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EVALUATION OF *LISTERIA INNOCUA* TRANSFER FROM PERSONAL PROTECTIVE EQUIPMENT (PPE) TO THE PLANT ENVIRONMENT AND EFFECTIVE SANITATION PROCEDURES TO CONTROL IT IN DAIRY PROCESSING FACILITIES

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by

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EVALUATION OF *LISTERIA INNOCUA* TRANSFER FROM PERSONAL PROTECTIVE EQUIPMENT (PPE) TO THE PLANT ENVIRONMENT AND EFFECTIVE SANITATION PROCEDURES TO CONTROL IT IN DAIRY PROCESSING FACILITIES

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Listeria monocytogenes can survive and grow under wet environmental conditions often encountered in dairy processing facilities. Pasteurization of milk kills *L. monocytogenes*; however, recent listeriosis outbreaks have linked post-pasteurization contamination from the food environment to the final product. One of the sources of microbial contamination may include employees and their personal protective equipment (PPE), which often become in contact with equipment and food contact surfaces. To understand this issue, this study evaluates *Listeria innocua*, as a surrogate for *Listeria monocytogenes*, transfer from PPE to food products and surfaces encountered in a dairy plant. Gloves, aprons, and boots were inoculated with *L. innocua* using Phosphate Buffer Saline (PBS) and skim milk as bacterial carriers. Overall, PPE contaminated with *L. innocua* in the presence of skim milk led to higher bacterial transfer to the surfaces under evaluation, than those inoculated using PBS. With PBS, consecutive touches led, for some PPE/surface combinations, to a decline in transfer. However, with skim milk no decline in transfer was observed between the PPE and the surfaces tested. This study also evaluated the effectiveness of chlorine, quaternary ammonia, and peroxyacetic acid (PAA) in reducing *L. innocua* contamination from different types of PPE. When sanitizers were used by themselves, the most effective was PAA. For all sanitizers tested,

effectiveness was great greatly reduced in the presence of organic matter. Due to the negative impact of organic matter in sanitizer effectiveness, cleaning regimes that included cleaning and scrubbing steps, followed by the use of a sanitizer, were evaluated. With the proposed cleaning regime more than 3-log reductions were achieved in the different types of PPE even when organic matter (skim milk inoculum) was present.

To Linda, Roy, Concepción and Alfredo.

TABLE OF CONTENTS

CHAPTER 1

CHAPTER 2

LIST OF TABLES

CHAPTER 1

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

APPENDIX A

CHAPTER 1

LISTERIA MONOCYTOGENES IN THE DAIRY PLANT ENVIRONMENT

1. *LISTERIA MONOCYTOGENES* AND LISTERIOSIS

Listeria monocytogenes is a Gram-positive, non-spore-forming rod-shaped bacterium. All *Listeria* genus members share these characteristics—*Listeria ivanovii*, *Listeria innocua*, *Listeria seeligeri*, *Listeria welshimeri, and Listeria grayi*, and the recently discovered species, *Listeria marthii* and *Listeria rocourtiae* (Cossart, 2011). However, only *L. monocytogenes* is pathogenic to humans and is the causative agent of foodborne infection listeriosis. There are 13 serotypes of *L. monocytogenes*, although most human listeriosis cases are caused by just four of these: 1/2a, 1/2b, 1/2c, and 4b (Buchanan et al., 2017).

Listeria monocytogenes is widespread in the environment and can be found in moist environments, soil, water, decaying vegetation, and animals (FDA, 2020). It also can grow and survive in food under harsh environmental conditions. It survives in a broad pH range (4.4-9.4), salt concentrations up to 14%, water activity as low as 0.92, and low temperatures ranging from 0.6 to 45℃ (Jordan, 2019; Lake, 2009). *Listeria* is also a facultative anaerobe and can grow in modified atmosphere packaged products, particularly those with extended shelf-life (United Fresh Produce Association, 2018). These characteristics allow *L. monocytogenes* to persist and multiply in various food products and food processing environments.

Listeriosis is the general term for the infection of *L. monocytogenes.* Most cases of listeriosis are sporadic and low incidence. In the U.S., it is estimated that it affects 1,600 people every year (CDC, 2020). However, its high hospitalization and mortality rates (~20%) primarily affects pregnant women, the elderly, and individuals with compromised immune systems (FDA, 2012). The infective dose is unknown and is likely

to vary, depending on the state of health of the individual affected (Jordan et al., 2018). There are two main types of listeriosis, non-invasive and invasive listeriosis. Non-invasive listeriosis (febrile listerial gastroenteritis) can include fever, diarrhea, muscle aches, nausea, and vomiting, mainly affecting healthy people. It shows a rapid onset (6-10 days), and outbreaks involved include high infection doses (Painter and Slutsker, 2007).

In comparison, invasive listeriosis affects high-risk individuals. An incubation time between two weeks and three months occurs in which the organism may cause septicemia and meningitis (McLauchlin et al., 2004) and lead to severe infection of the newborn, premature delivery, spontaneous abortion, or stillbirth in pregnant patients. Additional factors affecting the probability of illness include, but are not limited to, food matrixes and individual strain virulence (CAC, 2000).

The oral inoculum required to produce clinical infection is unknown; experiments in healthy mammals indicate that $\geq 10^9$ organisms are required (Farber and Peterkin, 1991). However, recent outbreaks have occurred with lower doses. The 2010-2015 Blue Bell ice cream outbreak indicated a low dose (average 8 MPN/g) in contaminated ice cream. Nevertheless, the case count for this outbreak involved patients who were previously hospitalized for other health issues and ate contaminated ice cream on various occasions. Nevertheless, research has shown a wide variation of virulence associated with *L. monocytogenes* isolates, depending on serotype and strain (Chen et al., 2006).

Currently, the regulations assume that all *L. monocytogenes* are equally pathogenic. This is reasonable as, at present, there is no test to distinguish between *L. monocytogenes* of different pathogenicity (Jordan et al., 2016) . Because the infective

dose is unknown, and it might vary in different types of people, the Food and Drug Administration (FDA) enforces a Zero-Tolerance policy for the presence of *L. monocytogenes* (*Lm*) in ready-to-eat (RTE) products. Unfortunately, according to Annual Surveillance Reports from the CDC, *Listeria's* incidence of infections has remained constant over the years (Tack et al., 2019).

2. FOODBORNE DISEASE OUTBREAKS CAUSED BY MILK AND DAIRY PRODUCTS

Listeria monocytogenes (*Lm*) is a ubiquitous foodborne pathogen, and its presence in dairy products may originate from raw milk or due to post-processing contamination from the plant environment. *L. monocytogenes* is a common contaminant, and its incidence from raw milk varies from 0% to 19.7% in the United States (Lee et al., 2019). The earliest reported listeriosis outbreak in the United States in 1985 associated with Latin-style cheese (queso fresco) was due to contaminated raw milk. Although the cheese was labeled as made from pasteurized milk, *L. monocytogenes (Lm)* was most likely introduced into the cheese through contaminated raw milk (Linnan et al., 1988).This outbreak is considered one of the country's deadliest foodborne illness outbreaks and resulted in 142 illnesses, 28 deaths, and 20 fetal losses (Jackson et al., 2018).

Jackson et al., 2018 gathered *L. monocytogenes* outbreak data from the Foodborne Disease Outbreak Surveillance System (FDOSS) in the United States from 1998 to 2014. Researchers found that 17 out of 58 *Lm* outbreaks reported were associated with soft cheeses. Non-commercial, homemade queso fresco was the leading cause of *Listeria* outbreaks in the early 2000s. For example, the 2000 outbreak affected eleven pregnant

women, and infection with *L. monocytogenes* resulted in five stillbirths, three premature deliveries, and two infected newborns (CDC, 2001). Contaminated raw milk was the source of microbial contamination. These findings-initiated information dissemination among the Hispanic population and the importance of food safety while pregnant. Awareness also involved law enforcement in regulating the sale of raw milk and dairy products made by unlicensed manufacturers (MacDonald et al., 2005).

Nevertheless, illness related to the consumption of nonpasteurized dairy products remains a public health problem in the U.S. (Langer et al., 2012). In 2014, raw chocolate milk was the source of a multi-state *Listeria* outbreak that caused two illnesses. This outbreak resulted in one death, from an elder individual. As a result, the CDC has highlighted the health risks associated with raw milk consumption among susceptible populations such as the young, pregnant, adults 65 and older, and people with weakened immune systems (FDA, 2017).

Even though *L. monocytogenes* is sensitive to heat, and pasteurization effectively eliminates the organism (Lianou And and Sofos, 2007); outbreaks associated with pasteurized dairy products have been documented. A list of documented outbreaks of *Listeria monocytogenes* in dairy products from the last 20 years in the U.S. is shown in **Table 1-1.** The outbreaks include data from pasteurized and unpasteurized milk and dairy products. Overall, the objective of pasteurization is to destroy harmful bacteria by thermal treatment. However, pasteurization does not protect against potential *Listeria* contamination from the dairy processing environment due to cross-contamination after the heating treatment.

Table 1-1. Characteristics of listeriosis outbreaks associated with raw and pasteurized dairy products, United States, 2000-2019.

Date	Dairy Product	State	Serotype	Inspectional Observations	A	B	C	Reference
2000	Mexican-style fresh soft cheese	North Carolina	4b	Contaminated unpasteurized milk	12	12	0	(CDC 2001)
2003	Raw milk Queso Fresco	Texas	4b		12		1	
2005	Raw milk Queso Fresco	Texas	1/2b		12		0	
2006	Pasteurized cheese (sheep's milk)	Oregon	4b		3	2	1	
2007	Pasteurized milk and flavored milks	Massachusetts	4b	The facility did not have an environmental monitoring program for *L. monocytogenes*	5	5	3	(CDC,2007)
2008	Mexican-style fresh soft cheese	Multistate	1/2a	*L. monocytogenes* was isolated from a vat gasket in a post pasteurization section of the cheese production line	8	8	0	(Jackson et al., 2018)
2009	Mexican-style fresh soft cheese	Multistate	1/2b	Inadequate sanitizing and cleaning operations	8	3	0	(FDA, 2009)
2010	Mexican-style fresh soft cheese	Multistate	1/2b	Buildings and structures are unsuitable for food-production purposes	6	5	1	
2010-2015	Ice cream	Multistate	(1/2b, 3b, 1/2a)	The plant is not constructed in such a manner as to prevent condensate from contaminating food and food-contact surfaces	10		3	(Buchanan et al., 2017)
2010-2015	Soft Cheese	Multistate			30	28	3	(FDA, 2016)
2011	Middle Eastern style cheese	Michigan	1/2b		2	2	1	
2011	Blue-veined cheese	Multistate	4b		15	1	0	
2012	Ricotta salata cheese (sheep's milk)	Multistate	1/2a	FCS sample (brush in washing machine) from the packaging area tested positive for *L. monocytogenes*	22	20	4	(Acciari et al., 2016)

Date	Dairy Product	State	Serotype	Inspectional Observations	A	B	C	Reference
2014	Soft Hispanic style cheese and sour cream	Multistate		*L. monocytogenes* harborage problem. Presence in trays, processing floor, spaces between floors and doors	5		1	FDA, 2015
2014	Raw chocolate milk	Multistate			2		1	CDC, 2016
2014	Soft Hispanic style cheese	United States		Unsanitary conditions at the company	8	7	1	CDC, 2014
2015	Mexican-style fresh soft cheese	Washington	1/2a		3	2	1	
2016-2017	Raw Milk soft cheese	Multistate		Poor employee hygiene practices, black and/or green mold in several places within the facility, and equipment in disrepair	8		2	FDA, 2017
2016-2019	Deli sliced meat and cheese	Multistate		.	10	10	1	CDC, 2019

Adapted from: (Jackson et al., 2018)
A: Case Count Number
B: Number of Hospitalizations
C: Number of Deaths

In 2007, pasteurized, flavored and unflavored, fluid milk from a local dairy was the source of contamination that led to five cases and three deaths (CDC, 2007). According to the FDA and the local health department, the local dairy met federal pasteurization standards. However, environmental samples from the finished product area tested positive for *L. monocytogenes*, suggesting that the product's contamination occurred after milk pasteurization. Unfortunately, the facility did not have an established environmental monitoring program for *Listeria* (Weisbecker, 2015).

Three years later, pasteurized dairy products were pointed out again as a vehicle of contamination. The 2010-2015 multi-state outbreak linked to ice cream from Blue Bell creameries made 10 people sick and resulted in three deaths. In 2015, five hospitalized patients from unrelated health problems consumed milkshakes prepared from Blue Bell contaminated products. Microbial analysis from the contaminated lots showed a 99.4% prevalence of *L. monocytogenes*, but at low levels (average was 8 MPN/g) (Chen et al., 2017). This outbreak indicated that the underlying health of a patient, immune status, and the medication they are taking is more important than the dose (Buchanan et al., 2017).

It is not clear how *Listeria* spp. entered the plant facility and contaminated the final product. However, the FDA inspectors found multiple anomalies in three Blue Bell ice cream facilities, including; *Listeria* spp presence in contact surfaces and environmental sites, inadequate cleaning and sanitizing procedures, improper food handling practices, and improper use of Personal Protective Equipment (PPE) (FDA, 2015). This indicates that pasteurized dairy products are susceptible to contamination if inadequate sanitary procedures and cross-contamination activities take place.

Dairy products have served as vehicles of *Listeria monocytogenes* in several listeriosis outbreaks in Europe, where raw milk and raw milk products were the major causes (Lunden, JM, Autio et al., 2003). However, listeriosis transmitted by pasteurized dairy products has also been documented (Lyytikäinen et al., 2000; Schoder et al., 2011). Therefore, *L. monocytogenes'* presence in dairy products is a continuing issue for processed dairy manufacturers. Between 1985 and 2019, there have been 40 confirmed significant listeriosis outbreaks associated with commercially pasteurized dairy products worldwide. In most cases, *L. monocytogenes* were found in niches in the dairy processing environment, and final product contamination was due to cross-contamination post the pasteurization step (Jordan et al., 2018).

3. *L. MONOCYTOGENES* IN THE DAIRY PLANT ENVIRONMENT

L. monocytogenes is a lethal foodborne pathogen that is widespread in the environment. Studies have shown its presence in the dairy supply chain from the dairy farm to the processing environment. At the farm, *L. monocytogenes* can be found in poor-quality silage, hay, bedding, and water (Bandelj et al., 2018). Healthy cattle frequently shed *L. monocytogenes* in feces, disseminating the pathogen into the farm environment. Transmission to bulk tank milks can be due to the pathogen's presence on the udder surface (Castro et al., 2018). Also, (Latorre et al., 2009) documented *L. monocytogenes'* presence in a bulk tank milk, initially caused by fecal or environmental contamination that established themselves in the milking harvesting system as a biofilm.

As a result, raw milk and other raw materials are potential sources of *L. monocytogenes.* However, upon arrival at a food processing facility, raw milk is handled

and heat-treated. Pasteurization temperatures have proven to inactivate the microorganism. Overwhelming evidence indicates that contamination of commercially processed foods with *Listeria monocytogenes* and other *Listeria* species occurs in the post pasteurization environment (Kornacki and Gurtler, 2007). *Listeria* has been found in dairy processing facilities, and its presence includes equipment, production areas, and even in food handlers' hands and personal protective equipment.

In 1995, Pritchard et al., gathered samples from 21 dairy plants, and 17/215 processing equipment sites (17.9%) tested positive for *L. monocytogenes,* including areas such as holding tanks, conveyor/chain systems, tabletops, and food product fillers. *L. monocytogenes* can also be present on food-contact surfaces. In 2013, Almeida et al., evaluated *L. monocytogenes* presence in a small-scale cheese plant using Pulsed Field Gel Electrophoresis (PFGE). Results showed that the same PFGE type was shared by isolates recovered from cheese, from the brush used to wash cheese, and from the cheese washing zone. Also, (Kabuki et al., 2004) found *L. monocytogenes* in a plastic connecting tube at the exit of a pasteurizer used to transfer milk to a coagulation vat and at a polytetrafluorethylene table in a Latin-style cheese plant**.** These findings reflect that contaminated tools and equipment close to or in contact with finished products indicate a potential threat for post-processing contamination.

L. monocytogenes has also been found in receiving, production, and storage areas. (Pritchard et al., 1995) reported environmental samples indicated contamination in coolers/ freezers and raw milk associated areas. The refrigerated, moist environment, coupled with organic soil deposition, allows *L. monocytogenes* to survive and grow (Ryser, 2007). Other Non-Food contact surfaces (NFCS) can also be a source of *L.*

monocytogenes. According to (D'Amico and Donnelly, 2009), NFCSs such as drains and floors are more commonly contaminated with *Listeria* spp., compared with those in contact with food such as processing equipment. *L. monocytogenes* finds favorable growth conditions on floors, and within food industry premises, notably in the cold and wet atmosphere of refrigerated rooms where non-psychrotrophic bacteria can only survive (Carpentier and Cerf, 2011).

People can be asymptomatic carriers of *L. monocytogenes*. (El-Shenawy, 1998) sampled *Listeria* spp. presence in food handlers from a processing facility. Results showed that *L. monocytogenes* and *L. innocua* were isolated from 11 people's nose/nasal secretions, from seven people's hands, and two people's faces. However, their Personal Protective Equipment (PPE) can also be contaminated. (Schoder et al. 2011) evaluated *Listeria* presence in farms that manufactured cheese from raw milk. Swabs from working boots had the highest *L. monocytogenes* prevalence with 51% positive samples. Also, (Dass et al., 2018) found a 4% *L. monocytogenes* relative abundance in boots from a fluid milk processing plant in the Midwest. Gloves and aprons can also be a potential *L. monocytogenes'* source. (Barancelli et al., 2013) found *L. monocytogenes* serotype 4b from worker gloves in a cheese manufacturing plant. As a result, the dairy processing environment can be a potential source of *L. monocytogenes*.

Its occurrence in the environment may pose a threat of *L. monocytogenes* transfer from the environment to the milk product, even though contamination routes are not always clearly identified (Jordan et al., 2018). Persistent strains or strains recurring in the environment over time can also be identified. Several studies in dairy processing facilities have shown *Listeria* persistence in environmental sites over the years (Ferreira et al. 2013).

Persistence can be related to *Listeria's* physiological characteristics, including attachment to various surfaces, biofilm formation, and sanitizer resistance (Lunden et al., 2000) (Pan et al. 2006). Nevertheless, failures of hygiene practices from personnel or the incorrect design of equipment or facilities may facilitate *L. monocytogenes* presence and persistence in dairy plants (Almeida et al., 2013;Carpentier and Cerf, 2011).

4. *L. MONOCYTOGENES* TRANSMISSION IN THE PROCESSING ENVIRONMENT

4.1 *L. monocytogenes* transmission

Most transmissions of listeriosis occur by contamination of foods, and transmission from infected animals to humans is possible. *L. monocytogenes* has the opportunity to enter the food chain at all points of the farm-to-fork and is a significant cause of post-processing contamination (Erkmen and Bozoglu, 2016). *Listeria* may enter the food processing environment from raw materials or the movement of people or equipment. Also, poor design of food equipment or the environment and insufficient control of personnel workflows can aid in microbial transmission (Muhterem-Uyar et al., 2015). **Figure 1-1** shows a flowchart of *L. monocytogenes* contamination in dairy products in a processing environment.

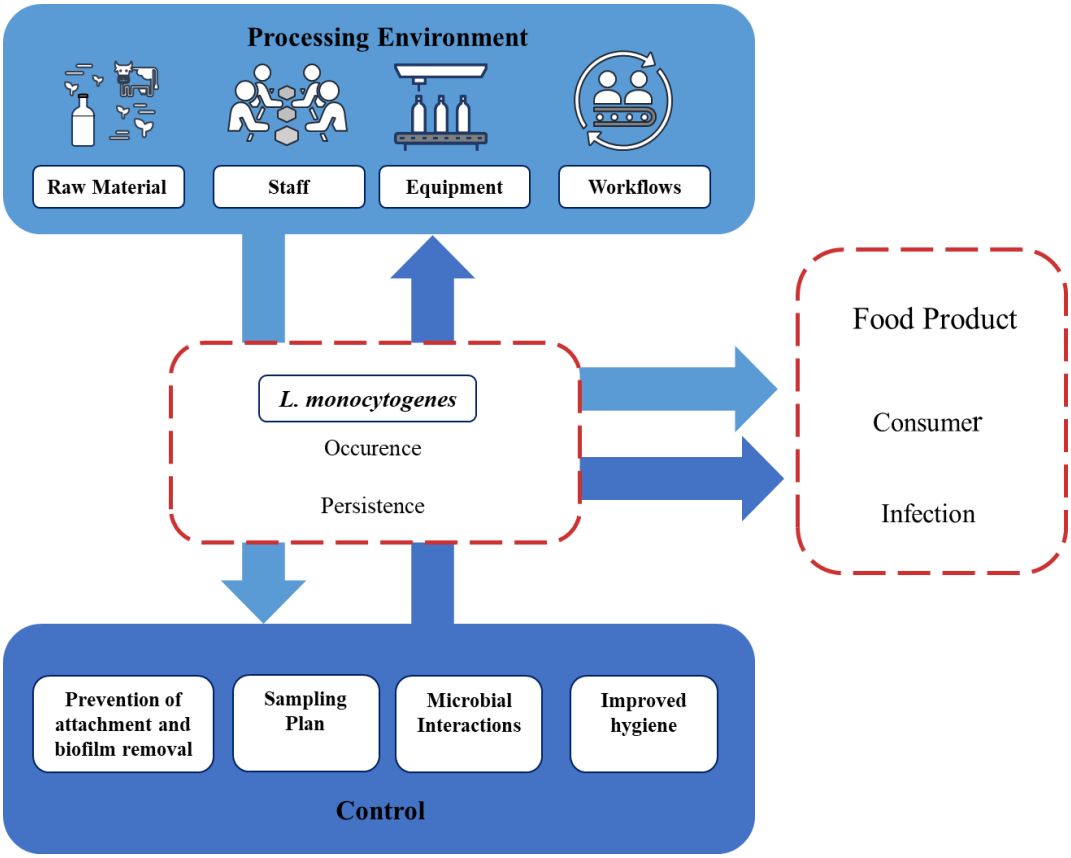

Figure 1-1: Adapted from (Jordan et al., 2016) Flow-chart of contamination of milk product processing environment with *L. monocytogenes.*

As previously discussed, raw milk is a potential source of *L. monocytogenes*. As a result, milk should be treated as if contaminated, and steps should be taken to prevent cross-contamination from raw ingredients to products that have been treated to eliminate or reduce the contamination (Tompkin, 1999). Also, the personnels' actions can transfer *L. monocytogenes*. When present in the environment, *L. monocytogenes* on employee hands and contact surfaces (such as hand tools, gloves, and aprons) can transfer from the processing environment into the finished food product (Tompkin, 1999).

Moreover, contamination of foods via hands is frequently mentioned in inspection reports during outbreak investigations. For example, during the Blue Bell *L.*

monocytogenes ice cream outbreak, the FDA observed several examples of employees touching non-food contact surfaces and food contact surfaces using the same pair of gloves (DHHS, 2015). Another scenario includes *L. monocytogenes* presence in equipment such as trolleys, conveyors, hoses, and forklift trucks. *L. monocytogenes* adherence to this equipment can aid in cross border spread in a food processing facility (Jordan et al., 2013). Also, equipment with rough welds or with hollow places can cause residue build-up facilitating *Listeria* growth and subsequent product contamination. Equipment should be easily dissembled for cleaning and sanitation to avoid growth niche/biofilm development (Kornacki, 2004)

Other well-known areas where *Listeria monocytogenes* can hide is in a freezer or cooler system and in air handling units. Condensation can form in these units if the relative humidity is high and air temperature differentials occur. Condensation on walls, ceilings, and behind pipes and conduit has been shown to promote *Listeria* establishment in food processing facilities (Kornacki, 2004;United Fresh Produce Association, 2018). Therefore, condensation on overhead structures can lead to contamination of food or food preparation surfaces (USDA, 2016).

Other methods of dissemination include the presence of *L. monocytogenes* in drains and floors. (Carpentier and Cerf, 2011) suggest that bacterial cells move naturally by themselves in liquids or displaced by aerosols caused by mechanical action during processing and cleaning operations. As a result, the recurrent presence of *L. monocytogenes* in drains may pose the risk of the airborne spreading of *Listeria* by an inadvertent water spray during cleaning into contact surfaces, equipment, and exposed product (Berrang and Frank, 2012). Also, improper maintenance and cleaning of

footbaths can be a cause of *Listeria* spp. survival or growth, resulting in cross-contamination of the factory environment by foot traffic (Kornacki, 2004).

4.2 *L. monocytogenes* control

Ultimately, control measures are needed to avoid *L. monocytogenes* contamination to finished products. The application of Current Good Manufacturing Practices (CGMPs) to the production of RTE foods can significantly minimize or prevent contamination of an RTE food with *L. monocytogenes.* In addition, dairy processing facilities should have a food safety plan in place that includes an analysis of hazards and risk-based preventive controls to minimize or prevent identified hazards. RTE producers need to be especially vigilant regarding *L. monocytogenes* since their products are directly consumed and do not undergo steps to destroy the organism. Nevertheless, one of the biggest hindrances to the prevention of *L. monocytogenes* contamination in food is the lack of awareness (Jordan et al., 2018).

5. SANITATION

Sanitation plays a vital role in preventing and controlling *L. monocytogenes* in food processing facilities. *L. monocytogenes* adherence to food contact surfaces is a source of concern in the dairy industry. Its survival and biofilm formation has been shown on stainless steel, polyethylene, rubber, PVC, and other surfaces found in the processing environment. This adherence is problematic since adhered cells are more difficult to remove mechanically from surfaces and are more resistant to disinfectants (Lunden et al., 2000). As a result, effective cleaning and sanitation procedures are needed for *L. monocytogenes* eradication. Effectiveness is achieved if proper sanitation sequencing is followed. Where cleaning or the complete removal of food soils using

appropriate detergent needs to be applied first while sanitizing or substantially reducing undesirable microbial populations should be done after (Burnett, 2017).

Sanitizers need to be registered by the US Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). EPA requires sanitizers to undergo efficiency tests, where test parameters differ for food-contact surface sanitizers and non-food-contact sanitizers (Fatica and Schneider, 2009). It is suggested that a three-log reduction (99.9%) is needed to target effective inactivation of attached or biofilm bacteria (Somers and Wong, 2004). The FDA must also approve the antimicrobial agent and its maximum usage level on surfaces to be used in a food facility. Their formulations and usage levels are listed in the Code of Federal Regulations (21CFR178.1010, 2019).

5.1 Sanitizers

A wide variety of sanitizers are used in the food industry. However, sanitizers containing quaternary ammonium compounds (QACs), peroxyacetic acid, or chlorine have been evaluated to control, reduce, and inactivate *L. monocytogenes* in various situations.

Chlorine

In its various forms, chlorine is the most commonly used sanitizer in food processing and handling applications (Schmidt, 2012). Chlorine compounds are very effective at killing a wide variety of microorganisms. Chlorine's antimicrobial activity has not been entirely determined; however, its mechanism of action includes protein synthesis disruption, oxidative decarboxylation of amino acids, and unbalanced metabolism after the destruction of crucial enzymes (Marriott et al., 2016). Nevertheless,

chlorine is adversely affected by the organic material presence, high pH values, and high temperatures. Also, the use of such high concentrations increases the risk of formation of potentially hazardous by-products or the production of off-tastes and odors, which are the main drawbacks of chlorination (Virto et al., 2005).

Quaternary ammonia

Quaternary ammonium compounds commonly called “quats,” are a group of chemicals in which four compounds surround a nitrogen atom (Montville, 2012). They are natural wetting agents with built-in detergent properties and are referred to as synthetic surface-active agents (Marriott et al., 2016). Their permanent positive charge makes them bind readily to the negatively charged surface of most microbes. Mechanisms of action include disruption of cell wall membrane, internal organ leakage, and enzyme inhibition (Chauret, 2014). Quaternary ammonia is effective at a high pH, has detergency and soil penetration abilities, and leaves a residual antimicrobial film. However, this would be a disadvantage in operations such as cultured dairy products and cheese, where microbial starter cultures are used (Schmidt, 2012).

Peroxyacetic acid

Peroxyacetic acid is an aqueous mixture of acetic acid as the active ingredient and hydrogen peroxide as a contributing factor in the formulation's additional oxidation capacity (Gawande et al., 2013). The action of both compounds depends on dissociation, where the lethal action of both compounds comes from the $OH^{\cdot}$ radical. This powerful oxidizer reacts with membrane lipids, DNA, and proteins (Montville, 2012). Peracetic acid is a highly biocidal oxidizer that maintains its efficacy in the presence of organic soil and has a pH tolerance range of 1-8. It also shows a lack of potentially hazardous

disinfection by-products (DBPs). (Fatica and Schneider, 2009; Rutala and Weber, 2013). Nevertheless, peroxyacetic sanitizers can be higher cost, have a strong, pungent smell, present corrosiveness, and lower effectiveness against yeasts and molds than other sanitizers (Marriott et al., 2016).

5.2 *L. monocytogenes* and sanitizers

Several studies have demonstrated that *L. monocytogenes'* reduction is different among various surfaces and sanitizers. **Table 1-2** shows log reductions of *L. monocytogenes* (biofilms) treated with commonly found sanitizers on different surfaces encountered in a plant facility. In several studies, Peroxyacetic Acid (PAA) achieved a high log reduction (Skowron et al., 2018; Korany et al., 2018; Hua et al., 2019). In comparison, sanitizer efficiency from chlorine and quaternary ammonia differed depending on surface type. Also, sanitizing efficiency can be affected by material hydrophobicity, material condition, organic matter presence, and biofilm age. (Krysinski et al., 1992; Park and Kang, 2017; Korany et al., 2018).

Regardless of which sanitizer is chosen to meet a facility's needs, sanitizer rotation in food processing facilities is highly advised. Biocides incorporated in food processing facilities provide such a powerful attack on the microorganisms that the development of resistance to adverse environmental conditions could happen (Marriott et al., 2016). *Listeria monocytogenes* is highly adaptable and is more prone to develop tolerance to QACs than oxidizers, such as chlorine-based compounds (Gregerson, 2009). The only available data regarding the resistance of *L. monocytogenes* to disinfectants applied in food production environments refers to genotypic resistance to quaternary ammonium compounds (QACs). Microorganisms that are frequently exposed to

subinhibitory concentrations of QACs and prolonged environmental persistence of certain strains may facilitate resistance development over time (Martínez-Suárez et al., 2016).

Table 1-2. Examples of inactivation (log reductions) of *L. monocytogenes* biofilms (48-72 h) when treated with commonly used sanitizers on different surfaces.

Sanitizer Tested	Stainless Steel	Rubber	Polyester	Polystyrene	PVC	LDPE
Chlorine (sodium hypochlorite)	(1-5 min, 0.5%) 1.97-3.55 (2 min, 100 ppm) 4.5 (10 min) 1.3 (5 min, 200 ppm) 3.8	(1-5 min, 0.5%) 1.79-2.21 (5 min, 200 ppm) 3.0	(10 min) <1	(1 min, 200 ppm) 2.57	(5 min, 200 ppm) 3.3	(5 min, 200 ppm) 2.7
Quaternarry Ammonia Campounds	(1 min, 200 ppm) 4 (1 -5 min, 0.5%) 4.06 - 5.01 (10 min) (> 4) (5 min, 400 ppm) 3.7	(1- 5 min, 0.5%) 1.72 -3.14 (5 min, 400 ppm) 3.0	(10 min) 1.4	(1 min, 400 ppm) 2.20	(1 min, 400 ppm) 3.7	(1 min, 400 ppm) 3.2
Peracetic acid (with or without hydrogen peroxide)	(1-5 min, 0.5%) 6.63 (10 min) (> 4) (5 min, 200 ppm) 4.5	(1-5 min, 0.5%) 5.10-5.70 (5 min, 200 ppm) 4.4	(10 min) 1.4	(1 min, 200 ppm) 3.85	(5 min, 200 ppm) 4.4	(5 min, 200 ppm) 4.3

Adapted from: (Jordan, 2019) Data compiled from: (Hua et al., 2019)(Korany et al., 2018)(Krysinski et al., 1992) (Skowron et al., 2018)

6. PPE MATERIALS AND USE IN THE FOOD INDUSTRY

Personal Protective Equipment (PPE), such as gloves, aprons, and boots are essential in the food industry. Besides being a means of protection for workers, it has also proven crucial for product safety. The U.S. Food and Drug Administration (FDA) advocates for an appropriate glove, apron, and footwear usage to prevent inadvertent contamination during processing. Gloves made of materials such as latex, nitrile, and polyethylene are the most common types of protective equipment used to prevent cross-contamination and transmission of pathogenic bacteria in the food industry (Oh et al., 2016). Aprons and work boots are also manufactured of these materials. Nevertheless, a wide variety of options can be selected depending on material composition, durability, puncture resistance, among other characteristics. **Table 1-3.** summarizes relevant information on material composition and important characteristics for PPE selection.

6.1 Materials detailed

Polyethylene

Polyethylene (PE) is a condensation of polymers of ethylene. There are several types of Polyethylene, low-density polyethylene (LDPE), high-density polyethylene (HDPE), and linear low-density polyethylene (LLDPE). Polyethylene is very sensitive to environmental stress (chemical and mechanical) and has poor heat-aging resistance. Polyethylene properties vary depending on the molecular structure and density (Zhong et al., 2018). Due to its high availability, PPE made of polyethylene generally are the least expensive.

Vinyl

Vinyl, otherwise known as polyvinyl chloride (PVC), is a widely produced synthetic plastic polymer. Vinyl is not molecularly cross-linked, in contrast to NRL or other types of synthetic latex such as nitrile. Because of this lack of cross-linking, vinyl individual molecules separate when the film is stretched or flexed (Ardagh, 2018). As a result, vinyl gloves have shown poor durability and low puncture resistance. Plasticizers are added to make vinyl flexible. However, these plasticizers contain Phthalates and Bisphenol A (BPA) who have adversely impacted human health. Phthalate plasticizers can be absorbed through worker's skin and quickly transfer to and contaminate food products. (Michaels, 2017). However, vinyl gloves are low cost and contain no protein allergens, which are considered to some extent, an alternative to latex (Michaels, 2004).

Latex

Natural rubber (NRL) known as cis-1,4-poly(isoprene) is contained primarily in the milky sap or latex of the *Hevea brasiliensis* tree (Bokobza, 2019). This natural product has a "coiled" molecular structure that allows for rigorous manipulation activities while maintaining the integrity and supporting the return (or rebound) to the original shape (Rego and Roley, 1999). As a result, latex gloves have proven to have excellent elasticity and dexterity and show good chemical barrier properties. Nevertheless, latex proteins can cause allergic reactions; thus, its use in the food industry has been limited.

Nitrile

Nitrile is used as a replacement for latex to prevent latex allergy. Nitrile is manufactured by synthetic polymers that provide specific characteristics for glove production. It contains Acrylonitrile, which provides permeation resistance

characteristics. The second component is Butadiene, which contributes to the glove's modulus, affecting elasticity, flexibility, and feel. The third major component, carboxylic acid, contributes to physical characteristics such as tensile strength and tear resistance (Rego and Roley, 1999). As a result, nitrile possesses good strength and durability characteristics. Nitrile gloves are recommended because they resist chemicals, including certain disinfectants, such as chlorine, and because nitrile is more environmentally friendly than latex (WHO, 2016).

Table 1-3. Comparison guide for glove types used in food processing/service facilities.

Material	Plastic (Poly)	Vinyl	Nitrile	Natural Rubber Latex
Composition/ Source	Polyethylene	Polyvinyl chloride (plasticized)	Acrylonitrile and butadiene	Cis 1.4 Polyisoprene *Hevea brasiliensis*
Strength and Durability	Very poor, weakest of all glove types. Easily breaks in use	Poor, weak, breaks and punctures easily in use	Good, possesses some puncture resistance	Good, strong, and durable
Puncture Resistance	Low tensile strength	Low tensile strength	Has puncture resistant properties	Strong, has some puncture resistant qualities.
Chemical Barrier Properties	Extremely poor protection, soluble in some solvents, including alcohols	Limited barrier protection; easily permeated by organic solvents, oils, and alcohols	Resist most solvents. Sensitive to alcohols and ketones	Good protection from most caustics and detergents; soluble to solvents such as alcohols.
Allergenicity	Contains no latex protein but contact dermatitis reported from additives.	Contains no proteins but some curing agents, chemical ingredients, and plasticizers.	Contains no proteins but has accelerators and other chemicals.	Contains protein and chemical allergens.
Fit and Comfort	Very limited fit and feel (baggy)	Loose cuff (baggy)	Tighter fit.	Very good comfortable fit due to its elasticity.
Cost Per Use	Very low	Low	Moderate	Low

Adapted from:(Michaels, 2004)

6.2 PPE Material and Microbial Contamination

PPE manufacturing involves the use of a wide range of materials. Nevertheless, PPE selection depends on the industry and its needs. However, recent studies investigated if the material type can contribute to microbial contamination in the food industry. (Michaels et al., 2019) evaluated bacterial transmission in polyethylene (PE), nitrile, latex, and vinyl gloves. Results showed that PE and nitrile gloves showed slight increases in transmissibility compared to clean hands. Latex gloves doubled that risk, and vinyl gloves had triple propensity to transfer microbial contamination and associated carrier soils, compared to clean hands. According to (Michaels et al., 2019), vinyl gloves are more hydrophilic and have a higher surface free energy unit (8 millinewtons/square meter). As a result, these surfaces have more energy to pick-up and spread microorganisms. Vinyl gloves are also more sensitive to glove punctures and cause cross-contamination concerns if leakages occur during food handling. Also, several studies showed that increasingly rough surfaces would cause a corresponding increase in microbial retention contributing to the adhesion of *L. monocytogenes* (Beltrame et al., 2015). Therefore, proper PPE selection needs to be evaluated to mitigate cross-contamination risks in food processing facilities.

7. REGULATIONS

With the emergence of *L. monocytogenes* as a foodborne pathogen, the Food and Drug Administration (FDA) and USDA have applied zero tolerance for the organism in all RTE foods. The absence of *L.* monocytogenes in five, 25 g of food, and in the processing environment, is required at all times (International Dairy Federation, 2013).

However, there is considerable debate about whether zero tolerance is warranted for *L. monocytogenes* (Montville, 2012). European countries such as Germany, France, and The Netherlands have a tolerance of below 100 CFU *L. monocytogenes* at the point of consumption (Nørrung, 2000). The current legislation for *L. monocytogenes* in the EU requires absence (10 × 25 g samples) for foods intended for infants and special medical purposes and allows presence at different levels depending on the ability of the food to support the growth of the bacterium. For RTE foods unable to support the growth of *L. monocytogenes*, the numbers should be < 100 CFU/g throughout the shelf-life of the product (5 × 25 g samples) (Jordan et al., 2018). Nevertheless, the FDA has accumulated evidence from refined risk assessments and from unexplained "low dose" outbreaks to continue the conservative zero-tolerance approach (Archer, 2018).

Several resources are available to help the food industry minimize *Listeria monocytogenes* risk and prevent foodborne outbreaks. The Innovation Center for U.S. Dairy Guidance to Control *L. monocytogenes* emphasizes establishing a well-designed "Pathogen Control Equation." The equation is based on different principles recommended to control *L. monocytogenes* in the dairy plant environment. Principles include i) separating raw from RTE, ii) following proper GMPs, iii) having proper sanitary equipment design, iv) effective cleaning and sanitation procedures, and v.) an effective Environmental Pathogen Monitoring Program (EMP) as critical factors to control and provide long-term stability for pathogen management programs. The FDA's Guidance for the Control of *Listeria monocytogenes* in Ready-To-Eat Foods also provides recommendations and highlights the importance of proper cleaning and sanitation in RTE facilities. These documents provide core principles to early control and

long-term stability for *L. monocytogenes* management (Innovation Center for U.S. Dairy, 2015).

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CHAPTER 2. EVALUATION OF THE TRANSMISSION OF *LISTERIA INNOCUA* FROM PERSONAL PROTECTIVE EQUIPMENT (PPE) TO THE PLANT ENVIRONMENT AND FOOD PRODUCTS

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ABSTRACT

L. monocytogenes is a ubiquitous foodborne pathogen that can grow and survive in the dairy processing environment. Due to *Listeria's* sensitivity to heat treatment, post-processing contamination is the likely source of dairy product contamination and the reason behind outbreaks associated with *L. monocytogenes*. Employees and their personal protective equipment (PPE) are potential vectors of microbial contamination in the food processing environment. The objective of this study was to evaluate *Listeria innocua* transference from PPE to food product and processing plant surfaces encountered in a dairy plant (i.e. cheese, cutting boards, stainless steel, and dairy brick floors). To mimic real scenarios, five combinations of PPE and surfaces of interest were selected. PPE was inoculated using either Phosphate Buffer Solution (PBS) or skim milk as the carrier for *L. innocua.* Transmission mediated by gloves and aprons were tested using a texture analyzer to apply a constant force (2.942 N) and time (5 s) mimicking one contact transfer. Additionally, boots inoculated with *L. innocua* were used to assess bacterial transmission to dairy brick floors. In general, contamination carried by organic matter led to higher transfer of *Listeria innocua* from PPE to the surfaces of interest, compared to tests done using PBS ($P < 0.05$). With PBS, consecutive touches from gloves to food products led to a decline in transfer, however *L. innocua* populations were never eliminated. When skim milk was used, no decline in transfer was observed in the different combinations of PPE and surfaces of interest. Considering common sampling points for different combinations of PPE-surfaces and skim milk as the carrier of

contamination, on average, gloves transferred 5.33 log CFU/in^2 *L. innocua* to queso fresco, and 4.28 log CFU/in^2 *L. innocua* to cheddar cheese. Other surface combinations involving food contact and non-food contact surfaces showed lower *L. innocua* transmission. A 4.01, 2.66, and 2.61 log CFU/in^2 average transmission was observed from gloves to cutting board, aprons to stainless steel, and boots to dairy tiles. In general, bacterial transference from PPE to food contact surfaces and food product were higher than those observed between PPE and non-food contact surfaces, emphasizing the risk associated with potential cross-contamination of final product.

1. INTRODUCTION

L. monocytogenes is an environmental pathogen that can contaminate foods and cause a mild, non-invasive illness (called listerial gastroenteritis) or a severe, invasive illness (called listeriosis) (Food and Drug Administration, 2017). *L. monocytogenes* causes approximately 1,600 cases of infection and 260 deaths annually in the United States (Scallan et al., 2011). Dairy products such as Mexican-style cheese made from raw milk, pasteurized milk, butter, and ice cream, have been implicated in multiple outbreaks (Jackson et al., 2011;Jordan et al., 2018). *L. monocytogenes* is widely distributed in the environment and has been isolated from a variety of sources, including soil, vegetation, silage, fecal material, sewage, and water. It's ubiquitous presence leads to the potential for contamination of the food processing environment, where occurrence and persistence of *L. monocytogenes* is frequent (Jordan et al., 2018).

L. monocytogenes can be transmitted into the processing facilities by raw materials, the workers, trucks, tools, cleaning materials, or machines (Reij and Den

Aantrekker, 2004). Also, poor facility design, inappropriate personnel movements and food workflows, and poor cleaning practices can lead to *Listeria* establishment (Muhterem-Uyar et al., 2015). People and the inappropriate use of personal protective equipment could contribute to cross-contamination to finish products. In 2004, Reij et al., evaluated hygienic practices in European processing plants, and food worker contact was the most significant cause of food contamination (9.2%), followed by cross-contamination from dirty equipment (5.7%) and contaminated food ingredients (3.4%) (Todd et al., 2010).

Generally, workers are well informed about personal protective equipment and the need to wear gloves when handling hazardous chemicals or agents to protect their hands. However, they may not be so well informed about the issues concerning glove use to avoid cross-contamination (Todd et al., 2010). In 2013, Barancelli et al. used Pulsed Field Gel Electrophoresis (PFGE) to evaluate *L. monocytogenes*' occurrence and routes of contamination in dairy processing facilities. In one dairy plant, highly similar pulsotypes were found on a cooling chamber floor and on food handlers' gloves, highlighting contamination routes from contaminated floors. In addition, in 2010, Blue Bell ice cream products were linked to 10 confirmed listeriosis cases and three deaths in four states. Inappropriate glove use was among FDA inspectors' main observations in the Blue Bell processing facilities (Department of Health and Human Services, 2015).

L. monocytogenes has also been identified on employee aprons and gloves, floormats, and drains in food processing facilities (Lappi et al., 2004). In 2013, a persistent *L. monocytogenes* strain was found on boots and floors of a changing room in a plant facility. *L. monocytogenes'* presence in the changing area and areas before a

footbath indicated possible dissemination methods throughout the processing facility. These results suggest the importance of controlling *Listeria's* movement through carriers that can allow transfer to food contact surfaces and compromise the final product.

The U.S. Food and Drug Administration (FDA) advocates for an appropriate glove, apron, and footwear usage to prevent inadvertent contamination during processing. Nevertheless, food worker education and training should be emphasized. Training should be designed to facilitate positive behavior and practices that seek to prevent contamination and opportunities for the growth of *L. monocytogenes* in RTE foods during their production, retailing, and handling (Luber et al., 2011). If managed correctly, with increased hand hygiene, best gloving practices, and implementation of adequate sanitation and cleaning protocols, *L. monocytogenes* contamination can be reduced (Michaels et al., 2019)

2. MATERIALS AND METHODS

2.1 Personal Protective Equipment

Personal Protective Equipment (PPE), such as aprons, gloves, and boots are essential in a food processing facility's daily activity. However, improper traffic patterns and food handling represent a risk of *L. monocytogenes'* transfer from PPE to different surfaces in dairy processing environments. As a result, the following PPEs were selected to determine *Listeria* transfer to other surfaces: a) Vinyl gloves b) Vinyl aprons, and c) food service PVC boots.

2.2 Test strains and Inoculum preparation

Stock cultures of two *Listeria innocua* strains **(**ATCC 33090 and ATCC 51742**)** were kept at -80°C in cryogenic vials containing Brain Heart Infusion broth (BHI; Acumedia, Lansing, MI) and 10% sterile glycerol. *Listeria innocua* was used as surrogate for *Listeria monocytogenes*. For inoculum preparation, 100 µL of each of the stock solutions were grown in 9 mL BHI tubes, followed by a 24 h incubation at 35℃. After two consecutive transfers, cultures were then transferred individually to 15-mL sterile conical tubes to be harvested by centrifugation at 4,000 x *g* / 4°C for 12 min (Sorvall™ ST 16R Centrifuge, Thermo Fisher Scientific Inc., Waltham, MA). The pellets were harvested and resuspended in both Butterfield's Phosphate-Buffered Dilution Water (PBS) and skim milk broth (Acumedia, Lansing, MI). Individual strains were resuspended in either of the broths and then mixed in equal quantities to form a two-strain *Listeria innocua* cocktail. The skim milk inoculum was used to evaluate the effect of organic matter on microbial protection. PPE inoculation consisted of both cell suspensions.

2.3 PPE Inoculation

Gloves and aprons were cut into 7.9 in^2 coupons using a sterile scalpel. This area was defined to match the area of the probe used to apply constant force and time in the transmission experiments. Before inoculation, the PPE was disinfected with a 70% ethanol solution to avoid interference from natural microflora. A spray nozzle was used to inoculate the PPE coupons to ensure even distribution. After inoculation, the aprons and gloves were allowed to dry. In contrast, boots were tested as a single piece. Boot soles were inoculated by submersion for one minute in a *Listeria innocua* cocktail. After inoculation, the footwear was positioned sole side up for complete drying. All the PPE

inoculation and drying steps were performed inside a biological safety cabinet in a BSL-2 Laboratory. Both inoculation procedures achieved a bacterial population of 7 log CFU/in^2.

2.4 Surfaces

These experiments evaluated different surfaces, including food products, food-contact surfaces (FCS), and non-food-contact surfaces (NFCS). Food products constituted of cheddar cheese and pasteurized queso fresco. Pre-sliced, packaged mild cheddar cheese and queso fresco blocks were purchased from a retail supermarket in Lincoln, Nebraska, and stored at 4° C before use. Queso fresco was aseptically sliced into square pieces to match cheddar cheese size and shape. Other surfaces included polyethylene (to represent cutting boards) and stainless steel, which were custom made into 9 in^2 coupons. The purpose was for, food products, cutting boards, and stainless-steel coupons to have similar shapes and sizes. Both surfaces were cleaned, dried, and later autoclaved for 30 minutes at 121°C before analysis. Alternatively, dairy tiles were not subsampled and were evaluated in place. Floors from a nonoperational dairy plant located in Lincoln, Nebraska, were evaluated for this study. These floors consisted of acid brick-dairy tiles that were thoroughly cleaned using a general-purpose alkaline detergent, rinsed with tap water, and sanitized with 70% ethanol before microbial transfer experiments were conducted

2.5 Contamination: PPE and Surface Combination

Depending upon the PPE, different cross-contamination patterns may occur in the processing plant. For this experiment, five PPE to surface contamination combinations were identified and evaluated. For example, gloves could contaminate cutting boards and

food products during packaging and handling. These PPE to surface combinations involve contamination of a finished product and Food Contact Surfaces (FCS), which are Zone 1 areas. As a result, combinations were classified according to PPE type and location using FDA's hygienic zoning identification for environmental monitoring. **Figure 2-1** shows a summary of the PPE/surface combinations. These experiments evaluated *Listeria* transference to environmental surfaces and products from a specific PPE item.

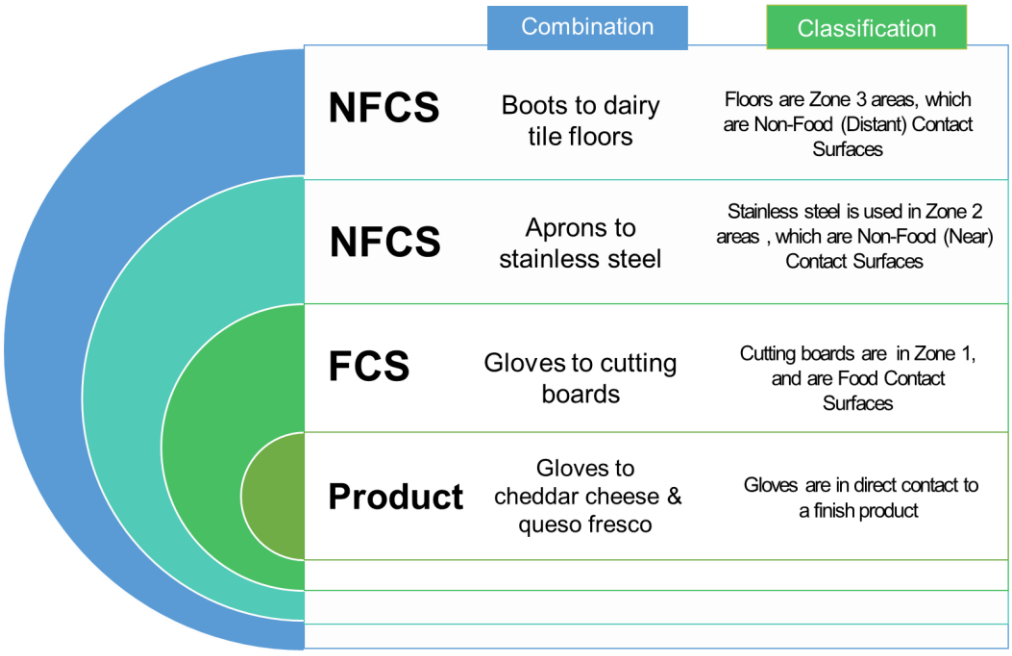

Figure 2-1. Summary of PPE/Surface combinations evaluated for this study

2.6 PPE mediated transfer of *Listeria innocua* to surfaces

Personal protective equipment, such as gloves, aprons, and boots, is essential to carry out the daily activities in the food processing industry. However, inadequate PPE use can lead to cross-contamination affecting not only food-contact and non-food-contact surfaces, but also finished food products. This study evaluated five PPE/surface combinations to simulate scenarios encountered in dairy processing facilities that could lead to cross-contamination. Nevertheless, variability associated with the translocation of

bacteria from one surface to another is a significant challenge for all researchers working to measure cross-contamination (Rodríguez and McLandsborough, 2007). In an attempt to reduce variability, each transfer was achieved using a constant contact force (2.942 N) and time (5 s) for four out of the five PPE/surface combinations. Those combinations included glove/queso fresco, glove/cheddar cheese, glove/cutting board, and apron/stainless steel. To reduce the experimental error, time and force were standardized for glove and apron mediated transfers of *L. innocua* to surfaces. The transmission was assessed by using a texture analyzer (Model TA-TX2, Texture Technologies; Scarsdale, NY) equipped with an A/OTC Ottawa cell probe. The instrument was calibrated to a five-second contact time and 2.942 N force. The PPE coupon (i.e. glove, apron) was placed so that the inoculated side contacted the surface (i.e., piece of cheese, stainless steel coupon). Simultaneously, the non-inoculated side was against the probe applying constant pressure and time as shown in **Figure 2-2**.

To evaluate the risk of contamination by multiple touches from a contaminated PPE, multiple cheese slices (or coupons of the surface of interest) were analyzed. The first touch was considered the first attempt and was evaluated for microbial counts. Subsequent touches (52 total attempts) were done to determine the microbial transfer over multiple touches. Samples for microbial analysis were gathered every three touches.

Surfaces containing transferred *Listeria* cells were placed in stomacher bags with 30 ml of 0.1% Buffer Peptone Water (BPW; Acumedia, Lansing, MI) and mechanically mixed in a Stomacher (Stomacher® 400, Seward Ltd, Bohemia, NY) for 2 minutes. Inoculated PPE pieces that acted as controls and the PPE used after the microbial transfer were also analyzed.

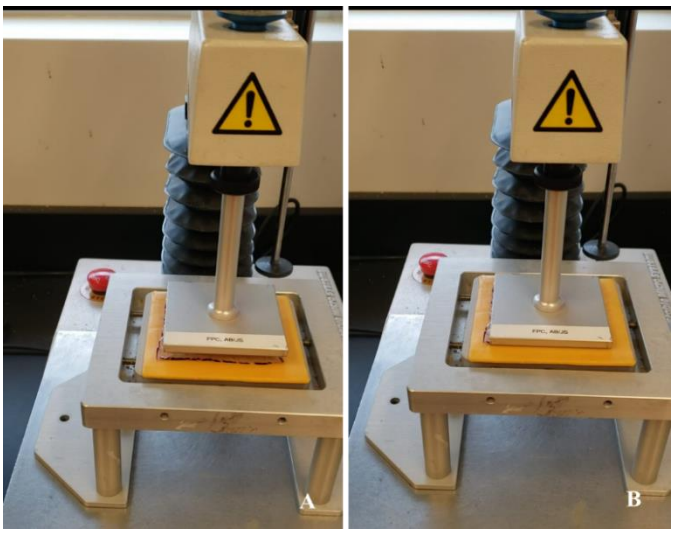

Figure 2-2. [A] Glove- mediated *Listeria innocua* transfer to a cheddar cheese slice. [B] A cell probe was calibrated to 2.942 N of force for 5 seconds while touching the food product.

Boot combination experiment consisted of a slightly different approach. The pressure was applied by a 170 lb volunteer who walked through the floor using the *L. innocua* inoculated boots. The individual stepped into sole stencils (59 in^2) that were placed in each step. Floor samples were collected using environmental sampling sponges (Nasco Whirl-Pak™ Fisher Scientific, Pittsburgh, PA) and hydrated with 15 ml of BPW. Excess buffer was removed from the sponge before floor sampling (**Figure 2-3**). Each "step" was sampled by wiping the 59 in^2 area horizontally using sterile gloves. The sponge was then turned over, and the opposite side and end were used to sample the same area vertically. The transfer evaluation consisted of 52 transfer steps; however, samples for microbial analysis were gathered for the first 10 touches, and after that, every three attempts. An individual boot was considered a replicate; thus, evaluation consisted of 4 replicates per inoculum. Inoculated boots that acted as controls, and boots used after *Listeria* transfer were also analyzed.

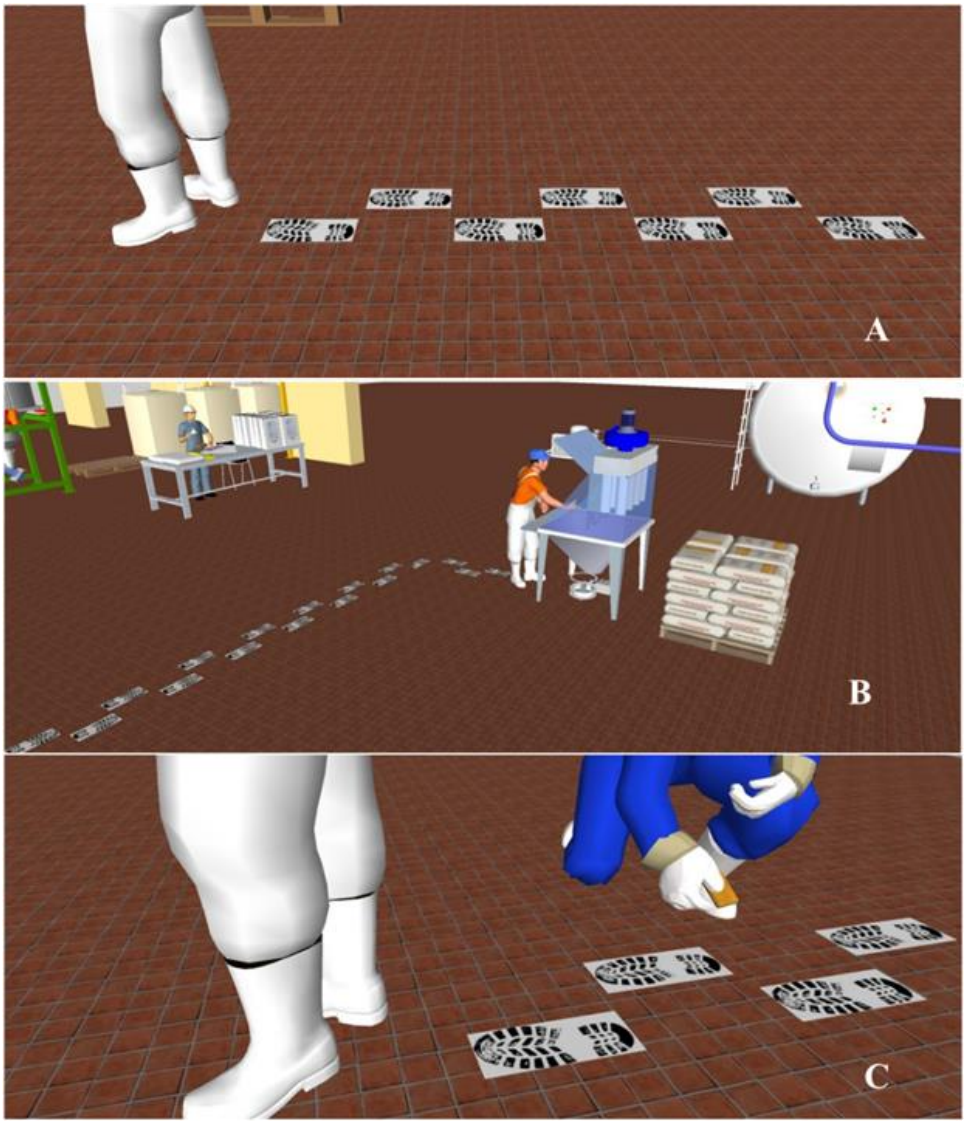

Figure 2-3. diagram of the transmission process; [A] individual walking using *Listeria innocua*—inoculated boots. [B] 52 steps were evaluated for transmission. [C] floor samples were collected using environmental sponges (A= 59 in^2).

2.7 Microbiological Analysis

The microbial analysis evaluated *Listeria innocua* transfer to different surfaces, and *Listeria* attachment to PPE before and after touches. After the samples were stomached, the solution was spread plated in Tryptic Soy Agar (TSA; Acumedia, Lansing, MI) with a Modified Oxford Listeria Agar (MOX; Acumedia) overlay after a three-hour resting period. All plates were then incubated at 30 °C for 48 h. Cell numbers

(*Listeria innocua*/CFU per square inch) were calculated based on PPE type and contact area to evaluated surfaces.

2.8 Statistical Analysis

Three independent replications were performed for the glove/queso fresco, glove/cheddar cheese, glove/cutting board, apron/stainless steel combinations, and four replications for the boot/dairy brick tile experiment. Data were statistically evaluated for each PPE/surface combination in the presence of PBS buffer or skim milk (organic matter). An ANOVA was performed using Statistical Analysis Software version 9.3 (SAS Institute, Cary, NC) to determine the significance of *Listeria innocua* transmission from each PPE to the surface of interest while considering the organic matter effect.

3. RESULTS AND DISCUSSION

3.1 *Listeria* transmission to food products and other surfaces

The bacterial transfer in all PPE/surface combinations were evaluated until the 52^{nd} touch. **Figure 2-4** shows *Listeria innocua* transfer from PBS and skim milk inoculated gloves to cheddar cheese slices. The inoculated PPE achieved a 7.06 log CFU/in^2 when the *Listeria* inoculum was prepared in PBS and a 7.57 log CFU/in^2 log when prepared in skim milk. The first touch achieved the highest bacterial transfer, with a 5.95 and 5.58 log CFU/in^2 of the population being transferred when the inoculum was prepared in PBS and skim milk, respectively.

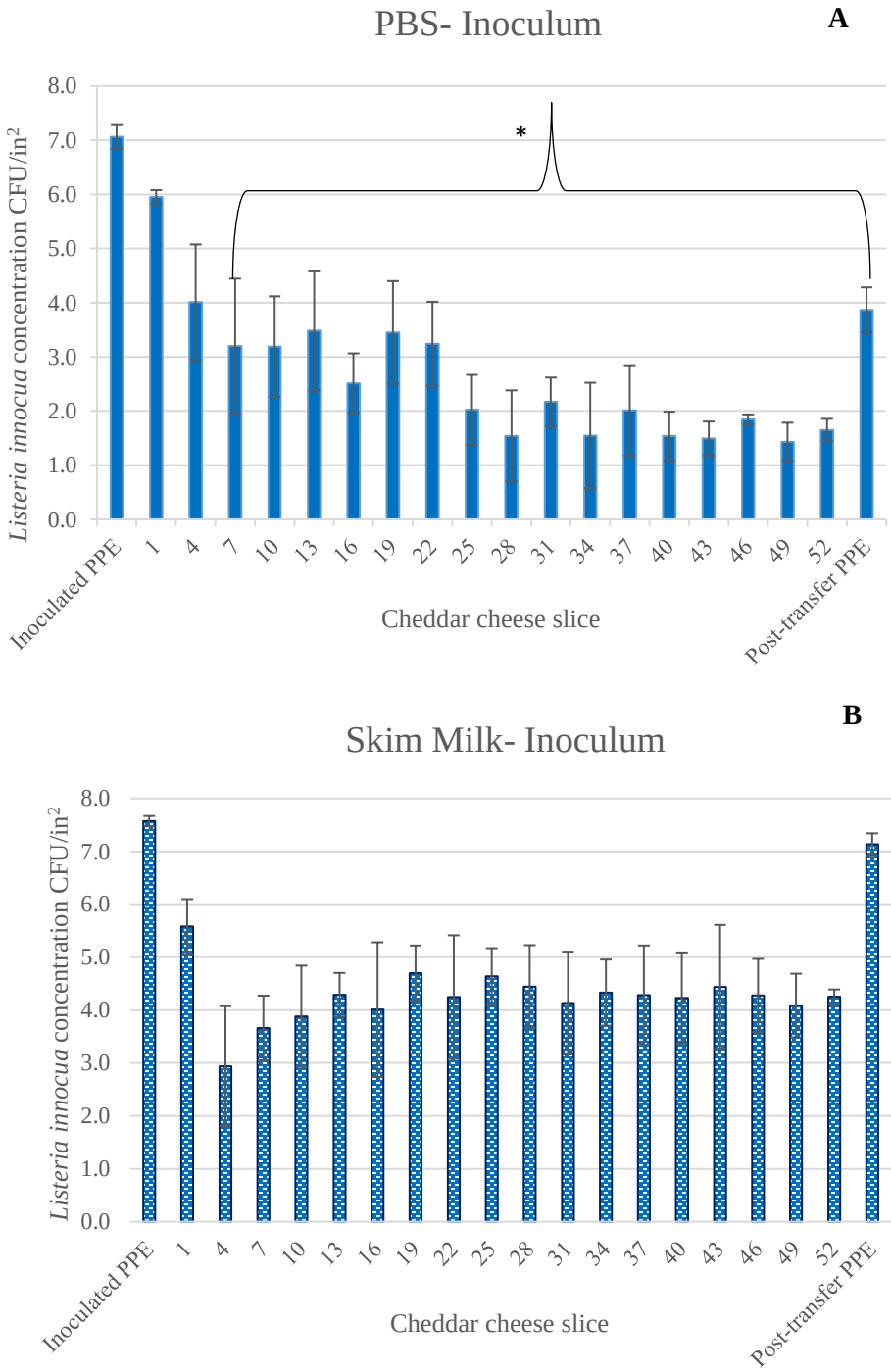

Figure 2-4. *Listeria innocua* transfer from gloves inoculated with PBS (A) and skim milk (B) to cheddar cheese slices. Asterisk (*) denotes statistical difference from the first transfer attempt ($P < 0.05$).

In the glove to cheddar cheese combination, the average transfer remained at 4.98 log CFU/in^2 until the 7th touch **(Figure 2-4 A**). From the 7th touch onward, there was an average 2.71 log CFU/in^2 decline in *Listeria* population when PBS was the bacterial carrier. However, when skim milk was the carrier; there were no statistically significant differences between the first and the other transfer attempts (**Figure 2- 4B**). A different scenario occurred with the queso fresco cheese (QFC) (**Figure 2-5**). In the PBS inoculum, a 5.91 log CFU/in^2 of *Listeria innocua* population was transferred in the first attempt. With this product, the average transfer remained at 4.50 log CFU/in^2 until the 34th touch. From then on, *L. innocua* decreased approximately 3 log units on average. When skim milk was the carrier, the first attempt transferred a 6.3 log CFU/in^2 of *Listeria innocua* to queso fresco. Statistical significance on transfers could only be observed at the 49th and 52nd transfer attempts, with a microbial population decline of approximately 1.7 log CFU/in^2.

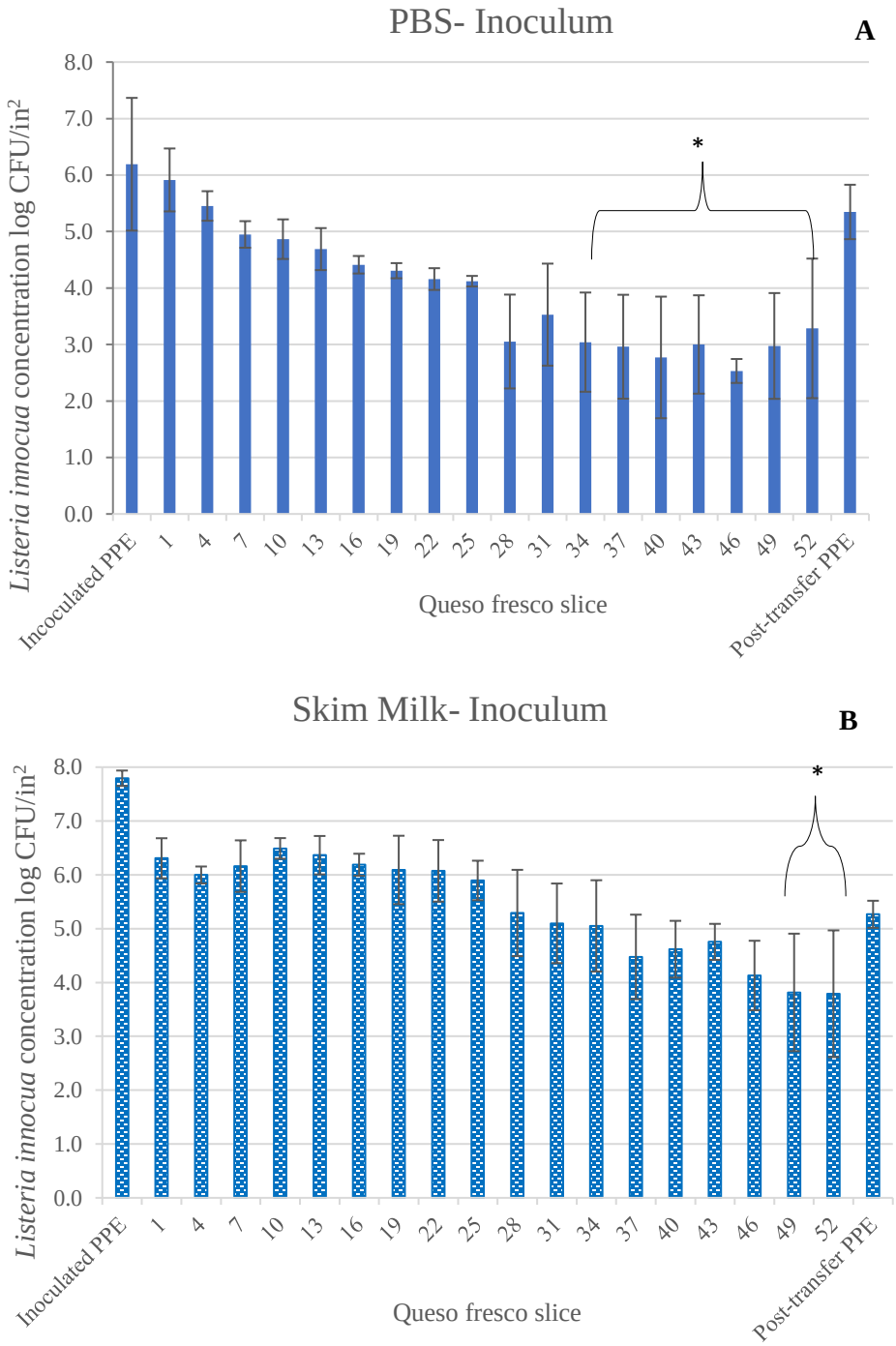

Figure 2-5. *Listeria innocua* transfer from gloves inoculated PBS (A) and skim milk to queso fresco slices. Asterisk (*) denotes statistical difference from the first transfer attempt ($P < 0.05$)

In this study, the contamination varied with inoculation method and food product. Cheeses contaminated using skim milk as bacterial carriers showed higher transfer of *Listeria innocua* than those contaminated using PBS. Also, results showed that it took more transfer attempts to achieve a *Listeria* population decline in queso fresco than in cheddar cheese. The transfer difference could be due to queso fresco's physical characteristics. Queso fresco is characterized by its high moisture, salt content as low as 1% and not greater than 3%, and near-neutral pH (Ibarra-Sánchez et al., 2017). In a study conducted by Rodríguez et al., (2007), stainless steel inoculated with a *L. monocytogenes* biofilm was used to evaluate microbial transfer to bologna and salami. Moisture present in the bologna created a liquid bridge or "capillary neck" between the dried biofilm and the food. This study hypothesized that if the food's moisture is higher than in the biofilm, the efficiency of the transfer of dried cells will increase due to capillary forces. As a result, the high moisture content and water activity of the product may have influenced *Listeria innocua* transmission from a contaminated glove to the product in the experiments reported here.

When other PPE/surface combinations were tested, the first touch also achieved the highest bacterial transfer, (4.78 and 5.71 log CFU/in^2 in cutting boards, 4.73 and 6.93 in stainless steel, and 1.70 and 4.32 log CFU/in^2 in dairy tiles, when PBS and skim milk were used as bacterial carriers, respectively). However, no statistically significant differences in *Listeria* transfer decline were observed from the first to subsequent touches (**Appendix A**).

3.2 Effect of organic matter

Transfer of *L. innocua* from contaminated PPE to the food product, cutting boards, stainless steel surfaces, and dairy tiles was evaluated with inoculum prepared using PBS and skim milk. Skim milk was used as a *Listeria innocua* carrier to evaluate transfer in a simulated dairy-processing scenario. **Figure 2-6** shows the average transfer of *Listeria* from PPE to different surfaces. Averages were calculated from the seventh touch onward to avoid the *Listeria innocua* concentration peak from the first transfer attempts from contaminated PPE. Results showed a statistically significant increase in the log CFU/in^2 values in the presence of milk for all PPE/Surface combinations.

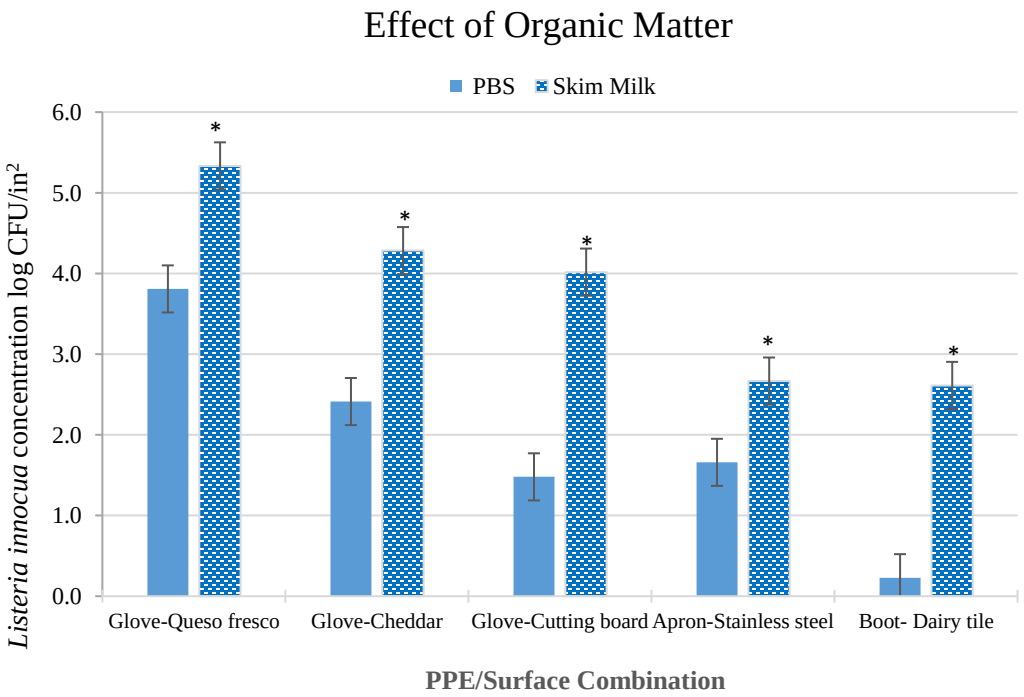

Figure 2-6 Average transfer of *Listeria innocua* from different PPE/surface combinations using PBS and skim milk as bacterial carries. Mean values within the same PPE/Surface combination with an asterisk (*) are significantly different from one another ($P < 0.05$).

The use of food soils or media simulating residues encountered by microorganisms in food processing facilities for laboratory studies is highly

recommended (Papaioannou et al., 2018). For example, the behavior of *L. monocytogenes* cells and biofilms has been previously found to be affected by the presence of organic matter. In 2015, Kuda et al., evaluated *L. monocytogenes* adherence and survival rates in diluted milk, soy milk, carrot, and laver nori extracts. This study suggested that small sediments of food, such as proteins and carbohydrates, increased *L. monocytogenes* resistance and adherence. As a result, a minimal amount of food residue can provide bacterial protection. Microbial protection is a relevant issue since the presence of organic matter can make sanitation procedures more complicated and can encourage cross-contamination in processing facilities (Overney et al., 2017; Korany et al., 2018)

3.3 Difference in *Listeria innocua* transfer among PPE/Surface combinations

Our results indicated that *Listeria innocua* transfer was significantly different depending upon PPE/Surface combinations, with higher transfer values observed in in glove-mediated transfers to food products with both skim milk and PBS inocula. **Figure 2-7** shows the average *Listeria* transfer from the 7^{th} to the 52^{nd} touch when skim milk was used as the microbial carrier (PBS followed a similar pattern). When comparing transfer to queso fresco and cheddar cheese, the glove to queso fresco combination showed a higher *Listeria innocua* transfer (5.33 log CFU/in^2), than glove to cheddar cheese (4.28 log CFU/in^2). According to Holle et al., (2018), queso fresco's susceptibility to bacterial contamination is partially due to its high pH and moisture content as well as *Listeria*'s tolerance for the salt content typically associated with this product. As a result, the high levels of transfer observed from gloves to queso fresco

classifies this PPE/surface combination as high risk for cross-contamination in a dairy environment.

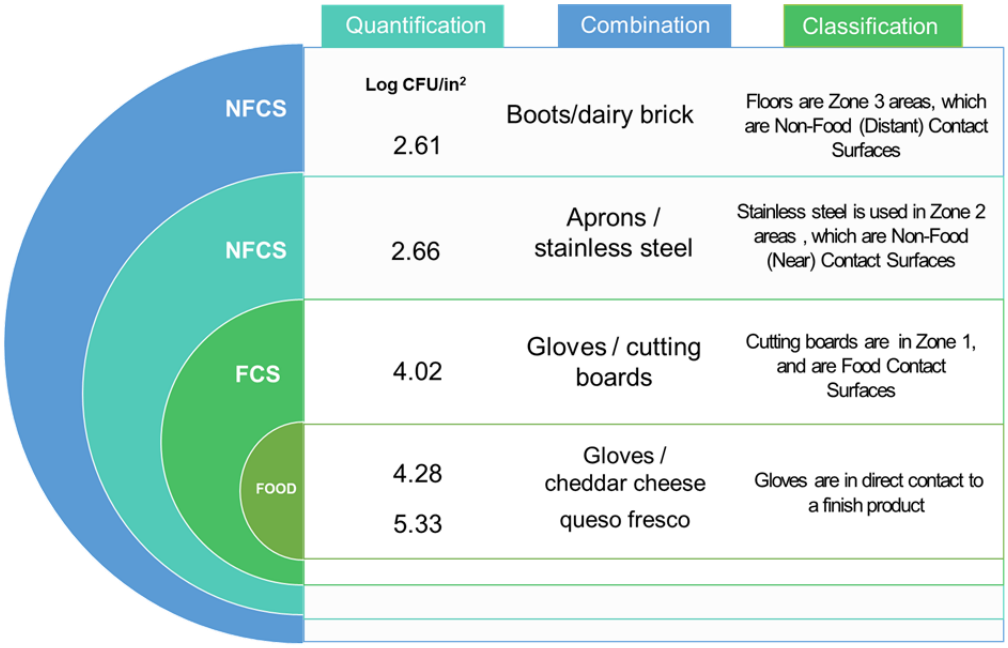

Figure 2-7 *L. innocua* transfer quantification (log CFU/in^2) in different PPE/surface combinations using skim milk inoculum.

Furthermore, queso fresco has been implicated in various listeriosis outbreaks worldwide, which may pose a significant risk to consumers (Jackson et al., 2018). Alternatively, cheddar cheese is a close-textured-hard cheese, with a maximum moisture content of 38%, 1.5–3% salt, and a final pH of 5.2-5.4 (Chandan, 2014). In 2014, Dalmasso and Jordan monitored *L. monocytogenes* growth in natural contaminated cheddar cheese, with *Listeria* never exceeding 20 CFU/g. Also, it was not detected after five months of ripening, showing that the ripening process in cheddar creates a hostile environment for pathogenic bacteria. Nevertheless, the microbial transmission to cheddar cheese reported here showed and average of 4.28 CFU/in^2; hence glove usage should be monitored in cheddar cheese packaging and handling

facilities. As a result, the risk associated with the transfer of gloves to queso fresco justifies the industry's need for more stringent food handling practices when producing high-moisture soft cheeses, followed by lower-moisture cheeses.

Following gloves to product combination, glove mediated transfer of *Listeria innocua* to cutting boards were quantified at 4.02 log CFU/in^2. This PPE/Surface combination is worrisome since cutting boards are food-contact surfaces, and their contamination could lead to finished product contamination. For example, in 2008, pasteurized cheese was linked to a *L. monocytogenes* outbreak in Canada that made 38 people sick, including 16 maternal-neonatal cases. Cross-contamination was supported by the isolation of *L. monocytogenes* in cutting boards and knives from an environmental analysis in 22 retail stores. Therefore, practices applied at the retail stores enabled the cross-contamination of cheeses during the cutting and/or packaging process (Gaulin et al., 2012).

According to the FDA's guidance for the control of *Listeria monocytogenes*, gloves are considered food-contact surfaces. When gloves are worn properly, the risk of pathogen transmission can be reduced considerably. However, glove use must be monitored carefully to ensure that it is appropriate for the required tasks (Todd et al., 2010). The results reported here showed a high level of transmission of *Listeria* from gloves to cheeses and therefore the risk associated with these activities is also high. Nevertheless, the FDA has established recommendations to avoid cross-contamination in RTE- facilities, including handwashing before glove usage and proper sanitation or replacement of gloves after touching any non-FCS.

For the non-FCS PPE/surface combinations (i.e. Apron to stainless steel), a 2.66 log CFU/in^2 transmission was observed. Several studies have evaluated *L. monocytogenes* transfer from stainless steel to food products and vice versa (Vorst et al., 2006;Rodríguez and McLandsborough, 2007). However, little research has been done to study the transfer of *L. monocytogenes* from aprons to stainless steel.

In 2002, Midelet and Carpentier, evaluated *L. monocytogenes* attachment to stainless steel and conveyor belt materials (polyvinyl chloride and polyurethane). Results showed that attachment was greater on polymers than on stainless steel, which explains why stainless-steel surfaces are easier to clean than polymers. Hence, stainless steel is widely recognized as an excellent material for the food industry and is used to construct vats, equipment, and tables. Nevertheless, the presence of *L. monocytogenes* on the surface of equipment and utensils is evident of its widespread occurrence in the meat and dairy processing industries (de Oliveira et al., 2010).

Interestingly, the lowest PPE to surface *Listeria innocua* transfer was found on a non-food-contact surface. A 2.61 log CFU/in^2 transmission was observed when boots were used to transfer bacterial contamination to dairy tiles and in a dairy processing environment. Based on these results, the boot to dairy tile combination was classified as a medium to low risk combination since it involves the contact of two non-food contact surfaces and low bacterial transference.

Nevertheless, *L. monocytogenes* has widely been isolated in places where standing/condensed water occurs, such as floors and drains (El-Shenawy, 1998) and in boots used in dairy processing facilities (Dass et al., 2018). As a result, proper Good Manufacturing Practices (GMP's) should be followed to avoid bacterial cells being

translocated by droplets caused by mechanical action during processing and cleaning (Carpentier and Cerf, 2011). Moreover, the FDA provides recommendations to minimize the potential for RTE food to become contaminated with *L. monocytogenes* through personnel actions. In the case of foot traffic, recommendations include the use of foamers and footbaths when personnel enter RTE-areas, a captive shoe policy, and proper sanitation and cleaning practices.

4. CONCLUSIONS

Based on the results obtained in this research, the highest *Listeria innocua* transfer from PPE to other surfaces occurred at initial transmission. This was observed when both skim milk and PBS were used as *L. innocua* carriers; however, the skim milk inoculum always led to a higher level of transmission. Initial transfers from glove to queso fresco and to cheddar cheese showed a 6.30 and 5.58 log CFU/in^2 of *Listeria innocua*, respectively. Other PPE and surface combinations showed a transfer of 5.71, 6.93, and 4.32 log CFU/in^2 of *L. innocua* from glove to cutting boards, apron to stainless steel, and boots to dairy tile, respectively.

When *Listeria* transfers occurred from gloves to food products, consecutive touches led to a decline in levels of transference. From the first touch mediated by gloves, a drop in *L. innocua* transmission occurred with every touch until the 7th one in cheddar cheese; while it took 34 consecutive touches to achieve the same effect in queso fresco. Other PPE and surface combinations did not show a decline in bacterial transfer, which highlights the risk associated with PPE mediated transmission of *L. innocua* to surfaces in dairy facilities. Also, the effect of organic matter on *L. innocua* transmission

is a matter of concern because milk solids are commonly found in the dairy processing environment.

Considering common sampling points for different combinations of PPE-surfaces and skim milk as the carrier of contamination, on average, gloves transferred 5.33 log CFU/in^2 *L. innocua* to queso fresco, and 4.28 log CFU/in^2 *L. innocua* to cheddar cheese. Other surface combinations involving food contact and non-food contact surfaces showed lower *L. innocua* transmission. A 4.01, 2.66, and 2.61 log CFU/in^2 average transmission was observed from gloves to cutting board, aprons to stainless steel, and boots to dairy tiles. In general, bacterial transference from PPE to food contact surfaces and food product were higher than those observed between PPE and non-food contact surfaces, emphasizing the risk associated with potential cross-contamination of final products

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CHAPTER 3

DETERMINATION OF AN EFFECTIVE SANITIZING PROCEDURE FOR *LISTERIA INNOCUA* IN PERSONAL PROTECTIVE EQUIPMENT USED IN DAIRY FACILITIES

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ABSTRACT

Listeria monocytogenes can survive and grow under wet environmental conditions often encountered in dairy processing facilities. The source of microbial contamination may include employees and their personal protective equipment (PPE), which often contact equipment and food contact surfaces. This study investigates the effectiveness of sanitizers in reducing *Listeria innocua.* contamination from different types of gloves, aprons, and boots. The PPE was inoculated with a two-strain cocktail of *Listeria innocua* and suspended in either Phosphate Buffer Saline (PBS) or skim milk to determine the potential effect of organic matter. With PBS, results showed 2.08 to 4.60 log CFU/in^2 reduction in the different types of aprons, with the peroxyacetic acid-based sanitizer showing the highest effectiveness. Different kinds of gloves showed a similar average reduction (2.15 – 4.16 log CFU/in^2). In comparison, the boots showed a 1.00 – 1.70 log CFU/in^2 reduction. With the skim milk as the carrier of potential contamination, the sanitizers achieved less than 1.00 log CFU/in^2 reduction in the aprons, showing no significant differences in effectiveness among them. Similarly, reduction levels of 0.5 to 0.8 and 0.7-1.0 log CFU/in^2 were observed in the different gloves and boots, respectively. Overall, the different sanitizers' antimicrobial activity was considerably diminished when used to sanitize PPE with high organic matter concentration. This highlights the negative impact of organic matter in sanitizer effectiveness and the need to include cleaning steps to achieve the desired reduction in the bacterial population. The inclusion of cleaning

regimes with and without mechanical action, achieved a ≥ 3- log CFU/in^2 reductions in the different types of PPE. This study highlights the importance of scrubbing as an essential step to reduce and control *Listeria* from PPE.

1. INTRODUCTION

In recent years, concerns regarding the safety of pasteurized dairy products have increased in the light of several food safety incidents involving contamination with *Listeria monocytogenes*. *Listeria monocytogenes* is widespread in the environment, commonly found in soil, water, and plant material. These bacteria can survive harsh environmental conditions such as low pH, salt concentrations up to 10%, and growth at refrigeration temperatures (Jordan, 2019). These characteristics allow *L. monocytogenes* to grow and survive in food processing plants and can potentially contaminate finished products.

Once the pathogen is established in the plant facility, it can be easily carried throughout the environment by materials, inappropriate personnel movement, and processing workflows (Muhterem-Uyar et al., 2015). Employee movement within food facilities can also significantly impact finished products' microbiological quality and safety (Kornacki and Gurtler, 2007). Observations by the FDA in *L. monocytogenes* outbreak investigations have shown *Listeria* spp. was present in equipment, on product contact surfaces, and in drains (Flynn, 2016). Also, poor employee cleaning/sanitizing practices and improper use of personal equipment has been highlighted (FDA, 2017). As a result, Personal Protective Equipment (PPE) worn by personnel can be a potential transmission route for cross-contamination.

Studies have shown *L. monocytogenes* can adhere and form biofilms on polyvinyl chloride (PVC), polyethylene, and rubber surfaces (Berrang et al., 2008; Takahashi et al., 2010; Korany et al., 2018). These materials are commonly used to make Personal Protective Equipment (PPE) such as aprons, gloves, and boots. Therefore, control of traffic patterns and proper sanitation and cleaning practices are needed to avoid cross-contamination issues in dairy processing facilities.

Listeria monocytogenes is sensitive to sanitizing agents commonly employed in the food industry. However, cell attachment to surfaces can provide protection against chemical sanitizers (Frank and Koffi, 1990). In 2018, Martinez et al. evaluated various sanitizers for *Listeria spp*. reduction from squeegees made of different materials. Results showed a 1-2 log CFU/in^2 reduction from chlorine, quaternary ammonium, and a combination of hydrogen peroxide and acetic acid sanitizers (Martinez et al., 2018).

Moreover, higher reductions were observed from rubber than foam squeegees. Nevertheless, these results did not meet sanitation efficiency since a 3-log reduction (99.9%) has been suggested to target effective inactivation of attached or biofilm bacteria (Somers & Wong, 2004; EPA, 2011). As a result, sanitizing and cleaning regimes must be assessed to verify, reduce, and control *Listeria* in PPE and tools.

2. MATERIALS AND METHODS

2.1 Materials

The everyday use of Personal Protective Equipment (PPE) such as gloves, aprons, and boots represent a potential source of cross-contamination in dairy facilities. These

items are essential in food operation and are often in contact with food contact surfaces (FCS) and food products. Thus, these items need to follow a standard cleaning procedure to avoid bacterial transfer from the PPE to other food and non-food contact surfaces. Therefore, the following PPEs were selected to determine the effectiveness of different sanitizers to remove *Listeria*.

Dairy facilities and food establishments use nitrile, latex, and vinyl gloves. Hence the three types of gloves evaluated for this study included: a) Nitrile Gloves (Glove 1); b) Latex Gloves (Glove 2); and c) Vinyl Gloves (Glove 3). Apron evaluation consisted of three types of aprons. The materials used were a) Vinyl (Apron 1); b) Polyethylene (Apron 2); and c) 65% Polypropylene/35% polyethylene (Apron 3). The last PPE evaluated was boots. In this study, evaluation consisted of PVC work boots with different tread patterns. Dunlop™ Onguard™ White PVC Boots (Boot 1) contain a Safety-Loc outsole with shallow lugs closer together and are categorized as having narrow treads. In contrast, the Honeywell PRO® Servus boots (Boot 2) with a Triple Density Technology outsole contain lugs that were deeper and wider apart. Thus, Boot 2 consisted of a broader tread pattern. All the boots used for the procedure were the same size.

2.2 Test strains and inoculum preparation

Listeria monocytogenes represents a biosafety hazard in the laboratory. Therefore, *Listeria innocua*, a non-pathogenic member of the same genus, was selected as a suitable candidate for this study. Preparation included a two strain *Listeria innocua* cocktail (ATCC33090 and ATCC 51742) to provide a range of strain susceptibilities to the experiment's conditions. Culture propagation was carried out in 9 mL Brain Heart Infusion broth (BHI; Acumedia, Lansing, MI), incubated at 30°C for 24

h (Heratherm™ IGS 180 Incubator, Thermo Fisher Scientific Inc., Waltham, MA). After propagation, all cultures were stored individually at -80°C in cryogenic vials containing 10% sterile glycerol. For inoculum preparation, 100 µL of each of the stock solutions were grown in 9 mL BHI tubes, followed by a 24 h incubation at 35℃. After two consecutive transfers, cultures were then transferred individually to 50-mL sterile conical tubes to be harvested by centrifugation at 4,000 x *g* / 4°C for 12 min (Sorvall™ ST 16R Centrifuge, Thermo Fisher Scientific Inc., Waltham, MA). The pellets were re-suspended in both Butterfield's Phosphate-Buffered Dilution Water (PBS) and skim milk broth (Acumedia, Lansing, MI). Skim milk was included to study the impact of high organic matter concentration on protecting the microorganisms present. Inoculation of the different types of PPE was carried out with both cell suspensions.

2.3 PPE Inoculation

Before inoculation, the PPE was sanitized with a 70% ethanol solution to avoid interference from natural microflora. Gloves and aprons were cut into 4 in^2 coupons using a sterile scalpel. The PPE coupons were spray- inoculated to ensure even distribution using a Dynalon™ Quick Mist™ HDPE spray nozzle. After inoculation, the aprons and gloves were allowed to dry. Alternatively, boots were tested as a single piece. Boot soles were immersed for one minute in a plastic tub that contained the *Listeria innocua* cocktail. After inoculation, the footwear was positioned sole side up for drying. All the PPE inoculation and drying steps were performed inside a biological safety cabinet in a BSL-2 Laboratory. **Figure 3-1** shows a summary of this protocol**.** Both inoculation procedures achieved a bacterial population 6-7 log CFU/in^2.

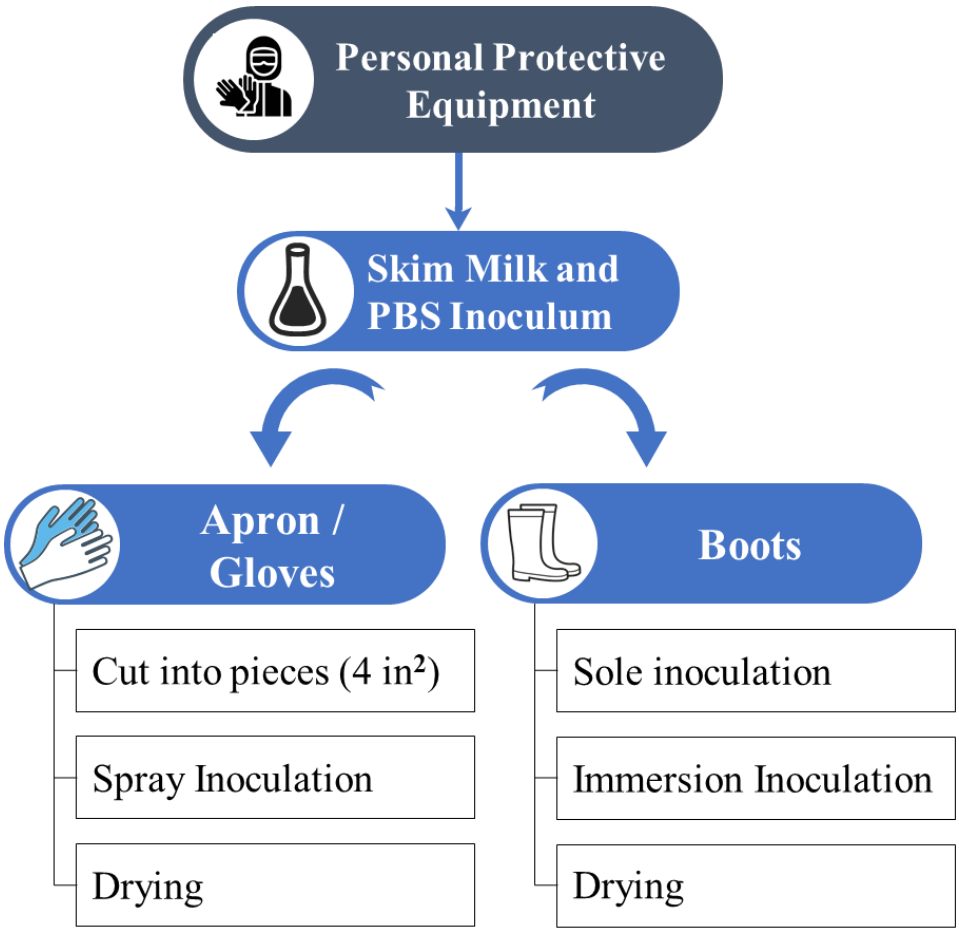

Figure 3-1. Inoculation procedures for different Personal Protective Equipment (PPE).

2.4 Cleaning and Sanitizing Protocols

After the gloves, aprons, and boots were inoculated with *Listeria innocua* and dried, multiple sanitizing agents were tested to determine which one would most efficiently kill the organism. This study used three sanitizers: a) a chlorine-based sanitizer commonly used in the dairy industry, b) a quaternary ammonia compound considered a good sanitizer against *Listeria monocytogenes* and, c) a peroxyacetic acid-based sanitizer, which is a recognized biofilm removal sanitizer. Sanitizer preparation included the use of deionized water to avoid any organic materials that could interfere with the sanitizer's available active forms. Manufacturers recommended a one-minute contact time for each sanitizer.

The concentrations of the solutions to be used were determined based upon the type of PPE treated. **Figure 3-2** shows a summary of this intervention. Gloves and aprons have direct contact with food contact surfaces (FCS). Therefore, to meet regulatory requirements, quaternary ammonia and chlorine concentrations for these PPE did not exceed 200 ppm, to prevent transferring these chemicals to food surfaces. According to manufacturers' instructions, a 0.20% concentration is recommended for the peroxyacetic acid sanitizer. Boots, however, required a higher concentration. Boots are associated with soiling material such as organic matter from processing plant floors that can reduce sanitizers' efficacy. Therefore, for quaternary ammonia and chlorine-based sanitizer levels used were 400 ppm in shoe baths. Peroxyacetic sanitizer preparation followed manufacturer recommendations.

The sanitizer solution's final desired concentration was checked by a titration method (iodometric titration) using a Quat Test Kit 317 for the quaternary ammonia-based sanitizer and an Oxidizer Test Kit 322 for the chlorine and peroxyacetic based sanitizers. The sanitizer manufacturer provided the test kit (Ecolab, St. Paul, MN). The kits rely on oxidizing agents' ability to produce "a back titration," and the kit manufacturer provides a correlation for each chemical. This method allows confirmation of the concentration of the sanitizing solution used in the experiments.

In addition to the three different sanitizers, a water rinse step was tested as a negative control. The water rinse evaluation consisted of a one-minute contact time. The negative control allowed for the comparison of the microbial reduction observed by placing the PPE in a water rinse (physical removal) versus the sanitizer (physical removal and bactericidal effect). Inoculated pieces without chemical or water rinse tests were also

evaluated as controls.

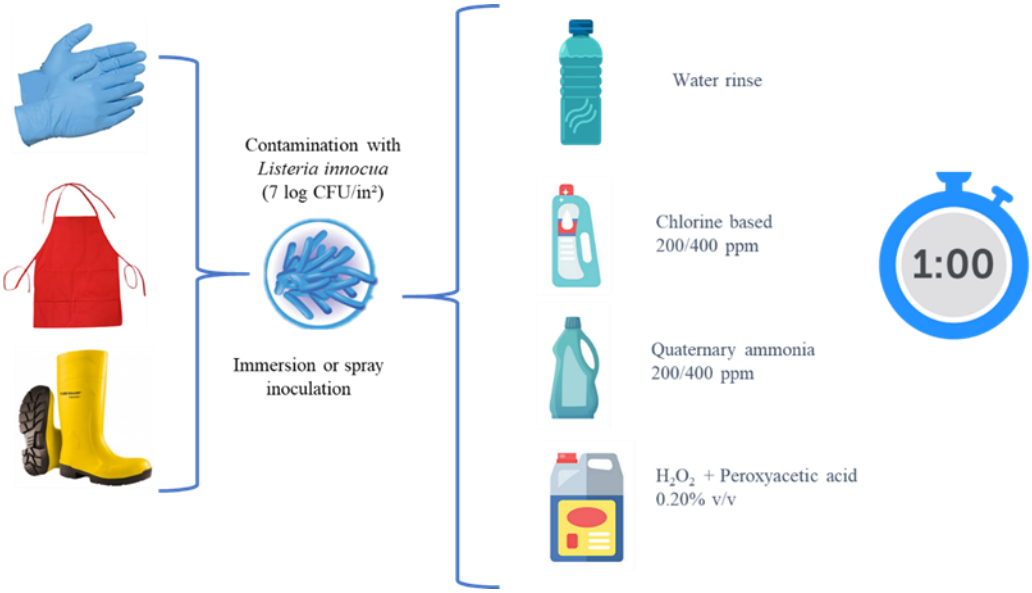

Figure 3-2: Diagram of the sanitizing procedure for PPE analysis.

After completing the sanitizer experiments, the next experiment evaluated consisted of a cleaning protocol. The cleaning protocol included a cleaning and mechanical action step. The protocol's purpose was to achieve a three-log reduction, the desired microbial reduction for proper cleaning and sanitation performance. As observed in **Figure 3-3**, two cleaning protocols were evaluated. The first step of Protocol One consisted of immersion cleaning with a 10-minute contact time. After the immersion, the evaluated PPE was pre-rinsed with deionized water and followed by a sanitizing treatment with a one-minute contact time. Cleaning Protocol Two consisted of the same steps as cleaning Protocol One, except that a one-minute scrubbing step was evaluated after soak cleaning and before water rinsing.

Two different types of cleaners were tested for both cleaning protocols. Cleaner 1 is a neutral, general-purpose cleaner with versatility, high solvent concentration, and low foaming formula that aid in rinsing. In comparison, Cleaner 2 is a chlorinated alkaline

cleaner that contains excellent alkaline builders that aid in soil removal. This product claims rapid soil-penetration, free-rinsing properties, and effectiveness under a variety of water hardness conditions. According to the manufacturers' directions for use, both cleaners need 5 to 15 minutes of soaking time. As a result, 10 minutes were chosen as adequate time for the cleaning protocols.

After the PPE was soaked in the cleaners and rinsed (Protocol 1) or soaked in a cleaner, scrubbed, and rinsed (Protocol 2), a sanitizer was used as the last step for both cleaning protocols. The sanitizer for the cleaning protocols was selected based on the sanitizing regime's conclusions. The sanitizer with the lowest effectiveness was selected to see if a three-log reduction could be achieved with the addition of cleaning and scrubbing steps. The decision was made based on the assumption that if a three-log reduction was achieved with the worse sanitizer, it should be outperformed using the best sanitizer.

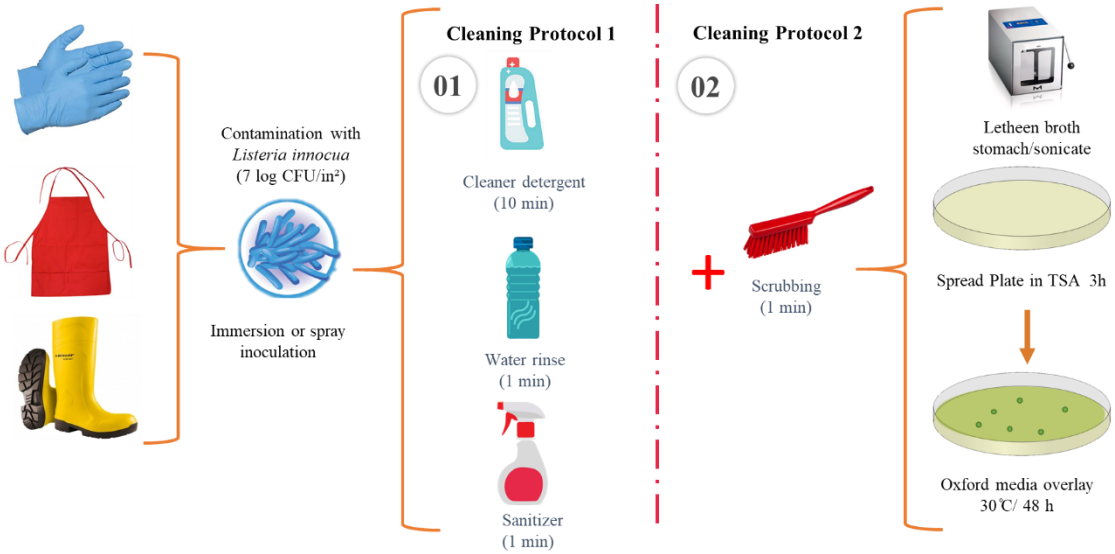

Figure 3-3: Cleaning Protocols for PPE analysis.

2.5 Microbiological Analysis

The microbial analysis was evaluated before and after the sanitation procedures. As shown in **Figure 3-3**, Letheen broth (Acumedia, Lansing, MI) was added after the PPE pieces were contaminated and treated with water, sanitizers, and the two different cleaning protocols. Letheen broth contains neutralizing agents that aid in microbial recovery and stabilization after chemical treatment. The samples were sonicated and later vortexed in one-minute intervals to aid in *Listeria innocua* detachment from the PPE materials. Finally, the solution was spread plated onto Tryptic Soy Agar (TSA; Acumedia, Lansing, MI) with a Modified Oxford Listeria Agar (MOX; Acumedia) overlay after a three-hour resting period. This procedure allowed the recovery of injured cells, and *Listeria innocua* was distinguishable as black colonies. All plates were then incubated at 30 °C for 48 h. Log reduction of *Listeria innocua* was calculated by subtracting the counts obtained after the application of the water and chemical treatments from the control (untreated sample).

2.6 Statistical Analysis

For each experiment, three independent replications were performed for each of the inoculum types. In each replication, evaluation consisted of two sub-samples to account for variation. Finally, results were analyzed to observe the reduction in *Listeria innocua* population after the cleaning protocols. Data were statistically evaluated for each sanitizer/cleaning regime in the presence of PBS buffer or skim milk (organic material). An Analysis of variance with a randomized complete block design was performed to determine the best cleaning agent (most effective) and the organic matter effect, using SAS

software version 9.4 (SAS Institute, Cary, NC). All statistical data were analyzed with a significance level of $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Sanitizer Intervention

Listeria innocua reduction in Aprons

Sanitizers' effect was evaluated on three types of aprons commonly used in the dairy industry. Butterfield's Phosphate-Buffered Saline (PBS), water was used in the inoculation procedures since water, e.g., condensation, has been recognized as a potential vehicle for cross-contamination with *Listeria monocytogenes* in dairy facilities. The inoculation procedure delivered a bacterial population of 6.86 log CFU/in^2 in control samples using PBS (data not shown). However, cross-contamination could also happen with milk as a vehicle of contamination. Therefore, to simulate this scenario, a skim milk broth was also used to inoculate the aprons. For these samples, an average bacterial population of 7.35 log CFU/in^2 was achieved (data not shown).

Figure 3-4 shows the water rinse's contamination reduction and the three different sanitizer effects on both inoculum types. On average, the reduction achieved by water rinsing was 0.81 log CFU/in^2 in the water-based inoculum and 0.44 log CFU/in^2 in the skim milk inoculum. The data showed no statistical differences between PBS and skim milk inoculum in the water rinse. Results indicate that cell dislodgment can occur from the material by rinsing in water, but no meaningful reductions were observed in the population levels.

Chlorine, a widely used sanitizer in the food industry, achieved a 2.20 log CFU/in^2 reduction when the inoculum used was prepared in PBS. A 2.46 log CFU/in^2 reduction was observed with the quaternary ammonia sanitizer, while the peroxyacetic acid showed a 4.57 log CFU/in^2 reduction. These results showed statistical significance; when PBS was used to apply the inoculum, the peroxyacetic (PAA) sanitizer was the most effective in reducing *Listeria innocua* on the different aprons tested. Similar results were obtained by Hua et al., 2019, who obtained a 4.0–4.5 log10 reduction using a 5-min treatment of 200 ppm PAA in low-density polyethylene (LDPE), polyvinyl chloride (PVC), polyester (PET), and rubber coupons. Peroxyacetic acid has proven to be a highly biocidal oxidizer. However, it has only found food-industry applications in recent years and is being promoted as a potential chlorine replacement (Schmidt, 2012; Gawande et al., 2013).

When the inoculum was applied using skim milk as the carrier, only a 0.78 to a 0.92 log CFU/in^2 reduction was observed with the three different sanitizers, showing no statistical differences in effectiveness among them. The amount of bacterial reduction achieved in this situation was much less than the inoculum delivered using PBS as the carrier. This difference highlights the presence of organic matter in reducing the effectiveness of the sanitizer. Additionally, milk tends to foul surfaces, leading to biofilm formation or physical protection for the microorganisms that might be present. Nevertheless, when the difference in log reduction between both inoculums is compared, the three different sanitizers showed a statistically significant difference in skim milk versus PBS (**Figure 3-4**).

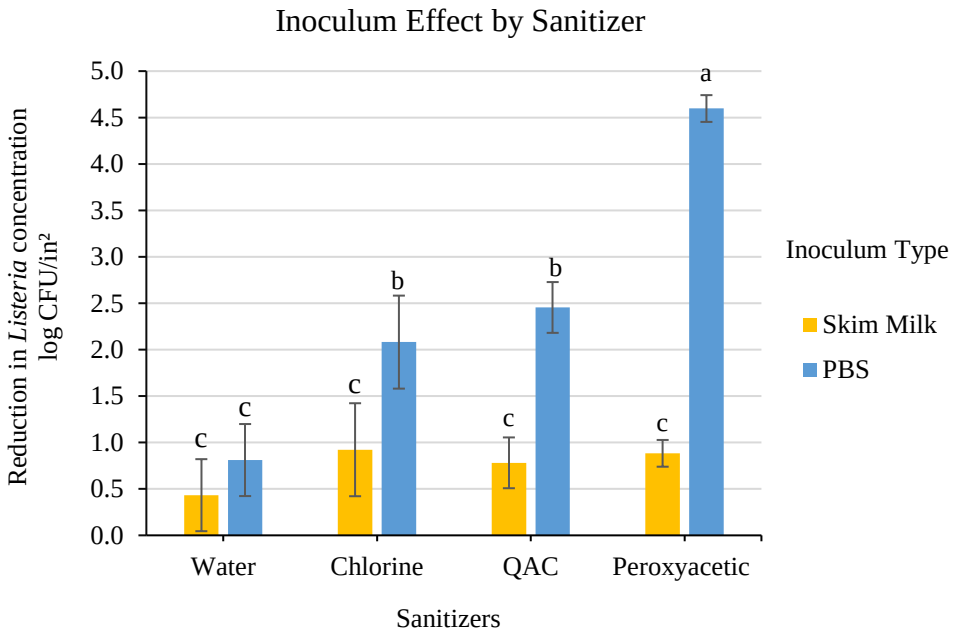

Figure 3-4. Average *Listeria innocua* reduction of the water rinse and sanitizers on aprons using PBS and skim milk inoculation. Mean values within the same water or sanitizer treatment that share the same letter are not significantly different from one another (P > 0.05)

When the type of material used for manufacturing the aprons is considered and the inoculum used is prepared in PBS, the Vinyl material (Apron 1) showed less than 1 log reduction using water rinse, a 2.64 and 2.77 log reduction with chlorine, and quaternary ammonia, respectively. The peroxyacetic acid (PAA) sanitizer achieved a 4.44 log CFU/in$^{2.}$ bacterial reduction. Therefore, in the absence of organic matter, peroxyacetic acid sanitizers performed adequate sanitation by achieving the desired three-log reduction. Water rinse, as expected, was not enough to remove bacterial contamination, as shown by the removal of 0.37 log CFU/in^2.

For the Polyethylene material (Apron 2) and the inoculum delivered with PBS, there was less than 1 log CFU/in^2 reduction observed with the water rinse, a 1.94 log reduction the chlorine sanitizer, and a 2.31 log reduction with the quaternary ammonia sanitizer. Peroxyacetic acid obtained the best results, with a 4.63 log CFU/in^2 reduction.

Similar to the Vinyl apron, the peroxyacetic acid sanitizer performed effective sanitation by achieving the desired three log reduction.

Aprons made with 65% polypropylene/35% polyethylene material (Apron 3) showed similar PBS inoculum results where the peroxyacetic acid achieved a 4.72 log CFU/in^2 reduction. As a result, all the aprons achieved the same log reduction pattern, and none of the apron materials were significantly different from one another (p-value < 0.05). This can be observed in **Figure 3-5.,** which shows the *Listeria innocua* reduction in aprons made of different materials using the three sanitizers in both inocula.

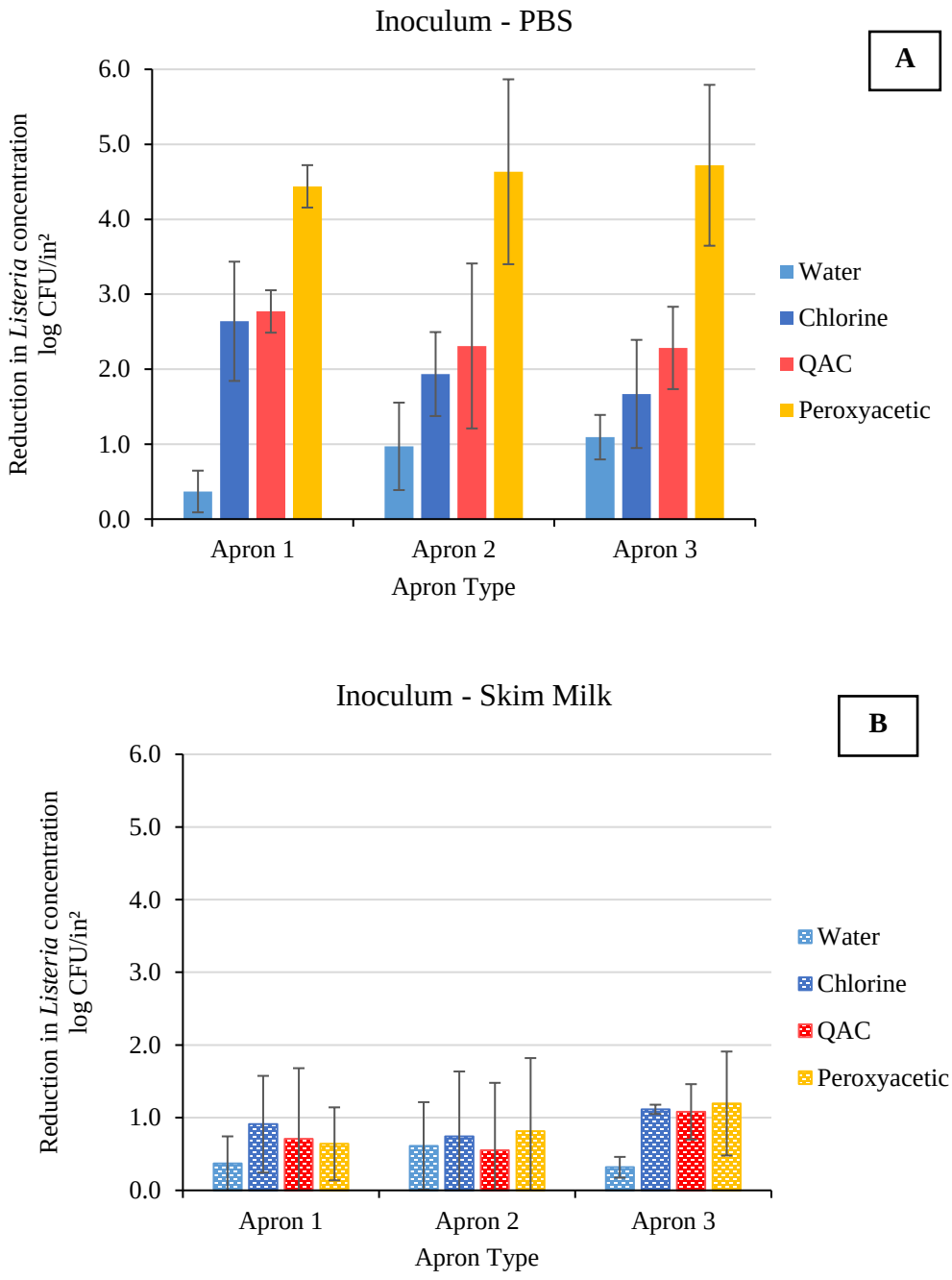

Figure 3-5. *Listeria innocua* reduction in Vinyl (Apron 1), Polyethylene (Apron 2), and 65% polypropylene/35% polyethylene (Apron 3) aprons using different sanitizers upon inoculation delivered using Butterfield-phosphate-dilution water (PBS) [A] or skim milk [B]

Listeria innocua reduction in Gloves

Once again, both PBS and skim milk were used to deliver the inoculum. The inoculation procedure achieved an average bacterial population of 6.53 log CFU/in^2 in control samples using PBS as the carrier. In contrast, the skim milk inoculum achieved a bacterial population of 7.6 log CFU/in^2 (data not shown). **Figure 3-6** shows the reduction achieved by the water rinse and the three different sanitizers. The reduction achieved using just water varied from 0.52 to 1.35 and 0.29 to 0.41 log CFU/in^2 in the PBS and skim milk inoculum.

Similar to the aprons, when the gloves were inoculated using PBS as a carrier, an average 1.52 – 5.5 log CFU/in^2 reduction was observed with the three different sanitizers. The Peroxyacetic Acid (PAA) showed the highest log reduction (5.5 log CFU/in^2). PAA has a broad antimicrobial activity, does not generate harmful by-products, and its fast-acting efficacy has made it more relevant in the disinfection of Personal Protective Equipment (Lemmer et al., 2017).

In comparison, a different scenario can be observed with the skim milk inoculum. All the sanitizers were affected by organic matter, reducing less than or equal to 1 log CFU/in^2. The data showed no statistical difference among sanitizer effectiveness in the different gloves for the Skim Milk Inoculum. However, when the difference in log reduction with both inoculums is compared, the water rinse and the three different sanitizers showed a statistically significant difference in PBS versus skim milk Inoculum (**Figure 3-6**).

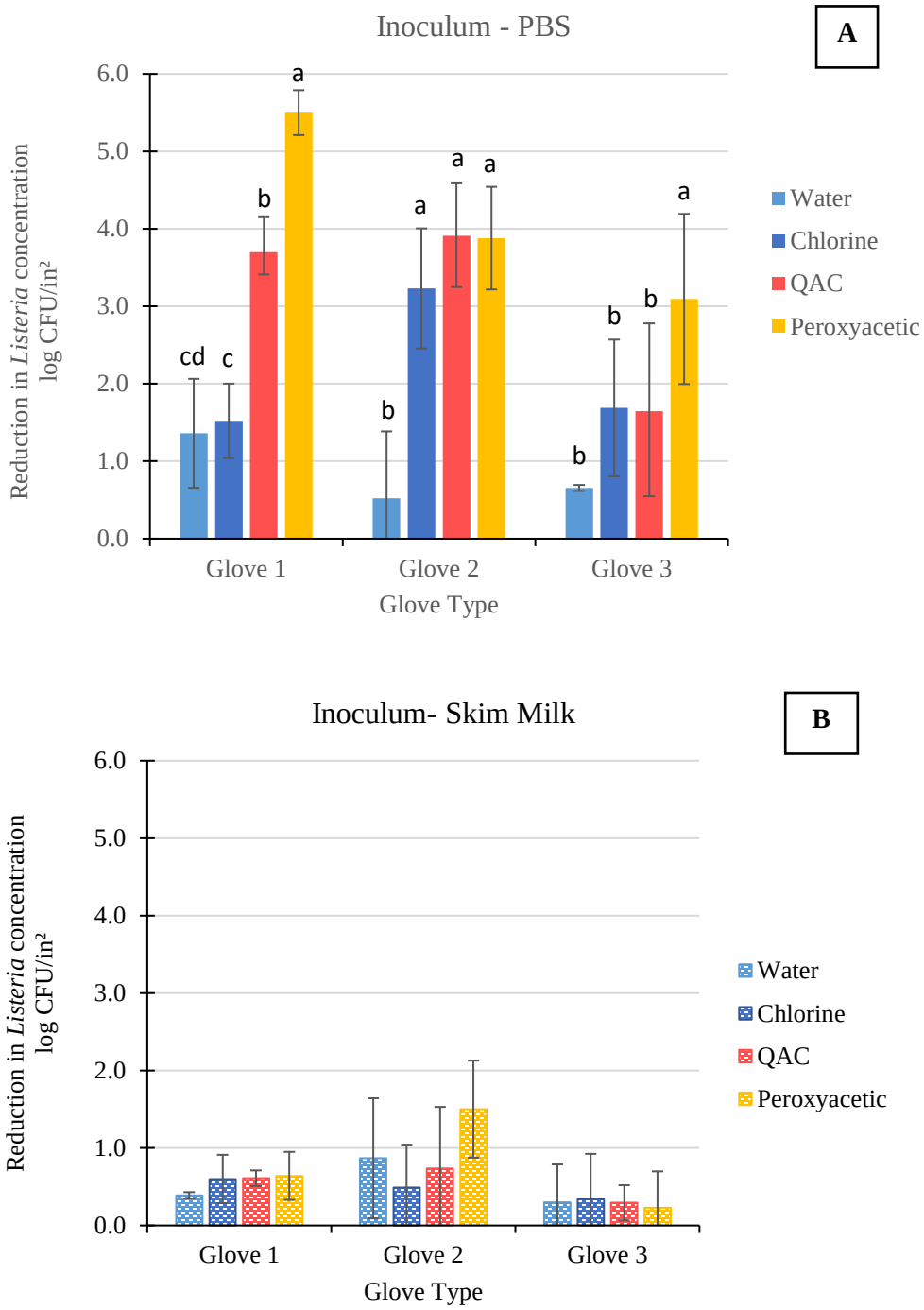

Figure 3-6. *Listeria innocua* reduction in Nitrile (Glove 1), Latex (Glove 2), and Vinyl (Glove 3) gloves using different sanitizers upon inoculation delivered using Butterfield-phosphate-dilution water (PBS) [A] or skim milk [B]. Mean values within the same water or sanitizer treatment that share the same letter are not significantly different from one another ($P > 0.05$)

On the other hand, data showed a statistical difference between sanitizer and glove type in the PBS inoculum. The efficacies of sanitizers against *L. innocua* populations varied on different surfaces. When the glove material is considered, and the inoculum used is prepared in PBS, the Nitrile material (Glove 1) showed a 1.36 log reduction CFU/$in^{2.}$ in the water rinse. A 1.52, 3.7, and 5.5 log reduction was observed in the chlorine, quaternary ammonia, and PPA sanitizer, respectively. With the PAA sanitizer outperforming the rest of the treatments. The Vinyl Glove (Glove 2) showed a similar situation, with a 3.09 log CFU/in^2 reduction in PAA, followed by a 1.68 and 1.64 log CFU/in^2 reduction in chlorine, and quaternary ammonia. However, all the sanitizers were better than the water in the Latex Glove (Glove 2), but there were no significant differences among them. McCarthy, 1996, evaluated sanitizer effectiveness against *L. monocytogenes* attached to latex gloves in the presence of phosphate-buffered saline and crab cooking water. Data showed nondetectable levels of *L. monocytogenes* in the gloves after treatment with chlorine and quaternary ammonia sanitizers (200 ppm concentration).

As a result, any of the three sanitizers can be used for the Latex gloves (made of rubber). Latex gloves have strong puncture resistance, adequate protection against caustics and detergents, and low cost. However, it contains proteins and chemical allergens, limiting their use among food handlers (Michaels, 2004). Contrarily, Nitrile and Vinyl gloves contain no proteins; therefore, allergenicity issues are eliminated. Nevertheless, Vinyl has limited chemical barrier properties and can easily be permeated by organic solvents, which could be an issue for Food Safety managers and handlers. Nitrile gloves resist most solvents; however, they are still sensitive to alcohols and

ketones. In the end, glove selection would depend on the food processing facility and its needs. When selecting gloves, essential features to review are break and abrasion resistance, durability, elasticity and resilience, tactile sensitivity, and heat dissipation (Luber et al., 2011).

Listeria innocua reduction on Boots

The last PPE evaluated was boots. The study evaluated PVC work boots with different tread patterns. Dunlop™ Onguard™ White PVC Boots (Boot 1) contain a Safety-Loc outsole with shallow lugs closer together and, therefore, categorized as having narrow treads. In contrast, the Honeywell PRO® Servus boots (Boot 2) with an outsole contain lugs that were deeper and wider apart (**Figure 3-7**). All the boots used for the procedure were the same size.

Figure 3-7. Narrow (Boot 1) and broader (Boot 2) tread pattern of industrial processing boots.

Both inoculation procedures (PBS and skim milk) achieved a bacterial population of 7.8 log CFU/in^2 in control samples (data not shown). **Figure 3-7** shows the reduction achieved by the water rinse and the three different sanitizers on the boots. Overall, averages showed that the reduction achieved by water rinsing was 1.36 log CFU/in^2 in the water-based inoculum and 1.05 log CFU/in^2 in the milk-based. The results demonstrate that some cells are dislodged from the outsole by rinsing in water and stomping in a foot mat, but no meaningful reductions are observed in the population levels.

Mean values for different treatments showed reductions varying from 1.36 to 2.30 log CFU/in^2 in the PBS inoculum. When the inoculum was applied using skim milk as the carrier, only a 1.05 to a 1.38 log CFU/in^2 reduction was observed with the three different sanitizers and boots, indicating no statistical differences in effectiveness among them. In general, the three treatments' efficacy to reduce the bacterial population on footwear soles did not achieve the desired three log reduction for none of the carriers (**Figure 3-8**). Burnett et al., 2013, evaluated *Citrobacter freundii*, *Pseudomonas fluorescens*, and *Serratia marcescens* reduction on industrial footwear with different tread patterns. However, no significant reductions in microbial populations on soles were observed upon treatment with aqueous and dry quaternary ammonia compounds and control samples. Decontamination with a mixture of alcohols and quaternary ammonia in aqueous and dry forms resulted in 2.3 and 3.5 log reductions, respectively. In conclusion, this study emphasizes the need for extensive cleaning protocols to reduce microbial populations in footwear.

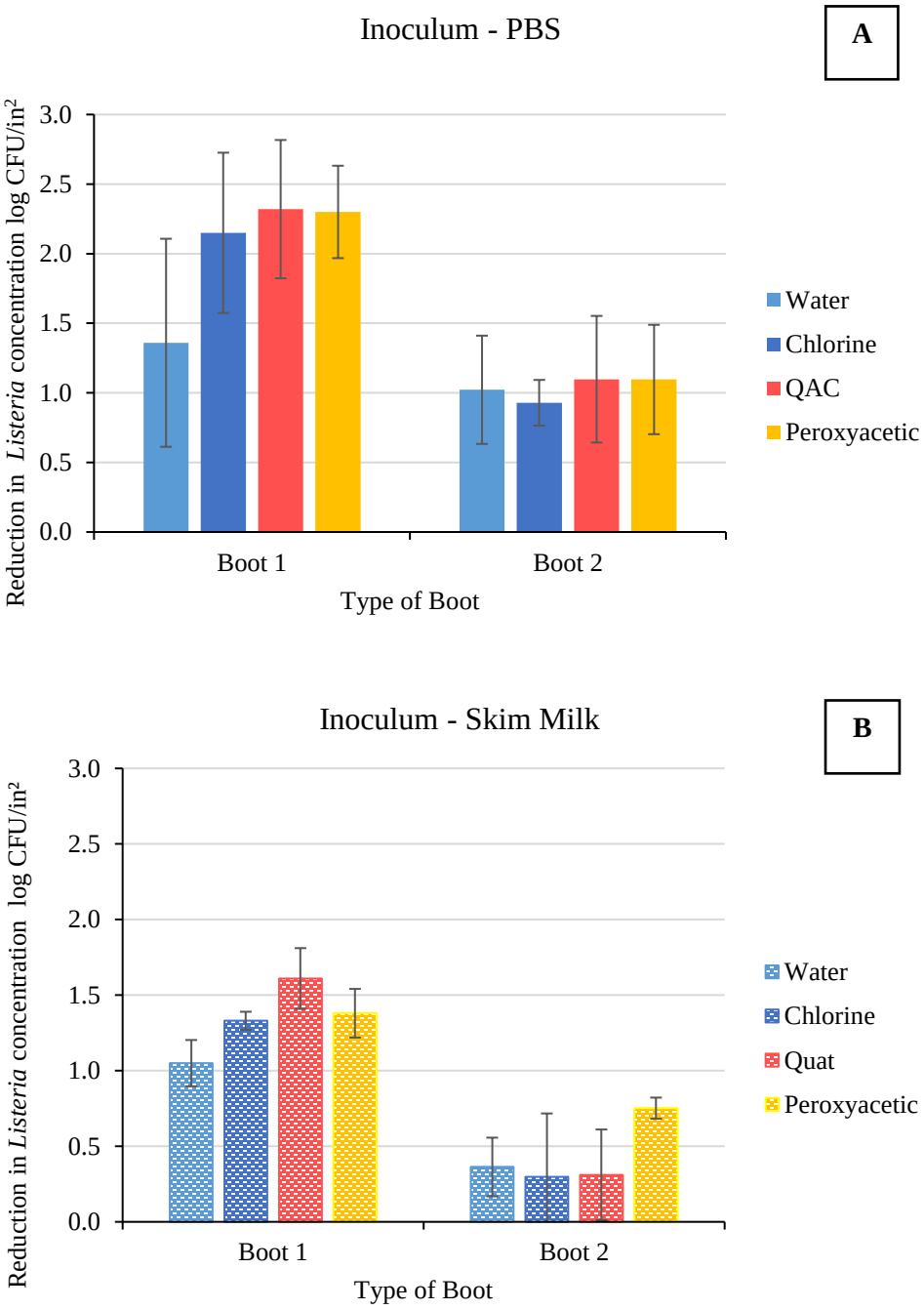

Figure 3-8. *Listeria innocua* reduction on boots with narrow (Boot 1) and broad (Boot 2) tread patterns using different sanitizers upon inoculation delivered either using Butterfield-phosphate-dilution water (PBS) [A] or skim milk [B].

However, when the difference in log reduction with both inoculums is compared, the water rinse and the three different sanitizers showed a statistically significant difference in PBS versus skim milk inoculum (**Figure 3-8**). Skim milk showed a statistically lower log CFU/in^2 reduction than PBS.

When the type of tread pattern is considered and the inoculum used is prepared in PBS, the narrow tread pattern (Boot 1) shows a 1.36 log reduction using water rinse and 2.15, 2.32, and 2.30 log CFU/in^2 reduction in the chlorine, quaternary ammonia, and PAA sanitizer. The wider boot (Boot 2) achieves a lower bacterial reduction (less than or equal to 1 log CFU/in^2) using the sanitizers. There is a statistically significant difference in bacterial reduction with the different tread patterns. The data suggests that broader and deeper lug boots are more challenging to clean. The skim milk inoculum follows the same conclusion.

3.2 Cleaning Regime

Listeria innocua reduction in Aprons

Additionally, an extensive cleaning procedure was evaluated using skim milk inoculum as the carrier in aprons. Cleaning protocols evaluated a neutral, general-purpose cleaner (Cleaner 1) and a chlorinated alkaline cleaner (Cleaner 2). The study included only skim milk as the *Listeria innocua* carrier for the cleaning regime since the sanitizer protocol for this inoculum did not achieve a three-log reduction. Data showed a 5.59 and a 6.57 log CFU/in^2 reduction in *Listeria* population using Cleaner 1 and Cleaner 1 plus scrubbing. On the other hand, a 4.25 log CFU/in^2 was achieved using Cleaner 2. This cleaning intervention showed a statistical difference between the other cleaning regimes (**Figure 3-9 [A]**). Cleaner 2 is a self-foaming, chlorinated alkaline cleaner. Chlorine is

incorporated into alkaline detergents not as a sanitizing agent but as a peptizing agent to aid in protein soil removal (Gilbert, 1982). However, high pH values, heavy organic materials, and low temperatures can affect chlorine's antimicrobial activity. Also, these cleaners have minimal sanitizing activity because of the reaction of chlorine with the soil being removed (Marriott et al., 2016). As a result, Cleaner 2's performance may have been affected due to the organic presence of the skim milk inoculum and water temperature. Nevertheless, mechanical action by scrubbing using Cleaner 2 achieved a 5.84 log CFU/in^2 reduction. The information highlights the effect of scrubbing on the bacterial reduction on aprons.

When the apron type is considered, the Vinyl Apron (Apron 1) showed a 5.91 log reduction using Cleaner 1 and a 6.74, 4.75, and 7.05 log CFU/in^2 reduction in the Cleaner 1 plus scrubbing, Cleaner 2, and Cleaner 2 plus scrubbing protocol, respectively. The Polyethylene (Apron 2) and 65% polypropylene/35% polyethylene (Apron 3) aprons showed similar results, with Cleaner 1 and scrubbing steps outperforming the Cleaner 2 protocol **(Figure 3-9 [B])**. As a result, none of the aprons materials were different from each other.

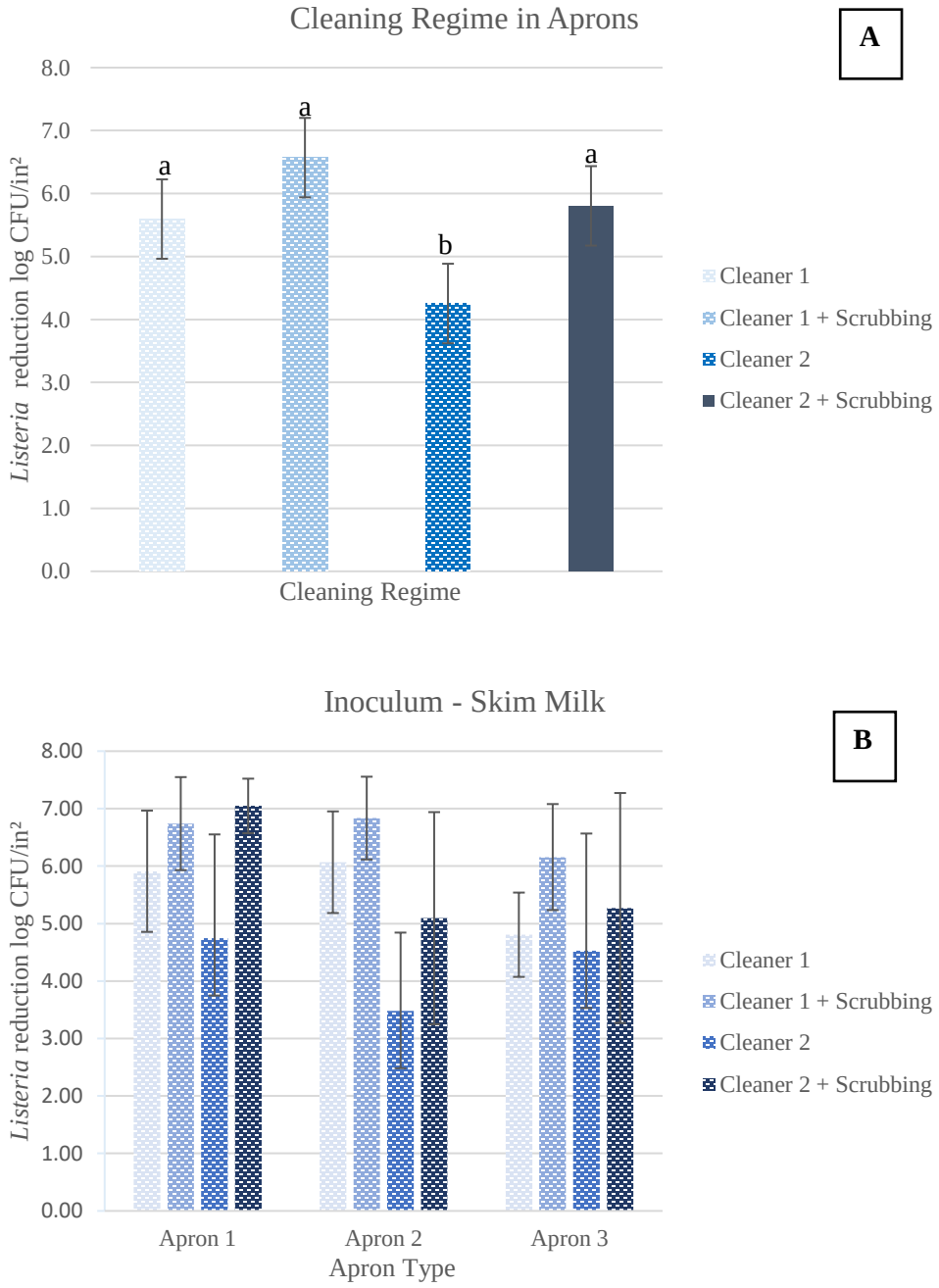

Figure 3-9. [**A**] Average *Listeria innocua* reduction in aprons with general-purpose (Cleaner 1) and chlorinated alkaline (Cleaner 2) cleaners with and without scrubbing steps. Mean values within the same cleaning regime that share the same letter are not significantly different from one another ($P > 0.05$). [**B**] *Listeria innocua* reduction in Vinyl (Apron 1), Polyethylene (Apron 2), and 65% Polypropylene/35% polyethylene (Apron 3) aprons using different cleaning regimes upon inoculation delivered using skim milk.

Listeria innocua reduction in Gloves

Like the aprons, the study included only the skim milk inoculum for the cleaning regime since the sanitizer protocol for this inoculum did not achieve a three-log reduction. Results showed a 5.89 and 6.24 log CFU/in^2 reduction in *Listeria* population using general purpose cleaner (Cleaner 1) and Cleaner 1 plus scrubbing. In comparison, chlorinated alkaline cleaner (Cleaner 2) achieved a 2.97 log CFU/in^2 reduction, showing significant statistical differences between Cleaner 1 plus the scrubbing step regime. Cleaner 2 plus scrubbing achieved a 5.55 log CFU/in^2 reduction, which also outperformed the chlorinated alkaline cleaner (Cleaner 2) (**Figure 3-10 [A]**). These results highlight the importance of mechanical action in cleaning protocols to reduce microbial populations. Campdepadrós et al., 2012 shared similar results in *L. monocytogenes* reduction on different Food Contact and Non-Food Contact Surfaces in a dessert processing facility. The evaluation consisted of cleaning protocols with mechanical action. *L. monocytogenes* showed a presence of 15.2% before cleaning, falling to 6.9% after cleaning. However, *Listeria* did not completely disappear and survived on floors that had undergone sanitizing treatments.

When the glove type is considered, the Nitrile Glove (Glove 1) showed a 5.74 log reduction using Cleaner 1 and a 6.59, 3.35, and 5.31 log CFU/in^2 reduction in the Cleaner 1 plus scrubbing, Cleaner 2, and Cleaner 2 plus scrubbing protocol, respectively. The Latex (Glove 2) and Vinyl gloves (Glove 2) gloves followed a similar pattern. Cleaner 2 achieved a 2.98 and 2.56 log CFU/in^2 reduction in latex and vinyl gloves (**Figure 3-10 [B]**).

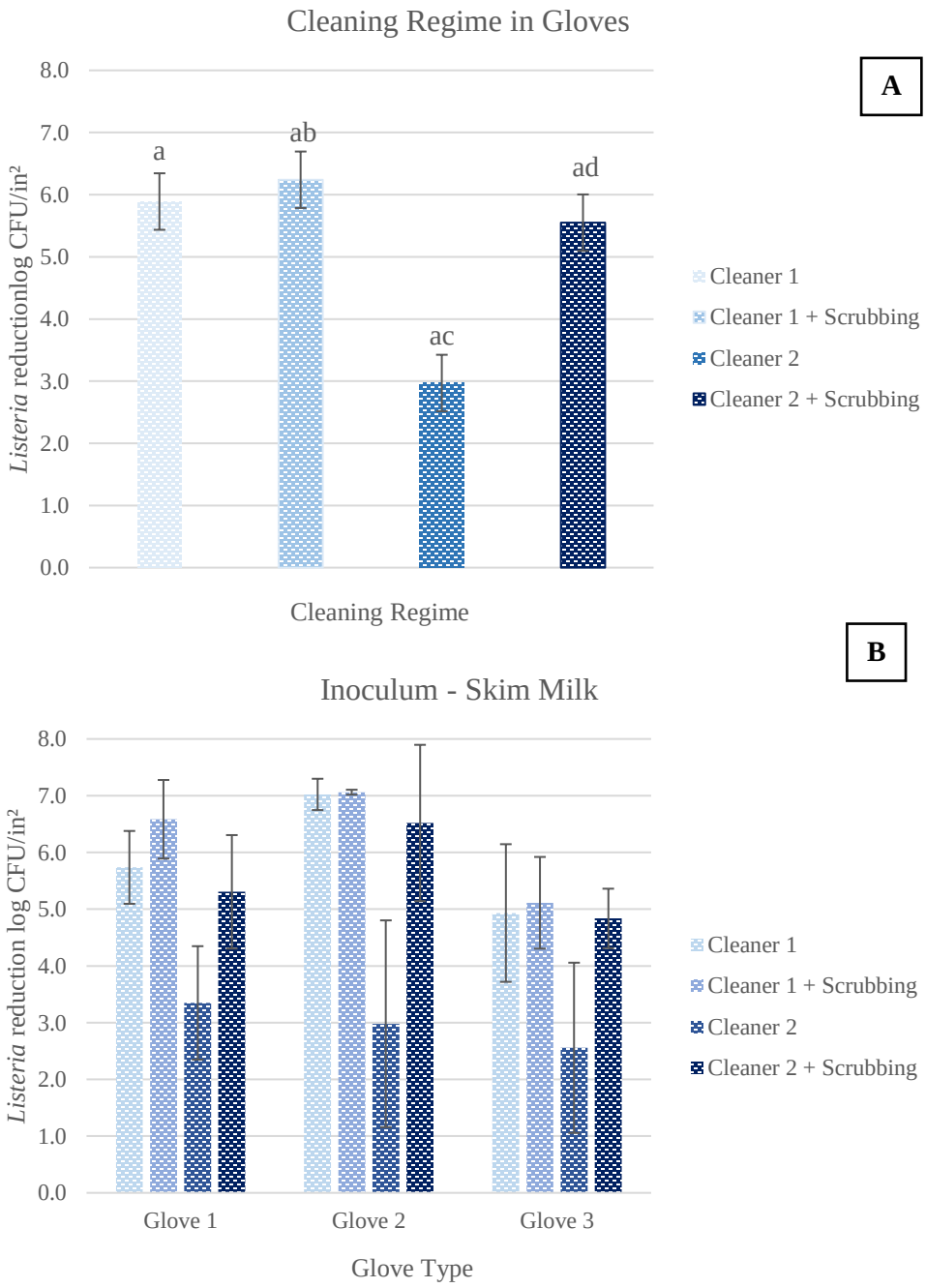

Figure 3-10. [**A**] Average *Listeria innocua* reduction in gloves with general-purpose (Cleaner 1) and chlorinated alkaline (Cleaner 2) cleaners with and without scrubbing steps. Mean values within the same cleaning regime that share the same letter are not significantly different from one another ($P < 0.05$). [**B**] *Listeria innocua* reduction in Nitrile (Glove 1), Latex (Glove 2), and Vinyl (Glove 3) gloves using different cleaning regimes upon inoculation delivered using skim milk.

Listeria innocua reduction in Boots

Cleaning Regime evaluation consisted of both PBS and Skim Milk Inoculum for the boots experiment. Sanitizing protocols for both inocula did not achieve a desired three-log reduction. **Figure 3- 11** shows *Listeria innocua* reduction in the different tread patterns for the PBS and skim milk inoculum. The Narrow Tread Pattern (Boot 1) achieved a 3.88, 4.30, 3.71, and 4.30 log CFU/in^2 reduction in the different cleaning regimes. The broader tread pattern (Boot 2) achieved similar results, with a 2.78, 3.55, 4.01, and 4.70 log reduction in the Cleaner 1, Cleaner 1 plus scrubbing, Cleaner 2, and Cleaner 2 plus scrubbing regimes, respectively. As a result, there is no statistical difference between the cleaning regimes and boot type for this experiment. However, when the difference in log reduction with both inoculums is compared, the cleaning regimes showed a statistically significant difference in PBS versus skim milk Inoculum (**Figure 3-11**). Skim milk shows a statistically lower log CFU/in^2 reduction than PBS. The cleaning regimes achieved a 2.39-3.07 and a 2.85-3.48 log CFU/in^2 reduction in Boot 1 and Boot 2. Once again, this highlights the effect of organic matter as interference for cleaning and sanitation protocols. Hua et al., 2019b also found that anti-*Listeria* efficacies of different sanitizers were diminished by organic matter presence in Low-density polyethylene (LDPE), polyvinyl chloride (PVC), polyester (PET), and rubber surfaces. Food residues from apple juice or milk protected the organisms against chemical treatments.

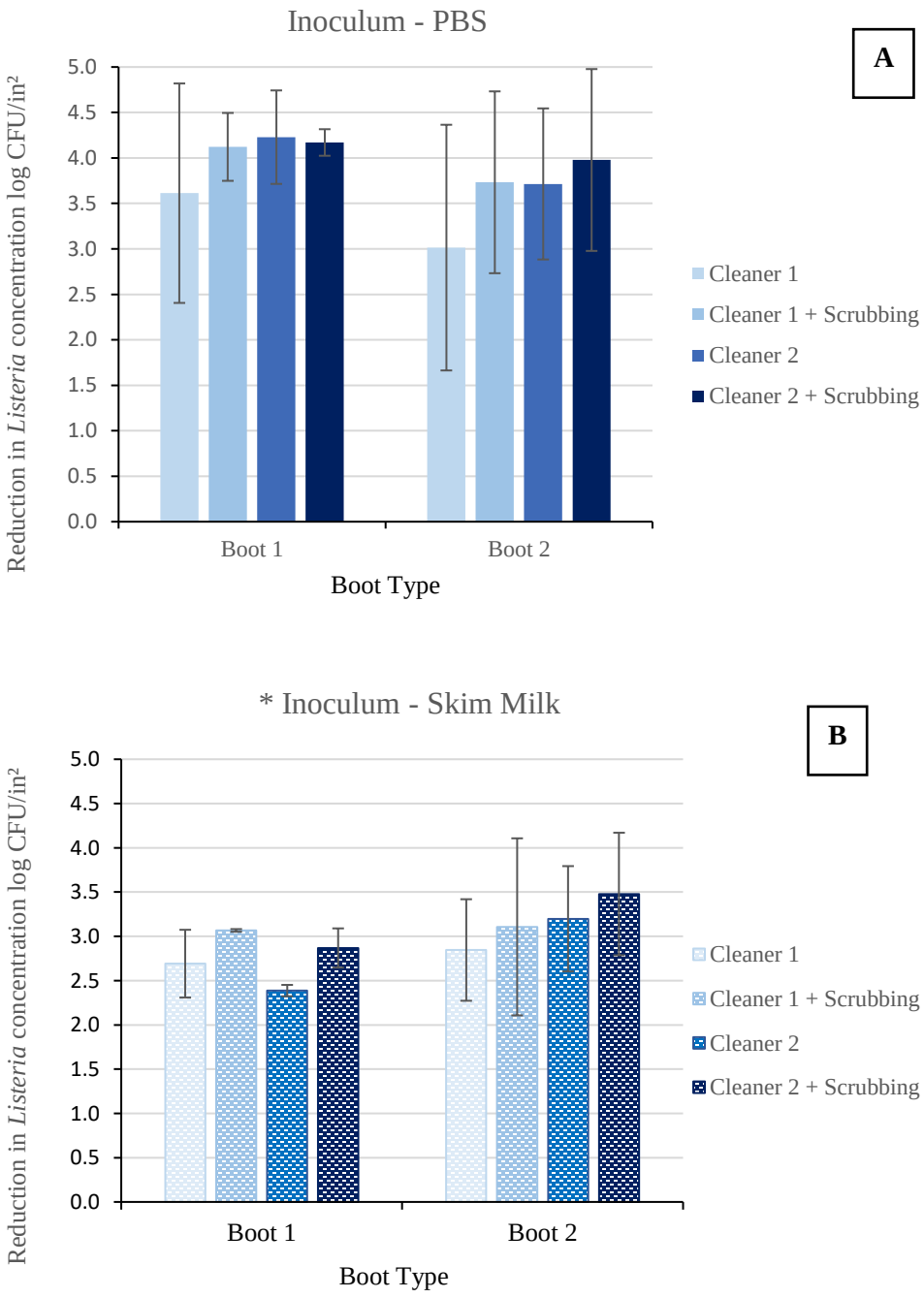

Figure 3-11. *Listeria innocua* reduction in boots with narrow (Boot 1) and broad (Boot 2) tread patterns using general-purpose (Cleaner 1) and chlorinated alkaline (Cleaner 2) cleaners with and without scrubbing steps upon inoculation delivered either using Butterfield-phosphate-dilution water (PBS) or skim milk. Where asterisk (*) shows statistical significance between the Inoculum type ($P > 0.05$)

4. CONCLUSIONS

- The results of this study revealed that the different sanitizers' antimicrobial activity is diminished when used to sanitize PPE with high organic matter concentration (e.g., inoculum in the presence of skim milk vs. PBS).
- When no organic matter was present, Peroxyacetic Acid (PAA) showed a 4.60 log CFU/in² reduction of *Listeria innocua* on aprons, proving to be the best sanitizer. However, PAA only achieved a 0.88 log CFU/in^2 reduction in the aprons inoculated with skim milk.
- With the PBS inoculum, PAA was the best sanitizer for nitrile (Glove 1) and vinyl (Glove 3) gloves, achieving a 5.50 and 3.09 log CFU/in^2 reduction, respectively. All the sanitizers proved to be better than a water rinse for the Latex (Glove 2) glove.
- Boots with narrow tread patterns (Boot 1) achieved higher log reductions than boots with broader tread patterns (Boot 2) with different sanitizers.
- A neutral, general-purpose cleaner (Cleaner 1) and scrubbing steps outperform a cleaning regime consisting of a chlorinated alkaline detergent (Cleaner 2) for aprons and gloves.
- The presence of skim milk leads to lower log CFU/in^2 reductions compared to PBS in boots treated with cleaning regimes.
- An extensive cleaning protocol is needed to reduce bacterial populations from PPE effectively.

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CONCLUDING REMARKS

Listeria monocytogenes can be present in personal protective equipment (PPE) due to environmental cross-contamination. The transmission of this foodborne pathogen could lead to contamination of food contact surfaces and finished products. This research evaluated the transfer of *Listeria innocua*, as a surrogate for *Listeria monocytogenes,* from personal protective equipment (PPE) to food products (cheddar cheese, queso fresco) and surfaces commonly encountered in dairy plants (i.e., cutting boards, stainless steel, and dairy tiles). The PPE tested included gloves, aprons, and boots that were inoculated using Phosphate Buffer Saline (PBS) and skim milk as *L. innocua* carriers. Results obtained from the present study showed that *Listeria innocu*a transfer was different depending upon the combination of PPE and surface of interest. Furthermore, higher transmission values and no decline after consecutive transfers were observed in the presence of skim milk.

In addition to transfer studies, effective sanitation procedures in PPE were tested to control and/or reduce *Listeria* contamination in dairy processing facilities. Results of the sanitation procedures revealed that when PBS was used as the *L. innocua* carrier, peroxyacetic acid proved to be the best sanitizer. Nevertheless, the antimicrobial activity of all sanitizers tested was significantly diminished in the presence of organic matter. Finally, the evaluation of an extensive cleaning protocol, showed that detergent and mechanical action (scrubbing), followed by the use of a sanitizer, is required to effectively reduce *L. innocua* from PPE in the presence of organic matter.

RECOMMENDATIONS AND FUTURE WORK

Dissemination of research

Listeria innocua transmission studies from PPE to food and food contact surfaces provided valuable quantifiable information about possible cross-contamination scenarios in a dairy plant. Also, by comparing multiple sanitizers and cleaning agents the best cleaning and sanitizing strategies were identified for PPE. One of the future immediate goals of this research is to provide outreach to the dairy processing industry by disseminating this information to a wide audience. As a result, the next step is to develop updated guidelines for managers and in-plant personnel. These guidelines will highlight the best practices to avoid, control, and/or reduce *Listeria* contamination from PPE to food products and surfaces. The information will take the form of downloadable flyers or posters that are easily understood and can be used as training documents for dairy plant personnel. In addition, for convenience to the industry these documents will be available in English and Spanish.

Future Work

Due to biosafety considerations, the present study used a surrogate for the transfer and sanitation experiments. Nevertheless, further work in the area may include the use of *Listeria monocytogenes* instead of *Listeria innocua.* Other potential directions for transfer studies, involving the evaluation of *Listeria* in a dairy processing environment, include further evaluation of the effects of extrinsic factors in microbial transfer. Such factors would include the impact of pressure, contact area and time, temperature, and moisture level. Better understanding these factors, may provide further insights on the

risk associated with *Listeria* transfers in the plant environment. In addition, future work could evaluate “new” and “previously used” PPE and surfaces. Corrosion, scratches, and wear may have an influence in transfer and/or provide protection during sanitation procedures. Other types of studies that could be performed include those where laboratory work is combined with field evaluations in dairy plants. Field evaluations may provide insights regarding the transfer of *Listeria* in complex systems. Furthermore, by using data from dairy plant investigations, findings obtained in the laboratory can be validated.

APPENDIX A

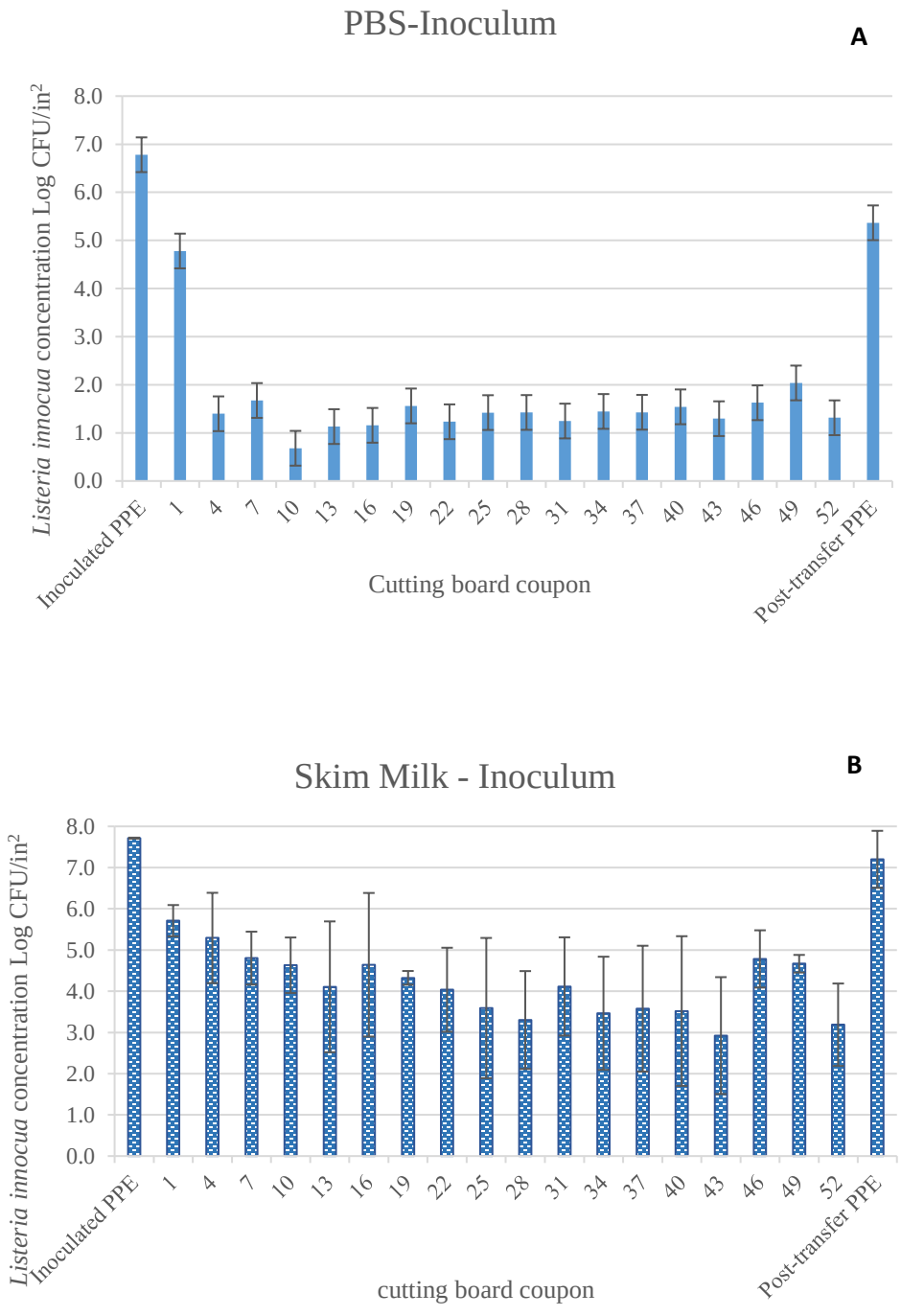

Figure 2-8. *Listeria innocua* transfer from PBS (A) and skim milk(B) inoculated gloves to cutting boards.

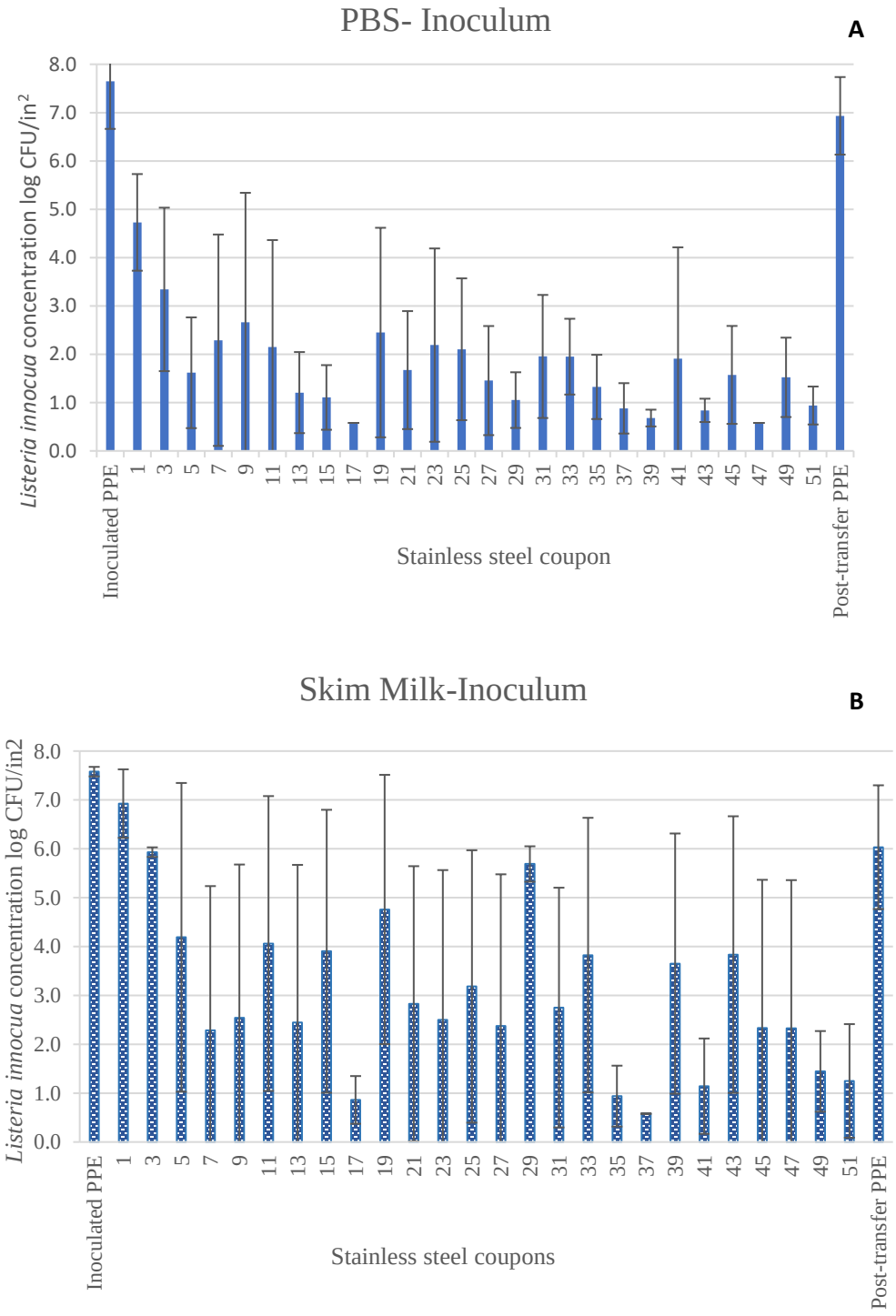

Figure 2-9. *Listeria innocua* transfer from PBS (A) and skim milk (B) inoculated aprons to stainless steel coupons.

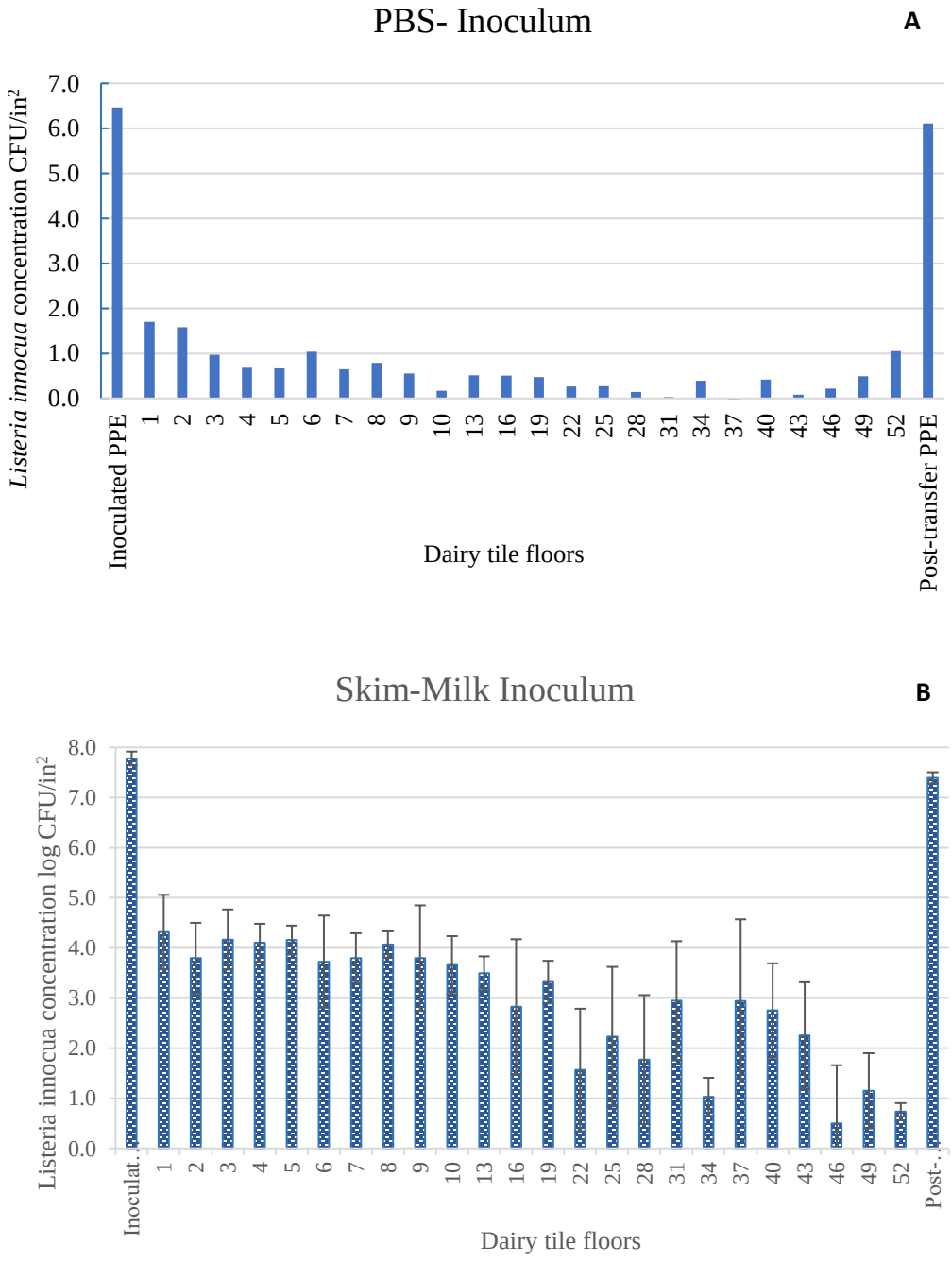

Figure 2-10 *Listeria innocua* transfer from PBS (A) and skim milk (B) inoculated PVC boots to dairy brick floors