately represent what is present in a food processing environment due to a complex microbial system present, environmental conditions, and biofilm resistance. For instance, a study by Pan, Breidt Jr., and Kathariou (2006) suggests that biofilms repeatedly exposed to sanitizers (peroxide, quaternary ammonium, and chlorine) in a simulated

food processing plant developed resistance to the sanitizers over a period of three weeks. This could lead to issues using laboratory-based parameters, such as choice of media and recovery methods, in applied industry settings. The crossover of applied industry methodologies and laboratory-based studies is an avenue that needs further research exploration and collaboration.

3.6. Surface sampling area

Another factor impacting environmental sampling is the surface area being sampled. The U.S. Department of Agriculture and the FDA encourages the swabbed sampling area to be 12” × 12” (30.48 cm × 30.48 cm), but if the surface area being sampled is smaller, the entire surface is expected to be swabbed (U.S. Food and Drug Administration 2015; U.S. Department of Agriculture 2014). When completing the environmental monitoring, one must consider what type of surface is being tested, as in, where the food contact surface is located within the processing facility. There are two primary types of surfaces areas of concern being sampled (1) food contact surfaces (and surrounding areas) where food is in a post-lethality processing environment or is a ready-to-eat (RTE) product and could be exposed to pathogens and (2) an environmental testing program being used to verify sanitation efficacy (U.S. Food and Drug Administration 2018c).

To support food safety professionals, the U.S. FDA (2017a) has recommended dividing surface locations into four zones which are described as follows: Zone 1 is the easiest to define due to its rigid definition as a food contact surface where it is certain to contaminate a product if a pathogen is present. Zone 2 is an area that if contaminated with a pathogen there is a likelihood that zone 1 could become contaminated due to cross-contamination via human or machine. Zone 2 surfaces are typically in the same room as zone 1 surfaces. Zone 3 becomes a slightly more complicated to define as it is an area that if contaminated with a pathogen there is a likelihood that zone 2 could become contaminated due to cross-contamination via human or machine. Zone 3 surfaces areas may not be in the same room as zones 1 and 2, such as a warehouse, but could still introduce pathogens via cross-contamination. Zone 4 proves the

smallest risk for introducing foodborne pathogens into the food supply as it represents areas outside of the protection of the processing facility such as break rooms, restrooms, and shipping and receiving. The “zone concept” is based on the probability of product contamination if a foodborne pathogen were to be present within that zone. This hygienic zone concept is summarized in Table 2.

Furthermore, this zoning categorization sampling of a food processing facility has helped validate the need for environmental monitoring. For instance, a thorough review by Malley, Butts, and Wiedmann (2015) encourages the control of *L. monocytogenes* by environmental sampling, implementation of “seek and destroy” processes, and improved hygienic equipment and plant design. The authors strongly suggested that environmental sampling should promote microbial testing of both food contact surfaces and other environmental areas (e.g., zones 2 through 4) (Malley, Butts, and Wiedmann 2015). In addition, a study by Beno et al. (2016) developed, implemented, and evaluated environmental monitoring programs for small cheese processing facilities and reported that only two of nine facilities studied did not have *Listeria* present within the facility (zones 2 through 4) indicating the need for complete environmental monitoring.

Part of FSMA is the verification of sanitation programs to ensure that the facility is maintained in a sanitary condition thus ensuring high quality and safety (U.S. Food and Drug Administration 2018c). Although cleaning and sanitizing may be the most severe stress that microorganisms experience in a typical food processing facility, this environmental monitoring step is usually completed after the sanitation process to verify the facility is held at standard sanitary conditions (Pan, Breidt, and Kathariou 2006). The size of the sanitation verification sampling area is based on the discretion of the company and sanitation standard operating procedures (SSOPs) and is not directly addressed in FSMA. The USDA Food Safety and Inspection Service states that environmental surface sampling can be used as sanitation

verification and recommends using the 12” × 12” (30.48 cm × 30.48 cm) sampling area (U.S. Department of Agriculture 2012).

4. Methods for recovery of microorganisms from surfaces

4.1. Laboratory-based studies on the recovery of microorganisms

A large variety of different materials and methods are currently in the marketplace for environmental monitoring. Many of these have been used in the recovery of microorganisms from food contact surfaces in laboratory-based studies under varying conditions. Disparities between studies include, but are not limited to, microorganisms examined, surface area sampled, environmental conditions, and inoculum level. In the following subsections, further exploration of these differences and their impact will be discussed. Table 1 presents a summary of the surface sampling studies.

4.1.1. Swabs

As mentioned in Section 1, published studies highlight the immense variability across surface sampling tools due to operator dependency, low recovery rates, and minimal reproducibility (Favero et al. 1968; Ismaïl et al., 2013; Moore and Griffith, 2007). Part of this variability can be attributed to the sampling device. Swabs of various material types have been longstanding devices in environmental monitoring hence the numerous studies using swabs as a collection device (U.S. Food and Drug Administration 2015; Dalmaso et al. 2008; Davidson et al. 1999).

For example, after observing the variability of swabs, Lutz et al. (2013) determined that swabs were ideal for small surface sampling yet a poor option for larger surface areas (≥100 cm^2) which impacts the recovery quality of the device. This could impact how industry members choose what devices to use for sampling certain surfaces (e.g., choosing swabs only for small niches). Furthermore, Rönnqvist et al. (2013) when assessing the recovery of hNoV from stainless steel found that the swabs (polyester, flocked nylon, cotton wool) were outperformed by microfiber cloth wipes (Table 1). The microfiber cloth wipes reached almost 80% hNoV

recovery from stainless steel whereas the various swab types peaked hNoV recovery just over 50% when using premoistened sampling devices. Although, when comparing recovery efficiencies of different swab materials (macrofoam, rayon, cotton, and polyester), Park et al. (2015) found that the recovery of hNoV GII.4 was not significantly different among cotton, polyester, and rayon materials when sampled from stainless steel and toilet seat surfaces. Conversely, the macrofoam swabs tested presented significantly higher recovery efficiencies of hNoV GII.4 when compared to the other swab types (Park et al. 2015). A thorough study completed by Keeratipibul et al. (2017) assessed recovery of bacterial cells from various surfaces and under different conditions using a variety of swab types. The authors go on to report that swab efficiency was significantly influenced by the swab type (Keeratipibul et al. 2017). For the swab efficiency of each swab type on a wet surface, polyurethane foam and cellulose sponge swabs provided the highest recovery efficiency (94.5 and 94.4%, respectively), followed by gauze and cotton swabs (90.3 and 84%, respectively) (Keeratipibul et al. 2017). On dry surfaces, although the recovery efficiencies were decreased across all sampling devices, cotton swabs exhibited the lowest swab efficiency (48.5%) (Keeratipibul et al. 2017). While swabs are the traditional environmental collection device, evidence is building against their efficacy for the recovery of bacterial cells and virus particles.

4.1.2. Sponges

Sponges are another tool commonly recommended for use in environmental monitoring (U.S. Food and Drug Administration 2015; Keeratipibul et al. 2017; Gómez et al. 2012). Sponges typically have a larger surface area than swabs and are used when sampling large areas, such as the 12” × 12” (30.48 cm × 30.48 cm) area mentioned in Section 3.6. Sponge sampling devices, like swabs, can be made of varying materials (e.g, cellulose, polyurethane) (Pearce and Bolton 2005). Sponges have been suggested as an alternative to the traditional swabs (Moore and Griffith 2002), but both sampling methods have relatively low recoveries of

microorganisms. Notably this tends to occur when few bacteria are present on the surface (Yan et al. 2007).

For example, research by Lahou and Uyttendaele (2014) evaluated three swabbing devices for detection of *L. monocytogenes* on various food contact surfaces. During this study, the authors inoculated stainless steel, neoprene rubber, and high-density polyethylene with *L. monocytogenes* at 10^2 CFU/250 cm^2—a relatively low inoculation level compared to other laboratory-based surface sampling studies (Lahou and Uyttendaele 2014). The sampling systems used in the study were 3M™ Sponge-Stick, Copan Foam Spatula, and 3M™ Enviro Swab. The authors reported detection capabilities of the sampling tools for *L. monocytogenes* (Lahou and Uyttendaele 2014). Table 1 reports the percent of positive (detected) *L. monocytogenes* enriched samples for the dried stainless steel coupons (Lahou and Uyttendaele 2014). In contrast to other studies, the sponge-stick indicated the lowest amount of positive samples (66%) whereas the foam spatula and traditional swab yielded higher amounts of positive samples of *L. monocytogenes* (89%) (Lahou and Uyttendaele 2014). When combined with additional data from the study, the authors concluded that the different swabbing devices possessed equal detection abilities although they are composed of different materials (Lahou and Uyttendaele 2014). Moore and Griffith (2002) postulated a similar result when comparing traditional hygiene swabs to sponges. They used various sampling tools to detect coliforms on surfaces and found that the sampling sponge was the least effective means of detecting coliforms on a wet surface. (Moore and Griffith 2002). The sponge resulted in a minimum detection limit of approximately 100 CFU cm^2, whereas all other test methods were able to detect the presence of less than 3.5 CFU cm^2. To address this result, the authors hypothesized that the poor performance of the sponge could be due to ineffective bacterial release (Moore and Griffith 2002). Sampling sponges are very absorbent and can take up a greater volume of liquid compared to swabs (Moore and Griffith 2002). Any bacteria picked up during the sampling process can potentially become trapped within the sponge matrix (Daley, Pagotto, and Farber

1995). Moore and Griffith (2002) elaborated that the repeated compressions of the sponge during mastication of the sample within a diluent perhaps only exacerbate the problem, as the bacteria that are released may become reabsorbed into the sponge. Further research is needed to characterize what mechanisms are reducing the number of microorganisms recovered and subsequently reported when using sponges for environmental monitoring.

4.1.3. Cloths and wipes

Technologies including cloths and wipes are also being used for surface sampling. Cloths and wipes have been employed in laboratory-based studies and used in routine environmental surveillance as new surface sampling methods (Turci et al. 2003). As an example, Park et al. (2015) compared macrofoam swabs to antistatic wipes on large stainless steel surface areas (161.3 cm^2 and 645 cm^2). The rates of virus recovery with a macrofoam swab from stainless steel coupons of 161.3 and 645 cm^2 were 7.08 ± 2.21% and 2.3 ± 0.5%, respectively. Comparatively, antistatic wipes were reported to have recovery rates at 0.33 ± 0.21% and 0.30 ± 0.10%, respectively (Park et al. 2015). The study concluded that macrofoam swabs had greater than 10-fold higher levels of hNoV recovery from large surface areas than antistatic wipes (Park et al. 2015). Lutz et al. (2013) compared the performance of contact plates, electrostatic wipes, swabs, and a novel roller sampling device for the detection of *Staphylococcus aureus* on environmental surfaces. When analyzed, the mean CFU recovery across differing inoculation concentrations was highest for the wipe and the novel roller (Lutz et al. 2013). At lower contamination levels ($< 6\times10^3$ CFU per 100 cm^2), the wipe performed better than the roller (Lutz et al. 2013). In fact, the overall results of this study revealed that the electrostatic wipe and swab sampling methods were superior to the other sampling devices across a range of diverse contamination levels of *S. aureus* (Lutz et al. 2013). A study by Yan et al. (2007) reported similar results. In that study, the authors examined the use of single-ply composite tissues (CT) in an automated optical assay (Soleris) for the recovery of *Listeria* from food contact surfaces. The CT-Soleris and traditional culture methods were evaluated for

recovery of *Listeria* from inoculated stainless steel and high-density polyethylene surfaces (Yan et al. 2007). The proportion of inoculated stainless steel surfaces that were positive for *L. monocytogenes* at inoculation levels of 10^4, 10^2, and 10 CFU per plate using the CT-Soleris and traditional *Listeria* culture methods was 100, 93, and 75% and 100, 80, and 75%, respectively, with no significant differences ($P > 0.05$) between the methods (Yan et al. 2007). Complicated by other co-variables, the authors concluded that, overall, the CT-Soleris method compared well to the traditional culture methods for the detection of *L. monocytogenes* (Yan et al. 2007). Overall, wipes and cloths have the potential to be very useful in environmental monitoring programs established by food manufacturers. However, additional validation studies are needed—as with all sampling tools—and possible steps forward have been outlined in Section 6.

4.1.4. Alternative and emerging sampling technologies

Other surface sampling devices are being used in the food industry in the recovery of microorganisms on food contact surfaces. Emerging sampling devices include sonicating swabs and minirollers, all made of various materials, and represent just few of the alternative sampling systems used to collect microbial loads (Moore and Griffith 2002). For example, Gómez et al. (2012) determined that a novel miniroller device coated with wool fiber–velour generated a higher percentage of recoveries of *L. monocytogenes* after stomaching and agitation (6.27± 1.62% and 5.05 ± 2.19%, respectively) than conventional sampling tools (e.g., swab, sponge). This novel miniroller device may provide an effective alternative to the “gold standard” methods, but further analysis will need to be done. Brank et al. (2017) conducted a study using modified ultrasonic toothbrushes as novel sonicating swabs to remove *L. monocytogenes* biofilms. Sonication is an established method to aid in the removal of biofilms from surfaces (Zips, Schaule, and Flemming 1990) while maintaining cell viability (Kang et al. 2007; Ismaïl et al. 2013; Assere, Oulahal, and Carpentier 2008). More specifically, Brank et al. (2017) used a sonicating swab and a standard swab to remove biofilms from a stainless steel surface. The

authors noted that unswabbed control samples exhibited a median area of biofilm coverage of 97.9% whereas the sonicating swab and the standard swab exhibited a median area of biofilm coverage of 1.1% and 70.4% after swabbing, respectively. Results indicated that significantly less biofilm was left behind from the sonicating swab. While these results using a sonicating swab are promising in the removal of biofilms and surface sampling, further studies are needed to test the sonicating swab under other environmental conditions and with additional microorganisms.

Next-generation sampling devices may include the application of materials such as shape memory gels (SMG). SMG are materials among an evolving family of smart polymers that are influenced by thermodynamic stimuli (Liu, Qin, and Mather 2007). SMG can hold a permanent shape, be manipulated to an alternate shape under specific stimuli, and subsequently relax to the original, stress-free condition via thermodynamic command (Liu, Qin, and Mather 2007). However, the utility of SMG systems considered to date has been limited by the functionality, scale and interactivity of the gel, supporting structure, and corresponding trigger. Innovative technologies are improving the way environmental monitoring takes place. Further research is needed to validate these tools for widespread acceptance and adoption within the food industry and regulatory sector. This future research is discussed to a greater extent in Section 6.

4.2. Industry applications and governing body recommendations

Food industry processors are not the only ones who employ environmental sampling, the United States' regulatory agencies also conduct routine microbiological testing. Regulatory agencies test environmental surfaces during inspections of food processing facilities as well as during foodborne disease outbreak investigations. Part of FSMA's prevention-based system to inhibit foodborne illnesses involves sampling within the U.S. food supply. The FDA currently employs three categories of microbiological sampling: product, environmental, and emergency response/emerging issues sampling (U.S. Food and Drug Administration 2018d). Routine

product and environmental sampling are not prompted by an outbreak, rather, they are a verification measure. The FDA samples finished food products, as well as in-process products and raw ingredients, to ensure they do not reach the marketplace if hazards are present (U.S. Food and Drug Administration 2018d). The FDA, like industry personnel, conduct environmental monitoring since a known mode of contamination is from cross-contamination in production facilities or transport vehicles as addressed in Section 2.1.

Prior to 2011, the FDA would assemble a response team once an outbreak was identified, and those respective staff would go back to their usual jobs once the response was over (U.S. Food and Drug Administration 2017c). Now, the FDA has developed a team to respond to foodborne disease outbreaks in the U.S., known as the Coordinated Outbreak Response and Evaluation (CORE) Network. According to the FDA's CORE Network Background Paper (2017), the CORE group is employed by personnel who are constantly looking for potential outbreaks in the U.S., investigating those outbreaks, and developing policies and guidance to prevent future outbreaks . The CORE employees are divided into three separate efforts: a Signals and Surveillance Team, three Response Teams, and a Post-Response Team.

The Signals and Surveillance Team is dedicated to early detection that will limit or prevent illness linked to products regulated by the FDA. If there is a foodborne outbreak, this information is passed on to one of the Response Teams. Once delegated, the FDA is responsible for finding the source of the outbreak and they must subsequently make certain that contaminated product is removed from retail. The Response Team works with other FDA field personnel as well as other state and local food safety and public health officials. Further sampling can be conducted to discern the probable cause of an outbreak of food contamination event known as an "environmental assessment" (U.S. Food and Drug Administration 2018b). Environmental assessments are used to identify how the environment can contribute to the introduction, cross-contamination, and spread of pathogens into the food supply (U.S. Food and

Drug Administration 2018b). Samples are taken from food as well as food contact and surrounding surfaces. After sampling, the specimens are processed by methods established in the FDA's Bacteriological Analytical Manual which is the agency's preferred and recommended laboratory procedures for microbiological analyses of foods (U.S. Food and Drug Administration 2017a). This recommendation of analytical methods is applicable to both governing bodies and industry laboratories and is discussed further in Section 5.

Once the foodborne illness outbreak case is concluded, the Post-Response Team analyze the data (U.S. Food and Drug Administration 2017c). This team looks at all impacting factors of the foodborne outbreak, from raw ingredient sourcing through distribution to gain an understanding as to why the outbreak occurred within the supply chain. Officials work to identify the source of an outbreak and how the contamination could be prevented in the future, such as improved environmental monitoring. Sourcing this information may lead to new research on how contamination can occur and invoke further studies within industry and academia in ways to prevent foodborne illnesses.

The food industry is arguably the most important player in food safety. As food producers, the food industry is the primary force in the prevention of foodborne illnesses. Through the creation and implementation of food safety plans, environmental monitoring is conducted based on hazard analysis and risk assessments as well as the rules and recommendations of the primary governing body. The food industry has long integrated innovative, science-based technology to improve product safety and lessen in-house product holding times (National Registry of Food Safety Professionals 2018). Industry members are continuously looking for ways to adapt their food safety plans, including implementation of environmental monitoring. Adaptations such as, advanced technologies like rapid pathogen detection and whole genome sequencing as well as transparency endeavors such as blockchain technology—an electronic system that maintains a permanent record of transactions online and is incapable of being altered or falsified after the event (Roberie 2018, Apte 2016).

With the ever-changing food safety adaptations, it is important for regulatory agencies and food industry members to understand what the most accurate detection methods are.

5. Detection of microorganisms recovered during environmental monitoring

5.1. Pathogenic microorganisms

Pathogenic microorganisms are of the utmost concern when monitoring the environment within a food processing facility. Pathogenic microorganisms present in the environment do not necessarily mean there is contaminated product, but an increased risk. As mentioned in Section 2.1, *L. monocytogenes* is most often introduced via the food processing environment and not raw contaminated product (Lin et al. 2006). Therefore, it is important to understand which microorganisms to monitor and how to monitor them. This subsection will address various environmental pathogenic microorganisms of concern and their detection methods.

5.1.1. Microorganisms

L. monocytogenes is a foodborne bacterial pathogen of significant concern to the food industry (Teixeira et al. 2007). *L. monocytogenes* is a Gram-positive bacterium, motile via flagella and can be found in all types of food products, particularly in dairy products and other ready-to-eat products (Teixeira et al. 2007). *L. monocytogenes* causes listeriosis primarily in the young, elderly, and immunocompromised. Manifestations of listeriosis include septicemia, meningitis, encephalitis, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion in the second/third trimester or stillbirth (Teixeira et al. 2007). Listeriosis in humans is uncommon, but serious with a case-fatality rate of 20 to 30% despite adequate antimicrobial action (Swaminathan and Gerner-Smidt 2007). *L. monocytogenes* grows slowly in temperatures as low as 2°C (Rocourt and Bille 1997), in environments of reduced water activity (0.92) (Nolan, Chamblin, and Troller 1992), at pH values from 4.4 to 9.4 as well as in NaCl concentrations up to 10% (te Giffel and Zwietering 1999). Many food products frequently have a water activity above 0.95 and that provides an environment which supports the growth of mold, yeasts, and bacteria thus negatively impacting

quality and safety (U.S. Food and Drug Administration 1984). Unfortunately, many of the characteristics typically suggested and implemented to reduce foodborne pathogens in RTE products and their environments are the same ones that *L. monocytogenes* cells are capable of surviving within and even growing.

Salmonella spp. are a group of pathogenic bacteria responsible for one of the most frequent foodborne diseases in the United States (Teixeira et al. 2007). *Salmonella* are a genus of facultative anaerobic, Gram-negative, rod-shaped bacteria from the Enterobacteriaceae family. Salmonellosis, the disease induced by *Salmonella*, results in fever, diarrhea, occasionally vomiting, and abdominal cramps for four to seven days following an up to 72 hour incubation period (Centers for Disease Control and Prevention 2018c). While there are many serotypes of *Salmonella*, *Salmonella enterica* serovars Enteritidis and Typhimurium are the *Salmonella* types most frequently associated with human disease (Liu et al. 2011). The primary reservoir for *S.* Enteritidis is shell eggs, as 80% of the *S.* Enteritidis outbreaks and up to 110,000 cases are attributed to contaminated eggs in the United States each year (Liu et al. 2011).

Salmonella can inhabit various food contact surfaces with different degrees of adhesion (Teixeira et al. 2007). During a study of bacteria adhesion to food contact surfaces by Teixeira et al. (2007), *Salmonella* strains strongly adhered to stainless steel and adhered to the lowest extent to polymeric materials. Such factors are important to consider when choosing sampling devices, considering the amount of pressure applied during sampling, and selecting surfaces to use within the processing facility. Environmental sampling of *Salmonella* within a plant is important for hygienic monitoring due to its frequency related to foodborne illnesses and prevalence in food processing environments.

Escherichia coli is a Gram-negative, facultative anaerobe, rod-shaped bacteria that is often found in the lower gastrointestinal tract of warm-blooded animals (Feng et al. 1998). Often, *E. coli* is not problematic, and in fact, it is part of the normal microbiota of the gastrointestinal

tract yet some strains are pathogenic to humans (Feng et al. 1998). Certain kinds of *E. coli*, such as STECs discussed in Section 2.2, induce illnesses in humans. Since *E. coli* is found in the lower gastrointestinal tract, it is often an indicator of fecal contamination due to its abundance in warm-blooded animals.

Additionally, there are pathogenic microorganisms of concern that are not currently regulated, namely enteric viruses. In the U.S., there is not a standardized environmental swabbing method available for human enteric viruses such as hNoV—the primary cause of foodborne disease illness in U.S. with notable transmission via contaminated environmental surfaces (Boone and Gerba 2007, Rzeżutka and Cook 2004, Hall 2012). This will be addressed further in Section 6.

5.1.2. Enrichment and standard culture methods

Standard culture methods are those where results are based on traditional plate count methods. Standard culture methods appear to have been used since the beginning of environmental monitoring programs (Adzitey and Huda 2010, Adzitey, Huda, and Ali 2013). After samples are taken from the food processing environment, an enrichment and growth period must occur to obtain results. Afterward, presumptive microorganisms must be confirmed via biochemical tests (Corry et al. 2003; Adzitey, Huda, and Ali 2013). The growth period of the microorganisms is typically over several days. Using standard culture methods alone is time consuming, delays the release of products, and delays corrective action in the event of pathogen contamination. Standard culture methods remain in the forefront of microbiological work because they detect only viable bacteria and provide bacterial isolates that can further be characterized and studied in depth (Engberg et al. 2000; Adzitey, Huda, and Ali 2013). Standard culture methods are required by regulatory agencies (e.g., FDA Bacteriological Analytical Manual, USDA/FSIS Microbiology Laboratory Guidebook) in the presence of pathogenic bacteria. After standard culture methods, biochemical and serological confirmation of the pathogen must be completed. Classical detection methods only detect the viable cells and, as

outlined in Section 4, the sampling device and methods used to recover cells during environmental monitoring ultimately determine the reported microbial load. This can potentially hinder the accuracy of the results reported in standard culture methods.

5.1.3. Enrichment and molecular-based analysis

More recently, surveillance of foodborne pathogens is achieved through a multifaceted combination of methods addressed in Section 5.1.4. and several polymerase chain reaction (PCR)-based assays (Adzitey and Corry 2011; Loncarevic et al. 2008; Aurora, Prakash, and Prakash 2009). Molecular-based assays are widely used in the surveillance of mutation and other genetic factors of foodborne pathogens (Adzitey, Huda, and Ali 2013). According to Adzitey et al. (2013), these molecular techniques help increase the understanding into the primary source of foodborne pathogens, the source of infection, and genetic diversity of the microorganisms. Molecular-based assays have the advantage over standard culture methods in that they are rapid, less labor intensive, more sensitive, and specific (Magistrado, Garcia, and Raymundo 2001; Keramas et al. 2004; Adzitey, Huda, and Ali 2013). However, organic matter and food residues present on the environmental surface, enrichment media, or DNA extraction solution can inhibit the sensitivity of PCR-based methods (Rossen et al. 1992, Wilson 1997). Numerous molecular-based detection and typing methods have been developed. Many of them are used to detect, differentiate, and type pathogens within the food industry. This improves the efficiency of specific pathogen identification, outbreak investigations, and epidemiological studies. In addition to the inhibitors mentioned, sampling devices, operator dependency and other environmental factors can alter the accuracy of the molecular-based detection methods. Even as assays become more accurate and sophisticated, it is important to remember that their outcomes depend on the quality of the device and methodology used to obtain the sample.

5.1.4. Direct detection analysis

Rapid method technology is a highly desired tool within the food industry. According to a thorough review of direct detection analysis methods by Mandal *et. al.* (2011), time and the

sensitivity of analysis are crucial when assessing the usefulness of microbial testing. Several factors must be considered when using direct analysis methods such as accuracy, speed, cost, and design (Mandal et al. 2011). The authors go on to categorize direct detection analysis into three groups: biosensors, immunological methods, and nucleic acid-based assays.

In the review, Mandal et al. (2011) discussed the limitations of direct analysis methods since the current uses are designed for preliminary testing. Negative results from direct analysis methods are definitive and require no further action whereas positive results from direct analyses are presumptive and must be confirmed with further testing. Rapid, direct methods are the future of food safety; to become successful, they must be bridged with further research between the sampling devices and the detection methods.

5.2. Microbial indicators

Indicator microorganisms are groups of indigenous microorganisms that are commonly tested for and used as indicators of overall food quality and the hygienic conditions present during food processing, and, to a lesser extent, as a potential presence of pathogens (Kornacki 2011). Indicator microorganisms are often monitored across all environmental sampling systems to determine if contamination is present. They can be detected using rapid methods in environmental monitoring. The rapid results are important for the food industry due to the perishable nature of food and help determine if further, species specific testing is needed. Food processors increasingly depend on rapid quality control tests that deliver results rapidly to support rapid product distribution (Jemmi and Stephan 2006). This subsection will address various indicator microorganisms targeted during environmental monitoring programs and their associated detection methods.

5.2.1. Target microorganisms and detection

An Aerobic Plate Count (APC) microbial enumeration is used as an indicator of general bacterial populations of a sample which can be an environmental surface or food product. APC does not discern types of bacteria in a sample, and is often used to observe sanitary quality,

adherence to current good manufacturing practices, and to a lesser extent, as an indicator of safety. APCs are poor indicators of safety since they do not correlate with the presence of pathogens in the sample (U.S. National Research Council Subcommittee on Microbiological Criteria 1985). However, samples that show unusually high (depending on product or time of sampling the surface) APCs may reasonably be assumed to be potential health hazards but require further analysis to determine if pathogens are present (U.S. National Research Council Subcommittee on Microbiological Criteria 1985). Large numbers of bacteria may be an indication of poor sanitation (U.S. National Research Council Subcommittee on Microbiological Criteria 1985). Conversely some products, such as fermented foods, naturally have a high APC. Notably, low APC numbers do not correspond to an absence of pathogenic microorganisms. Often, it is necessary to further test for specific pathogens before determining the product safety.

Coliform is a term often used to describe enteric, lactose-fermenting bacteria. It should be noted that coliform is not an official taxonomic classification, but remains as a descriptor of a group of Gram-negative, facultative anaerobic, rod-shaped, lactose-fermenting bacteria (Feng et al. 1998). In 1914, the United States Public Health service determined that the presence of coliforms would be an accurate interpretation of sanitation (Feng et al. 1998). Even though coliforms were easy to detect, their association with fecal contamination was questionable because some coliforms occur naturally in environmental samples (Feng et al. 1998; Odonkor and Ampofo 2013). This can lead to false positive indicators of fecal contamination where fecal matter may not have been present (Feng et al. 1998). Therefore, the fecal coliform classification was introduced. Fecal coliforms ferment lactose at an elevated incubation temperature of 45.5°C (Feng et al. 1998). The improved fecal coliform group consists mostly of *E. coli* (along with some other enteric organisms).

Currently, all three groups are used as indicators (coliform, fecal coliform, *E. coli*) but in different applications (Feng et al. 1998; Odonkor and Ampofo 2013). As designated in the U.S.

FDA Bacteriological Analytical Manual [BAM] (Feng et al. 1998), detection of coliforms is used as an indicator of sanitary quality of water or as a general indicator of sanitary conditions in the food-processing environment. *E. coli* is used to indicate recent fecal contamination or unsanitary processing (Feng et al. 1998; Odonkor and Ampofo 2013). Failure to detect *E. coli* in a food, however, does not assure the absence of enteric pathogens (Mossel 1967; U.S. National Research Council Subcommittee on Microbiological Criteria 1985). Furthermore, *E. coli* are not always restricted to the gastrointestinal tract and can survive in the food processing plant environment and subsequently contaminate foods. Under those circumstances, the presence of *E. coli* in a post-processing environment does not necessarily indicate fecal contamination, but can indicate either process failure (e.g., heat treatment) or post-processing contamination from cross-contamination (U.S. National Research Council Subcommittee on Microbiological Criteria 1985).

Another common microorganism to screen for is *Listeria*-like organisms. *Listeria*-like organisms are those that hydrolyze esculin in Fraser broth. Typical black colonies on MOX agar could indicate *Listeria* spp., *Enterococcus* spp., *Lactobacillus* spp., and others (Kornacki et al. 1993; Yan, Gurtler, and Kornacki 2006; Kornacki 2011). The presence of *Listeria*-like organisms can identify if cleaning and sanitation are adequate as well as indicate the pathogen risk in a post-processing environment (Kornacki 2011). However, finding *Listeria*-like organisms in an environment does not necessarily correspond to the presence of pathogens (Kornacki 2011). Therefore, if *Listeria*-like organisms are present, all suspected product should still be tested as appropriate for *L. monocytogenes* in accordance with the U.S. FDA or USDA guidance to ensure the safety of the product (Kornacki 2011).

5.2.1.1. Petrifilm-based culturing

Film-based analytical plates are alternatives to poured agar dishes discussed in the previous subsection. They consist of rehydratable nutrients that are embedded into a film along with a gelling agent (Odonkor and Ampofo 2013). After incubation, the colonies can be counted

directly from the film system like traditional culture-based poured agar dishes. Petrifilm™ is made for a variety of microorganisms including those discussed in the previous subsection. This variety of film-based systems allows for food safety teams to analyze environmental monitoring samples with minimal preparatory work. In environmental monitoring, Petrifilm™ can be used to identify the presence of aerobic microorganisms, *E. coli*/coliforms, and environmental *Listeria* spp. within the food processing environment (Nyachuba and Donnelly 2007; Nelson et al. 2013; Linton, Eisel, and Muriana 1997). Petrifilm™ has been shown to give results equivalent to traditional culture methods and is widely used in food microbiology as an indicator assay (Nelson et al. 2013; Silbernagel et al. 2003; Nyachuba and Donnelly 2007).

5.2.2. ATP Bioluminescence assay

All living organisms contain adenosine triphosphate (ATP) (Mandal et al. 2011). The bioluminescent system that measures the presence of ATP in a sample uses an enzyme system known as luciferin-luciferinase (Vasavada 2001; Lappalainen et al. 2000). The total light output of the sample is directionally proportional to the amount of ATP present in the sample (Mandal et al. 2011). ATP sampling devices are used to monitor the efficacy of a sanitation system within the food industry (Vasavada 2001; Lappalainen et al. 2000). According to an article published in Food Safety Magazine by Vasvada (2001), there are several companies with ATP test systems for sanitation monitoring on a very rapid basis in hand-held designs from 20 seconds to 1 minute. A drawback to using ATP assays is it assesses the total ATP produced by both microorganisms and the ATP present in the sample or food residue (Vasavada 2001). Vasavada (2001) also reported that ATP levels may vary depending on the metabolic activities of the organisms as well. ATP is a useful indicator for total microbial loads on an environmental surface but is not useful as a validatable measure or indicator of pathogenic microorganisms in a food processing facility (Hammons et al. 2015; Osimani et al. 2014).

6. Future research

The food industry and microbiological technologies continue to advance. Streamlined communication, accelerated sample and data collection, and in-line microbiological methods are desirable for regulatory agencies and food processors. Benefits offered by implementation of disruptive technologies in these areas drive developments in environmental monitoring. Future microbiological research can be described as “precision food safety” (Kovac et al. 2017). Development of precision food safety is based on genomics and related tools that allow for a more precise approach to detection, characterization, and identification of pathogenic microorganisms (Kovac et al. 2017; Xu 2017; Den Besten et al. 2017). Data accumulated through use of “omic” technologies (e.g., genomics, metabolomics, proteomics, and transcriptomics) could lead to a transition from current food safety concepts identified in this review to novel food safety concepts with impacts similar to those used in personalized human medicine (Xu 2017; Kovac et al. 2017). For example, future microbial detection and characterization strategies for environmental monitoring could be based on the metabolome of the target of concern as opposed to its genome or other highly specific cellular components (e.g., surface antigens). As discussed in Xu et al. (2017), the metabolome is able to reflect the phenotype of a given biological system as it is the final downstream product of gene expression; however, research to has primarily focused on discerning quality aspects of food as opposed to safety. Even still, over a decade ago Chen et al. (2007) applied mass spectrometry—a common analytical method in metabolomics—combined with extractive electrospray ionization for the detection of *E. coli* in spinach using the bacterium’s metabolomic fingerprint. Miniaturization of electronics and optics as well as advances in wireless power and data transmission are supporting development and use of portable spectroscopic equipment. These include promising technologies such as surface enhanced Raman scattering (SERS) (Zhao et al. 2018) that permit detection and characterization of single molecules and cells. This allows for the possibility of *in situ* analysis of microorganisms on food contact surfaces, which appears to be on the horizon.

For an in-depth review on the state-of-the-art in spectroscopic and spectral imaging techniques for the detection of microorganisms in food and food manufacturing environments refer to the recent publication by Wang et al. (2018). Overall, the rapid pace of advancement of analytical tools and methods is anticipated to dramatically improve the accuracy, sensitivity, and speed of identifying environmental pathogens.

As the microbiological detection assays improve, environmental monitoring tools will also need to advance. As demonstrated in this review, there are gaps in the accuracy and dependability of sampling devices. Next-generation sampling tools, as discussed in Section 4.1.4., will need further research and development as well as subsequent validation to be implemented in the food industry. Sampling devices should be similar or better in accuracy than those currently used and, ideally, compensate for the inevitable operator variability.

The improved environmental monitoring tools and assays should be used to assess foodborne pathogens, including enteric viruses. As previously discussed, human enteric viruses cause the most foodborne related illnesses worldwide due to low infectious dose and highly transmissible nature (Siebenga et al. 2009). The ingestion of as little as 18 to 1000 viral particles can lead to infection (Kambhampati, Koopmans, and Lopman 2015). Enteric viruses, including hNoV, are spread by vomiting or fecal shedding and have a greater chance of transmission the longer the virus is able to survive outside the host via environmental surfaces (Turnage and Gibson 2017). In addition to person-to-person transmission of hNoV, contaminated food, water, and aerosolized particles can also deliver an infectious dose (Kambhampati, Koopmans, and Lopman 2015). Foodborne viruses can withstand and survive in gastrointestinal tracts, contaminated water, frozen foods, and environmental surfaces for weeks or months (Food Safety Preventive Controls Alliance 2016). While foodborne pathogenic bacteria are readily regulated throughout the food supply, the regulation of enteric viruses is lacking. Furthermore, a standard approach has not been developed to recover enteric viruses from environmental surfaces (Turnage and Gibson 2017). Environmental monitoring studies

with the inclusion of novel assays, next-generation sampling devices, and standardized methodologies are needed to provide crucial data for the advancement of environmental monitoring programs.

7. Conclusions and recommendations

Studies of environmental monitoring tools vary throughout the literature. The variability in results possibly exists due to numerous factors outlined in this review including, but not limited to, the sampling devices, surface types, and evaluated microorganisms. Many surface sampling studies focus on swabs of varying compositions while there is limited research evaluating and validating other possible novel sampling devices. For this reason, researchers may have difficulty choosing the most applicable variables for a study due to the range of environmental conditions, tools, and methods used across literature. These difficulties are even more prevalent when sampling for human enteric viruses.

The following recommendations are based on our review to aid researchers in moving towards the optimization and standardization of environmental monitoring tools in the food industry:

- Concentrations and volumes of microorganisms need consistency and should include standard low ($10 – 10^2$) and high ($10^4 – 10^6$) inoculum levels.
- Systematic evaluation and characterization of materials currently used in environmental sampling devices (e.g., polyurethane foam, polyester, cellulose).
- Studies need to be conducted in one standard unit of sampling area based on current industry standards.
- Environmental monitoring studies should use current environmental pathogens of concern or strain-specific surrogates that represent environmental pathogens.

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9. Tables

Table 1. Selected laboratory-based studies on the recovery of microorganisms from food contact surfaces

Apparatus	FCS type	MO + load	Conditions	Buffer	DL or % Recovery	Reference
Minirollers: white microfiber, 100% wool fiber–velour, 100% white polyamide fiber, white high-density foam, high-density foam flocked with polyamide fiber of 3 mm	Stainless steel (100 cm^2)	*L. monocytogenes* (human origin) • 10^5 CFU	Immediate (wet)	0.1% peptone water	% recovery (stomached) • Microfiber: 3.53% ± 1.17% • Wool: 6.27% ± 1.62% • Polyamide: 3.31% ± 0.10% • Foam: 2.32% ± 0.48% • Flocked: 3.69% ± 0.64%	(Gómez et al. 2012)
Swabs: cotton, polyurethane foam Sponges: cellulose, gauze	Various (100 or 900 cm^2)	*S. enterica* ser. Typhimurium, *E. coli, S. aureus, L. monocytogenes* • 10^5 CFU	Immediate (wet) or 1 hr. drying	Buffered peptone water	% recovery • Wet: 88% to 93% • Dry: 55% to 66%	(Keeratipibul et al. 2017)
Swabs: Cotton-tipped	Various (25 cm^2)	*S. enterica* ser. Typhimurium • 10^6 CFU	Immediate to 6 hr. drying	Ringer solution (10 ml)	Recovery (CFU/25 cm^2) • Immediate: 10^4 to 10^6 • 1 hr.: 10^2 to 10^3	(Moore, Blair, and McDowell 2007)

Swabs: rayon Wipes: electrostatic	Stainless steel (100 cm^2)	*S. aureus* • 6 to 6×10^5 CFU	24 hr. drying	PBS-Tween 20 (10 ml or 50 ml)	Swab (CFU/100 cm^2) DL • 7.6 to 4.3×10^3 Wipe (CFU/100 cm^2) DL • 7 to 5.7×10^3	(Lutz et al. 2013)
Swabs: flocked nylon, cotton, polyester Cloths: microfiber	Latex, plastic, stainless steel (25 cm^2)	hNoV GII.4 • 100 to 1,000 PCR Units	Overnight drying at RT	PBS or 50mM glycine (2 ml)	Swabs (% recovery, stainless steel)[a] • 20 to 60% Cloths (% recovery, stainless steel) • 79% ± 10%	(Rönnqvist et al. 2013)
3M Sponge-Stick, Copan foam spatula, 3M Enviro Swab	Various (250 cm^2)	*L. monocytogenes* • 10^2 CFU	Immediate (wet) or 1 hr. drying	Buffered peptone water (10 ml)	% positive (dry, stainless steel, enriched) • Sponge-Stick: 66% • Foam spatula: 89% • Swab: 89%	(Lahou and Uyttendaele 2014)
Swabs: cotton, polyester, rayon, polyurethane foam Wipes: antistatic	Stainless steel (645 cm^2)	hNoV GII.4 • 6.2 $\log_{10}$ RNA copies	1 to 48 hr.	Swabs: PBS + Tween 80 (2.5 ml) Wipes: Ringer solution (10 ml)	Macrofoam (DL on stainless steel) • 3.5 $\log_{10}$ RNA copies per 645 cm^2	(Park et al. 2015)

[a] Polyester swab performed best, but not significantly different.

FCS: food contact surface; hNoV: human norovirus; DL: detection limit; MO: microorganism; PBS: phosphate buffer saline; RT: room temperature

Table 2. Characterization of hygienic zones

Zones	Description	Example
Zone 1	Food contact surfaces	Table surfaces, slicers, pipe interiors, tank interiors, and conveyor belts
Zone 2	Non-food contact surfaces near food and food contact surfaces	Equipment housing or framework, and some walls, floors, or drains in the immediate area of FCSs
Zone 3	Remote non-food contact surfaces that are in or near the processing areas and could lead to potential contamination of zones 1 and 2	Forklifts, hand trucks, and carts that move within the plant and some walls, floors, or drains not in the immediate vicinity of FCSs
Zone 4	Remote non-food contact surfaces in areas outside of the processing range	Locker rooms, cafeterias, and break rooms outside the production or stored product area

Table adapted from U.S. FDA (2017b).

Chapter 3: Factors impacting microbial release from environmental monitoring tools

Abstract

The U.S. FDA Food Safety Modernization Act Preventive Controls for Human Food Rule underlines the importance of an effective environmental monitoring (EM) program. EM is used to determine harborage sites of microorganisms on processing equipment, assess effectiveness of sanitation programs, and prevent transmission of foodborne pathogens. This study characterizes commercially-available polyurethane foam (PUF) and cellulose (CELL) EM tools for their efficacy in the release of foodborne pathogens from their sponge matrices. Specifically, the objectives of this study were to 1) compare the ability of EM tools to release microorganisms into a recovery eluent, 2) characterize EM tool performance at decreasing inoculum concentrations, and 3) assess the impact of various operators during the processing of EM samples. Two bacteria (*Listeria monocytogenes*, *Salmonella* Typhimurium) and one human norovirus surrogate (Tulane virus [TV]) were compared at decreasing inoculum levels utilizing two elution techniques (mechanical stomacher, manually by operator), and across six operators. Data indicated that EM tool material composition impacted the release of microorganisms (p=0.0001), where the PUF EM tool released TV more readily than the CELL EM tool. Conversely, the decreasing inoculum levels did not statistically differ in the release of microorganisms from the EM tool matrices. In addition, no significant difference was found between the machine stomacher and manual elution by human operator or between operators. Overall, the study provides a detailed characterization of two commercially-available EM tools, and the differences identified in this study can be used to improve the effectiveness of EM programs.

1. Introduction

In 2011, the U.S. Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) was enacted and is currently implemented via seven major rules to enhance the safety of the food supply. The FDA-FSMA Final Rule for Preventive Controls for Human Food (PCHF) addresses the importance of environmental monitoring (EM) and stresses the implementation of an effective EM program (U.S. FDA, 2020a). Environmental monitoring is designed to: 1) verify that preventive controls are reducing the risk of pathogen cross-contamination from surfaces to the food; 2) identify harborage sites of microorganisms, and 3) ultimately, prevent the transmission of pathogens (U.S. FDA, 2020a; 2020b). To monitor the presence of microorganisms, EM uses sampling tools (e.g., swabs, sponges) to recover microorganisms from food contact surfaces and the adjacent non-food contact surfaces within the food processing environment. Ultimately, the choice of EM sampling tool used is up to the food manufacturer.

Under FDA-FSMA, regulatory rules and guidance have been developed to help food manufacturers create EM programs using best practices, but there is no standardization for the EM tools themselves. The EM tools can vary in material as well as size and shape. As reviewed by Jones et al. (2020) and Turnage and Gibson (2017), these tools vary in their ability to recover and release microorganisms. Most of the current EM research focuses on the recovery, or the capture of microorganisms from a surface, but do not characterize the tools any further. A holistic approach to characterization of the EM tools can provide valuable insight into the potential limitations of currently available EM tools, including microbial release capacity. At the time of this study, there have only been two studies published regarding the release of microorganisms from specific EM tools (Jones and Gibson, 2020; Keeratipibul et al., 2017). Moreover, most EM characterization studies generally focus on bacteria and provide limited insight into the recovery and release of foodborne viruses such as human norovirus (HuNoV)—the primary cause of foodborne illnesses in the U.S. with an estimated 5.5 million cases

annually (Koopmans and Duizer 2004; Scallan et al. 2011). Thus, it is essential to further characterize EM tools for the recovery and release of human enteric viruses.

The role of the operator (i.e., the person processing the EM sample) is another aspect that is poorly characterized. This is especially critical when direct quantification is desired as opposed to presence/absence testing (i.e., direct enrichment of the swab itself) (FDA, 2015; 2017). For instance, some ISO/IEC 17025 accredited laboratories use a homogenizing elution device such as a stomacher while others rely on laboratory technicians to manually elute the sample for pathogen detection (personal communication). While laboratory personnel within ISO/IEC 17025 accredited laboratories are required to meet certain competency levels (ISO, 2017), there are no published data that specifically characterize differences between human operators and a mechanical elution device with respect to pathogen quantification. Moreover, if manual elution is used, there are no published data on the variation of microbial recovery from EM tools among human operators. To effectively characterize risk related to cross contamination in the food processing environment, these fundamental questions about EM sampling must be answered.

The present study aimed to characterize polyurethane foam (PUF) and cellulose (CELL) EM tools currently used in the food industry for their ability to release microorganisms. The objectives of this study were to 1) determine and compare the ability of PUF and CELL devices to release *Listeria monocytogenes*, *Salmonella* Typhimurium, and Tulane virus (TV), 2) identify EM tool performance differences at decreasing inoculum concentrations, and 3) assess the impact of various operators during the manual, hand-massage processing of EM samples.

2. Materials and Methods

2.1. Sampling devices

Two environmental sampling devices were used in this research (Figure 1). The PUF EZ Reach Sponge Sampler (henceforth referred to as ‘PUF’; World Bioproducts, Woodinville, WA) moistened in 10 mL of 1 × PBS was selected to study the release of microorganisms. To

compare the impact of manufacturer materials, the CELL EZ Reach Sponge Sampler (henceforth referred to as 'CELL') moistened in 10 mL of 1 × PBS was also selected to study the release of microorganisms. The dimensions of both sponges are 1.5 in × 3 in (3.81 cm × 7.62 cm). If a processor were swabbing a surface, both EM tools selected would be used to sample larger surface areas, (≤ 2 ft^2 [≤ 0.185 m^2]) per the manufacturer's recommendations.

2.2. Processing aids

To compare the impact of elution methods, two processing aids were used. One processing aid was a stomacher (Stomacher 400 Circulator; Seward, Worthing, United Kingdom). The other methodology had a person (henceforth referred to as 'operator') manually 'hand-massage' the sponges to recover the released microorganisms in the eluent. Six operators were recruited to process the sponges manually. Each operator was given a training demonstration one-on-one. Additionally, the operators were individually observed and timed while processing their samples. Each operator was familiar with microbiological techniques, and a demonstration of the method was provided by the researchers as a teaching tool.

2.3. Microorganisms

2.3.1. Virus cultivation and detection

Tulane virus was provided by Dr. Jason Jiang (Cincinnati Children's Hospital Medical Center, Cincinnati, OH) and propagated in monkey kidney cells LLC-MK2 (ATCC CCL-7; Manassas, VA). Tulane virus stocks were titered via plaque assay (Jones and Gibson, 2020). Aliquots of the TV were stored at -80°C. For each plaque assay, the methods described by Jones and Gibson (2020) were used. The TV stock concentration was approximately 5×10^5 PFU/mL.

2.3.2. Bacteria cultivation and detection

L. monocytogenes and *S.* Typhimurium were used in this study. The *S.* Typhimurium (ATCC 14028; American Type Culture Collection, Manassas, VA) was streaked from a frozen 50% glycerol stock onto a tryptic soy agar (TSA; NEOGEN Culture Media, Lansing, MI) plate

using a sterile inoculation loop and incubated overnight (18 to 24 h) at 37°C. The *L. monocytogenes* serotype 4b (strain FSL R9-5506) was kindly provided by Dr. Martin Wiedmann at Cornell University. *L. monocytogenes* was streaked from a frozen 50% glycerol stock onto brain heart infusion agar (BHI; BD, Franklin Lakes, NJ) plate and incubated overnight at 37°C. Further bacterial culture preparation was completed, as described by Gibson et al. (2019). Each culture was combined and diluted in buffered phosphate water to prepare the two-strain cocktail inoculum for each decreasing inoculum level from 10^6 to 100 colony forming units per mL (CFU/mL) of both microorganisms. Experiments were replicated and samples were plated in duplicate.

For the detection of *S.* Typhimurium, 100 µL of each dilution was plated onto Xylose Lysine Tertigol™ 4 agar (XLT4) plates (Hardy Diagnostics, Santa Maria, CA) followed by incubation for 24 h at 37°C. *L. monocytogenes* were enumerated by plating 100 µL of serially diluted sample onto Oxford Listeria Agar Base with modified Oxford *Listeria* supplement (MOX; NEOGEN Culture Media), and the plates were incubated for 48 h at 35°C. Viable cells were reported as CFU/mL. Experiments were replicated and samples were plated in duplicate.

2.4. Evaluation of microbial release from EM tool based on processing aid

The bacterial and virus studies were completed separately. Inoculum levels for bacteria ranged from approximately 10^6 to 100 CFU/sponge. The PUF and CELL sponges were hydrated with 10 mL of 1 × PBS. The saturated sponge was manually squeezed so excess fluid was expelled from the sponge in the sample bag. Then, 500 µL of the bacterial cocktail was pipetted directly onto the moist PUF and CELL sponges. Direct inoculation of the PUF and CELL sponges was necessary for evaluation of microbial release without the additional variable of recovery from surfaces. The sponge was placed back into the sample bag with excess eluent. Bacteria were recovered by machine stomaching or manual elution processes within 5 min of inoculation. Sponges were processed via stomaching (Seward) for 1 min at 230 rpm. The manual elution process was completed by moderate pressure being applied to the sponge for 1

min to remove as much eluent as possible from the sponge. After processing, any eluent held in the sponge was squeezed out, and the total recovered volume was measured and recorded. The total recovered volume was approximately 5 mL for each sample, where the PUF eluent recovered ranged from 4.0 to 5.8 mL, and CELL ranged from 3.2 mL and 5.8 mL. The samples went without enrichment and were plated onto selective agar for bacterial quantification.

For the virus studies, 500 µL of the TV inoculum was directly applied onto the PUF and CELL sponges hydrated with 10 mL of 1 × PBS. Inoculum levels ranged from approximately 10^5 to 100 PFU/sponge, and samples were processed as described above for bacteria. Samples were analyzed as described in Sections 2.3.1. and 2.3.2. for TV and bacteria, respectively. All experiments were replicated and analyzed in technical duplicates.

2.5. Influence of operator on microbial release

To compare the influence of various operators, the manual elution process was completed with six operators (Section 2.2). The bacterial and virus studies were completed separately where inoculum levels ranged from 1.38 to 6.05 × 10^4 CFU/sponge or PFU/sponge. Five hundred µL of the bacterial cocktail was directly inoculated onto the PUF and CELL sponges hydrated with 1 × PBS. The manual elution process was completed as described in Section 2.4. The total recovered volume was measured and recorded. The samples went without an enrichment period and were plated onto selective agar. For the TV studies, 500 µL of the TV inoculum was directly inoculated onto the PUF and CELL sponges hydrated with 1 × PBS. Samples were processed as described previously (Section 2.4). Samples were analyzed for TV and bacteria as described in Sections 2.3.1. and 2.3.2., respectively. All experiments were replicated and analyzed in technical duplicates.

2.6. Data analysis

The data (N=220) are reported in percentages for comparison and visual convenience. The percent release of two bacteria (*L. monocytogenes*, *S.* Typhimurium) and one human norovirus surrogate (TV) were compared at decreasing inoculum levels, with two EM tools and

two elution techniques (mechanical stomacher, manually by operator). The operator variability study was conducted at single inoculum level compared across six operators. All experiments were replicated and analyzed in duplicate.

The percentages represent the number of microorganisms recovered in the respective assay after EM tool processing compared to the number of microorganisms directly inoculated onto a premoistened EM tool. The comparison of mean release by EM tool type and processing aid were analyzed via Student's *t*-test whereas the multiple comparisons of the means across inoculum levels, microorganisms, and operators were analyzed via Tukey's honest significant difference (HSD) test. Any statistically significant differences were defined by $p \leq 0.05$. All data analyses were completed in JMP® Pro 14 (SAS Institute, Inc., Cary, NC) statistical software.

3. Results

3.1. Release of microorganisms from EM tools

When comparing the EM tool matrices, the PUF released microorganisms significantly ($p < 0.0001$) more efficiently than the CELL EM tool. When combining all variables and simply comparing the EM tools, the PUF sponges released 42.60% ± 1.90% of the microorganisms inoculated on the sponge compared to 30.06% ± 1.79% from the CELL matrices (data not shown). This information can aid decision making within the industry and regulatory sectors when selecting an EM tool type.

To compare the release of microorganisms, all inoculum levels, both elution methods, and both EM tools were combined to assess how the microorganisms interact with the sampling devices. Interestingly, *L. monocytogenes* and TV were not significantly different from each other, while *S.* Typhimurium did vary significantly (Table 1). The mean release of *S.* Typhimurium was 46.69% ± 2.43% compared to 31.26% ± 1.50% and 29.98% ± 2.72% for *L. monocytogenes* and TV, respectively. When comparing the release of *L. monocytogenes* and TV to *S.* Typhimurium, both *p*-values were < 0.0001. Overall, these data suggest that once microorganisms are introduced to the EM tool matrices, they are held intrinsically from

thereafter. Additionally, these data also indicate that *S*. Typhimurium is released more readily from the matrices of the devices compared to *L. monocytogenes* and TV evaluated in the present study.

The impact of decreasing inoculum levels on the release of microorganisms was also compared. To specifically understand the influence of inoculum level on release, the authors combined all microorganisms, EM tools, and elution methods. The mean release (%) and standard error (SE) are reported in Table 2. As the inoculum levels decreased, the percentage of released microorganisms did not drastically differ.

Last, the influence of EM tool type and microorganism type on the mean release of microorganisms was analyzed. Overall, as previously indicated, the data reveal that the PUF EM tool releases microorganisms more readily when compared to the CELL EM tool. More specifically, when analyzing the CELL EM tool, the release of all microorganisms varied significantly from each other ($p < 0.001$) (Figure 2). Conversely, regarding the PUF EM tool, the only significant difference in microbial release was between *S*. Typhimurium and *L. monocytogenes* ($p = 0.0232$). In Table 3, the data are further characterized by inoculum level category, EM tool type, and microorganisms. These data suggest that the composition of the matrices impacts the release of the microorganism.

3.2. Impact of processing aids

Based on personal communication with food industry members, the effect of the processing aids on the release of microorganisms was compared. When comparing the two elution methods across all microorganisms, inoculum levels, and EM tools, the data indicate there is no significant difference ($p = 0.1373$) between using a stomacher or human operator. The mean release with a standard error was 34.13% ± 2.30% and 38.24% ± 1.5% between the stomacher and operator, respectively (data not shown).

3.3. Influence of operator variability

In the present study, differences between operators were also investigated in order to characterize any operator effect on downstream detection and quantification. The authors compared multiple operators (n = 6), combining all microorganisms and sponge types at a single inoculum level. Percent microbial release ranged from 43.55 to 52.41% across operators with standard errors from 3.58 to 5.39%. These data indicate that the release of microorganisms was not impacted by the human operator as there were no significant differences ($p > 0.05$) across operators.

4. Discussion

The effectiveness of EM programs within the food industry is dictated by multiple variables, both intrinsic and extrinsic. Intrinsic to an EM program is the sampling device itself. While there is an abundance of data on the ability of EM tools to recover microorganisms from surfaces, there is limited data specific to the release of microorganisms from EM tools. Interestingly, nearly two decades ago, Moore and Griffith (2002) hypothesized that microbial release capacity was the root cause for the poor performance of some EM tools. Moreover, most research has focused on the recovery of bacterial pathogens even though human enteric viruses, such as HuNoV, account for more than half of all foodborne illnesses annually in the U.S. Last, practical aspects of EM program workflows tend to be neglected in the current literature including the impact of standard processing aids (mechanical versus manual elution) and operators (i.e., analytical laboratory personnel) on the release of microorganisms from sampling devices when direct quantification is desired. In the present study, data were generated that provide a more holistic characterization of currently available EM tools utilized within the food industry.

Previous studies have demonstrated the influence of surface sampling device composition on subsequent microbial yield (Jannsson et al. 2020; Rose et al. 2004; Keeratipibul et al. 2017; Park et al. 2015). However, as identified in Jansson et al. (2020), the results across

studies for similar sampling devices are often inconsistent. Interestingly, most previous research has investigated sampling methods and sampling device material related to the recovery of bacteria from food product surfaces, such as poultry and beef carcasses (Barkocy-Gallagher et al., 2002; Pearce and Bolton, 2005; Martínez et al., 2010). Moreover, various factors may cause these inconsistencies, such as surface type, surface condition (e.g., presence of organic material, new versus old), inoculum drying time, surface area, target microorganism, differences in sampling technique, and analytical method used for microbial detection (Rawlinson et al. 2019; Jones et al. 2020). Notably, the influence of these factors on sampling device performance is frequently based on the recovery of microorganisms from a surface first without characterizing a device's ability to release microorganisms in the absence of the surface as an additional variable. For instance, Faille et al. (2020) indicated that biofilm pieces (i.e., food particulates, biological material, etc.) become entrapped in the swab material during surface sampling, potentially impacting the release of microorganisms. However, the authors did not specifically investigate the effect of organic material on microbial release (Faille et al. 2020). The results of the present study indicate that the release capacity of EM tools differs significantly by both sponge material composition and type of microorganism.

For bacterial release, Keeratipibul et al. (2017) previously reported the efficiency of release from directly inoculated EM tools composed of the same materials used in the present study—cellulose and polyurethane foam. However, it is unknown the brand or dimensions of the EM tools which could range from a traditional swab bud or to a sponge, similar to the present study. Keeratipibul et al. (2017) inoculated each EM swab with 10^5 CFU of *S.* Typhimurium, *Staphylococcus aureus*, *Escherichia coli*, or *L. monocytogenes* and reported microbial mean release percent efficiencies ranging from 95.7% to 99.3% and 94.1% to 99.1% for CELL and PUF EM tools, respectively—importantly, these are based on log-transformed data. The present study found that mean bacterial release at 'high' (10^5 to 10^6 CFU/sponge) inoculation levels were observed and ranged from 9.92% to 90.32% and 10.36% to 61.82% for CELL and PUF

EM tools, respectively (data not shown). The values reported here for release of 10^5 CFU, when log-transformed, are similar to those reported by Keeratipibul et al. (2017) with recovery efficiency ranging from 89.7 to 96.4% and 91.2 to 93.2% for CELL and PUF EM tools, respectively. Unfortunately, decreasing inoculum levels for evaluating release efficiency were not reported by Keeratipibul et al. (2017). Similarly, Moore and Griffith (2007) investigated bacterial release after direct inoculation of sampling devices with 5×10^3 CFU *E. coli* or *S. aureus*. The authors reported mean percent release capacity for *S. aureus* and *E. coli* ranging from 74.6% to 88.6% and 30.4% to 52.8%, respectively, across all sampling devices when using BPW as the eluent. However, the sampling devices were not comparable to those used in the present study or by Keeratipibul et al. (2017); thus, directly comparing these results is problematic.

For viral release from directly inoculated EM tools, the release capacity of the tools in the present study was significantly different between the two EM tool types. In the present study, the mean percent release of TV from the CELL EM tool was 14.91% ± 1.99% compared to PUF which was 45.05% ± 3.38% (Figure 2). These results align with previous reports on the superior performance of macrofoam swabs (i.e., PUF-based swabs) to recover viruses from surfaces compared to other sampling device materials such as polyester, cotton, and rayon (Park et al. 2015). Moreover, PUF-based sampling devices have also been shown to recovery bacteria from surfaces at a greater efficiency (Gilbert et al., 2014; Rose et al., 2004). The data in the present study concur with previous studies comparing polyurethane and cellulose-based EM tools where PUF EM tools released significantly more ($p<0.0001$) microorganisms than the CELL EM tool.

Of additional importance is the differential release of bacteria and viruses from EM tools, as observed in the present study. Here, we reported a statistically significant difference in average release efficiency between *L. monocytogenes* and TV compared to *S.* Typhimurium when all data were combined (i.e., inoculum levels, elution method, and swab type) (Table 1).

To our knowledge, this is the first study to compare the release of bacteria and viruses from surface sampling devices. Reasons for these differences could be due simply to microbial size, with bacterial cells being 10 to 20 times larger than a typical enteric virus particle; thus, viruses may become more easily ‘trapped’ in the EM tool material. Another possibility could be the specific interaction between the microorganism and the EM tool material. For example, non-enveloped viruses typically hold a net-negative charge at an environmentally relevant, or neutral, pH (Michen and Graule, 2010). If the virus interacts with the material via electrostatic forces, it could be more difficult to recover the virus particles. As such, viruses may need a more specialized elution buffer for release from the EM tools due to potential electrostatic binding. More specifically, it may be advantageous to manipulate virus charge through the application of an elution buffer with a more basic pH as well as one containing proteinaceous components similar to the approach to viral elution from environmental and food matrices (Knight et al., 2013; Stals et al., 2012).

The final component of the present study involved the evaluation of operator variability on microbial release when a manual processing aid is applied. As introduced previously, some accredited laboratories use a stomacher while others rely on laboratory technicians to manually elute the sample for pathogen detection and quantification (personal communication). Prior to this study, published data were not available on the potential differences between 1) human operators and a mechanical elution device (e.g., a stomacher) or 2) across human operators utilizing manual elution methods. For the most part, studies evaluating EM tool performance often utilize processing aids that are not typical to commercial laboratories, such as a vortex mixer. Furthermore, mechanized recovery methods have largely been focused on elution of microorganisms from food matrices and carcass swabs, not environmental sampling tools (Martínez et al., 2010; Sánchez et al., 2012; Rohde et al., 2015; Kim et al., 2012). In the present study, the authors compared the difference between manual elution by a human operator and the utilization of a stomacher. Notably, no significant difference was observed between the

elution methods. These data can help inform laboratories in the food sector in processing EM samples.

As reviewed by Turnage and Gibson (2017), previous studies on the recovery of viruses from surfaces have typically shown that higher viral densities result in higher mean recovery efficiencies while lower viral density often results in both lower and greater inconsistency in recovery efficiency (Scherer et al. 2009; Tung-Thompson et al. 2017). The same holds true for bacterial densities. According to Jones et al. (2020), studies often inoculate at high concentrations (>10^4 CFU) and yield consistently high recovery efficiencies, while the same methods do not perform as well when applied to lower inoculation levels. However, within this study, the data do not indicate significant differences in the release of microorganisms at decreasing inoculum levels (Table 2). The lack of corroboration with previously reported data on the effect of microbial concentrations and recovery efficiency may be attributed to any number of variables. For studies on viruses, these differences may be in part because of virus selection. Previous studies have used various human norovirus surrogates—including TV used in the present study—in addition to human norovirus.

There are a few limitations to the present study. Foremost, this study was performed in a controlled laboratory setting, whereas, within the food industry, recovery of microorganisms from EM tools would be done in a commercial laboratory with potential processing delays as investigated previously (Jones and Gibson, 2020). In addition, the release efficiency of the EM tools evaluated here only accounts for one factor in the complexity of EM programs. Moreover, this study is focused on identifying differences between EM tools, microorganisms, and elution methods as a first step in the characterization in EM tools but is currently limited to speculation in the reasoning for these differences. Last, as mentioned previously, TV was selected as the HuNoV surrogate in this study as opposed to human norovirus. In recent years, the use of TV as a HuNoV surrogate has been established as a common and suitable surrogate (Arthur and Gibson, 2015). TV and HuNoV share similar attributes, such as genetic identity as they are both

caliciviruses and recognition of histo-blood group antigen (HBGA) receptors (Farkas et al. 2010). Importantly, TV is easily replicable in cell culture systems—another key factor in HuNoV surrogate selection, especially when direct detection of infectious virus particles is desired (Li et al. 2012). However, the authors acknowledge that extrapolation of results between TV and HuNoV should be considered with caution.

This study lays the foundation for future research designed to characterize EM tools further. As outlined in the present study, there are significant differences amongst microorganisms, EM tools, and inoculum levels. The noticeable yet insignificant differences between processing aids and human operators indicate that future research and optimization should focus on the EM tool or on specific microbial detection assays. Additionally, future research should investigate the mechanisms behind these described differences. Last, potential research should also explore the influence of extrinsic factors, such as temperature and humidity, on the recovery of microorganisms using various EM tools. The data collected in this study will help guide EM research to improve the safety of our food supply.

5. Conclusions

Environmental monitoring is a required but largely undefined area in the food industry. Frequent EM in the food supply helps prevent pathogenic contamination of food and subsequent foodborne disease. However, EM is only as good as the EM tools used. This study identified fundamental differences in the efficiency and utilization of select EM tools currently used in the food industry. Specifically, notable differences between microorganism type and EM tool composition. Data suggesting that variability between operators and processing aids is inconsequential in the release of microorganisms helps narrow the research scope to improve EM. Future research inquiries should include:

- Exploration and visualization of microorganisms on varying EM tools pre- and post-processing.

- Investigations on the influence of extrinsic factors on microbial recovery from surfaces subject to EM programs.
- Expansion of studies to include other relevant pathogenic foodborne microorganisms.

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7. Tables

Table 1. Comparison of mean release (%) from environmental monitoring tools by microorganism

Microorganisms	Mean release (%) [b]	Standard Error	Significance [a]
Salmonella Typhimurium	46.69	2.43	A
Listeria monocytogenes	31.26	1.50	B
Tulane virus	29.98	2.72	B

[a] Levels not followed by the same letter are significantly different.
[b] All inoculum levels, both elution methods, and both EM tools were combined. N=220.

Table 2. Analysis of mean release (%) from environmental monitoring tools by decreasing inoculum levels

Inoculum Level (CFU/sponge or PFU/sponge) [a]	Mean release (%) [b]	Standard Error
1.5 to 8.8×10^6 [c]	28.14	2.99
1.1 to 8.8×10^5	40.21	3.18
1.4 to 9.5×10^4	36.95	2.06
1.2 to 6.5×10^3	37.87	3.16
2.5 to 9.5×10^2	32.95	3.06

[a] Range of inoculum levels
[b] All microorganisms, both elution methods, and both EM tools were combined. N=220.
[c] 10^6 inoculum level does not include Tulane virus

Table 3. Percentage release of microorganisms by environmental monitoring tool and inoculum level.

Inoculum Level [a]	*Listeria monocytogenes*				*Salmonella* Typhimurium				Tulane virus			
	CELL		PUF		CELL		PUF		CELL		PUF	
	Mean % Release	(SE)	Mean % Release	(SE)	Mean % Release	(SE)	Mean % Release	(SE)	Mean % Release	(SE)	Mean % Release	(SE)
Low	23.69	(3.59)	43.80	(6.52)	27.24	(8.44)	55.00	(6.30)	15.63	(6.58)	32.35	(4.77)
Medium	29.72	(2.80)	35.37	(2.42)	46.02	(2.58)	47.54	(7.88)	15.42	(2.34)	46.29	(3.88)
High	24.79	(2.23)	32.68	(4.08)	63.15	(5.37)	43.37	(3.05)	13.19	(1.68)	55.25	(8.78)

SE = standard error, CELL = cellulose, PUF = polyurethane foam

[a] High, Medium, and Low inoculum level correspond to 10^6 to 10^5, 10^4 to 10^3 and ≥ 10^2 CFU/sponge or PFU/sponge, respectively.

8. Figures

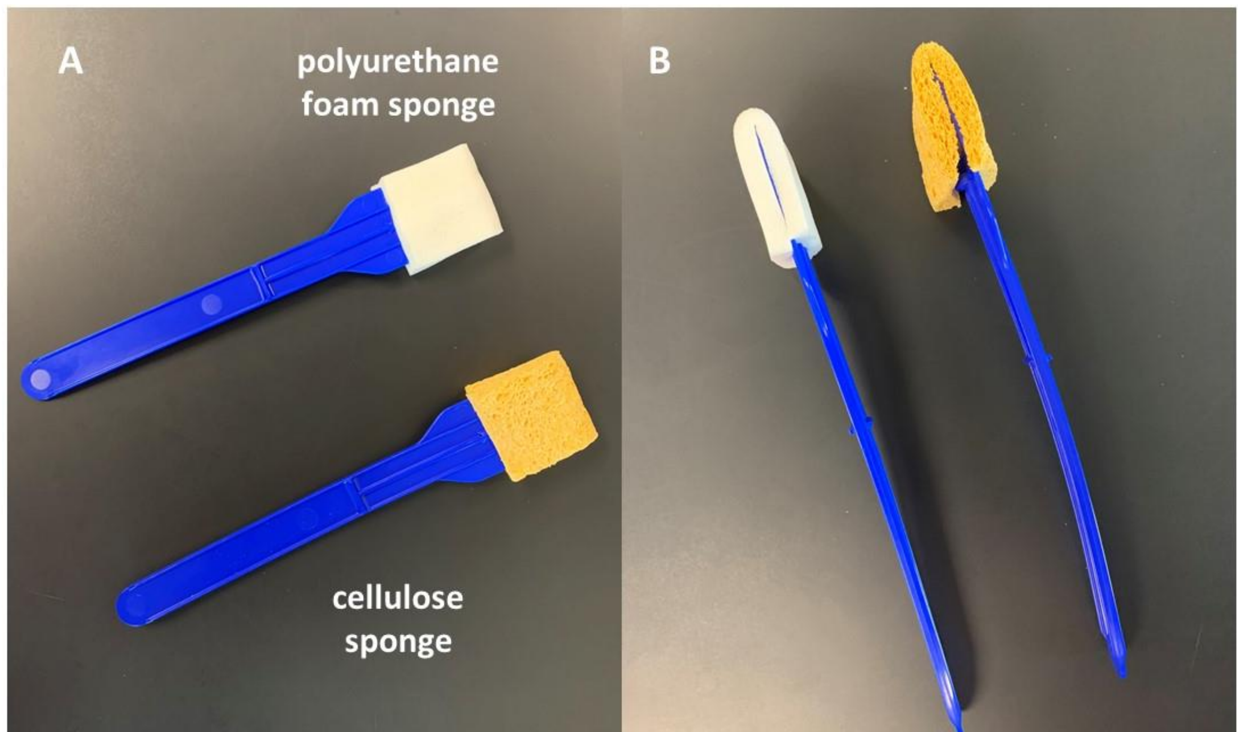

Figure 1. Environmental monitoring tools used in the present study. A) Comparison of the EM tools. Both sides of the sponge are utilized when swabbing the surface. The handle of the sponge twists off, and only the sponge is placed back into the sterile bag with eluent broth. B) Comparison of sponge sampling devices on their side. This image compares the thickness of the dry sponge material.

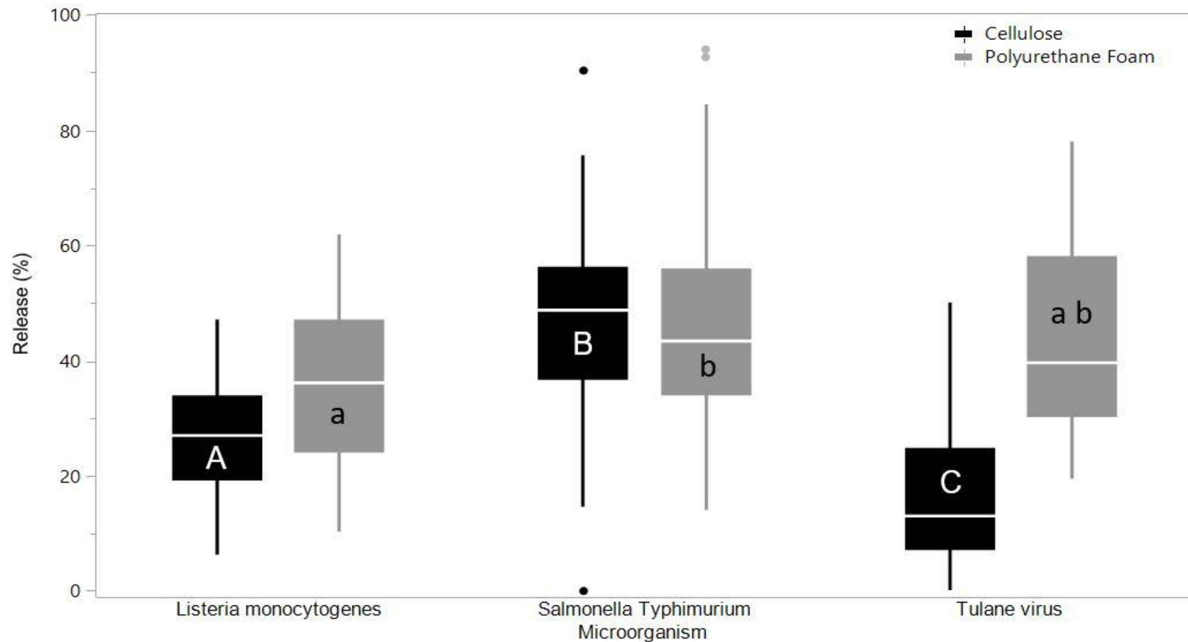

Figure 2. Percentage release of microorganisms by environmental monitoring tool type. In the box plots, the boundary of the box closest to 0 indicates the 25th percentile, a white line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whisker indicate outliers outside the 10th and 90th percentiles. Different capital letters (A-C) and lowercase letters (a-c) indicate significant differences (p<0.05) in release of microorganisms from CELL and PUF EM tools, respectively. All inoculum levels and both elution methods were combined (N=220).

Chapter 4: Temperature, Time, and Type, Oh My! Key Factors Impacting *Salmonella*, *Listeria*, and Virus Recovery from Surfaces

Abstract

Environmental monitoring (EM) programs are designed to detect the presence of pathogens in food manufacturing environments with the goal of preventing microbial contamination of food. Nevertheless, limited knowledge exists regarding the influence of environmental conditions on microbial recovery during EM. This study utilizes a commercially-available polyurethane foam (PUF) EM tool to determine the influence of environmental factors on the recovery of foodborne pathogens. The specific objectives of this study were to determine if environmental conditions and surface composition impact the recovery of sought-after microorganisms found in food processing environments. These data are compared across 1) microorganism type, 2) surface type, 3) environmental temperature and relative humidity, and 4) exposure time. Two bacteria (*Listeria monocytogenes*, *Salmonella* Typhimurium) and one human norovirus surrogate (Tulane virus [TV]) were inoculated onto three non-porous surfaces (polypropylene, stainless steel, neoprene). Surfaces were held in an environmental chamber for 24 or 72 h at 30°C/30%, 6°C/85%, and 30°C/85% relative humidity (RH). Data indicate that microbial recovery from environmental surfaces significantly ($p \leq 0.05$) varies by microorganism type, environmental conditions, and exposure time. For instance, all microorganisms were significantly different from each other, with the greatest mean log loss being TV and the lesser loss being *L. monocytogenes* at 4.94 ± 1.75 $\log_{10}$ PFU/surface and 2.54 ± 0.91 $\log_{10}$ CFU/surface, respectively. Overall, these data can be used to improve the effectiveness of EM programs and underscores the need to better comprehend how EM test results are impacted by food manufacturing environmental conditions.

1. Introduction

In the U.S., there are approximately 48 million cases of foodborne disease annually *(27, 28)*. The 2011 Food Safety Modernization Act (FSMA) designated the U.S. Food and Drug Administration (FDA) to implement seven major rules to enhance food supply safety to mitigate the risk of foodborne illness. Specifically, the FDA-FSMA Final Rule for Preventive Controls for Human Food (PCHF) directs the requirement of environmental monitoring (EM) and emphasizes the importance of an effective EM program in food processing facilities *(35, 37)*. This requirement for an EM program also applies to some facilities covered by the FDA-FSMA Final Rule for Preventive Controls for Animal Food. Across the food industry, EM is intended to 1) inhibit the transmission of pathogens, 2) detect harborage sites of microorganisms, and 3) verify that in-place preventive controls are minimizing the risk of pathogen cross-contamination from surfaces to the food *(36, 37)*. To survey the presence of microorganisms, EM uses sampling tools (e.g., sponges, wipes, swabs) to recover microorganisms from food-contact surfaces and the adjacent non-food contact surfaces within the processing environment. Notably, the EM sampling tool used is decided by the food manufacturer.

Under FDA-FSMA, regulatory rules and guidance for industry have been published to help food manufacturers create attentive EM programs, but there is no standardization for the EM tools themselves. Additionally, academic and industry stakeholders have published guides to help navigate EM program development, such as the *3M Environmental Monitoring Handbook for Food and Beverage Industries (31).* Generally, EM tools can vary in material composition as well as size and shape. Reviews by Turnage and Gibson *(32)*, Jones et al. *(10)*, and De Oliveira Mota et al. *(2)* highlight that these tools differ in their capacity to recover and release microorganisms. Most of the current EM research focuses on the recovery, or the capture, of microorganisms from a surface under ideal microbial growth conditions but does not characterize the tools any further. A comprehensive approach to evaluating the EM tools can provide valuable insight into the potential limitations of currently available EM tools, including

performance under varying environmental conditions (i.e., temperature and relative humidity) and across microorganism types (e.g., bacteria as well as viruses) *(11)*.

There is great value in strategically evaluating the influence of environmental conditions on EM tool performance and their impact on EM programs. Often, EM tools are marketed to food manufacturers with little indication of the environmental conditions where the EM tools will be deployed. Food product recalls connected to pathogen presence in the processing environment occur across the food chain, not a single commodity area. Moreover, regulatory agencies employ microbiological testing of environmental surfaces during inspections of food processing facilities and foodborne disease outbreak (FBDO) investigations. For example, in 2021, an FBDO of *Salmonella enterica* subsp. *enterica* serovar Typhimurium was associated with packaged salad greens and initiated a product recall *(40)*. However, further investigation by regulators found *Salmonella* Liverpool in the production environment triggering another wave of recalls *(40)*. In 2020, an expansive international FBDO of *S.* Newport in bulk, whole onions were based on regulatory investigations and the risk of cross-contamination from surfaces within the processing facilities *(39)*. In the same year, *Listeria monocytogenes* found on packing equipment during routine EM was the reason for two separate multistate recalls due to the risk of cross-contamination *(38)*. Overall, non-typhoidal *Salmonella* serovars and *L. monocytogenes* result in both significant financial loss and product loss each year due to the risk of cross-contamination between surfaces and food *(22)*.

There are varying approaches to EM across numerous segments of production and service industries—from food to healthcare. These approaches primarily involve using a swabbing device to recover microorganisms from a surface followed by a detection assay. The efficacy of a given pathogen EM program is influenced mainly by microbial attachment to the surface, surface characteristics, EM tool used (e.g., size and material), and surface area sampled (e.g., decrease in microbial recovery as area increases), as reviewed by Jones et al. (2020). Further characterization of EM sampling must be addressed to improve EM programs

for the food industry and reduce the risk of FBDOs and recalls. Thus, the present study aimed to determine if environmental conditions and surface composition impact the recovery of microorganisms found in food processing environments. The factors considered include 1) microorganism type, 2) surface type, 3) environmental temperature and relative humidity, and 4) exposure period. Upon completion, factors that are the most influential in the EM recovery of microorganisms were identified.

2. Materials and Methods

2.1. Bacteria cultivation and detection

L. monocytogenes and *S.* Typhimurium were both used in the present study. Both bacteria were recovered from glycerol stock as described by Jones and Gibson *(11)*. The *S.* Typhimurium (ATCC 14028; American Type Culture Collection, Manassas, VA) was streaked from a frozen 50% glycerol stock. The *L. monocytogenes* serotype 4b (strain FSL R9-5506) was kindly provided by the Institute for the Advancement of Food and Nutrition Sciences *L. monocytogenes* strains collection at Cornell University. *L. monocytogenes* was streaked from a frozen 50% glycerol stock. Further bacterial culture preparation was completed, as Gibson et al. *(5)* described. Each culture was combined and diluted in buffered phosphate water to prepare the two-strain cocktail inoculum level at 10^9 colony forming units per mL (CFU/mL) of both microorganisms. Experiments were replicated, and samples were plated in duplicate. To detect the *S.* Typhimurium and *L. monocytogenes*, the authors followed detection methods described by Jones and Gibson *(11)*. Experiments were replicated, and samples were plated in duplicate.

2.2. Preparation, cultivation, and detection of Tulane virus

Tulane virus (TV) was provided by Dr. Jason Jiang (Cincinnati Children's Hospital Medical Center, Cincinnati, OH) and propagated in monkey kidney cells LLC-MK2 (ATCC CCL-7; Manassas, VA). Tulane virus stocks were cultivated, stored, and titered via plaque assay as described Jones and Gibson *(9)*. The TV stock concentration was approximately 5×10^5 PFU/mL. For each plaque assay, the methods described by Jones and Gibson *(9)* were used.

2.3. Surface types and preparation

Sterile coupons (144 in^2 [929 cm^2]) of unpolished stainless steel 304 (Rose Metal Products, Inc., Springfield, MO), food-grade white neoprene rubber sheets (AllState Gasket Inc., Deer Park, NY), and flexible polypropylene cutting mats (Better Kitchen Products, Amazon Storefront, Seattle, WA) were used. The FDA and the Department of Agriculture (USDA) encourage the swabbed sampling area to be 144 in^2 (929 cm^2) or more *(33, 34)*. Clean, dry surfaces were saturated with 70% ethanol, wiped with paper towels, and spent 1 h under the UV-C lights in the Class II Biological Safety Cabinet before inoculation.

Following treatment (see *Environmental treatments*) and recovery (see *Recovery of microorganisms from surfaces*), surfaces were also decontaminated. Each surface was saturated with 70% ethanol, wiped with paper towels, washed in warm, soapy water (Dawn Liquid Dish Soap, Proctor & Gamble, Cincinnati, OH), then rinsed with DI water and air-dried.

2.4. Surface inoculation

For bacteria surface inoculation, the cocktail inoculum was 10^9 CFU per surface. The 929 cm^2 surfaces were spot inoculated with 40 25-uL spots (total volume [V_t] = 1 mL) of inoculum. For TV surface inoculation, the inoculum level was 10^6 PFU per surface area. The 929 cm^2 surfaces were spot inoculated with 40 25-uL spots (V_t = 1 mL) of TV. All inoculum spots were randomly dispersed evenly over the surface and allowed to completely dry in the Class II Biosafety Cabinet for 1 h. After drying, all surfaces were placed into the EM chamber for 24 or 72 h, as described in *Environmental treatments*.

2.5. Environmental treatments

An environmental chamber was utilized to control relative humidity (RH) and temperature (Caron Model 7000-10-1, Marietta, OH). RH is the amount of water vapor present in air expressed as a percentage of the amount needed for saturation at the same temperature. Inoculated surfaces were placed into the EM chamber and exposed to 6°C/85% RH, 30°C/85% RH, or 30°C/30% RH for 24 h and 72 h, then sampled.

2.6. Environmental monitoring sampling device

One environmental sampling device was used in this research (Figure 1). The PUF EZ Reach Sponge Sampler (hereby referred to as 'PUF'; World Bioproducts, Woodinville, WA) premoistened in 10 mL of 1 × PBS was selected. The dimensions of the sponge are 1.5 in × 3 in (3.81 cm × 7.62 cm). For use within the food industry, the EM tool selected would be used to sample larger surface areas (≤ 2 ft^2 [≤ 0.185 m^2]) per the manufacturer's recommendations.

2.7. Recovery of microorganisms from surfaces.

After the exposure period, the PUF sponges were used to sample the entire area of the 929 cm^2. To sample the entire surface using the EM tool, the authors pressed and dragged the pre-moistened sampling device across the surface. Then, once the surface was sampled, the authors flipped the sampling device and repeat the sampling process; thus, the entire surface was sampled.

After sampling, the EM tool was aseptically placed back into the sample bag with the 10 mL of eluent and immediately stomached (Stomacher 400 Circulator; Seward, Worthing, United Kingdom) for 1 minute at 230 rpm. After processing, the sponge and eluent were recovered as described by Jones and Gibson *(11)*. The total recovered volume was approximately 5 mL for each sample. These samples went without enrichment and were plated onto selective agar for bacterial quantification (see *Bacteria cultivation and detection*) and analyzed via plaque assay for TV detection (see *Preparation, cultivation, and detection of Tulane virus*).

2.8. Data analysis

For comparison, the data (N=216) are reported in logarithmic loss ($\log_{10}$ CFU/surface or $\log_{10}$ PFU/surface). The log loss of two bacteria (*L. monocytogenes*, *S.* Typhimurium) and one human norovirus surrogate (TV) were compared utilizing one EM tool on three surface types with three environmental condition combinations over two exposure periods. All experiments were replicated and analyzed in duplicate.

The reported data represent the logarithmic loss of microorganisms in the respective assay after EM tool sampling compared to the initial number of microorganisms directly inoculated onto the sterile surface. The comparison of mean loss by exposure time period was analyzed via *t*-test whereas the multiple comparisons of the means across surface types, microorganisms, and environmental conditions were analyzed via Tukey's honest significant difference (HSD) test. Any statistically significant differences were defined by $p \leq 0.05$. All data analyses were completed in JMP® Pro 16 (SAS Institute, Inc., Cary, NC) statistical software.

3. Results

3.1. Comparing the log loss between microorganisms

To compare the log loss of microorganisms, all surface types, exposure periods, and environmental conditions were combined to assess the differences between microorganisms during the EM recovery process. All microorganisms were significantly different from each other (Table 1), with the greatest mean log loss being TV and the lesser loss being *L. monocytogenes* at 4.94 ± 1.75 $\log_{10}$ PFU/surface and 2.54 ± 0.91 $\log_{10}$ CFU/surface, respectively.

3.2. The impact of surface type on the recovery of microorganisms

The authors combine all microorganisms, exposure periods, and environmental conditions to understand the influence of surface material types found in the food industry. The mean log loss and standard deviation (SD) are reported in Table 2. Overall, the surface type did not significantly impact the recovery of microorganisms. However, when focusing on specific microorganisms, some significant differences became evident.

When analyzing the recovery of *L. monocytogenes* from different surface types, there was a significantly greater mean log loss on the polypropylene mat (2.80 ± 0.90 $\log_{10}$ CFU/surface) and stainless steel (2.76 ± 0.88 $\log_{10}$ CFU/surface) compared to the neoprene surface (2.06 ± 0.79 $\log_{10}$ CFU/surface) (Figure 2). Meanwhile, the recovery of *S.* Typhimurium demonstrated different results. The mean log loss of *S.* Typhimurium was significantly different between stainless steel and the polypropylene mat with a mean log loss of 4.52 ± 1.31 $\log_{10}$

CFU/surface and 3.45 ± 1.50 $\log_{10}$ CFU/ surface (Figure 2). Conversely, the surface material type for TV did not significantly impact the recovery (Figure 2).

3.3. Impact of exposure period on recovery

Exploring the influence of the exposure period on microbial recovery is important. The authors combined all microorganisms, surface types, and environmental conditions to visualize the impact of exposure time. Overall, there was a significant difference ($p < 0.0001$) between 24 h and 72 h with a mean log loss of 3.19 ± 1.50 $\log_{10}$ CFU or PFU/surface and 4.52 ± 1.76 $\log_{10}$ CFU or PFU/surface, respectively (Table 3). For individual microorganisms, all were significantly different between 24 and 72 h (Table 3).

3.4. Understanding the influence of environmental conditions on microbial recovery

Lastly, the authors compared the environmental conditions across all microorganisms, surface types, and exposure periods. Data indicate that the influence of RH and temperature at 85% RH and 6°C was significantly different from 85% RH and 30°C and 30% RH and 30°C (Table 4). The 85% RH and 6°C environmental conditions resulted in a lesser loss of microorganisms (2.54 ± 1.00 $\log_{10}$ CFU or PFU/surface) compared to 85% RH and 30°C (4.74 ± 1.77 $\log_{10}$ CFU or PFU/surface) and 30% RH and 30°C (4.23 ± 1.59 $\log_{10}$ CFU or PFU/surface). The aforementioned significant differences held true when focusing specifically on *L. monocytogenes* and *S.* Typhimurium (Figure 3). However, for TV, each environmental condition was significantly different from the other (Figure 3).

4. Discussion

With a significant public health burden and a costly impact on the food industry, food safety and microbiology have largely shifted to a preventive model over the last decade with the enactment of FDA's FSMA (*37*). While testing finished food products has been the status quo, it is rarely enough to assure product safety due to the heterogeneous distribution of pathogens in foods and thus cannot represent the absence of contamination throughout the processing

facility *(2, 8)*. The effectiveness of EM programs within the food industry is dictated by multiple variables, both intrinsic and extrinsic. The intrinsic quality of an EM program is the sampling device itself which has been examined previously by Jones and Gibson *(11).* Extrinsically, EM programs are impacted by environmental conditions (i.e., relative humidity, temperature), surface types, period of time in the environment, and the target microorganisms. Presently, there is a dearth of literature examining extrinsic conditions and how they influence EM programs. This study aimed to characterize how EM was impacted when detecting three microorganisms of importance under varying conditions over time. These data are critical for EM tool standardization, especially when direct quantification of microorganisms is desired.

Previously, researchers have studied the recovery of microorganisms from surfaces while comparing different types of EM tools *(6, 13, 15, 17)*; utilizing one genus of microorganism *(6, 15, 17)*; and comparing different surface types *(13, 14, 15, 17)*. For instance, Lahou and Uyttendaele *(14)* studied the influence of surface type on the recovery of *L. monocytogenes* using three types of EM tools. The authors inoculated three surface types (stainless steel, rubber, high-density polyethylene) with 10^2 CFU/mL of *L. monocytogenes* and sampled them immediately after inoculation or after one hour of air-drying. The EM tools utilized by Lahou and Uyttendaele *(14)* were the 3M™ Sponge-Stick, 3M™ Enviro Swab, and Copan foam spatula. Like many researchers, the authors reported their results using a positivity rate based on presence/absence rather than direct quantification *(7, 12).* Regardless, the samples collected immediately after inoculation resulted in 100% detectable *L. monocytogenes* compared to a range of 88.9% to 96.3% after one hour, depending on the EM sampling tool. Lahou and Uyttendaele *(14)* also determined a significant effect (p=0.026) between the surface types, where on the rubber surface, *L. monocytogenes* was always detected compared to the stainless steel where *L. monocytogenes* was not detected 11.1% of the time. This is similar to the present study when comparing the recovery of *L. monocytogenes* across surface types; the neoprene surface is significantly different from the polypropylene mat and stainless steel surface.

Overall, the present study does not find significant differences between the surface types (Table 2). However, significant differences appear when the authors compare specific microorganisms and surface types (Figure 2). Research has previously shown that surface properties can directly influence the recovery of microorganisms—an issue to consider when sampling many surface types in a food processing facility *(18, 19, 25, 26, 32).* Interestingly, Keeratipibul et al. *(13)* also studied the influence of surface type on the recovery of bacteria and found no significant differences between the two surface types (stainless steel and polyester urethane). However, surface types and their properties are not the only influential extrinsic factor in microbial recovery.

Recently, De Oliveira Mota et al. *(2)* published a scoping literature review addressing the current state of EM research. Of the 69 publications analyzed, when a single microorganism was selected to study, the majority of research focused on *L. monocytogenes.* This selection is not surprising due to the well-documented and ubiquitous nature of *L. monocytogenes* in food processing environments *(21, 41).* However, the authors found a limited number of studies focusing on the recovery of *Salmonella* spp. and did not report any literature regarding the recovery of human enteric viruses or their surrogates in an EM setting *(2)*. In recent years, there has been an increase in non-*Listeria* microorganisms found in food processing environments, resulting in recalls and/or foodborne disease outbreaks. For instance, outbreaks in low-moisture food products (e.g., flour, tahini, dried coconut, cereals) over the last ten years have resulted in a seek-and-destroy dogma for microorganisms other than *Listeria* spp. *(1)*. Nevertheless, published EM research has not reflected this change and need within the food industry.

In 2017, Keeratipibul et al. *(13)* published a study addressing swabbing techniques on the recovery of bacteria from food-contact surfaces. The researchers investigated several variables, including swab type, surface type, and microorganism type. The authors utilized four microorganisms to evaluate recovery: *Escherichia coli*, *S.* Typhimurium, *L. monocytogenes*, and *Staphylococcus aureus***.** Similar to Lahou and Uyttendaele *(14)*, the authors swabbed the

surfaces immediately after inoculation (e.g., wet) as well as after a one-hour drying time (e.g., dry). Keeratipibul et al. *(13)* found significant differences between the Gram-positive (*L. monocytogenes*, *S. aureus*) and Gram-negative (*E. coli*, *S.* Typhimurium) microorganisms after sampling the dry inoculated surfaces; however, these differences were not shown for the wet surfaces. In the present study, three microorganisms were evaluated: *L. monocytogenes*, *S.* Typhimurium, and TV. When comparing recovery, there were significant differences between all three microorganisms ($p < 0.05$). The present findings align with Keeratipibul et al. *(13)*, where the Gram-positive microorganism, *L. monocytogenes*, resulted in a 2.54 ± 0.91 mean $\log_{10}$ CFU/surface loss compared with the Gram-negative *S.* Typhimurium, which had a 4.08 ± 1.58 mean $\log_{10}$ CFU/surface loss (Table 1). The present study also included a human norovirus surrogate, TV, which resulted in the greatest loss in recovery at 4.94 ± 1.75 mean $\log_{10}$ CFU/surface. When seeking out specific pathogens in food processing environments, these differences across microorganisms are important to consider.

Differences in recovery between bacteria and viruses and even bacteria Gram types can likely be attributed to inherent structural differences amongst the microorganisms. For instance, Gram-positive bacteria are characterized by their thick peptidoglycan layer cell wall, whereas Gram-negative bacteria have an outer membrane comprising lipopolysaccharides, phospholipids, and a thin peptidoglycan layer *(30)*. Similar differences are found in enteric virology. For example, Scherer et al. *(29)* described differences when recovering two human enteric viruses from the same surface type. The authors indicated that the varying recovery rates recorded between the two viruses might be due to the abilities of the viruses to differentially adhere to the surfaces *(29).* These inherent differences amongst microbial types and how they likely influence EM programs is an area that warrants further exploration.

Much of the knowledge on microbial recovery and EM is based on swabbing after a very short contact time post-inoculation. While this may provide initial microbial recovery estimates, these data do not necessarily reflect most microbial niches within the food industry, where

microorganisms can persist over time. The present study found significant differences overall, and between all individual microorganisms between the two exposure periods (Table 3). While these data are unsurprising, resulting in a decrease in recovered microorganisms from 24 to 72 hours, it is important to note that most EM research is conducted in ideal, laboratory-based conditions. This increase in the mean log loss of microorganisms is likely a multi-factor issue. For example, in the absence of nutritive matter or organic material, microorganisms are possibly dying or becoming viable-but-non-culturable (VBNC) *(16).* Moreover, the longer period of time microorganisms remain undisturbed and attached to the surface, the greater the likelihood that attachment becomes irreversible *(42).* While the surface types and length of time microorganisms are attached to the surface influence recovery, the surrounding environment is also an important factor in microbial recovery.

Environmental conditions vary across the food sector due to the diversity of the food supply. Temperature and RH have been explored as influential elements in microbial persistence *(3, 20, 23)* but often have not been a factor considered in EM research. In the present study, the authors aimed to fill that knowledge gap by exposing inoculated surfaces to three temperature and RH combinations. These data demonstrate significant differences between the cooler temperature of 6°C/85% RH compared to the 30°C/85% RH and 30°C/30% RH, which has similar $\log_{10}$ CFU/surface losses with 4.75 and 4.73 $\log_{10}$ CFU/surface, respectively (Table 4; Figure 3). These data indicated that the temperature was more influential compared to the RH. While not based on EM tool recovery, Redfern and Verran *(24)* arrived at similar conclusions when studying the effect of temperature and RH on the survival of *L. monocytogenes* on stainless steel. Within their study, the authors observed that the lower the temperature (4°C), the more *L. monocytogenes* cells were recovered, regardless of RH *(24).* However, while Redfern and Verran *(24)* reflect similar effects of temperature and RH to the present study, the authors tested at time points less than 24 hours.

The authors in the present study have identified a few limitations in this work. Within the food industry, post-sampling recovery of microorganisms from the EM tools would be completed in a commercial laboratory with potential processing delays, as investigated previously by Jones and Gibson *(9)*, not in a controlled laboratory setting. Additionally, the data presented in this study only account for the recovery of microorganisms from surfaces and the EM tool on sterile surfaces, absent of any other complex factors found in the food industry, such as organic matter or microbial die-off. Finally, TV was selected as the HuNoV surrogate in this study. TV and HuNoV share similar attributes, namely genetic identity, as they are both caliciviruses and recognize histo-blood group antigen receptors *(4)*. The authors of this study recognize that the extrapolation of results between TV and HuNoV should be examined carefully.

5. Conclusions

Environmental monitoring is a mandated but nonspecific requirement within the food industry. These EM programs help prevent pathogen contamination of food and, consequently, foodborne disease transmission. However, EM programs are only as good as the data collected during monitoring. The authors of this study identified the differences in extrinsic factors found in EM programs and how they influence the microbial recovery data. Data suggest that variability amongst environmental conditions is consequential in the recovery of microorganisms. Future EM research should:

- Expand to include additional relevant foodborne pathogens
- Investigate the differences between microorganism types further
- Explore the influence of complex extrinsic factors (e.g., organic matter, biofilms)
- Visualize microorganisms on EM tools and on surfaces pre- and post-swabbing and processing using microscopy

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7. Tables

Table 1. Comparison of log loss between microorganisms during environmental monitoring recovery

Microorganisms	Log Loss (CFU/surface or PFU/surface)[a]	Standard Deviation	Significance[b]
Listeria monocytogenes	2.54	0.91	A
Salmonella Typhimurium	4.08	1.58	B
Tulane virus	4.94	1.75	C

[a] All surface types, exposure periods, and environmental conditions were combined. N=216.
[b] Levels not followed by the same letter are significantly different.

Table 2. Influence of surface type of microbial recovery

Surface Type	Log Loss (CFU/surface or PFU/surface)[a]	Standard Deviation	Significance[b]
Neoprene	3.73	1.95	A
Polypropylene Mat	3.71	1.69	A
Stainless Steel	4.12	1.63	A

[a] All microorganisms, exposure periods, and environmental conditions were combined. N=216.
[b] Levels not followed by the same letter are significantly different.

Table 3. Impact of time on microbial recovery by microorganism

	24 h		72 h		
Microorganism	Log Loss (CFU/surface or PFU/surface)	Standard Deviation	Log Loss (CFU/surface or PFU/surface)	Standard Deviation	Significance[a]
Listeria monocytogenes	2.25	0.71	2.82	1.01	$p = 0.0073$
Salmonella Typhimurium	3.15	1.05	5.02	1.47	$p < 0.0001$
Tulane virus	4.17	1.83	5.72	1.28	$p < 0.0001$
Overall[b]	3.19	1.50	4.52	1.76	$p < 0.0001$

[a] Significant differences were defined by $p \leq 0.05$.
[b] All microorganisms, surface types, and environmental conditions were combined. N=216.

Table 4. Effect of environmental conditions on microbial recovery

Relative Humidity (%)/Temperature (°C)	Log Loss (CFU/surface or PFU/surface)[a]	Standard Deviation	Significance[b]
85/30	4.75	1.29	A
30/30	4.73	1.57	A
85/6	2.77	0.95	B

[a] All microorganisms, exposure periods, and surface types were combined. N=216.
[b] Levels not followed by the same letter are significantly different.

8. Figures

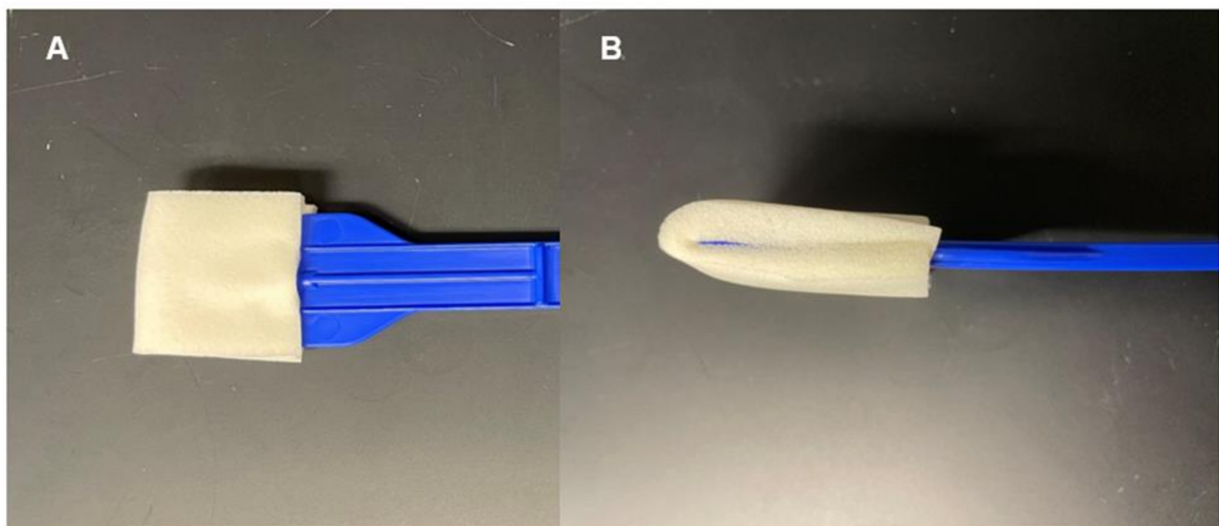

Figure 1. Polyurethane foam environmental monitoring tool used in the present study. A) Displaying the features of the environmental monitoring tool. Both sides of the sponge are utilized when swabbing the surface. The handle of the sponge twists off, and only the sponge is placed back into the sterile bag with eluent broth. B) Displaying the sampling device on its side. This image exhibits the thickness of the dry sponge material.

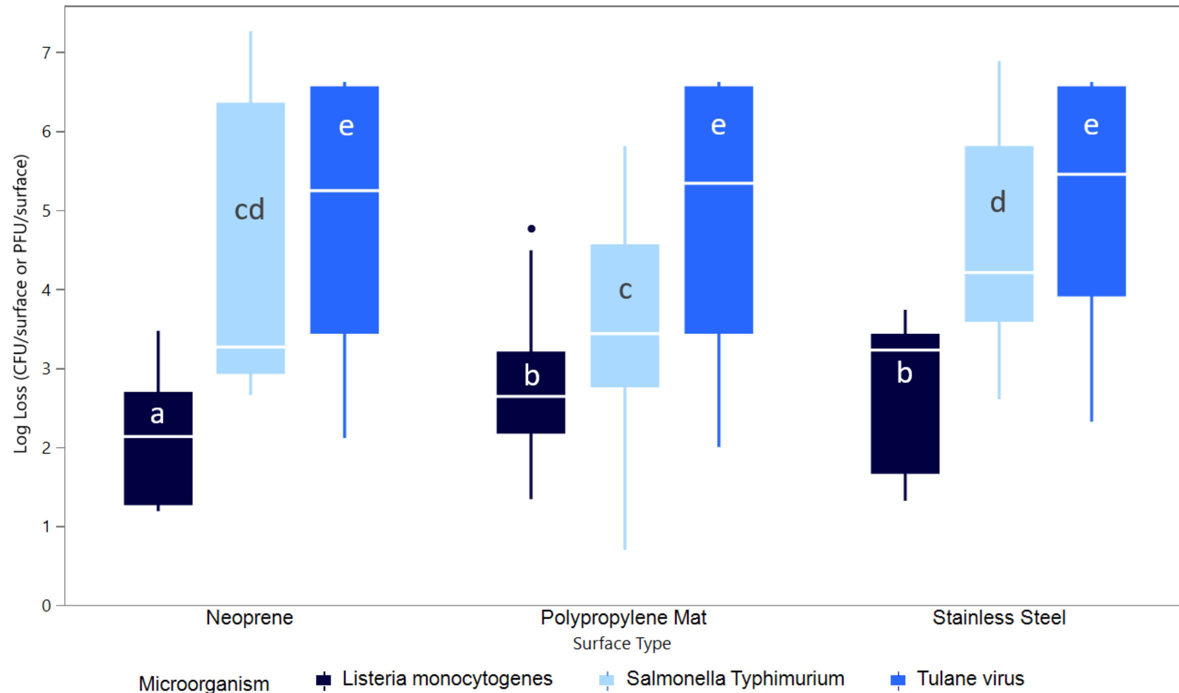

Figure 2. The influence of surface type on each microorganism examined. In the box plots, the boundary of the box closest to 0 indicates the 25th percentile, a white line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whisker indicate outliers outside the 10th and 90th percentiles. Different letter ranges (a-b; c-d; e) indicate significant differences ($p < 0.05$) between the same microorganism (e.g., Listeria monocytogenes) across surfaces. Exposure periods and environmental conditions were combined (N = 216).

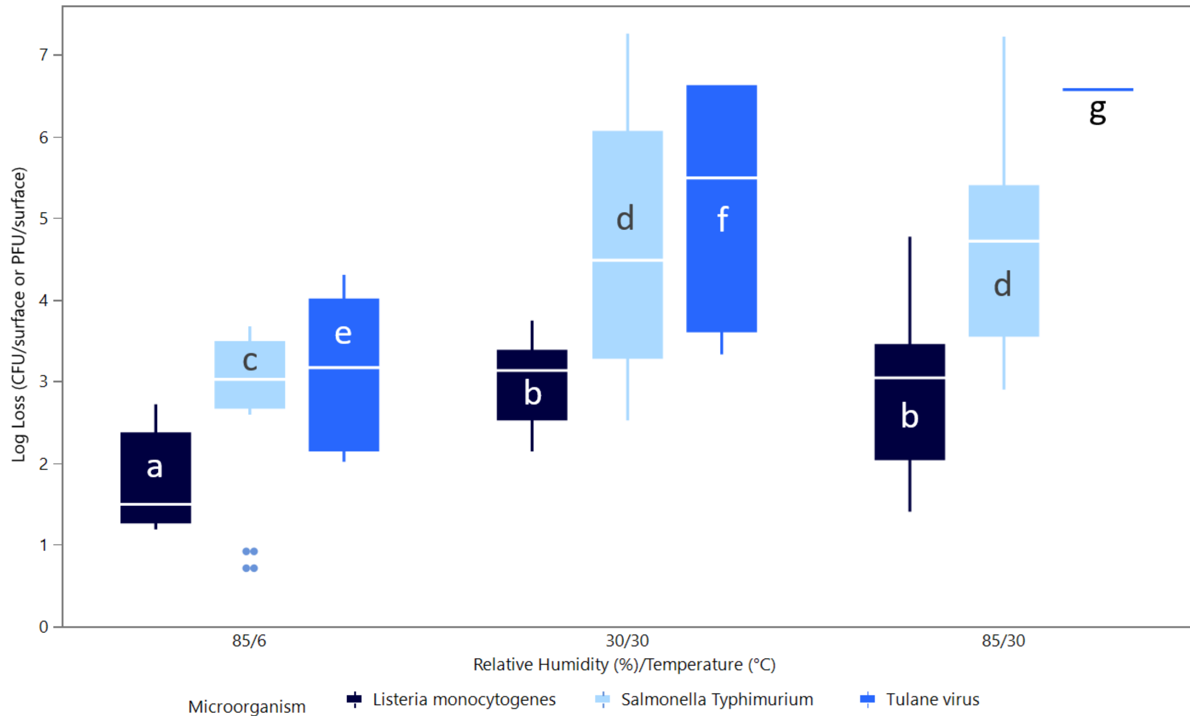

Figure 3. Environmental conditions and the impact on each microorganism examined. In the box plots, the boundary of the box closest to 0 indicates the 25th percentile, a white line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whisker indicate outliers outside the 10th and 90th percentiles. Different letter ranges (a-b; c-d; e-g) indicate significant differences ($p<0.05$) between the same microorganism (e.g., Listeria monocytogenes) across environmental conditions. Exposure periods and surface types were combined (N = 216).

Chapter 5: Type of Food Residue Impacts Recovery of *Listeria* during Environmental Monitoring

Abstract

Listeria monocytogenes has become a major pathogen of concern throughout the food supply chain. *L. monocytogenes* outbreaks are mostly sporadic and reported in developed countries. However, even though the number of illnesses is typically low, the fatality rate of listeriosis is high at approximately 20 to 30%. Over the last decade, there have been approximately one to four *L. monocytogenes* outbreaks in the US annually. However, the presence *of L. monocytogenes* in food products and the processing environment has resulted in far more food product recalls. Detection of *L. monocytogenes* in the food processing environment is completed through environmental monitoring (EM) programs. These EM programs are developed to seek-and-destroy microorganisms during processing and serve as verification of sanitation program efficacy. Additionally, in the event of a foodborne disease outbreak (FBDO) with an associated product or facility, food firms and regulatory agencies conduct in-depth environmental microbial sampling within the implicated facility to seek out the source of the causative microbial agent. During this root cause analysis process, areas that have not recently gone through cleaning and sanitation and may contain the presence of food residues on the surface are sampled. As a result, the present study aimed to determine if organic matter (i.e., food residues), and more specifically the type of organic matter, impacts the recovery of *L. monocytogenes* found in food processing environments. The factors considered here included four types of food residue (lettuce rinsate, blended lettuce, lowfat milk, and whole milk) compared to the presence of no food residue over time. Additionally, the authors determined if the act of EM sampling can aid in spreading *L. monocytogenes* across food processing surfaces. Data suggest that there is little variability amongst food residue types overall. However, differences begin to appear at the 24 h sampling period. Moreover, the authors determined that EM sampling can spread *L. monocytogenes* on the surfaces found in

processing facilities. Overall, these data are key in the improvement and development of EM protocols and FBDO investigation practices.

1. Introduction

Listeria monocytogenes was first recognized as a foodborne pathogen in the early 1980s (Schlech et al., 1983). Characterized by its hardiness in various food environments, such as low temperatures, low moisture content, and high salt concentrations, *L. monocytogenes* has become a major pathogen of concern throughout the food supply chain (de Noordhout et al., 2014). *L. monocytogenes* outbreaks are mostly sporadic and reported in developed countries. However, even though the number of illnesses is typically low, the fatality rate of listeriosis is high at approximately 20 to 30% (FDA, 2020b). In the US, an estimated 1,600 individuals become sickened with listeriosis each year with approximately 260 deaths (CDC, 2022). Global estimates are approximately 23,150 illnesses and 5,463 deaths annually (de Noordhout et al., 2014). Listeriosis often occurs in at-risk, immunocompromised groups, including pregnant women, fetuses, neonates, immunocompromised individuals, and older adults (CDC, 2022; Lamont et al., 2011). In addition to the public health burden, *L. monocytogenes* also takes a toll on the food industry.

Over the last decade, there have been approximately one to four *L. monocytogenes* outbreaks in the US annually. However, the presence *of L. monocytogenes* in food products and the processing environment has resulted in far more food product recalls. For instance, in 2019, 55 food recalls were issued by the US Department of Agriculture (USDA) and the US Food and Drug Administration (FDA) due to the presence of *L. monocytogenes* (USDA, 2020; FDA, 2022). Most *L. monocytogenes* food recalls are in ready-to-eat (RTE) food products, such as deli meat, raw vegetables, fruits, and dairy products (FDA, 2020b). As a ubiquitous microorganism, once introduced into the food processing environment, *L. monocytogenes* are difficult to remove permanently.

Detection of *L. monocytogenes* in the food processing environment is completed through environmental monitoring (EM) programs. Environmental monitoring programs are a required part of the FDA's Food Safety Modernization Act (FSMA) (FDA, 2015a; 2020a). These EM programs are developed to seek-and-destroy microorganisms during processing and serve as verification of sanitation program efficacy. This is completed with the use of EM sponges or swabs. Additionally, in the event of a foodborne disease outbreak (FBDO) with an associated product or facility, food firms and regulatory agencies conduct in-depth environmental microbial sampling within the implicated facility to seek out the source of the causative microbial agent. This results in sampling in areas that have not recently gone through cleaning and sanitation and may contain the presence of food products or ingredients on the surface.

Despite being a required activity, the efficacy of a pathogen EM program is influenced mainly by microbial attachment to the surface, surface characteristics, EM tool used (e.g., size and material), and surface area sampled and is well-established to be inconsistent across these factors, as reviewed by Jones et al. (2020). Further characterization of EM sampling must be addressed to improve EM programs for the food industry and reduce the risk of FBDOs and recalls. While there is research regarding the recovery of microorganisms from surfaces, there is little data regarding the impact of organic matter (e.g., food ingredients, product) on microbial recovery from surfaces. As a result, the present study aimed to determine if organic matter, and more specifically the type of organic matter, impacts the recovery of *L. monocytogenes* found in food processing environments. The factors considered included four types of food residue, or organic matter, compared to the presence of no food residue over time. Additionally, the authors determined if the act of EM sampling can aid in spreading *L. monocytogenes* across food processing surfaces. In conclusion, the most influential factors in the EM recovery in the presence of food residue and movement of *L. monocytogenes* were identified.

2. Materials and Methods

2.1. Bacteria cultivation and detection

The present study utilized *L. monocytogenes* serotype 4b (strain FSL R9-5506) generously provided by the Institute for the Advancement of Food and Nutrition Sciences *L. monocytogenes* strains collection at Cornell University. Stored in a frozen 50% glycerol stock, bacterial culture preparation was completed as Gibson et al. (2019) described. The overnight *L. monocytogenes* culture was titered at approximately 10^9 colony forming units per mL (CFU/mL). Post-centrifugation, the pelleted *L. monocytogenes* was held at room temperature (approximately 20°C) for less than 1 h until the food residue could be prepared (see *Organic matter*). 'No food residue' experiments and the microbial distribution experiments (see *Microbial distribution across the surface*) used 1 × PBS to resuspend the pellet. After sampling, to detect *L. monocytogenes,* the authors followed detection methods outlined by Jones and Gibson (2021). Experiments conducted in this study were replicated, and samples were plated in duplicate.

2.2. Organic matter

The four types of food residue were used in the present study. Greenhouse-grown Boston whole lettuce heads (Tanimura & Antle Fresh Foods, Inc., Salinas, CA) were purchased at a local grocery store. The lettuce was immediately held at refrigeration temperatures and was used within 48 hours of the purchase date to ensure freshness. Two different methods were used to prepare the lettuce food residue for inoculation, hereby known as 'blended lettuce' and 'lettuce rinsate.'

The authors also tested two types of fluid milk in the present study. Ultra-High Temperature pasteurized whole milk held in Tetra Pak® cartons (Horizon Organic, Broomfield, CO) and Ultra High Temperature pasteurized lowfat milk held in Tetra Pak® cartons (Horizon Organic) were both purchased from a local grocery store. Both shelf-stable milk types were held at room temperature and are hereby known as 'whole milk' and 'lowfat milk.'

2.2.1. Blended lettuce preparation

The first layer of exterior lettuce leaves was removed and discarded to prepare the blended lettuce. Then, 10 g of lettuce were weighed and added to a standard kitchen blender (Oster 12 Speed All-Metal Drive Blender, Sunbeam Products Inc., Boca Raton, FL), where 25 mL of 1 × PBS was added to the blender. The lettuce was then blended on the 'liquefy' setting for 2 min. During this time, a sterile spatula was used to scrape the blender wall to ensure all lettuce leaf particles were being blended. After blending, the blended lettuce was then poured into a 50 mL centrifuge tube. To prepare the *L. monocytogenes* blended lettuce inoculum, 10 mL of the blended lettuce was used to resuspend the *L. monocytogenes* pellet by vortexing.

2.2.2. Lettuce rinsate preparation

The first layer of exterior lettuce leaves was removed and discarded to prepare the lettuce rinsate. Then, 10 g of lettuce were weighed and added to a sterile filtered stomacher bag with 25 mL of 1 × PBS. The lettuce was then stomached for 1 min at 230 rpm (Stomacher 400 Circulator; Seward, Worthing, United Kingdom). After stomaching, the lettuce rinsate was poured from the filtered portion of the stomacher bag into a 50 mL centrifuge tube. To prepare the *L. monocytogenes* lettuce rinsate inoculum, 10 mL of the lettuce rinsate was used to resuspend the *L. monocytogenes* pellet by vortexing.

2.2.3. Milk preparation

To utilize the whole milk and lowfat milk, each hermetically-sealed individual carton was vigorously shaken for 1 min. Immediately after shaking, 10 mL of the whole milk or lowfat milk was added to the 50 mL centrifuge tube with the pelleted *L. monocytogenes* and vortexed. After use, the whole milk or lowfat milk carton was immediately discarded.

2.3. Sampling device

One environmental sampling device was used in this research. The polyurethane foam EZ Reach Sponge Sampler (referred to as the 'sponge'; World Bioproducts, Woodinville, WA) was moistened in 10 mL of 1 × PBS. The dimensions of the sponge are 1.5 in × 3 in (3.81 cm ×

7.62 cm). For use within the food industry, the EM tool selected would be used to sample larger surface areas (≤ 2 ft^2 [≤ 0.185 m^2]) per the manufacturer's recommendations.

2.4. Surfaces

2.4.1. Preparation

Sterile coupons (144 in^2 [929 cm^2]) of unpolished stainless steel 304 (Rose Metal Products, Inc., Springfield, MO) were used in the present study. The FDA and the USDA promote the swabbed sampling area to be 144 in^2 (929 cm^2) or more (FDA, 2015b; USDA, 2014). Clean, dry surfaces were saturated with 70% ethanol, wiped with paper towels, and spent 1 h under the UV-C lights in a Class II Biological Safety Cabinet prior to inoculation.

2.4.2. Inoculation

To inoculate the stainless steel surface, the *L. monocytogenes* inoculum was 10^9 CFU per surface. The 929 cm^2 surfaces were spot inoculated with 40 25-uL spots (total volume [V_t] = 1 mL) of inoculum. All inoculum spots were randomly dispersed evenly over the surface in the Class II Biosafety Cabinet. Samples were taken at 0 min, 1 h, and 24 h.

To determine if microorganisms are distributed from EM sampling, a 6 cm × 6 cm square in the corner of the surface was outlined with a red wax pencil (Crayola, New York, NY). Within the square, small, randomly dispersed inoculum spots (total volume [V_t] = 1 mL) were added and allowed to dry in the Class II Biosafety Cabinet.

2.4.3. Environmental treatment

An environmental chamber was used to control relative humidity (RH) and temperature (Caron Model 7000-10-1, Marietta, OH). RH is the amount of water vapor present in air expressed as a percentage of the amount needed for saturation at the same temperature. Inoculated surfaces were placed into the EM chamber, exposed to 30°C/85% RH for 1 h and 24 h, then sampled. In RTE food manufacturing environments, where *L. monocytogenes* is frequently found, the temperature conditions can range from refrigeration temperatures (4 to 6°C) to warmer than the average room temperature (20 to 30°C) and often with a high relative

humidity (personal communications). Thus, the authors selected the environmental conditions of 30°C/85% RH.

2.4.4. Decontamination

After treatment (see *Environmental treatment*) and recovery (see *Recovery of microorganisms)*, surfaces were decontaminated. Each surface was saturated with 70% ethanol, wiped with paper towels, washed in warm, soapy water (Dawn Liquid Dish Soap, Proctor & Gamble, Cincinnati, OH), then rinsed with DI water and air-dried.

2.5. Recovery of microorganisms

After the environmental exposure period, the sponges were used to sample the entire area of the 929 cm^2. The authors pressed and dragged the pre-moistened sampling device across the surface to sample the entire surface using the EM tool. Then, once the surface was sampled, the authors flipped the sampling device and repeated the sampling process; thus, the entire surface was sampled.

Post-sampling, the EM tool was aseptically placed back into the sample bag with the 10 mL of eluent and immediately stomached (Seward Stomacher 400 Circulator) for 1 min at 230 rpm. After processing, the sponge and eluent were recovered as described by Jones and Gibson (2021). The total recovered volume was approximately 5 mL for each sample. These samples went without enrichment and were plated onto selective agar for bacterial quantification (see *Bacteria cultivation and detection*).

2.5.1. Microbial distribution across the surface

Once the inoculum dried in the designated area (Figure 1) and was sampled as described in *Recovery of microorganisms,* the surface was rinsed with 10 mL of 1 × PBS and scraped with a cell scraper outside the inoculated region. These samples went without enrichment and were plated onto selective agar for bacterial quantification (see *Bacteria cultivation and detection*).

2.6. Data analysis

For comparison, the data (N=60) are reported in logarithmic loss (log_{10} CFU/surface). The log loss of *L. monocytogenes* was compared with four food residue types and one with no food residue utilizing one EM tool on one surface with one environmental condition across three exposure periods. All experiments were replicated and analyzed in technical duplicate.

The reported data represent the logarithmic loss of microorganisms after EM tool sampling compared to the initial number of microorganisms directly inoculated onto the sterile surface. The multiple comparisons of the means across food residue types and exposure times were analyzed via Tukey's honest significant difference (HSD) test. Any statistically significant differences were defined by $p \leq 0.05$. All data analyses were completed in JMP® Pro 16 (SAS Institute, Inc., Cary, NC) statistical software.

3. Results

3.1. Comparing the log loss between organic matter types

To directly compare the influence of food residue types, the authors combined all sampling exposure periods (0 h, 1 h, 24 h). After conducting statistical analysis, the authors determined no statistical difference between the food residue types overall. However, at the 24 h sampling period, the no food residue samples varied significantly from the blended lettuce and lettuce rinsate samples (Table 1). The mean log loss of no food residue was 0.81 log loss CFU/surface, whereas the blended lettuce and lettuce rinsate samples lost 1.18 ± 0.33 and 1.20 ± 0.08 log loss CFU/surface, respectively. The mean log loss between food residue types at times 0 h and 1 h were not significantly different from each other.

3.2. Analysis of log loss within organic matter types

To analyze the impact of time on log loss, the authors compared each food residue type against itself over time (Figure 2). For all food residue types and no food residue, except for lettuce rinsate, the log loss followed the same pattern: significant differences between the 0 and

1 h samples compared to the 24 h samples. With lettuce rinsate, each sampling time point was significantly different from the other.

3.3. Spreading of *L. monocytogenes* across the surface

The authors determined that the sampling of dried *L. monocytogenes* does spread microorganisms across the surface as the sample is being collected. After inoculating with approximately 9 log CFU/surface, the log recovery from the sponge and post-sampling rinse from the surface was 6.38 ± 0.16 CFU/sponge and 2.93 ± 0.13 log CFU/surface respectively (data not shown).

4. Discussion

Listeria monocytogenes poses a significant public health burden and costly impact on the food industry; thus, food regulatory agencies and food firms have predominantly shifted towards a preventive food safety model to inhibit the presence of *L. monocytogenes*. While much of the microbiological testing in the food supply has justly focused on finished food products, these results do not show the full microbiological picture in the food supply (IDF, 2020; Mota et al., 2021). Due to the diverse nature of the food supply, the effectiveness of EM programs can be impacted by many variables. While the recovery of microorganisms is a topic that has been explored, the authors have identified a gap in the literature investigating the influence of food residue and type of food residue on EM program efficacy. Presently, the authors of this study sought to characterize how EM recovery of *L. monocytogenes* was impacted by the presence of different food residues. Moreover, the authors explored the concept that EM swabbing can spread *L. monocytogenes* across environmental surfaces. The presented data are crucial for improving EM programs, particularly when direct quantification of *L. monocytogenes* is desired.

While efforts to keep the environment in food processing facilities clean and sanitized are required and of the utmost importance, keeping a pristine environment is typically not feasible. Thus, during processing shifts, facility environmental surfaces can become soiled with

food residue from the processing of food products and ingredients. Food contamination with *L. monocytogenes* often occurs in food processing environments, where many factors can contribute to the survival, spread, and contamination of food (Garner & Kathariou, 2016; Kuda et al., 2015; Overney et al., 2016; 2017; Takahashi et al., 2011). For instance, in 2011, during the investigation of the U.S. listeriosis outbreak associated with cantaloupes, the FDA determined that the transfer of *L. monocytogenes* from equipment surfaces to melons in the processing facility was a possible route of contamination (FDA, 2011). Moreover, Nyarko et al. (2018) determined that *L. monocytogenes* persisted on surfaces soiled with cantaloupe extract (crushed seedless flesh and rind) greater than on clean surfaces at 25°C between zero and 14 days. Even very small amounts (ranging from pure extracts to 10^{-4} diluted) of extracted food residues can increase the persistence of *L. monocytogenes* on environmental surfaces (Kuda et al., 2015).

Understanding that food residue can increase persistence, the authors of the present study wanted to better understand how the presence of food residue influences microbial recovery of *L. monocytogenes*. To the best of the authors' knowledge, there has not been published research considering this aspect of EM. For instance, in 2020, Faille and co-authors determined that biofilm 'pieces' (e.g., food particles, biological matter, etc.) are dislodged during surface sampling and are absorbed into the swab matrices. The authors considered if these entrapped particles impact the release of microorganisms from the EM tool. However, the authors did not explore the impact of food residue on microbial release or recovery (Faille et al., 2020).

In 2019, Lane studied the influence of food residue and *L. monocytogenes* on adenosine triphosphate (ATP) readings. Jones et al. (2020) reviewed that all living organisms contain ATP. ATP sampling devices are frequently used to monitor the efficacy of a sanitation system within the food industry (Lappalainen et al., 2000; Vasavada 2001,). Lane (2019) researched ATP measurements for postharvest surface cleanliness evaluation. Suspended leafy greens

(romaine, spinach, red cabbage) were used as food residue inocula onto stainless steel, HDPE plastic, and bamboo wood coupons to represent postharvest surfaces. Unsurprisingly, the author's data showed that the concentration of *L. innocua* and leafy greens on a surface had a significant effect on the ATP device reading when swabbing the soiled surfaces ($p < 0.0001$). While ATP has specific uses, it is not useful as an indicator of pathogenic microorganisms in a food processing facility (Hammons et al., 2015, Osimani et al., 2014). Lane (2019) investigated the influence of food residue in food industry environmental scenarios but did not explore the impact of food residues on microbial recovery and quantification.

In the present study, the author chose four food residue types: lettuce rinsate, blended lettuce, whole milk, and lowfat milk. Each food residue was chosen intentionally. For example, over the years, research has been conducted on the impacts of milk and milk proteins on microbial adhesion and persistence on surfaces (Barnes et al., 1999, Helke et al., 1993, Helke & Wong, 1994). Research has proven that food particles, including milk, obstruct the adhesion of microorganisms to surfaces but facilitate persistence and growth (Bernbom et al., 2019; Takahashi et al., 2011). Kyere et al. (2019) determined that *L. monocytogenes* can rapidly colonize lettuce, emphasizing the importance of preventing foodborne contamination from the environment.

However, despite the obvious differences in components between the food residue types tested in the present study, there were no significant differences between food residue types overall when combining all factors. When studying specific sampling points, at 24 h, significant differences were found between the blended lettuce (1.18 ± 0.33 log loss CFU/surface) and lettuce rinsate (1.20 ± 0.08 log loss CFU/surface) samples compared to the no food residue (0.81 ± 0.14 CFU/surface) (Table 1). As previously discussed, research indicates that the presence of organic matter may inhibit microbial attachment to surfaces. This leads to the question of whether the food residue is obstructing the release of microorganisms from the EM sampling tool.

The log loss followed the same pattern within each food residue type, except for lettuce rinsate. Significant differences appeared between 0 and 1 h samples compared to the 24 h samples (Figure 2). Interestingly, within each food residue type and comparing food residue types, the significant differences in recovery begin to appear at the 24 h sampling period timepoint (Table 1; Figure 2). With little research on this topic, future work should address if differences in food residue types appear post-24 h.

While EM program efficacy and microbial persistence vary widely based on the environment in the food processing facility, the act of swabbing the surfaces remains constant. Most EM tool manufacturers recommend utilizing the entire surface of the moistened swab or sponge to sample the surface; thus, this typically means covering the entire sampling surface in a two-directional checkered pattern (3M, 2012; World Bioproducts, 2022). In the present study, the authors questioned whether the act of swabbing the surface could spread microorganisms across the EM surface.

The authors inoculated a specific area, sampled per manufacturer recommendations, and then sampled the surface post-swabbing outside of the inoculation zone to answer this question. The authors determined that if there are *L. monocytogenes* present in a specific place on the surface, EM sampling does spread *L. monocytogenes* to areas of the surface where it was not originally present. These data suggest a risk of spreading *L. monocytogenes* throughout the processing facility by dislodging the microorganisms during the sampling process. The present study aligns with a study by Martinez and co-authors (2021) where the authors used a squeegee to remove condensation from overhead surfaces, a common practice in the food industry. If *L. innocua* is present in the condensation, then using a squeegee tool spreads the bacteria across the surface. The authors found that the *L. innocua* traveled at least 40.64 cm (16 in) on surfaces due to condensation removal. The present study recommends enhanced cleaning and sanitation measures to be put in place post-swabbing to hinder the spread of *L. monocytogenes*.

There are a few limitations in the present study. Primarily, this study was conducted in a controlled-environment laboratory setting. This is in direct contrast to the food industry, where the recovery of microorganisms from sponges would be completed in a commercial laboratory with potential processing delays, as explored previously by Jones and Gibson (2020). Additionally, the present study only focused on one microorganism type and did not account for the complex microbial matrices often associated with the environment.

5. Conclusions

Environmental monitoring programs are a required yet vaguely defined area within the food industry. These programs aim to prevent cross-contamination from the environment to foods and are used as a cleaning and sanitation verification tool. Additionally, EM swabbing takes place during the production cycle and during FBDO investigations, where surfaces may not be pristinely clean. The authors of the present study sought to explore the impact of different food residues in the recovery of *L. monocytogenes*. Data suggest that there is little variability amongst food residue types overall. However, differences begin to appear at the 24 h time period. Moreover, the authors determined that EM sampling can spread *L. monocytogenes* on the surfaces found in processing facilities. In EM programs, future research should consider:

- Exploring the effect of other complex extrinsic matrices (e.g., biofilms)
- Researching the impact of organic matter types over longer time periods
- Examining microorganisms on EM tools and surfaces pre- and post-swabbing and processing using advanced microscopy methods
- Expanding to include other relevant foodborne pathogens

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7. Tables

Table 1. Comparison of log loss between organic matter types at each sampling time point

Sample (mean log loss (CFU/surface))*

	No Organic Matter		Blended Lettuce		Lettuce Rinsate		Lowfat Milk		Whole Milk		Overall	
Exposure Time (h)**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	0.35^{a}	0.05	0.43^{a}	0.07	0.35^{a}	0.06	0.29^{a}	0.11	0.36^{a}	0.07	0.36	0.08
1	0.48^{b}	0.12	0.51^{b}	0.10	0.59^{b}	0.11	0.54^{b}	0.24	0.55^{b}	0.18	0.53	0.14
24	0.81^{c}	0.14	1.18^{d}	0.33	1.20^{d}	0.08	0.95^{cd}	0.04	1.11^{cd}	0.08	1.05	0.21
Overall	0.55^{e}	0.22	0.71^{e}	0.38	0.71^{e}	0.40	0.59^{e}	0.32	0.67^{e}	0.35	0.65	0.14

* Levels not followed by the same letter are significantly different.

** Statistical comparison is compared within the rows of the table.

8. Figures

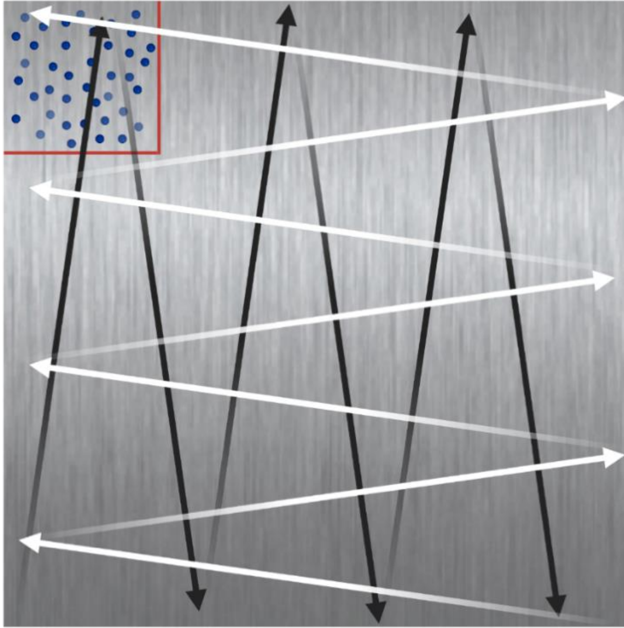

Figure 1. Inoculation and sampling pattern utilized in *Listeria monocytogenes* distribution experiments. The authors inoculated a specific area (6 cm × 6 cm) in the corner of the surface and allowed inoculum to dry. Then, the authors sampled with the environmental monitoring sponge, per manufacturer recommendations. Finally, the authors sampled the surface post-swabbing outside of the inoculation zone utilizing a cell scraper and a rinse method.

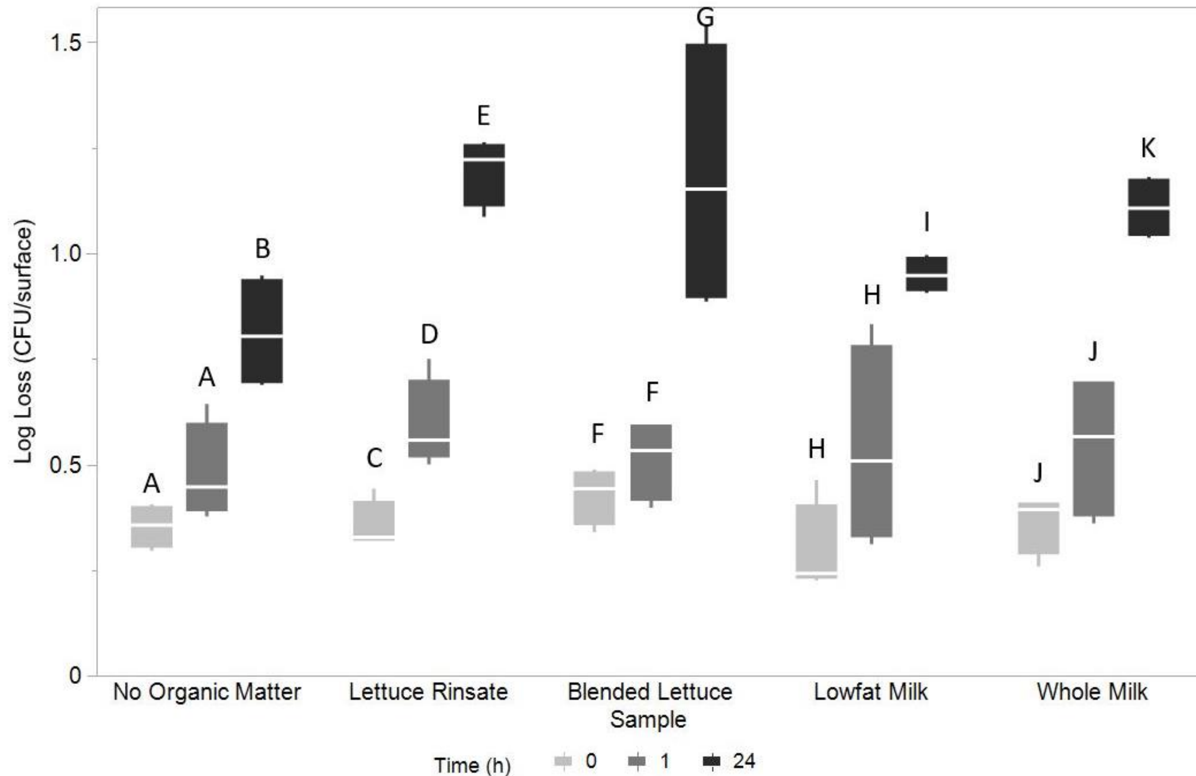

Figure 2. Log loss (CFU/surface) of *Listeria monocytogenes* by organic matter type over time. In the box plots, the boundary of the box closest to 0 indicates the 25th percentile, a white line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whisker indicate outliers outside the 10th and 90th percentiles. Different letter ranges (a-b; c-e; f-g; h-i; j-k) indicate significant differences ($p < 0.05$) of the log loss (CFU/surface) of *Listeria monocytogenes* between the same organic matter type (e.g., blended lettuce) over time.

Chapter 6: Food Residue Types Influence the Recovery of *Salmonella* Typhimurium from Surfaces

Abstract

Non-typhoidal *Salmonella* is responsible for 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths in the U.S. annually. Historically, *Salmonella enterica* subspecies *enterica* serovars have been linked to raw meat products, eggs, produce, and other minimally processed products. However, *Salmonella* recalls and foodborne disease outbreaks (FBDO) are increasingly linked to low water activity (A_w) foods and the production environment. Environmental monitoring (EM) programs are designed to prevent cross-contamination, verify cleaning and sanitation systems, and are used as a tool for seeking-and-destroying pathogens during FBDOs (FDA, 2015; 2020c). While EM is frequently conducted on clean and sanitized surfaces, there are instances of random EM sampling during processing and FBDO investigations where surfaces may contain organic matter, or food residues, in the production environment. These investigations may take place when food residues are present on the environmental surfaces. Thus, this study aimed to determine if organic matter, specifically the type of organic matter, impacts the recovery of *S.* Typhimurium found in food processing environments. The factors considered included three types of food residue (powdered infant formula, all-purpose flour, and whole milk dairy powder) compared to the presence of no organic matter over time (0, 1, 24 h). Additionally, the authors determined if environmental sampling facilitates spreading of *Salmonella* across food processing surfaces. Significant differences in the mean log loss of *S.* Typhimurium ($p<0.05$) between all food residue types at 1 h and 24 h sampling times were observed. Moreover, within each food residue type, significant differences were found over time. The authors also concluded that environmental surface swabbing could spread *S.* Typhimurium on surfaces found in food processing environments. Overall, data indicate that the type of food residue impacts the recovery of *S.* Typhimurium during

environmental sampling which is highly relevant for the development of EM programs and the success of FBDO investigations.

1. Introduction

Non-typhoidal *Salmonella* is responsible for 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths in the U.S. annually (CDC, 2022). Globally, the burden of foodborne non-typhoidal *Salmonella* is immense, resulting in approximately 80.3 million illnesses and 155,000 deaths worldwide (Majowicz et al., 2010). *Salmonella*, part of the Enterobacteriaceae family, is a Gram-negative, facultative anaerobe that is linked to the gastrointestinal tract of animals (Coburn et al., 2007). Historically, *Salmonella enterica* subspecies *enterica* serovars have been linked to raw meat products, eggs, produce, and other minimally processed products (CDC, 2021). However, *Salmonella* recalls and foodborne disease outbreaks (FBDO) have been increasingly linked to low water activity (A_w) foods (Bourdichon et al., 2021).

Water activity is the amount of free, unbound water in a food product; thus, available for microbial growth (Codex Alimentarius, 2015). Ranging from zero to one, A_w is a ratio between the vapor pressure of the food and the vapor pressure of distilled water under identical conditions. Low A_w foods are considered to have a A_w less than 0.85 (Codex Alimentarius, 2015). The most common foodborne pathogens cannot grow at a A_w of 0.85 or below, including *Salmonella*, which requires a A_w of 0.94 for growth (FDA, 2018). However, *Salmonella* is known to persist in low A_w food products.

Powdered food products have been linked to *Salmonella* foodborne illnesses and *Salmonella*-positive food samples resulting in significant food recalls. For example, *Salmonella* FBDOs have been linked to infant formula for decades, resulting in expansive recalls and deaths (Rowe et al., 1987; Threlfall et al., 1998; Brouard, et al., 2007; Jourdan-da Silva et al., 2018; FDA, 2022a). Moreover, in the last ten years, the U.S. Food and Drug Administration (FDA) has administered five major recalls of bulk wheat and tree nut flours (FDA, 2019a; 2019b;

2020b; 2020d; 2021). Other low A_w foods, such as whole tree nuts, have been linked to *Salmonella* FBDO outbreaks and recalls (FDA, 2020a; 2020e). Due to this evolving threat, there has been a significant amount of research conducted on the persistence and thermal resistance of *Salmonella* in low A_w foods and environments (Beuchat et al., 2011; Gruzdev et al., 2011; Finn et al., 2013; Koseki et al., 2015; Sekhon et al., 2021; Wei et al., 2021; Michael et al., 2022). This persistence in low A_w foods and environments was best demonstrated in the recurrent outbreak of *Salmonella enterica* serotype Agona in 1998 and 2008 in dry, unsweetened cereal manufactured by the same company at the same processing facility (Russo et al., 2013). Despite being a decade apart, the outbreak strains from the 1998 and 2008 were linked (Russo et al., 2013). Regardless of the persistence of *Salmonella* in the food processing environment, there is a dearth of research regarding surface sampling in the presence of low A_w food residues.

Environmental monitoring (EM) is a required surface sampling program promulgated by the FDA's Food Safety Modernization Act (FDA, 2015; 2020c). Environmental monitoring programs are designed to prevent cross-contamination, verify cleaning and sanitation measures, and be used as a tool for seek-and-destroying pathogens during FBDOs (FDA, 2015; 2020c). While EM is frequently conducted on clean and sanitized surfaces, there are instances of random EM sampling during processing and *Salmonella*-positive samples and FBDO investigations where surfaces may contain organic matter, or food residues, in the production environment. *Salmonella*-positive samples and FBDO investigations result in an in-depth sampling of the processing facility and equipment in order to find the reservoir of the pathogenic microorganism. Despite being a required program, the EM rules and guidelines are vague, as discussed by Jones et al. (2020), Bourdidan et al. (2021), and Mota et al. (2021),

The release of microorganisms from EM tools and the recovery of microorganisms from surfaces are important areas of research that have been explored (Moore et al., 2007; Margas et al., 2014; Landers et al., 2014; Keeratipibul et al., 2017; Jones et al., 2021). However, this

type of research is primarily conducted on sterile surfaces where no organic matter or food residue is present. As a result, there is no data on the influence of organic matter (e.g., product, food ingredients) on the recovery of *Salmonella*. The research conducted in the effort to characterize EM programs can improve future EM programs and reduce the risk of FBDOs in the food industry. Thus, this study aimed to determine if organic matter, specifically the type of organic matter, impacts the recovery of *Salmonella* found in food processing environments. The factors considered included three types of food residue compared to the presence of no food residue over time. Additionally, the authors determined if EM sampling facilitates the spread of *Salmonella* across food processing surfaces. In conclusion, the most influential factors in the EM recovery in the presence of food residue and movement of *Salmonella* were identified.

2. Materials and Methods

2.1. Bacteria cultivation and detection

This study used *Salmonella enterica* serotype Typhimurium (ATCC 14028; American Type Culture Collection, Manassas, VA). *Salmonella* Typhimurium was stored in a frozen 50% glycerol stock, and the bacterial culture preparation was achieved by following methods described by Gibson et al. (2019). The overnight *S.* Typhimurium culture was titered at approximately 10^9 colony forming units per mL (CFU/mL). After centrifugation, the pelleted *S.* Typhimurium was resuspended into 10 mL of 1 × PBS and held at room temperature (approximately 20°C) for less than 1 h until the food residue samples could be prepared (see *Organic matter*). 'No food residue' experiments and the microbial distribution experiments (see *Microbial distribution across the surface*) utilized 1 × PBS to resuspend the pellet. After sampling to detect *S.* Typhimurium, the authors followed detection methods defined by Jones and Gibson (2021). Experiments conducted in this study were replicated, and samples were plated in duplicate.

Three types of food residue were utilized in the present study. Infant formula (Infant Formula Milk-Based Powder with Iron, Parent's Choice™, Bentonville, AR), dried whole milk

dairy powder (Horizon Organic, Broomfield, CO), and all-purpose flour (Great Value, Walmart Inc., Bentonville, AR) were purchased at a local grocery store. For all three food types, the preparation of the inoculated food residues is the same and adapted from Wei et al. (2021).

One hundred grams of food was weighed and poured into a stomacher bag with no filter. The 10 mL of *S.* Typhimurium inoculum was held in a 15 mL centrifuge tube. The centrifuge tube cap was removed and replaced with a fingertip sprayer (2 fl oz (59 mL) Fingertip Sprayer, Equate™, Bentonville, AR). Inside the Class II Biological Safety Cabinet, the entire inoculum was spritzed directly into the food sample, then the stomacher bag was sealed with tape. The inoculated food was manually hand-massaged for 10 min, breaking apart any large clumps or food residue along the wall of the bag. After 10 minutes, the bag of inoculated food was laid flat, and the food was distributed evenly along the bag. A rubber mallet was then used to break up clumps until no clumps remain. The inoculated food was then held at room temperature for 24 h.

2.2. Sampling tool

One type of environmental sampling device was used in this research. The polyurethane foam EZ Reach Sponge Sampler (referred to as the 'sponge'; World Bioproducts, Woodinville, WA) was moistened in 10 mL of 1 × PBS. The dimensions of the sponge are 1.5 in × 3 in (3.81 cm × 7.62 cm). The EM tool selected would be used to sample larger surface areas (≤ 2 ft^2 [≤ 0.185 m^2]), per the manufacturer's recommendations, for sampling in the food industry.

2.3. Surfaces

2.3.1. Preparation

Sterile coupons (144 in^2 [929 cm^2]) of unpolished stainless steel 304 (Rose Metal Products, Inc., Springfield, MO) were used in the present study. The FDA and the USDA encourage the swabbed sampling area to be 144 in^2 (929 cm^2) or more (FDA, 2015; USDA, 2014). Clean, dry surfaces were saturated with 70% ethanol, wiped with paper towels, and spent 1 h under the UV-C lights in the Class II Biological Safety Cabinet before inoculation.

2.3.2. Inoculation

To inoculate the stainless steel surface, the *S.* Typhimurium inocula ranged from 10^7 to 10^8 CFU per surface. The inoculated food residue was weighed out at 1.5 g per sample. The 1.5 g of inoculum was passed through a sterile, fine-mesh sieve onto the 929 cm^2 stainless steel surface in an off Class II Biological Safety Cabinet with the sash pulled down. Samples were taken at 0 min, 1 h, and 24 h.

To determine if microorganisms are further dispersed during EM sampling, a 6 cm × 6 cm square in the corner of the surface was outlined with a red wax pencil (Crayola, New York, NY). Within the square, small, randomly dispersed inoculum spots (total volume [V_t] = 1 mL) were added and allowed to dry in the Class II Biological Safety Cabinet.

2.3.3. Environment treatment

An environmental chamber was used to control relative humidity (RH) and temperature (Caron Model 7000-10-1, Marietta, OH). RH is the amount of water vapor present in air expressed as a percentage of the amount needed for saturation at the same temperature. Inoculated surfaces were placed into the EM chamber, exposed to 30°C/30% RH for 1 h and 24 h, then sampled. In powdered food manufacturing, where *Salmonella enterica* is frequently detected, the temperature conditions can range from 20 to 30°C and often with low relative humidity (personal communications). Thus, the authors selected the environmental conditions of 30°C/30% RH.

2.3.4. Decontamination

After treatment (see Environmental treatment) and recovery (see Recovery of microorganisms), surfaces were decontaminated. Each surface was saturated with 70% ethanol, wiped with paper towels, washed in warm, soapy water (Dawn Liquid Dish Soap, Proctor & Gamble, Cincinnati, OH), then rinsed with DI water and air-dried.

Recovery of microorganisms

After the environmental exposure period, the sponges were used to sample the entire area of the 929 cm^2. The authors pressed and dragged the pre-moistened sponge across the surface to sample the entire surface using the EM tool. Then, once the surface was sampled, the sampling device as the flipped, and the sampling process was repeated; thus, the entire surface was sampled.

After surface sampling, the EM tool was aseptically placed back into the sample bag with the 10 mL of eluent and immediately stomached (Seward Stomacher 400 Circulator) for 1 min at 230 rpm. After processing, the sponge and eluent were recovered, as described by Jones and Gibson (2021). The total recovered volume was approximately 5 mL for each sample. These samples went without enrichment and were plated onto selective agar for bacterial quantification (see *Bacteria cultivation and detection*).

2.4. Microbial distribution across the surface

Once the inoculum dried in the designated corner and was sampled as described in *Recovery of microorganisms,* the surface was rinsed with 10 mL of 1 × PBS and scraped with a cell scraper outside the inoculated region. These samples went without enrichment and were plated onto selective agar for bacterial quantification (see *Bacteria cultivation and detection*).

2.5. Data analysis

For comparison, the data (N=48) are reported in logarithmic loss (log_{10} CFU/surface). The log loss of *S.* Typhimurium was compared with three food residue types and one with no food residue utilizing one sponge on one surface with one environmental condition across three exposure periods. All experiments were replicated and analyzed in technical duplicate.

The reported data represent the logarithmic loss of microorganisms after EM tool sampling compared to the initial number of microorganisms directly inoculated onto the sterile surface. The multiple comparisons of the means across food residue types and exposure times were analyzed via Tukey's honest significant difference (HSD) test. Any statistically significant

differences were defined by $p \leq 0.05$. All data analyses were completed in JMP® Pro 16 (SAS Institute, Inc., Cary, NC) statistical software.

3. Results

3.1. Evaluating the log loss between organic matter types

To compare the impact of food residue types on EM sampling, all sampling exposure periods (0 h, 1 h, 24 h) were pooled. After conducting statistical analysis, it was determined that the only significant difference was between no organic matter (3.90 ± 2.24 mean log loss CFU/surface) and all other food residue types (Table 1). The loss of *S.* Typhimurium on the no organic matter samples was significantly greater than the food residue samples, as shown in Figure 1.

At each sampling time period, the food residue types and no organic matter were statistically compared to each other (Table 1). At the 1 h and 24 h sampling time periods, all samples were significantly different from each other (Table 1). However, at sampling time 0 h, the only two samples that were significantly different from each other were the infant formula and whole milk dairy powder, which had a 0.57 ± 0.10 and 1.09 ± 0.40 mean log loss CFU/surface, respectively.

3.2. Assessment of log loss within organic matter types

To assess the influence of time on the log loss of *S.* Typhimurium, each food residue was compared against itself over time (Figure 2). For no organic matter and all-purpose flour, each sampling time period was significantly different ($p \leq 0.05$) from each other. The 24 h sample was significantly different from the 0 h and 1 h time points for infant formula. The whole milk dairy powder was similar at time 0 h and 24 h and significantly different at time 1 h.

3.3. Distribution of *Salmonella* Typhimurium across the surface

It was determined that the environmental sampling of dried *S.* Typhimurium does spread microorganisms across the surface as the sample is being collected. After inoculating with approximately 8 log CFU/surface, the log recovery from the sponge and post-sampling rinse

from the surface was 5.77 ± 0.10 CFU/sponge and 2.70 ± 0.08 log CFU/surface, respectively (data not shown).

4. Discussion

Non-typhoidal *Salmonella* is a significant public health burden and poses a detrimental and costly impact on the global food industry. To protect consumers and reduce the risk of contaminated food products from entering the marketplace, food regulatory agencies have focused on enforcing a preventive food safety system to hinder the presence of *Salmonella* in the food supply. Before the shift in dogma from reactionary to preventive, much of the microbiological testing in the food industry was justifiably focused on sampling finished food products. However, it is well known that finished product sampling results do not represent all microbiological activity in the food supply (IDF, 2020; Mota et al., 2021).

Environmental monitoring programs are one of the tools utilized in the food industry to better understand the microbiological activity and niches in food manufacturing facilities (FDA, 2017; 2020c). However, even though EM is a mandatory activity, the effectiveness of a pathogen EM program is influenced by many factors. Namely, extrinsic factors such as the EM tool selected (e.g., material, size), surface characteristics, microbial attachment to the surface, and surface area sampled, as examined by Jones et al. (2020). While the aforementioned areas of EM research have been explored, the present study aimed to address the lack of available information on the influence of food residue and the type of food residue on EM programs. Specifically, it was hypothesized that food residue and food residue types impact the EM recovery of *S.* Typhimurium. Additionally, the study aimed to determine if EM swabbing can spread and distribute *S.* Typhimurium across stainless steel environmental surfaces. The data collected in this study are critical for improving EM program implementation and accuracy, especially when direct quantification of *S.* Typhimurium is chosen.

Even though cleaning and sanitation programs are set in place, maintaining a spotless food manufacturing environment is not feasible. During production, environmental surfaces can

become soiled with food residues during the processing of food products and ingredients. Widespread *Salmonella* contamination in food products is frequently linked to the food processing environment, where extrinsic factors influence the persistence and distribution of pathogens (Cahill et al., 2008). For example, FBDO investigative environmental sampling during numerous milk powder and powdered infant formula outbreaks found that EM samples from the manufacturing environment were *Salmonella*-positive (Cahill et al., 2008). However, the presence of *Salmonella* in the low A_w foods and food manufacturing environments is unsurprising. Research shows that *Salmonella* can survive in low A_w foods, such as stored wheat flour, for at least 360 days at approximately 20°C in a resealable bag (Michael et al., 2022). In fact, *Salmonella* has been demonstrated to be more resistant to intervention measures, such as thermal treatments, in whole milk dairy powder (Sekhon et al., 2021). Knowing that the presence of food residues can influence the persistence of *Salmonella*, the present study aimed to explore how the food residues on surfaces influence the recovery of *S.* Typhimurium. To the best of our knowledge, there has been no published research exploring this characteristic in EM.

In the present study, three food residue types were selected as organic matter: infant formula, all-purpose flour, and whole milk dairy powder. The selected food residue types have been either 1) linked to a FBDO caused by *Salmonella* (Rowe et al., 1987; Threlfall et al., 1998; Brouard, et al., 2007; Jourdan-da Silva et al., 2018; FDA, 2019a; 2019b; 2020b; 2020d; 2021; 2022a) or 2) have been shown that *Salmonella* can persist in the product or the processing environment of that product over time (Sekhon et al., 2021; Michael et al., 2022). Interestingly, there were no significant differences between the food residue types when combining all factors in the present study. Overall, the significant difference was between the no organic matter and all other individual food residue types (Table 1). At sampling time 0 h, the two samples that were significantly different from each other were the infant formula and whole milk dairy powder. Whereas, at 1 h and 24 h sampling points, all food residues and no organic matter were all

significantly different from each other (Table 1). Therefore, since significant differences appear at later sampling time points, future research should explore if the differences in food residue types continue post-24 h. These data concur with Moore et al. (2007) who found that the presence of artificial organic matter (tryptic soy broth + 5% horse serum) led to a greater recovery of *S.* Typhimurium compared to no organic matter from 0 to 6 h at ambient temperatures. Similarly, de Cesare and co-authors (2003) found that, overall, the presence of artificial organic matter (trypticase soy broth) slowed the time it took for a five-serovar *Salmonella* cocktail to result in a three-log reduction compared to no organic matter which was a mean 2,092 min (34.9 h) versus 687 min (11.5 h), respectively.

Visually, the behavior of the food residue types varied (Figure 1). All-purpose flour, infant formula, and no organic matter resulted in mean log losses of *S*. Typhimurium over time (Figure 1). However, the whole milk dairy powder data suggest either 1) a greater dislodging of *S*. Typhimurium from the surface at 24 h compared to 1 h, or 2) *S*. Typhimurium began to replicate and increase in number under these conditions. Significant differences were observed within each food residue type (Figure 2). Each food residue type had different patterns of microbial mean log losses, as visualized in Figure 2. These data suggest that food residue types impact the recovery of *S*. Typhimurium causing the bacteria to behave differently over time. Speculatively, these differences could be due to nutrient content differences among various food types. For instance, research has shown that proteins (Hirai et al., 1991), fats (Craven et al., 1975; Lehmacher et al., 1995; Werber et al., 2005), and food particle density (Li Cari and Potter, 1970; Miller et al., 1972) can all influence the persistence of *Salmonella* in low water activity. Conversely, Sekhon et al. (2021) found that over 180 days in resealable bags and plastic secondary containers at ambient temperatures, there were no differences in the survival of five-serovar *Salmonella* cocktail between non-fat dry milk powder and whole milk dairy powder. Future research should continue to explore the reasoning behind these differences, especially in a food processing-like environmental setting.

Environmental program efficiency and microbial persistence vary based on the environmental conditions within the food processing facility. Meanwhile, the methods utilized in swabbing the surfaces remain the same. Environmental monitoring tool manufacturers direct users to sample the surface while maximizing the surface of the moistened swab or sponged used. To do this, the user must typically follow a two-directional checkered pattern that covers the entire surface (3M, 2012; World Bioproducts, 2022). Following this guidance, the present study aimed to determine if the act of swabbing the surface with a moistened sponge could further the spread and distribution of *S.* Typhimurium across the surface. Indeed, *Salmonella* was spread from the initial point of inoculation to clean areas of the surface via swabbing. These data suggest a risk of spreading *S.* Typhimurium throughout the food processing environment by dislodging the microorganisms during the sampling process. This is a concern, especially in FBDO scenarios, where the presence of a pathogen is detected. For example, in the 2022 outbreak of *Cronobacter sakazakii* in powdered infant formula, the food firm and the FDA both conducted extensive environmental sampling on food and non-food contact surfaces. During the FBDO outbreak investigation, the food firm identified at least 20 instances of *Cronobacter*-positive environmental samples between February 6th and February 20th, 2022 (FDA, 2022b). Without extensive cleaning and sanitation measures post-swabbing, these sampling events could contribute to the distribution of pathogens in the environment.

As for constraints, there are some limitations in the present study. Foremost, this study was performed in a controlled-environment laboratory setting. This is in immediate contrast to the food industry, where the recovery of microorganisms from sponges would be completed in a commercial laboratory with likely processing delays, as explored previously by Jones and Gibson (2020). Furthermore, the present study only focused on one microorganism type and did not account for the complex microbial matrices often found within the environment.

5. Conclusions

Environmental monitoring programs are an integral part of food safety initiatives employed by the food industry but are largely undefined in how they are implemented. While routine EM sampling takes place after cleaning and sanitation, surfaces can also be sampled during food processing and FBDO investigations where food ingredient residue may be present. This study aimed to determine if food residues impact the recovery of *S.* Typhimurium. Data indicate variability amongst the food residue types, especially at 1 h and 24 h time points. Additionally, the authors concluded that EM swabbing could spread *S.* Typhimurium on surfaces found in food processing environments. To continue characterizing and improving EM programs and protocols, future research should include:

- Determining the impact of complex extrinsic matrices (e.g., biofilms)
- Exploring the impact of organic matter over longer time periods
- Utilizing advanced microscopy to visualize microorganisms on EM tools and surfaces pre- and post-swabbing

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7. Tables

Table 1. Evaluating the log loss of each organic matter type by sampling time point

	Sample (mean log loss (CFU/surface))*									
	No Organic Matter		All-Purpose Flour		Infant Formula		Whole Milk Dairy Powder		Overall	
Exposure Time (h)**	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	1.00[ab]	0.03	0.71[ab]	0.15	0.57[b]	0.10	1.09[a]	0.40	0.84	0.29
1	4.62[c]	0.19	1.14[d]	0.05	0.58[e]	0.10	1.80[f]	0.12	2.04	1.61
24	6.10[g]	0.12	1.51[h]	0.21	0.89[i]	0.10	1.19[j]	0.09	2.42	2.21
Overall	3.90[k]	2.24	1.12[L]	0.37	0.68[L]	0.18	1.36[L]	0.40	1.76	1.69

* Levels not followed by the same letter are significantly different.
** Statistical comparison is compared within the rows of the table.

8. Figures

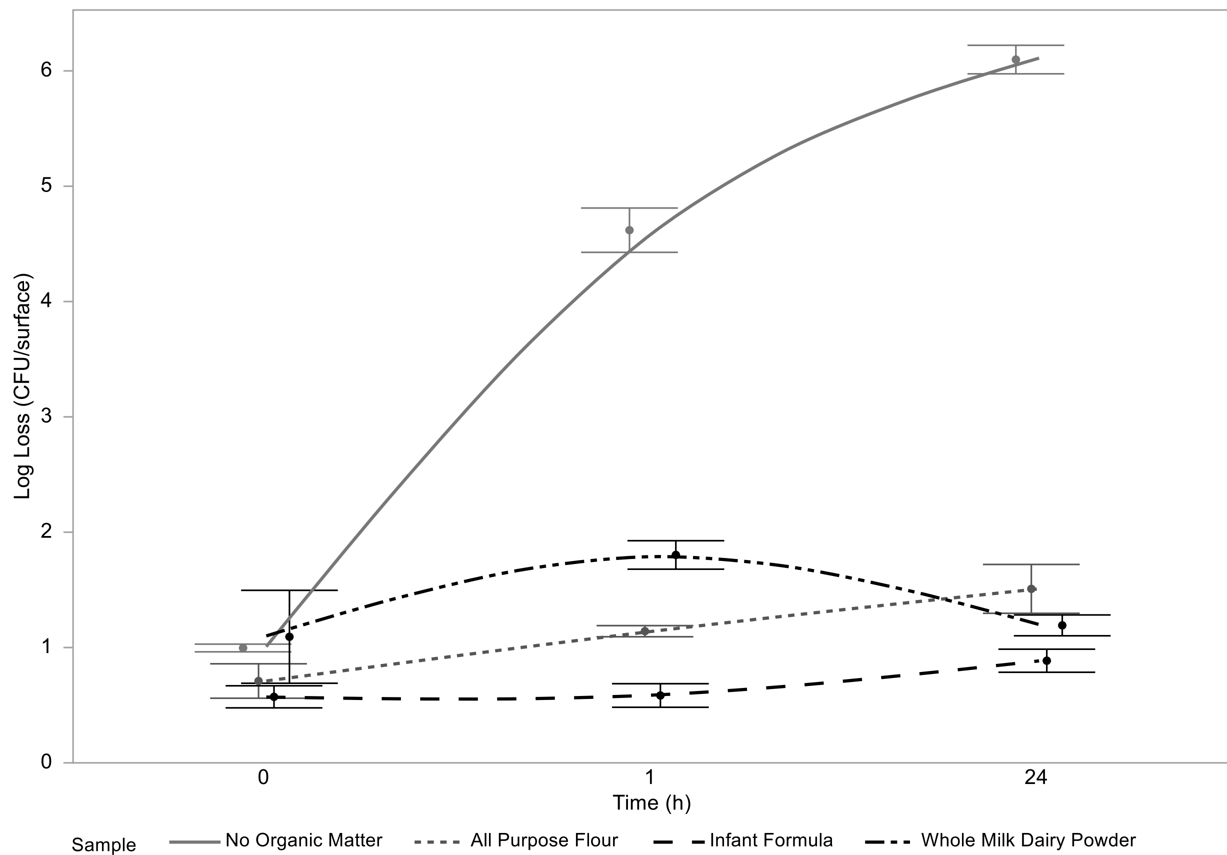

Figure 1. The log loss of *Salmonella* Typhimurium over time. Each error bar is constructed using 1 standard deviation from the mean.

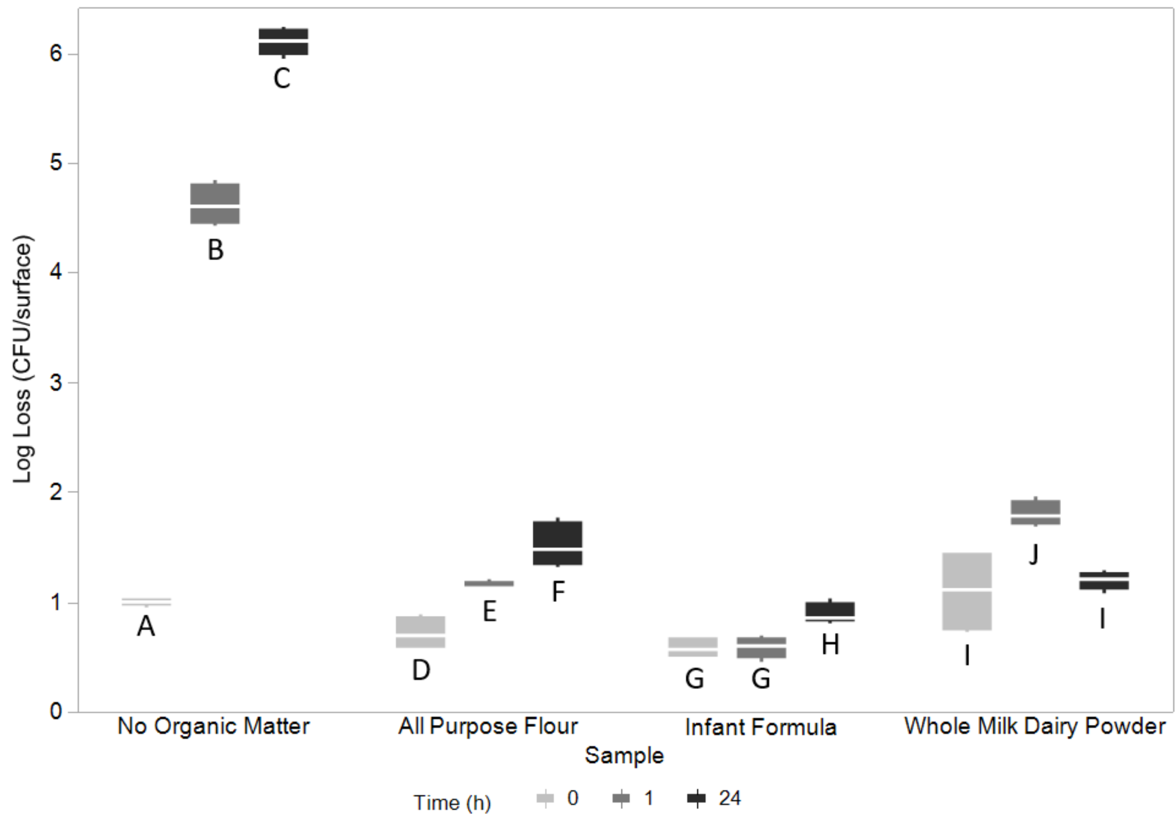

Figure 2. The log loss of Salmonella Typhimurium within the same organic matter type over time (h). In the box plots, the boundary of the box closest to 0 indicates the 25th percentile, a white line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whisker indicate outliers outside the 10th and 90th percentiles. Different letter ranges (a-c; d-f; g-h; i-j) indicate significant differences ($p < 0.05$) of the log loss (CFU/surface) of Salmonella Typhimurium within the same organic matter type over time.

Chapter 7: Conclusions

With one in six Americans becoming ill every year due to foodborne disease, improving food safety is of utmost importance (Scallan et al., 2011a; 2011b). Environmental monitoring (EM) is part of the critical infrastructure of enhancing food safety in the U.S. However, despite being a required activity, many gaps in EM standardization exist. This dissertation aimed to characterize the most influential factors in EM to increase the effectiveness of EM programs in the food industry.

Studies regarding EM and microbial recovery vary throughout the literature. The variability in results possibly exists due to numerous factors including, but not limited to, the EM sampling tools, environmental conditions, and target microorganisms. Many surface sampling studies focus on the recovery of a variety of microorganisms; however, there is limited research evaluating and validating novel sampling devices or diverse EM tool matrices. For this reason, industry members may have difficulty choosing the most appropriate EM tool for their food processing facility due to the range of environmental conditions, microorganisms of concern, and methods used across EM research. These challenges are even more predominant when sampling for human enteric viruses.

Here, when evaluating the release of microorganisms, data indicated that EM tool material composition impacted the release of microorganisms ($p = 0.0001$), whereas the polyurethane foam EM tool released TV more readily than the cellulose EM tool. Conversely, the decreasing inoculum levels did not statistically differ in the release of microorganisms from the EM tool matrices. In addition, no significant difference was found between the machine stomacher and manual elution by human operator or between operators. These findings provide a detailed characterization of two commercially-available EM tools, and the distinctions identified in this study can be used to improve the effectiveness of EM programs.

Next, surface type and environmental conditions further highlighted the differences in EM efficacy. Specifically, microbial recovery was compared across 1) microorganism type, 2)

surface type, 3) environmental temperature and relative humidity, and 4) exposure time (i.e., time on the surface). Data indicated that microbial recovery from environmental surfaces significantly ($p \leq 0.05$) varies by microorganism type, environmental conditions, and exposure time. For instance, all microorganisms were significantly different from each other, with the greatest mean log loss being TV and the lesser loss being *L. monocytogenes* at 4.94 ± 1.75 log PFU/surface and 2.54 ± 0.91 log CFU/surface, respectively. Overall, these data can be used to improve the success of EM programs and underscores the need to better comprehend how EM sampling results are impacted food manufacturing environmental conditions.

Lastly, specific microorganisms and food residues were studied to understand the nuances in pathogen-food associations and varying environmental conditions found in the food industry. As a result, the study aimed to determine if food residues, specifically the type of food residue, impacted the recovery of *L. monocytogenes* found in food processing environments. The factors considered included four types of food residue (lettuce rinsate, blended lettuce, lowfat milk, and whole milk) compared to the presence of no food residue over time (0, 1, 24 h). Data suggest that there is little variability amongst food residue types overall. However, differences begin to appear at the 24 h sampling period.

A similar study was conducted with *S*. Typhimurium and low water activity foods (all-purpose flour, powdered infant formula, and whole milk dairy powder) compared to the presence of no food residue over time (0, 1, 24 h). Significant differences in the mean log loss of *Salmonella* Typhimurium ($p<0.05$) were observed between all food residue types at 1 h and 24 h sampling times. Moreover, significant differences were found within each food residue type over time. Overall, these data indicate that the type of food residue impacts the recovery of *S.* Typhimurium and *L. monocytogenes* during environmental sampling, which is highly relevant for the development of EM programs and the success of FBDO investigations.

Environmental monitoring programs are a key factor in the implementation of preventive food safety system. Whilst being a required monitoring activity, many gaps exist in the research

and regulations surrounding EM. This dissertation identified and filled some of the gaps found in EM in the food industry. More specifically, this research focused on three primary objectives: 1) identifying the factors that influence the release of microorganisms from EM tools, 2) evaluating the environmental factors that impact the recovery of microorganisms, and 3) studying the effects of organic matter, or food residues, on microbial recovery from surfaces. These findings will better inform food safety researchers and the food industry by providing them with the data and tools to make more scientifically-sound decisions when developing EM programs.

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