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REVIEW



Swabbing the surface: critical factors in environmental monitoring and a path towards standardization and improvement

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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.

KEYWORDS

Cross-contamination;
environmental monitoring;
food contact surface;
Listeria;
Salmonella; norovirus

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 harbor-age 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, McDade, Robertsen, Hoffman, and Edwards, 1968; Ismail 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 towards the development of standard methodologies for surface sampling.

Background

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).

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 1990s response to *E. coli* O157:H7 involved in a fast food 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, Rubinelli, Park, Carbonero, et al. 2016). 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. 2016b). 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 nonpathogenic 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, Sampredo, and Hedberg (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.

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 2016; Sarvikivi et al. 2012; Centers for Disease Control and Prevention 2013). 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 (Ismail 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.

Factors impacting the recovery of microorganisms from surfaces

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 (Briand 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 (Mafu et al. 1990, 1991; Turnage and Gibson 2017). 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.

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

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 flopped with polyamide fiber of 3 mm	Stainless steel (100 cm ²)	<i>L. monocytogenes</i> (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% % recovery • Wet: 88% to 93% • Dry: 55% to 66%	(Gómez et al. 2012)
Swabs: cotton, polyurethane foam Sponges: cellulose, gauze	Various (100 or 900 cm ²)	<i>S. enterica</i> ser. Typhimurium, <i>E. coli</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> • 10 ⁵ CFU	Immediate (wet) or 1 hr. drying	Buffered peptone water		(Keeratipibul et al. 2017)
Swabs: Cotton-tipped	Various (25 cm ²)	<i>S. enterica</i> ser. Typhimurium • 10 ⁶ CFU <i>S. aureus</i> • 6 to 6 × 10 ⁵ CFU	Immediate to 6 hr. drying 24 hr. drying	Ringer solution (10 ml) PBS-Tween 20 (10 ml or 50 ml)	Recovery (CFU/25 cm ²) • Immediate: 10 ⁴ to 10 ⁶ • 1 hr.: 10 ² to 10 ³ 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	(Moore, Blair, and McDowell 2007) (Lutz et al. 2013)
Swabs: rayon Wipes: electrostatic	Stainless steel (100 cm ²)		Overnight drying at RT	PBS or 50mM glycine (2 ml)	Swabs (% recovery, stainless steel) ^a • 20 to 60% Cloths (% recovery, stainless steel) • 79% ± 10% % positive (dry, stainless steel, enriched) • Sponge-Stick: 66% • Foam spatula: 89% • Swab: 89% Macrofoam (DL on stainless steel) • 3.5 log ₁₀ RNA copies per 645 cm ²	(Rönnqvist 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	Immediate (wet) or 1 hr. drying	Buffered peptone water (10 ml)		(Lahou and Uyttendaele 2014)
3M Sponge-Stick, Copan foam spatula, 3M Enviro Swab	Various (250 cm ²)	<i>L. monocytogenes</i> • 10 ² CFU	1 to 48 hr.	Swabs: PBS + Tween 80 (2.5 ml) Wipes: Ringer solution (10 ml)		(Park et al. 2015)
Swabs: cotton, polyester, rayon, polyurethane foam Wipes: antistatic	Stainless steel (645 cm ²)	hNoV GII.4 • 6.2 log ₁₀ RNA copies				

^aPolyester 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.

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, 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, Monk, and Butler (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 conveyor belt surfaces. Comparable results were found by Krysinski, Brown, and Marchisello (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.

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. 2018; 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.

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 cm² 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).

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, 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.

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. Food and Drug Administration (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

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. Food and Drug Administration (2017b).

×30.48 cm) sampling area (U.S. Department of Agriculture 2012).

Methods for recovery of microorganisms from surfaces

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.

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, McDade, Robertsen, Hoffman, and Edwards, 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 ($\geq 100\text{ cm}^2$) 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, Rönqvist 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.

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 $10^2\text{ CFU}/250\text{ cm}^2$ —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 cm², whereas all other test methods were able to detect the presence of less than 3.5 CFU cm². 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.

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 cm² and 645 cm²). The rates of virus recovery with a macrofoam swab from stainless steel coupons of 161.3 and 645 cm² were $7.08 \pm 2.21\%$ and $2.3 \pm 0.5\%$, respectively. Comparatively, antistatic wipes were reported to have recovery rates at $0.33 \pm 0.21\%$ and $0.30 \pm 0.10\%$, 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 ($<6 \times 10^3$ CFU per 100 cm²), 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 10⁴, 10², 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.

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 ($6.27 \pm 1.62\%$ and $5.05 \pm 2.19\%$, 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. Branck 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, Ismail et al. 2013, Assere, Oulahal, and Carpentier 2008). More specifically, Branck 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.

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 & 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.

Detection of microorganisms recovered during environmental monitoring

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.

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 20–30% 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 warmblooded 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 warmblooded 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.

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.

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, Huda, and Ali (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.

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.

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.

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).

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).

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 Vasavada (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).

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 (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 has primarily focused on discerning quality aspects of food as opposed to safety. Even still, over a decade ago Chen, Wortmann, and Zenobi (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, Li, and Xu 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, Pu, and Sun (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.

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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