

**VALIDATING THE EFFICACY OF COMMERCIAL FOAMING CLEANER AND  
SANITIZER FOR CONTROLLING *LISTERIA INNOCUA* (SURROGATE FOR  
*LISTERIA MONOCYTOGENES*) IN DRAINS AND POTENTIAL TRANSLOCATION  
FROM THE DRAIN TO THE FOOD CONTACT SURFACES**

by

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## Abstract

*Listeria monocytogenes* is known to be an environmental contaminant in food processing facilities. Floor drains in processing environments harbor *Listeria* spp. due to continuous presence of humidity and organic substrates. The cleaning and washing activities undertaken may translocate the bacterial cells from the drain to the surrounding environment, thus contaminating food products being produced.

This study validates the effectiveness of Johnson Diversey 'Eliminex' Foaming Drain Cleaner and Johnson Diversey 'Final Step' 512 sanitizer for inhibition of *Listeria monocytogenes* in drain surfaces and evaluates the potential for translocation of *L. monocytogenes* from drains to food contact surfaces in the surrounding environment using *Listeria innocua* as a surrogate. A 7x 7 x 8 feet flexi glass chamber was built in which a 10 inch diameter drain mounted on an aluminum cabinet was placed. The drain was inoculated with the surrogate organism, *L. innocua*, at specific time intervals and then treated with the given chemicals. Sponge samples were taken and bacterial populations were recovered on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TALMOX). Stainless steel coupons (6.4 x 1.9 x 0.1 cm) were hung at 3 different heights 1, 3 and 5 feet inside the chamber and cell translocation from the drain on to the stainless steel coupons was studied.

Reductions up to 4 Log CFU/area or ml were seen at the drain surface, drain crate, drain pipe and wash water for both free cells and cells entrapped in biofilms. Treatment had a significant effect ( $p < 0.05$ ) on the reduction of bacterial cells. The wash water showed the greatest reduction from 8 Log CFU/ml to est. 0.23 Log CFU/ml. The given cleaner and sanitizer were found to be effective for reducing *Listeria* spp. on drain surfaces. Results for the second part indicated translocation at all three heights with percentage translocation ranging between 2-17%. Significantly higher translocation ( $p < 0.05$ ) was seen at 1 foot, followed by 3 feet and 5 feet indicating the closer the height to the drain, the greater the number of bacterial cells that are able to transfer from the drain to the surrounding environment.

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## **Dedication**

I would like to dedicate this accomplishment to my beloved mother Mrs. Surinder Kaur. I am proud to be your daughter, love you very much, and I miss you a lot. I wish you were here with me on this day.

## Introduction

*Listeria monocytogenes* is one of the ‘Big Four’ pathogens that are of utmost concern in the food industry today, others being *Salmonella*, *Campylobacter*, and pathogenic *Escherichia coli*. A number of outbreaks associated with consumption of Mexican style cheese, pasteurized milk, coleslaw, ready –to-eat foods contaminated with *Listeria monocytogenes* have been reported. The Centers for Disease Control and Prevention (CDC) estimate 2500 cases of foodborne listeriosis every year in the United States primarily affecting the very young, the elderly, and immunocompromised populations, including pregnant women, diabetics, transplant recipients and cancer patients, and 500 of these 2,500 cases result in death. The organism can easily be isolated from air, water, soil, natural microflora, commercial dairies, and food processing facilities. The threat posed by *L. monocytogenes* is due to its ubiquitous nature and ability to grow over a wide range of temperatures (0-45°C) including refrigeration temperatures.

*Listeria* is known to be an environmental contaminant in food processing facilities. The continued existence of conditions such as flowing water, humidity, organic substrates, ideal temperature, ample nutrients and suitable attachment surfaces favor the growth and proliferation of this microorganism in the food processing environments. Food products have known to be contaminated with *L. monocytogenes* as a result of recontamination after they have been processed due to its presence in the environment.

Floor drains in processing environments are an important harborage for *Listeria* due to continuous presence of humidity and organic substrates. Drains are difficult to clean because listeriae adhere and become entrapped onto the drain surface in slimy covering known as biofilm that protects the *Listeria* and makes the cleaning and sanitizing procedures ineffective. *Listeria* present in the drains may transfer from drains onto the food contact surfaces, thus contaminating the food being processed. Migration of the organism may occur from drains to food through workers and food handlers, contaminated equipment and high pressure cleaning and scrubbing activities undertaken in food processing environments.

The objective of this study was to validate the effectiveness of Johnson ‘Eliminex’ Foaming Drain Cleaner and Johnson Diversey ‘Final Step’ 512 sanitizer for inhibition of *L*

*monocytogenes* and to evaluate the potential for translocation of *Listeria* from drains onto food contact surfaces in the surrounding environment using *L. innocua* as a surrogate organism. To accomplish this objective, a 7x 7 x 8 feet flexi glass chamber was built in which a 10 inch diameter, painted cast iron drain mounted on a 2 x 3 feet aluminium cabinet was placed. The drain was inoculated with the surrogate organism, *L. innocua* at specific time intervals and then treated with the given chemicals. Sponge samples were taken and the reduction in bacterial populations was calculated by obtaining differences between treated and non treated samples. Stainless steel coupons (6.4 x 1.9 x 0.1 cm) were hung at 3 different heights 1, 3 and 5 feet inside the chamber and cell translocation from the drain on to the stainless steel coupons was studied as a result of aerosols generated due to cleaning and washing activities.

This study is important to assess the importance of the use of cleaners and sanitizers in food processing environments to eliminate, control or reduce microbial pathogens. The study identifies the cause of food product contaminated with *Listeria* spp. post processing. It shows how floor drains in processing environments can be a major cause of contamination of the product along the processing line. The close proximity of the drain and the area where the food is being handled and processed, can lead to the food product being contaminated with *Listeria* spp. after processing.

# CHAPTER 1 - Literature Review

## 1.1 Introduction

*Listeria monocytogenes* is a gram positive, motile, non spore-forming, acapsular, facultatively anaerobic, microaerophilic, non branching, regular, short rod shaped bacterium. It was first described by Murray et al. in 1926 (46) and has been recognized as a human pathogen since 1929 (52). The diphtheroid-like rod measures 1.0 to 2.0  $\mu\text{m}$  by 0.5  $\mu\text{m}$ . When grown at 20 to 25°C, Listeriae exhibit characteristic “tumbling motility” predominantly by means of peritrichous flagella (29). The bacterium can grow over a wide temperature range from 1°C to 45°C, with an optimum of 30°C to 37°C. The pH range for growth of this organism is 4.4 to 9.4 and water activity is  $\geq 0.92$  (34). *Listeria* can multiply in high salt (up to 10% sodium chloride) or bile (57). It grows readily on blood agar, producing incomplete  $\beta$  – hemolysis (18).

Six species of *Listeria* are recognized: *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii* and *L. grayi*. The species further give rise to 17 serovars which are characterized by possession of specific antigens. *L. monocytogenes* is the primary pathogenic species, is represented by 13 serovars based on cellular O and flagellar H antigens, but almost all diseases are due to types 4b, 1/2a and 1/2b (8). *L. innocua* is regarded as the non pathogenic variant of *L. monocytogenes* and is represented by only three serovars. Studies have shown that *L. innocua* can be used as a surrogate for *L. monocytogenes* in aerosol studies (64).

*Listeria monocytogenes* is ubiquitous in nature. It is widely distributed naturally, associates closely with animals that feed on plant material and can grow over a wide range of environmental conditions. It has been isolated from a variety of sources, including soil, water, silage, feces, plants and sewage (12). *L. monocytogenes* is known to exist widely in food processing environments (52) and can survive for long periods of time in foods, production facilities, processing areas and surrounding environments. Although commonly found in both plant and animal raw foods, it is also present in cooked foods if contaminated during post-processing handling. It has been isolated in foods such as raw meat, fermented and cooked sausages, raw and pasteurized fluid milk, cheeses, ice cream, vegetables, raw and cooked poultry, raw and smoked seafood (52). Due to growth and survival over a wide range of environmental conditions such as refrigeration temperatures, low pH and high salt concentration,

*L. monocytogenes* can overcome food preservation and safety barriers, and pose a potential risk to human health (25).

An estimated 76 million people contract foodborne illnesses in the United States every year (43). Being so widely distributed in the environment, humans and animals can come in contact with *L. monocytogenes* frequently through a variety of sources. *L. monocytogenes* is the causal agent of serious food borne illness called Listeriosis, an infection with high hospitalization rates for those who become ill. It is estimated that 2,500 cases of listeriosis occur in the United States every year, primarily affecting the very young, the elderly, and the immunocompromised populations, including pregnant women, diabetics, transplant recipients and cancer patients (10), and 500 of these 2,500 cases result in death. The annual incidence of listeriosis decreased by 44% between 1989 and 1993; 38% decline was revealed in an analysis of the incidence trend from 1996 to 2002. However, in 2002, an outbreak resulted in 54 illnesses, 8 deaths, and 3 fetal deaths in 9 states in United States as a result of consumption of turkey meat contaminated with *L. monocytogenes* (10). Human listeriosis is typically acquired through ingestion of contaminated food. Other modes of transmission include from mother to child through placenta or through infected birth canal and cross infection in neonatal nurseries. Listeriosis begins often with influenza-like symptoms, and sometimes diarrhea, however manifestations are host dependent. Symptoms might progress to include high fever, severe headache, and neck stiffness. Listeriosis can lead to septicemia, meningitis, and spontaneous abortion. When listeric meningitis occurs, the overall mortality may be as high as 70%, from septicemia 50%, from perinatal/neonatal infections greater than 80%. However, the mother usually survives when infected during pregnancy (59). Cervical and generalized lymphadenopathy are associated with adults, thus the disease seem to resemble infectious mononucleosis. Many different types of foods have been implicated in the outbreaks of listeriosis. In 1985, consumption of Mexican-style cheese caused over 142 cases of listeriosis, including 48 deaths (38). Between 1983 and 1987, consumption of contaminated Vacherin Mont d'Or soft-ripened cheese resulted in 31 deaths (41). In the United Kingdom, it was concluded that paté was a significant cause of increase in incidence of listeriosis between 1987 and 1989 (42).

Microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *L. monocytogenes* (7). The products with regard to *L. monocytogenes* that are

of the highest concern are those where the organism may be present in high numbers in the raw material, where it is consumed without further processing, where there is no process to control or reduce it or where it may grow in the finished product.

The pathogenicity of *L. monocytogenes* is manifested in two forms, invasive and non-invasive disease. The virulence of the infecting organism, susceptibility of the host and size of the inoculum influences the nature of disease. Transmission of *L. monocytogenes* by food first requires penetration of the organism through the intestine (3). Intracellular multiplication can occur in various types of cells. Several virulence factors have been identified that allow the intracellular functionality of *L. monocytogenes*. The bacterial cell possesses a surface protein called internalin. This protein reacts with a receptor on macrophages and cells lining the intestine called E-cadherin. This induces its own ingestion. The entry in to the non macrophage cells appears to be promoted by a membrane lipoprotein. Listeriolysin O, the major virulence factor, along with phospholipases, enables listeria to escape from the phagosome and avoid intercellular killing. Once bacterium becomes free in the cytoplasm, it can divide and propel itself to the cell membrane by inducing host cell polymerization and subsequently invade adjacent macrophages. Thus, through this kind of cell life cycle, *L. monocytogenes* is able to move from cell to cell, evading exposure to antibodies, complement, or neutrophils. Host susceptibility to *L. monocytogenes* primarily depends on cell-mediated immunity, and listeriosis mostly occurs in individuals with impaired cell-mediated immunity due to disease processes, medications or pregnancy. Although the infectious dose of listeriosis in humans is not known, host susceptibility most likely influence the inoculum size that can cause the infection (56).

Once the organism penetrates the lining of the gastrointestinal tract, phagocytosis occurs (24). It is through phagocytes that the organism is transported to the different parts of the body. Invasive listeriosis occurs in immunocompromised individuals and disease manifestation may include meningitis, septicemia, meningoencephalitis, abscesses of brain, spinal cord, osteomyelitis, septic arthritis, fever, ataxia, seizures, depressed consciousness and altered mental status. Neonates are at particular risk of acquiring invasive listeriosis from infected mother. Neonatal listeriosis is divided into early and late onset disease syndromes. Early onset listeriosis occurs as a result of intrauterine infection, which leads to clinical illness in the newborn at birth or shortly thereafter. Sepsis or respiratory distress may result from early-onset infection. On the contrary, late-onset disease occurs several days to weeks after the birth. Case fatality rates appear



to be lower in late-onset disease as compared to early-onset disease. The transmission route for late-onset disease is poorly understood. Infection may be acquired during passage through the birth canal or as a result of nasocomial transmission.

The non-invasive listeriosis results in febrile gastroenteritis with fever, diarrhea and vomiting. The median incubation period prior to onset of symptoms for the non invasive form is short, typically 18 to 20 h (15). It occurs in otherwise healthy adults, although the infectious dose, the host and bacterial characteristics that determine the severity of this form of infection still remain unclear.

This organism can survive under many adverse environmental conditions including refrigeration temperatures, as compared to other non-sporeforming bacteria that cause foodborne illnesses. The low infective doze, resistance to stress conditions along with ability to multiply and colonize on processing equipment makes *L. monocytogenes* a distinguished threat to the food industry.

The Food Safety and Inspection Service (FSIS) has published a Federal Register notice comprising guidelines for establishment producing processed products that are susceptible to *L. monocytogenes* contamination and sampling plans. The LM Rule lays down compliance guidelines to control *L. monocytogenes* in post lethality exposed ready-to-eat meat and poultry products. This rule was published in 9 CFR part 430 (Code of Federal Regulations) on June 6, 2003 and became effective on October 6, 2003. The rule underlines compliance with one of the three alternatives in post lethality exposed ready-to-eat products:

1. Alternative 1 requires the use of post lethality treatment (which may be antimicrobial agent or process) to reduce or eliminate *L. monocytogenes* and an antimicrobial agent or process to suppress or limit the growth of the pathogen.
2. Alternative 2 requires the use of either post lethality treatment or an antimicrobial process to control the growth of *L. monocytogenes*.
3. Alternative 3 requires application of a post lethality treatment or antimicrobial process to control *L. monocytogenes* is not required in the post lethality exposed product. The pathogen in its post lethality processing environment is controlled with the use sanitation control measures, which may be incorporated in the production establishment's Hazard Analysis Critical Control Point (HACCP) plan, Sanitation Standard Operating Procedures (SSOP) or prerequisite program.

The post lethality treatments that may be used are steam pasteurization, hot water pasteurization, radiant heating, high pressure processing etc.

## **1.2 Biofilm Formation**

When bacteria attach to a surface in an aqueous environment, they begin to excrete a slimy substance that can anchor them to different kinds of surfaces such as stainless steel, plastic, nylon, rubber, ultra high density polyethylene etc. This slimy mucilagenous coat is known as a biofilm. A biofilm constitutes microbes colonizing on the surface and associated polymers. The matrix of biofilm consists largely of water, 98-99% according to Characklis (11), 50-95% according to Flemming et al. (21), and the remainder is an assortment of extracellular polymers such as polysaccharides, glycoproteins etc. The microorganisms are not uniformly distributed in the biofilm. It is a film of microcolonies with water channels between them.

Attachment of organisms and biofilm formation has been commonly reported by number of researchers in food processing facilities due to the existence of favorable conditions i.e flowing water, suitable attachment surfaces , ample nutrients and raw materials or the environment supplying the inocula itself, that allows colonization of microbes.

It has been suggested that flagellation and motility play a role at the cell level at various stages of biofilm formation. Studies have reported that flagella, the locomotive organelle of bacteria, also serves as an adhesive structure (44). Study by Vantanyoopaisarn et al. in 2000 (61) revealed that flagella acts as an adhesive structure during early stages of attachment of *L. monocytogenes* on different surfaces under static conditions.

This biofilm coating protects the bacterial cells against environmental stress, offer resistance to cleaning and disinfection activities and are difficult to eradicate or remove as compared to free living cells. Bacterial attachment and biofilm formation is known to occur in a variety of environments such as food, industrial, marine, and medical. *Listeria* spp. have this unique capability of forming a biofilm and is known to attach and grow on different kinds of surfaces. The cleaning of surfaces in food processing facilities is rendered difficult due to the ability of *L. monocytogenes* to form biofilms (65). Cells in the biofilms have shown significantly more resistance to sanitisers and disinfectants than the planktonic cells (1, 50). Comparative studies between attached bacterial cells and the unattached cells (planktonic cells) showed that when microorganisms attach to different surfaces, their resistance to the cleaning and

disinfecting chemicals used for sanitation purposes increases (37, 45). Also, studies have shown that the tolerance of microorganisms in biofilms for heat treatment increased after attachment (21).

*Listeria monocytogenes* attached to stainless steel and other surfaces within 20 min of contact time (40). The adherence ability reinforces the view of *Listeria* as a microorganism that is able to populate widespread niches. Numerous studies have evaluated the cell attachment and biofilm formation of *Listeria* on stainless steel, rubber, plastic, ultra high molecular weight polyethylene. Blackman and Frank (5) found that *Listeria* grew on stainless steel, teflon, nylon, and polyester for 7 to 18 d, whereas its biofilm formation was supported at 21° C but was reduced at 10° C.

The time available for the biofilm formation depends on the frequency of cleaning activities undertaken in a processing unit. Food contact surfaces may typically be cleaned several times a day or at the end of each shift, however, the environmental surfaces such as walls and drains may only be cleaned once per week. Clearly, there is more time for a biofilm to develop on the environmental surfaces. Gibson et al. in 1995 (26) found that although bacterial cells readily attached to the food contact surfaces in the processing facilities, but extensive surface colonization and biofilm formation only occurred on environmental surfaces. Contaminated food contact surfaces contribute to the source of microbes transferred to the food product in contact or passing over the surface. Environmental surfaces such as walls, floors, drains may be regarded as indirect sources of contamination. Microbial cells may be transferred to the food product by vectors such as air, personnel, and cleaning systems (32, 33). The cleanliness and hygiene of the food contact surfaces and the surrounding environment therefore, affects the quality of the end product being processed.

Cell viability of the *L. monocytogenes* biofilms may vary with different strains of this microorganism. These strain differences are due to either variability in composition of extracellular polymeric substances or different cell physiology of the strains. Researchers at the University of Guelph evaluated growth of two *L. monocytogenes* strains, Murray and 7148, in biofilms and analysed the relationship between culturable and viable-but-non-culturable (VBNC) cells and found that culturable cells of Murray reached to Log 5 CFU/cm<sup>2</sup> within 2 days while *L. monocytogenes* 7148 required 4 days to reach the same cell numbers.

### **1.3 Incidence of *Listeria* spp. in Food Processing Facilities**

Bacteria have shown to enter foods as a result of contact with contaminated surface (17). There is enough evidence which indicates that contamination of commercially processed food products with *L. monocytogenes* and other *Listeria* spp. occurs in the post processing environments rather than as a result of organisms surviving the processing operation. *L. monocytogenes* is also known to be frequently associated with the raw materials used in the food processing facilities, so there is a constant reintroduction of the organism into the plant environment (16). Many factors contribute to the growth of microorganisms in food processing environments, including moisture, nutrients, pH, oxidation-reduction potential, temperature, presence or absence of inhibitors, microbial interactions, and time. Moisture plays an increasingly important role and considerably helps in survival of bacterial cells on different surfaces. Processing plant structures, including equipment, as well as maintenance, repair, and practices that entrap moisture often result in microbial niche development (20).

Numerous sampling studies have been conducted to assess the prevalence of *Listeria* spp. in different food production and processing facilities (Table 1, 2, 3). Samples were taken from the floor, drains, processing equipment, food contact surfaces and environment. Significant findings included the recovery of *L. monocytogenes* from the floor drains in all the food establishments tested hence emphasizing its control in floor drains of food production and processing facilities to prevent contamination of food products being produced.

**Table 1 Prevalence of Listeriae in samples from the slaughter floor environment of two beef processing plants**

Sampling Site		<i>Listeria spp.</i> Prevalence (%)	<i>L. monocytogenes</i> Prevalence (%)
Floor drains, before operation	Plant A	0/50 (0.0)	0/50 (0.0)
	Plant B	0/50 (0.0)	0/50 (0.0)
Floor drains, late in operation	Plant A	2/74 (2.7)	0/74 (0.0)
	Plant B	4/75 (5.3)	1/75 (1.3)
Product contact surfaces, before operation	Plant A	0/49 (0.0)	0/49 (0.0)
	Plant B	0/50 (0.0)	0/50 (0.0)
Product contact surfaces, late in operation	Plant A	0/74 (0.0)	0/74 (0.0)
	Plant B	1/75 (1.3)	0/75 (0.0)
Brisket saw, before operation	Plant A	0/20 (0.0)	1/20 (0.0)
	Plant B	1/11 (9.1)	1/11 (9.1)
Trolleys , late in operation	Plant A	1/73 ( 1.4)	0/73 (0.0)
	Plant B	2/73 ( 2.7)	0/73 (0.0)

*Source:* Rivera- Betancourt, M., S.D. Shackelford, T.M. Arthur, K.E. Westmoreland, G. Bellinger, M. Rossman, J.O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *J. Food Prot.* 67:295-30 (53, 54)

**Table 2 Prevalence of Listeriae in fabrication floor environmental samples in two beef processing plants**

Sampling Site		<i>Listeria</i> spp. Prevalence (%)	<i>L. monocytogenes</i> Prevalence (%)
Product contact surfaces, before operation	Plant A	0/100 (0.0)	0/100 (0.0)
	Plant B	6/99 (6.1)	6/99 (6.1)
Product contact surfaces, late in operation	Plant A	0/150(0.0)	0/150(0.0)
	Plant B	25/148 (16.9)	25/148 (16.9)

*Source:* Rivera- Betancourt, M., S.D. Shackelford, T.M. Arthur, K.E. Westmoreland, G. Bellinger, M. Rossman, J.O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *J. Food Prot.* 67:295-302 (53, 54).

**Table 3 Prevalence of Listeriae in four smoked-fish processing plants; percentage positive samples (total samples taken)**

<b>Sampling Site</b>	<b>Plant 1</b>	<b>Plant 2</b>	<b>Plant 3</b>	<b>Plant 4</b>	<b>Plants Total</b>
<b>Environmental surfaces</b>					
<i>L.monocytogenes</i>	29.8 (131)	7.0 (143)	14.4 (153)	0 (126)	12.8 (553)
<i>Listeria</i> spp. including <i>L.monocytogenes</i>	42.7 ( 131)	31.5 (143)	24.2 (153)	10.3 (126)	27.3 (553)
<b>Food Contact Samples</b>					
<i>L.monocytogenes</i>	6.1 (33)	0 (33)	12.5 (32)	0 (27)	4.8 (125)
<i>Listeria</i> spp. including <i>L.monocytogenes</i>	12.1 ( 33)	27.3 (33)	21.9 (32)	0 (27)	16.0 (125)
<b>Floor Drains</b>					
<i>L.monocytogenes</i>	60.0 (30)	7.3 (41)	30.3 (33)	0 (27)	23.7 (131)
<i>Listeria</i> spp. including <i>L.monocytogenes</i>	80.0 (30)	31.7 (41)	42.4 (33)	33.3 (27)	45.8 (131)

*Source:* Thimothe, J., K.K. Nightingale, K. Gall, V.N. Scott, and M. Wiedmann. Tracking of *Listeria monocytogenes* in smoked fish processing plants. *J. Food Prot.* 67:328-341 (53, 54)

## 1.4 Drains in Food Processing Facilities

The open nature of the drains clearly indicates that they are continuously challenged by a wide range of microbes, which vary depending on the site of the drain. Floor drains in processing environments are an important harborage for *Listeria* due to continuous presence of humidity and organic substrates. A study by Cox et al. (12) that involved sampling of 15 processing facilities in Netherlands indicated 50% positive samples for *L. monocytogenes* in floor drains of a fluid dairy factory, 100% in an ice cream factory, 45.2% in an Italian-style cheese factory, 66.7% in a frozen food factory, and 53.8% in a potato processing factory. Similar studies on incidence of *Listeria spp.* in several Western European blue and soft cheese factories, and ice cream factory show drain contamination from 22% to up to 100% with *Listeria* populations  $\geq 10^6$  CFU/g or ml (13).

Drains are difficult to clean because listeriae adhere and become entrapped onto the drain surface and form a biofilm that protects the *Listeria*. The bacteria entrapped in biofilms found in food processing environments can be very difficult to eliminate. Bacterial attachment and subsequent survival involves interactions between a bacterial cell, a surface, and the surrounding microenvironment. Temperature, relative humidity, soil and the surface affect the behavior of surface attached bacterial cells. The nature of the attachment surface affects the efficacy of sanitizers. The study by Wong (62) emphasizes the importance of the interactions between bacteria and the surfaces in specific food processing environments and the impacts of the surface associated bacteria on cleaning and sanitizing to develop more effective measures to control and prevent biofilm formation.

*Listeria* present in the drains may transfer from drains onto the food contact surfaces, thus contaminating the food being processed. Migration of the organism may occur from drains to food through workers and food handlers, contaminated equipment and high pressure cleaning and scrubbing activities undertaken in food processing environments. The aerosols generated as a result of high pressure cleaning and washing activities (40-60 psi commonly in processing environments) undertaken may translocate bacterial cells from the drain on to food contact surfaces in the surrounding environments. Based on this, the second part of this study was designed.



## 1.5 Control of *Listeria* spp. in Food Processing Environments

**Table 4 Limiting conditions for *Listeria monocytogenes* growth**

Parameter	Values Reported (FDA, 1998)
Minimum water activity	0.92
Minimum pH	4.4
Maximum pH	9.4
Maximum % NaCl	10
Minimum temperature	-0.4°C ( 31.3°F)
Maximum temperature	45.0°C ( 113°F)

Source : Center for Food safety and Applied Nutrition, USDA, 2006.

The traditional approaches to control microbial hazards linked with food products include frequent inspection and monitoring of facilities and operations, employee education, training programs and extensive microbiological testing of raw ingredients and unfinished and finished products. An effective cleaning and sanitation program is required to be in place in food processing plants to control contamination of the food products. Different kinds of cleaning and sanitation chemicals are used along with technologies like ozone decontamination of food processing environments. Cleaning is the complete removal of food soil with the use of a chemical compounds with appropriate detergent properties under recommended conditions. Sanitation is an act of maintaining a clean condition in a food-handling situation in order to prevent disease and other potentially harmful contaminants. A physical or chemical agent that reduces microorganism contamination levels on inanimate environmental surfaces is called a sanitizer. There are two classes of sanitizers:

1. Sanitizers on non–food contact surfaces – Performance standards for these sanitizers require a reduction of target microorganism by 99.9% or 3 logs after 5 min of contact time.
2. Sanitizing rinse on previously cleaned food contact surfaces - Performance standards for these sanitizers require a reduction of target microorganism by 99.999% or 5 logs after 30 sec of contact time (59).

Phage treatment is also one of the control measures that is being employed for *Listeria* control. Numerous studies on successful phage treatment of food stuffs contaminated with *Listeria* have been published (38). But this method does not seem to be practically feasible in food processing environments.

The apparent increase in number of recalls due to *L. monocytogenes* may be due to possible adaptation of *L. monocytogenes* to food production and processing environments. One such adaptation may be the development of resistance to sanitizers and disinfectants (49). Biocides, used as a part of thorough combined cleaning and disinfection program, are an essential weapon in the food industry hygiene armory to control pathogenic and spoilage microorganisms (30). A study by Holah et al. (31) has shown that conditions are likely to be present in food production and processing facilities that may give rise to persistent strains of *L. monocytogenes* and *Escherichia coli*. The nature of this developed persistence however, is not due to the resistance developed against cleaners and disinfectants but may be majorly due to physical adaptation (surface attachment, biofilm formation, reduced growth rate) to the entire range of environmental conditions with temperature, pH, nutrient supply and moisture level fluctuations, and frequency of cleaning and disinfection etc. Antimicrobial resistance can either be intrinsic (natural) property of an organism or extrinsic, acquired by mutation or by means of plasmids (51). However, research by Pan et al. in 2006 evaluating the resistance of *L. monocytogenes* biofilms to sanitizing agents in a simulated food processing environment showed that resistance of the treated biofilms to the sanitizing agents may be due to the attributes of extracellular polymeric substances and is not an intrinsic attribute of the cells in the biofilm (47). Additionally, in the processing environments, it is unlikely that *L. monocytogenes* is present as a single species culture. There are numerous other bacterial species present. The presence of *Pseudomonas* and *Flavibacterium* spp. has been reported which have known to enhance *L. monocytogenes* numbers on a surface (6, 19, 55) and resistance of the cells to cleaners and sanitizers (6).

Research work has been done on controlling *Listeria* spp. by competitive exclusion bacteria in floor drains. Studies have demonstrated that two lactic acid competitive exclusion bacterium isolates, *Lactococcus lactis* subsp. *Lactis* C-1-92 and *Enterococcus durans* 152, originally obtained from the biofilms in the floor drains have bactericidal effect on *Listeria monocytogenes* and inhibit the biofilm formation at 4 to 37 °C (63). Metabolites of *Enterococcus*

spp., used as starter cultures for meat fermentations, have been documented to be bactericidal to *L. monocytogenes* (58).

## **1.6 Surrogates for Research**

Evaluation and validation studies on control or intervention processes such as physical or chemical decontamination may utilize microorganism or representative material that serves as an alternate for target pathogen in order to prevent the introduction of harmful organisms into the production or processing facilities. Such organisms are referred to as Surrogates. These are generally taxonomically, physiologically and ecologically related to the pathogens or other target microorganisms. The use of surrogates by processing facilities is extremely important to ensure microbiological safety of the process (9). For instance, surrogates have been used in canning industry to validate the destruction of *Clostridium botulinum* spores. *Bacillus cereus* and *B. thuringensis* have been used as suitable surrogates for *B. anthracis*. The characteristics of a surrogate organism include non pathogenic nature, same inactivation characteristics as that of the target, durability similar to the target, easily enumerated and differentiated, does not establish itself as spoilage problem, attachment characteristics same as that of target, and genetic stability. Several research studies have been conducted on surrogates for *L. monocytogenes* which have established the use of *Listeria innocua* as a suitable surrogate for *L. monocytogenes* (8, 22, 48). Hence, *L. innocua* was chosen appropriate for this study.

## 1.7 Research Objectives

This research study had two main objectives:

1. To validate the effectiveness of Johnson 'Eliminex' Foaming Drain Cleaner and Johnson Diversey 'Final Step' 512 sanitizer for control of *Listeria monocytogenes* contamination in floor drains using *Listeria innocua* as surrogate.
2. To evaluate the potential for translocation of *Listeria monocytogenes* from drains on to the food contact surfaces in the surrounding environment using *Listeria innocua* as surrogate.

For conducting this study, a 7 x 7 x 8 feet flexi glass chamber was built to set up a simulated food processing environment. A commercial drain was placed inside the chamber. The drain was inoculated with 7-8 log CFU/ml of *Listeria innocua*. Cleaning and washing activities were simulated. The given chemicals were applied and reduction in the bacterial cells was calculated.

For the second part of the study, stainless steel coupons were hung inside the chamber at different heights. Cleaning and washing activities were simulated. The translocation of the bacterial cells, in to the surrounding environment, as a result of aerosols generated due to cleaning and washing activities was evaluated.

## **1.8 Johnson Diversey 'Eliminex' Foaming Drain Cleaner**

It is a chlorinated high alkaline liquid drain cleaner with clinging foam. It utilizes clinging foam technology and has been recommended safe for use on soft metals, such as brass, aluminium etc. The hazardous component in the formulation is Sodium hypochlorite, 0.1-0.5% by weight (LD50 oral-5800 mg/k mouse)

### **Physical and chemical properties:**

Physical State – Liquid

Appearance – Aqueous solution

Color – Light Yellow

Odor – Chlorine

Solubility – Soluble

Dilution pH – 11.0

Flash point - >200°F or > 93°C

For use, the product container is connected to a high pressure water hose and dispenser knob is adjusted to high flow position. The diluted product is sprayed into the drain for 2-10 seconds and allowed to stand for 60 seconds. Then the drain is thoroughly rinsed and allowed to dry.

## **1.9 Johnson Diversey 'Final Step' 512 Sanitizer**

It is a chlorinated ammonium compound consisting of N-alkyl dimethyl benzyl ammonium chlorides (5-10% by weight), N-alkyl dimethyl ethylbenzyl ammonium chlorides (5-10% by weight), and ethyl alcohol (0.1-1.5% by weight)

### **Physical and chemical properties:**

Physical State – Liquid

Appearance – Aqueous solution

Color – Pink

Odor – Characteristic

Solubility – Soluble

pH – 7.8

Flash point - >200°F or > 93°C

Specific gravity – 0.998 g/ml

Density – 8.3 lbs/ gal

The sanitizer is applied after the cleaner, allowed to sit for 30 sec and is then rinsed off thoroughly.

## 1.10 Growth Media

A number of studies have been conducted to develop methods for isolation and identification and developing suitable media for detection of *Listeria monocytogenes*. These methods are based on conventional microbiological techniques consisting of time consuming enrichment, isolation and then confirmation. Enrichment techniques require several days before the confirmed results are obtained. Chemicals with selective properties, antimicrobials, nutritive supplements are added to different media to make them selective for the detection of specific microorganisms. However, these selective media are formulated to select for healthy target organisms. Selective agents like organic dyes, antibiotics, bile salts, surfactants etc. may inhibit the repair of the injured cells of the target microorganism. *L. monocytogenes* is susceptible to injury on exposure to heat, freezing temperature, drying, irradiation, chemicals. Cells exposed to environmental stresses undergo sublethal physiological and structural changes, which can reveal a decrease in cell viability when traditional culture methods are used (4). Sublethal injury is defined as the reduction in ability of the cells to grow on a selective media, despite being recoverable on a non selective media. Selective agars may not allow the growth and enumeration of sublethally injured cells as injured cells may fail to resuscitate when plated directly on a selective media. Kang and Fung (36) developed the Thin Agar Layer (TAL) method to recover the injured cells, and improve selectivity and recovery. This method involves the use of 14 ml of non selective media (Tryptic Soy Agar, TSA) to overlay a prepoured, pathogen specific, selective medium.

In this study, Modified Oxford Medium (MOX, Difco) was used as a selective medium for the recovery of *Listeria* spp. and Thin Agar Layer Modified Oxford Medium (TAL/MOX) was appropriately chosen to recover injured cells. Bacto Oxford Medium Base is prepared according to the formulation of Curtis et al. (14) who described the medium originally and its use for selective isolation of *Listeria* spp. The ingredients including Columbia agar base combines pantone, bitone, and tryptic digest of beef heart as a source of nitrogen, carbon, amino acids and vitamins, agar as solidifying agent, sodium chloride to maintain osmotic balance, ferric ammonium citrate for differentiation of *Listeria* spp.. Selectivity is obtained due to the presence of Lithium Chloride which inhibits the growth of enterococci. The selectivity is increased by

addition of moxalactam and colistin methane sulfonate or colistin sulfate as the Oxford Antimicrobial Supplement.

In this study, in addition to MOX and TAL/MOX, Tryptic Soy Agar (TSA) was used as the basic growth medium to obtain the total aerobic colony counts.



## **CHAPTER 2 - Materials and Methods**

### **Part 1 - Validating the effectiveness of Johnson Eliminox Foaming Drain Cleaner and Johnson Diversey 'Final Step' 512 sanitizer**

#### **2.1 Bacterial Cultures and Inoculum Preparation**

The bacterial cultures used in this study included four strains of *Listeria innocua* (ATCC 33091, 51742, 49595, and 33090). The cultures were obtained from the American Type Culture Collection (ATCC). The sources for the freeze dried isolates of *Listeria innocua* ATCC 33091, ATCC 51742, ATCC 49595, and ATCC 33090 were feces of healthy pregnant women, an existing strain, plant derived foodstuff (cabbage), and cow brain respectively. The lyophilized microorganisms were transferred in to 9 ml Tryptic Soy Broth (TSB, Difco) individually, vortexed to mix the suspension well and incubated at 35°C for 24 h. For use of culture as inoculum, each strain was combined into a single mixed culture suspension to obtain a four strain cocktail of *L. innocua*. A 7-8 log CFU/ml culture suspension was used for inoculation purposes. The cell density of this suspension was determined by serially diluting the pure culture which was grown in TSB, and plating on duplicate Modified Oxford Media Agar (MOX, Difco) plates. The bacterial cell counts were obtained after incubating the plates at 35°C for 48 h.

#### **2.2 Preparation of Drain Surface**

A 10 inch diameter, circular, painted cast iron was used in this study. It was mounted on 2x3 feet 090" aluminum with two part white epoxy finish cabinet. The drain placed in a 316 stainless bowl, a schedule 40 PVC male 4 inch adapter screwed into the drain and was fitted with a 40 PVC pipe (manufactured by RGF Ltd.). A 5 gallon polyethylene bucket was used to collect drain wash water.

Picture 1 shows the drain set up. The drain set up was placed in a 7x7x8 feet closed flexi glass chamber.

**Figure 1 Drain Setup**



## **2.3 Inoculation of the Drain**

The drain was inoculated with meat slurry at regular intervals in order to simulate the normal conditions of drain surfaces in a food processing facility.

## **2.4 Treatment of Drain**

Drain surface was treated with Johnson Diversey 'Eliminex' Foaming Drain Cleaner, allowed to sit for 60 seconds and then Johnson Diversey 'Final step' 512 was applied as per manufacturer's instructions.

## **2.5 Sampling**

Surface samples were taken using sponge method by swabbing the drain surface, drain grate and drain pipe. The wash water was also plated.

## **2.6 Procedure**

The experiment was performed in four sets:

1. Non Inoculated Non Treated
2. Non Inoculated Treated
3. Inoculated Non Treated
4. Inoculated Treated.

For validating the effectiveness of the Johnson Diversey 'Eliminex' Foaming Drain Cleaner and 'Final Step' 512 Sanitizer a set of four experimental combinations were designed, Non Inoculated, Non Treated; Non Inoculated, Treated; Inoculated, Non Treated; and Inoculated, Treated. In these combinations, the inoculated set refers to the use of *Listeria innocua* to inoculate the drain, and the treated set refers to the use of the given chemicals manufactured by Johnson Diversey and applied according to the manufacturer's instructions.

Each of these experimental combinations was individually performed during 8 hour and 48 hour period of study, and the sampling was done at the end of the 8<sup>th</sup> and 48<sup>th</sup> hour. Meat slurry was used in order to mimic the natural conditions in a processing environment where waste raw materials, leftovers, trimmings or meat or produce are washed down the drain during the processing operation. In a normal setting, this may occur 2-3 times during a shift at the processing and production lines. The average normal duration of working shift in any production

and processing facility is 8 hour. Hence, the total duration of the study was chosen as 8 hour long and meat slurry was added at the 0, 4 and 8 h. Also, washing of the drain with a high pressure hose (40 psi) was done at the same intervals, further incorporating the typical conditions of a processing environment in order to better prove the applicability and the effectiveness of the chemicals being tested. The 48 hour period was chosen to determine the efficacy of chemicals against *Listeria* spp. biofilms.

### ***2.6.1 Experiment sets***

#### ***Non Inoculated Non Treated***

The drain was inoculated with meat slurry at 0, 4, and 8 h. For the preparation of meat slurry, 10 g of ground beef 80:20 (All Natural Ground Beef Chuck) was taken in a stomacher bag. To this, 100 ml of water was added and then stomached for 1 min. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 4 h. The process was repeated at 8 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi) and then sampled. Sponge (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using ‘L’ shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h. Bacterial counts were taken and reported as CFU/area.

#### ***Non Inoculated Treated***

The meat slurry was added to the drain at 0, 4, and 8 h. For the preparation of meat slurry, 10 g of ground beef 80:20 (All Natural Ground Beef Chuck) was taken in a stomacher bag. To this, 100 ml of water was added and then stomached for 1 min. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with

slurry at 4 h. The process was repeated at 8 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi). The Johnson Diversey Eliminox Foaming Drain Cleaner was then applied and allowed to sit for 60 sec before the Johnson Diversey “Final Step” 512 Sanitizer was used as per manufacturer’s instructions. Sponge (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using ‘L’ shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h. Bacterial counts were taken and reported as CFU/area.

### ***Inoculated Non Treated***

The drain was inoculated with meat slurry at 0, 4, and 8 h. For the preparation of meat slurry with *Listeria innocua* culture, 10 g of ground beef 80:20 (All Natural Ground Beef Chuck) was taken in a stomacher bag. To this, 90 ml of water was added and then stomached for 1 min. To prepare the culture cocktail, 2.5 ml of each strain of *Listeria innocua* was combined together and vortexed. 10 ml of this *Listeria innocua* cocktail of was mixed with stomached meat and water mixture. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry containing *Listeria innocua* at 4 h. The process was repeated at 8 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi) and then sampled. Sponge (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using ‘L’ shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h.

The plates were examined for typical *Listeria* colonies on MOX. The bacterial counts taken, were reported as CFU/area.

### ***Inoculated Treated***

The drain was inoculated with meat slurry at 0, 4, and 8 h. For the preparation of meat slurry with *Listeria innocua* culture, 10 g of ground beef 80:20 (All Natural ground Beef Chuck) was taken in a stomacher bag. To this, 90 ml of water was added and then stomached for 1 min. To prepare the culture cocktail, 2.5 ml of each strain of *Listeria innocua* was combined together and vortexed. 10 ml of this *Listeria innocua* cocktail of was mixed with stomached meat and water mixture. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry containing *Listeria innocua* at 4 h. The process was repeated at 8 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi). The Johnson Diversey ‘Eliminex’ Foaming Drain Cleaner was then applied and allowed to sit for 60 sec before the Johnson Diversey ‘Final Step’ 512 Sanitizer was used as per manufacturer’s instructions. Sponge (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using ‘L’ shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h. The plates were examined for typical colonies of *Listeria* on MOX. The bacterial counts taken, were reported as CFU/area.

### ***2.6.2 Efficacy against Biofilms***

Biofilms are a constant concern in food processing environments. *Listeria* is known to attach to different kinds of surfaces and grow and proliferate to form biofilms. In this study, biofilms were developed on the drain surface during a 48 h period as biofilms are known to develop on surfaces in 24-48 hours.

### ***Non Inoculated Non Treated***

The drain was inoculated with meat slurry at 0, 8, 12, 24, 36 and 48 h. For the preparation of meat slurry, 10 g of ground beef 80:20 (All Natural Ground Beef Chuck) was taken in a stomacher bag. To this, 100 ml of water was added and then stomached for 1 min. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0, 8, 12, and 24 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 36 h. The process was repeated at 48 h. The drain was then allowed to sit for 30 min and washed again with water (40psi) and then sampled. Sponge (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using ‘L’ shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h. Bacterial counts were taken and reported as CFU/area.

### ***Non Inoculated Treated***

The drain was inoculated with meat slurry at 0, 8, 12, 24, 36 and 48 h. For the preparation of meat slurry, 10 g of ground beef 80:20 (All Natural Ground Beef Chuck) was taken in a stomacher bag. To this, 100 ml of water was added and then stomached for 1 min. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0, 8, 12, and 24 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 36 h. The process was repeated at 48 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi). The Johnson Diversey ‘Eliminex’ Foaming Drain Cleaner was then applied and allowed to sit for 60 sec before the Johnson Diversey ‘Final Step’ 512 Sanitizer was used as per manufacturer’s instructions. Sponge (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in

sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h. Bacterial counts were taken and reported as CFU/area.

### ***Inoculated Non Treated***

The drain was inoculated with meat slurry at 0, 8, 12, 24, 36 and 48 h. For the preparation of meat slurry with *Listeria innocua* culture, 10 g of ground beef 80:20 (All Natural Ground Beef Chuck) was taken in a stomacher bag. To this, 90 ml of water was added and then stomached for 1 min. To prepare the culture cocktail, 2.5 ml of each strain of *Listeria innocua* was combined together and vortexed. 10 ml of this *Listeria innocua* cocktail of was mixed with stomached meat and water mixture. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0, 8, 12, and 24 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry containing *Listeria innocua* at 36 h. The process was repeated at 48 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi) and then sampled. Sponge (18 oz. "Speci Sponge", 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h. The plates were examined for typical *Listeria* colonies on MOX. The bacterial counts taken, were reported as CFU/area.

### ***Inoculated Treated***

The drain was inoculated with meat slurry at 0, 8, 12, 24, 36 and 48 h. For the preparation of meat slurry with *Listeria innocua* culture, 10 g of ground beef 80:20 (All Natural ground Beef



Chuck) was taken in a stomacher bag. To this, 90 ml of water was added and then stomached for 1 min. To prepare the culture cocktail, 2.5 ml of each strain of *Listeria innocua* was combined together and vortexed. 10 ml of this *Listeria innocua* cocktail of was mixed with stomached meat and water mixture. Then another 900 ml of water was added to this mixture to make it to 1 liter. This slurry prepared was then poured into drain at 0, 12, and 24 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry containing *Listeria innocua* at 36 h. The process was repeated at 48 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi). The Johnson Diversey 'Eliminex' Foaming Drain Cleaner was then applied and allowed to sit for 60 sec before the Johnson Diversey 'Final Step' 512 Sanitizer was used as per manufacturer's instructions. Sponge (18 oz. "Speci Sponge", 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) samples from drain surface, drain crate and drain pipe were taken. Sampled sponges were placed in sterile bags with 20 ml Lethen Broth (Difco). Five serial dilutions were made and plated on Tryptic Soy Agar (TSA, Difco), Modified Oxford Medium (MOX, Difco), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). The wash water from the drain collected in a bucket was also diluted serially and plated on TSA, MOX, and TAL/MOX plates. The plates were incubated at 35°C for 48 h. The plates were examined for typical colonies of *Listeria* on MOX. The bacterial counts taken were reported as CFU/area.

## 2.7 Statistical Analysis

The log values obtained from three replications based on the variables of the study were analyzed using SAS (Version 9.1.2, 2004). The variables in this study included the treatment set, location, media and time (hour) for evaluating their interaction to obtain the reduction in log counts of the bacterial cells. There were four sets of treatments, Non Inoculated Non Treated, Non Inoculated Treated, Inoculated Non Treated, and Inoculated Treated. Four different locations were sampled, drain surface, drain crate, drain pipe, and wash water. The counts were obtained on three different media TSA, MOX, and TAL/MOX and the study was carried out for 8h and 48 h duration.

## **CHAPTER 3 - Materials and Methods**

### **Part 2 - Evaluating the potential for translocation of *Listeria monocytogenes* from drains onto food contact surfaces in the surrounding environment using *Listeria innocua* as surrogate**

#### **3.1 Bacterial Cultures and Inoculum Preparation**

The bacterial cultures used in this study included four strains of *Listeria innocua* (ATCC 33091, 51742, 49595, and 33090). The cultures were obtained from the American Type Culture Collection (ATCC). The sources for the freeze dried isolates of *Listeria innocua* ATCC 33091, ATCC 51742, ATCC 49595, and ATCC 33090 were feces of healthy pregnant women, an existing strain, plant derived foodstuff (cabbage), and cow brain respectively. The lyophilized microorganisms were transferred in to 9 ml Tryptic Soy Broth (TSB, Difco) individually, vortexed to mix the suspension well and incubated at 35°C for 24 h. For use of culture as inoculum, each strain was combined into a single mixed culture suspension to obtain a four strain cocktail of *L. innocua*. A 7-8 log CFU/ml culture suspension was used for inoculation purposes. The cell density of this suspension was determined by serially diluting the pure culture which was grown in TSB, on duplicate Modified Oxford Media Agar (MOX, Difco) plates. The bacterial cell counts were obtained after incubating the plates at 35°C for 48 h.

#### **3.2 Preparation of Drain Surface**

A 10 inch diameter, circular, painted cast iron was used in this study. It was mounted on 2x3 feet 090" aluminum with two part white epoxy finish cabinet. The drain placed in a 316 stainless bowl, a schedule 40 PVC male 4 inch adapter screwed into the drain and was fitted with a 40 PVC pipe (manufactured by RGF Ltd.). A 5 gallon polyethylene bucket was used to collect drain wash water. Photograph 1 shows the drain set up. The drain set up was placed in a 7x7x8 feet closed flexi glass chamber.

### **3.3 Preparation of Surfaces**

Polished stainless steel coupons (6.4 x 1.9 x 0.1 cm) were washed with Fisherband Sparkleen detergent (Fisher Scientific) and autoclaved.

### **3.4 Inoculation of the Drain**

The drain was inoculated with meat slurry at regular intervals in order to simulate the normal conditions of drain surfaces in a food processing facility.

### **3.5 Cleaning and Washing Activities**

The aerosols generated as a result of washing and cleaning activities using high pressure hose were believed to be the cause of translocation of bacterial cells that are present in the drain, into the surrounding environment.

### **3.6 VIP for *Listeria***

VIP for *Listeria* (BioControl Systems, Inc.) is a one step detection method for *Listeria monocytogenes* and related *Listeria* species in foods, ingredients, and environmental samples. It is an AOAC approved method 997.03. VIP for *Listeria* is based on antigen-antibody reaction. If *Listeria* is present a antigen-antibody-chromogen complex is formed that is read on the kit. It ensures a high level on sensitivity and specificity for *Listeria*. For working with this test kit, 1ml of the broth from 24 h enriched samples is transferred into a clean test tube and heated at 100°C for 5 min to inactivate. The tubes are then cooled to 25-37°C before testing. A positive test shows a distinct line in the test verification window. Absence of control line indicates an invalid test result.

Figure 2 VIP for *Listeria* spp.



For studying the translocation, 3 different heights were chosen, 1 foot, 3 feet and 5 feet, based on the distance between the floor drains and the food contact surfaces and equipment in the surrounding environment in food processing facilities. The inoculation of the meat slurry was done in order to mimic the conditions existing in the food production and processing facilities where waste material is washed down in to the drains between and after shifts. The sampling was done at the end of 8 hours based on the usual duration of a shift in any production facility and 48 hours where biofilms were studied. Stainless steel is commonly used in construction of food contact surfaces in food processing environments. Also, previous studies have indicated that *Listeria* spp. attaches to the stainless steel surfaces fairly easy and quickly. Hence, coupons made out of stainless steel were hung inside the chamber at 3 different heights and used for sampling. Also, the walls of the chamber were marked at these same heights, 1 and 3 feet and were sampled as the aerosols generated during the cleaning and washing of drain directly contacted the walls.

### **3.7 Procedure**

In order to study the potential for translocation of *Listeria* from drains to the food contact surfaces, polished stainless steel coupons (6.4 x 1.9 x 0.1 cm) were hung surrounding the drain, inside the flexi glass chamber at three different heights, 1, 3, 5 feet above the drain surface. Autoclaved coupons with binder clips were passed through 1 ml pipettes and placed on cooling racks hung at 1, 3, and 5 feet with nylon thread strings inside the chamber. A total of 12 racks (4 per height) were hung inside the chamber. On each of these racks a set of 3 coupons was placed. Therefore, a total of 12 coupons were used for each height at which the translocation was studied. The inside walls of the chamber were marked for specific areas (3.6 x 7.6 cm, 28.88 cm<sup>2</sup>) at height 1 and 3 feet above the drain surface for sampling purposes. This study was performed for 8 and 48 hour period, each consisting of 4 sets, Non Inoculated Non Treated, Non Inoculated Treated, Inoculated Non Treated, and Inoculated Treated.

#### ***Non Inoculated Non Treated (8 h)***

The drain was inoculated with meat slurry at 0, 4, and 8 h. The meat slurry was prepared as in Part 1 of the study. The slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 4 h. The process was repeated at 8 h. The drain was then allowed to sit for 30 min and washed again with water

(40 psi). The coupons hung inside the chamber which were spray misted due to the cleaning activities were collected in individual sterile plastic bags. 100 ml of Listeria Enrichment Broth (LEB, Difco) was added to each of these bags containing stainless steel coupons. The previously marked areas on the inside walls of the chamber were sampled using sponge method. Sponges (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) with 20 ml peptone (Difco) were used to take samples at 1 and 3 feet. Sampled sponges were placed in sterile bags with 20 ml peptone. The diluent from the sampled sponges was plated on Modified Oxford Medium (MOX, Difco) plates in duplicates. 0.1 ml from each dilution was plated on the pre-poured spread plates and spread using ‘L’ shaped spreaders (Fisher Scientific). After plating the sponge diluents, 50 ml of the Listeria Enrichment Broth (LEB, Difco) was added to the sponges for enrichment. The plates, coupons and the enriched sponges were incubated at 35°C for 48 h. After 48 hours of incubation, the turbid broths were streaked on to the pre-poured MOX plates and then incubated at 35°C for 48 h. If black colonies were seen on the MOX plates, those were taken as presumptive positive for *Listeria*. Typical *Listeria* colonies from the MOX plates were picked up and grown in 9 ml Tryptic Soy Broth (TSB, Difco) test tubes for 48 h at 35°C. To confirm the presence of *Listeria*, the turbid TSB test tubes were taken and rapid VIP Listeria Test was performed on them. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

### ***Non Inoculated Treated (8 h)***

The drain was inoculated with meat slurry at 0, 4, and 8 h. The meat slurry was prepared as in Part 1 of the study. The slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 4 h. The process was repeated at 8 h. The drain was then allowed to sit for 30 min and washed with water (40 psi). The Johnson Diversey ‘Eliminex’ Foaming Drain Cleaner was then applied and allowed to sit for 60 sec before the Johnson Diversey ‘Final Step’ 512 Sanitizer was used as per manufacturer’s instructions. The coupons hung inside the chamber which were spray misted due to the cleaning activities were collected in individual sterile plastic bags. 100 ml of Listeria Enrichment Broth (LEB, Difco) was added to each of these bags containing stainless steel coupons. The previously marked areas on the inside walls of the chamber were sampled using sponge method. Sponges (18 oz. “Speci Sponge”, 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) with 20 ml peptone

(Difco) were used to take samples at 1 and 3 feet. Sampled sponges were placed in sterile bags with 20 ml peptone. The diluent from the sampled sponges was plated on Modified Oxford Medium (MOX, Difco) plates in duplicates. 0.1 ml from each dilution was plated on the pre-poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). After plating the sponge diluents, 50 ml of the Listeria Enrichment Broth (LEB, Difco) was added to the sponges for enrichment. The plates, coupons and the enriched sponges were incubated at 35°C for 48 h. After 48 hours of incubation, the turbid broths were streaked on to the pre-poured MOX plates and then incubated at 35°C for 48 h. If black colonies were seen on the MOX plates, those were taken as presumptive positive for *Listeria*. Typical *Listeria* colonies from the MOX plates were picked up and grown in 9 ml Tryptic Soy Broth (TSB, Difco) test tubes for 48 h at 35°C. To confirm the presence of *Listeria*, the turbid TSB test tubes were taken and rapid VIP Listeria Test was performed on them. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

#### ***Inoculated Non Treated (8 h)***

The drain was inoculated with meat slurry at 0, 4, and 8 h. The meat slurry containing *Listeria innocua* cocktail was prepared as in Part 1 of the study. The slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 4 h. The process was repeated at 8 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi). The coupons hung inside the chamber which were spray misted due to the cleaning activities were collected in individual sterile plastic bags. 100 ml of Listeria Enrichment Broth (LEB, Difco) was added to each of these bags containing stainless steel coupons. The previously marked areas on the inside walls of the chamber were sampled using sponge method. Sponges (18 oz. "Speci Sponge", 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) with 20 ml peptone (Difco) were used to take samples at 1 and 3 feet. Sampled sponges were placed in sterile bags with 20 ml peptone. The diluent from the sampled sponges was plated on Modified Oxford Medium (MOX, Difco) plates in duplicates. 0.1 ml from each dilution was plated on the pre-poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). After plating the sponge diluents, 50 ml of the Listeria Enrichment Broth (LEB, Difco) was added to the sponges for enrichment. The plates, coupons and the enriched sponges were incubated at 35°C for 48 h. After 48 hours of incubation, the turbid broths

were streaked on to the pre-poured MOX plates and then incubated at 35°C for 48 h. If black colonies were seen on the MOX plates, those were taken as presumptive positive for *Listeria*. Typical *Listeria* colonies from the MOX plates were picked up and grown in 9 ml Tryptic Soy Broth (TSB, Difco) test tubes for 48 h at 35°C. To confirm the presence of *Listeria*, the turbid TSB test tubes were taken and rapid VIP Listeria Test was run on them. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

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TSB test tubes were taken and rapid VIP Listeria Test was performed. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

### ***Non Inoculated Non Treated (48 h)***

The drain was inoculated with meat slurry at 0, 8, 12, 24, 36 and 48 h. The meat slurry was prepared as in Part 1 of the study. The slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 8 h. The process was repeated at 12, 24, 36, and 48 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi). The coupons hung inside the chamber which were spray misted due to the cleaning activities were collected in individual sterile plastic bags. 100 ml of Listeria Enrichment Broth (LEB, Difco) was added to each of these bags containing stainless steel coupons. The previously marked areas on the inside walls of the chamber were sampled using sponge method. Sponges (18 oz. "Speci Sponge", 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) with 20 ml peptone (Difco) were used to take samples at 1 and 3 feet. Sampled sponges were placed in sterile bags with 20 ml peptone. The diluent from the sampled sponges was plated on Modified Oxford Medium (MOX, Difco) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). After plating the sponge diluents, 50 ml of the Listeria Enrichment Broth (LEB, Difco) was added to the sponges for enrichment. The plates, coupons and the enriched sponges were incubated at 35°C for 48 h. After 48 hours of incubation, the turbid broths were streaked on to the prepoured MOX plates and then incubated at 35°C for 48 h. If black colonies were seen on the MOX plates, those were taken as presumptive positive for *Listeria*. Typical *Listeria* colonies from the MOX plates were picked up and grown in 9 ml Tryptic Soy Broth (TSB, Difco) test tubes for 48 h at 35°C. To confirm the presence of *Listeria*, the turbid TSB test tubes were taken and rapid VIP Listeria Test was run on them. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

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and washed with water (40 psi). The Johnson Diversey 'Eliminex' Foaming Drain Cleaner was then applied and allowed to sit for 60 sec before the Johnson Diversey 'Final Step' 512 Sanitizer was used as per manufacturer's instructions. The coupons hung inside the chamber which were spray misted due to the cleaning activities were collected in individual sterile plastic bags. 100 ml of Listeria Enrichment Broth (LEB, Difco) was added to each of these bags containing stainless steel coupons. The previously marked areas on the inside walls of the chamber were sampled using sponge method. Sponges (18 oz. "Speci Sponge", 3.8 x 7.6 cm; Nasco Laboratory, Fort Atkinson, WI) with 20 ml peptone (Difco) were used to take samples at 1 and 3 feet. Sampled sponges were placed in sterile bags with 20 ml peptone. The diluent from the sampled sponges was plated on Modified Oxford Medium (MOX, Difco) plates in duplicates. 0.1 ml from each dilution was plated on the pre poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). After plating the sponge diluents, 50 ml of the Listeria Enrichment Broth (LEB, Difco) was added to the sponges for enrichment. The plates, coupons and the enriched sponges were incubated at 35°C for 48 h. After 48 hours of incubation, the turbid broths were streaked on to the prepoured MOX plates and then incubated at 35°C for 48 h. If black colonies were seen on the MOX plates, those were taken as presumptive positive for *Listeria*. Typical *Listeria* colonies from the MOX plates were picked up and grown in 9 ml Tryptic Soy Broth (TSB, Difco) test tubes for 48 h at 35°C. To confirm the presence of *Listeria*, the turbid TSB test tubes were taken and rapid VIP Listeria Test was performed. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

### ***Inoculated Non Treated (48 h)***

The drain was inoculated with meat slurry at 0, 8, 12, 24, 36 and 48 h. The meat slurry containing *Listeria innocua* cocktail was prepared as in Part 1 of the study. The slurry prepared was then poured into drain at 0 h. The drain was washed with high pressure water hose (40 psi) and again poured with slurry at 8 h. The process was repeated at 12, 24, 36, and 48 h. The drain was then allowed to sit for 30 min and washed again with water (40 psi). The coupons hung inside the chamber which were spray misted due to the cleaning activities were collected in individual sterile plastic bags. 100 ml of Listeria Enrichment Broth (LEB, Difco) was added to each of these bags containing stainless steel coupons. The previously marked areas on the inside walls of the chamber were sampled using sponge method. Sponges (18 oz. "Speci Sponge", 3.8 x

7.6 cm; Nasco Laboratory, Fort Atkinson, WI) with 20 ml peptone (Difco) were used to take samples at 1 and 3 feet. Sampled sponges were placed in sterile bags with 20 ml peptone. The diluent from the sampled sponges was plated on Modified Oxford Medium (MOX, Difco) plates in duplicates. 0.1 ml from each dilution was plated on the pre-poured spread plates and spread using 'L' shaped spreaders (Fisher Scientific). After plating the sponge diluents, 50 ml of the Listeria Enrichment Broth (LEB, Difco) was added to the sponges for enrichment. The plates, coupons and the enriched sponges were incubated at 35°C for 48 h. After 48 hours of incubation, the turbid broths were streaked on to the pre-poured MOX plates and then incubated at 35°C for 48 h. If black colonies were seen on the MOX plates, those were taken as presumptive positive for *Listeria*. Typical *Listeria* colonies from the MOX plates were picked up and grown in 9 ml Tryptic Soy Broth (TSB, Difco) test tubes for 48 h at 35°C. To confirm the presence of *Listeria*, the turbid TSB test tubes were taken and rapid VIP Listeria Test was run on them. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

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ml of the *Listeria* Enrichment Broth (LEB, Difco) was added to the sponges for enrichment. The plates, coupons and the enriched sponges were incubated at 35°C for 48 h. After 48 hours of incubation, the turbid broths were streaked on to the pre-poured MOX plates and then incubated at 35°C for 48 h. If black colonies were seen on the MOX plates, those were taken as presumptive positive for *Listeria*. Typical *Listeria* colonies from the MOX plates were picked up and grown in 9 ml Tryptic Soy Broth (TSB, Difco) test tubes for 48 h at 35°C. To confirm the presence of *Listeria*, the turbid TSB test tubes were taken and rapid VIP *Listeria* Test was performed on them. Positive test kits were taken as confirmation for the presence of *Listeria* in the samples.

### **3.8 Statistical Analysis**

For statistical analysis, Single Factor Model with binomial distribution was used and the data obtained was analyzed using GENMOD procedure (SAS 9.1.2, 2004). The analysis was done to get the probability for positive test coupons obtained as a result of translocation of bacterial cells from the drain to the stainless steel coupons.

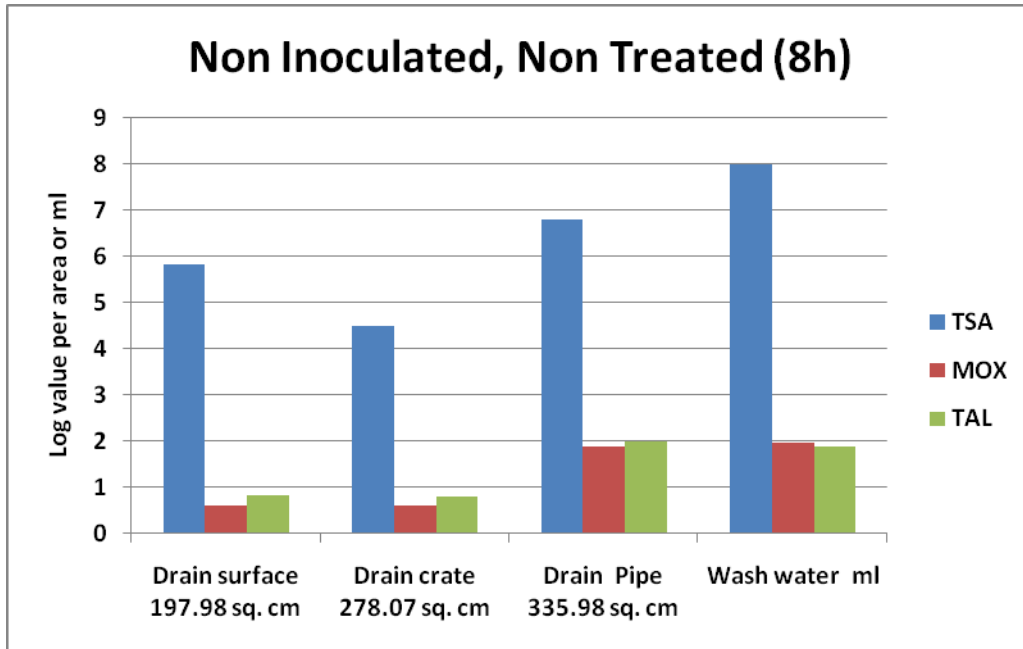
## CHAPTER 4 - Results and Discussion

### 4.1 Part 1

Figure 3 shows the average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TAL/MOX) from four sampling sites, drain surface, drain crate, drain pipe and wash water from Non Inoculated, Non Treated set during the 8 h study. Highest bacterial cells were recovered from the wash water as all the meat slurry inoculated in to the drain at different time intervals was washed down in to the drain during cleaning and washing step to collect wash water. 8 log CFU/ml was seen on TSA while MOX and TAL/MOX showed low counts, 1.96 log CFU/ml and 1.9 log CFU/ml respectively. Bacterial colony counts obtained from the drain surface, with fixed sampling area of 197.98 cm<sup>2</sup>, were 5.84 log CFU/area on TSA, est. 0.61 log CFU/area on MOX and est. 0.83 log CFU/area on TAL/MOX. Sampling of drain crate of area 278.07 cm<sup>2</sup> showed 4.5 log CFU/area on TSA, 0.6 log CFU/area on MOX and 0.8 log CFU/area on TAL/MOX while drain pipe of fixed sampling area of 335.98 cm<sup>2</sup> showed 6.8 log CFU/area on TSA, 1.9 log CFU/area on MOX and 2 log CFU/area on TAL/MOX. A comparison of the bacterial cell recoveries on MOX and TAL/MOX shows that TAL/MOX allows better recovery by acting as a resuscitating media to allow for recovery of injured cells.

Figure 4 shows the average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe and wash water from Non Inoculated, Treated set during 8 h study. Comparing with the non inoculated, non treated set, the counts obtained from the non inoculated treated set were far lower. The greatest reduction was seen in case of wash water where the count on TSA reduced from 8 log CFU/ml to est 0.23 log CFU/ml for wash water and there was zero recovery on MOX and TAL/MOX. Drain surface showed 5.03 log CFU/area on TSA and est. 0.23 log CFU/area and est.0.39 log CFU/area respectively on MOX and TAL/MOX. Sponge samples from the drain crate showed 4.43 log CFU/area on TSA while there was no *Listeria* recovery on both MOX and TAL/MOX plates. Low counts of 3.56 log CFU/area on TSA and est. 0.23 log CFU/area on MOX and est. 0.39 log CFU/area on TAL/MOX were seen from samples taken from the sponging the fixed area of the drain pipe.

**Figure 3 Average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe, and wash water from Non Inoculated, Non Treated (8h) set**



**Figure 4 Average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TALMOX) from drain surface, drain crate, drain pipe, and wash water from Non Inoculated, Treated (8h) set**

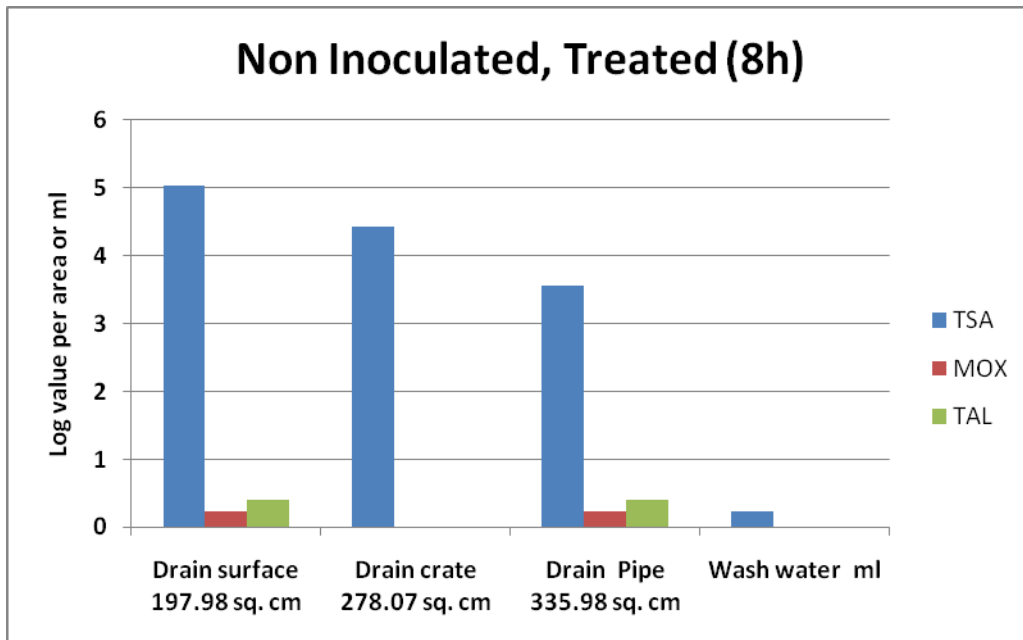
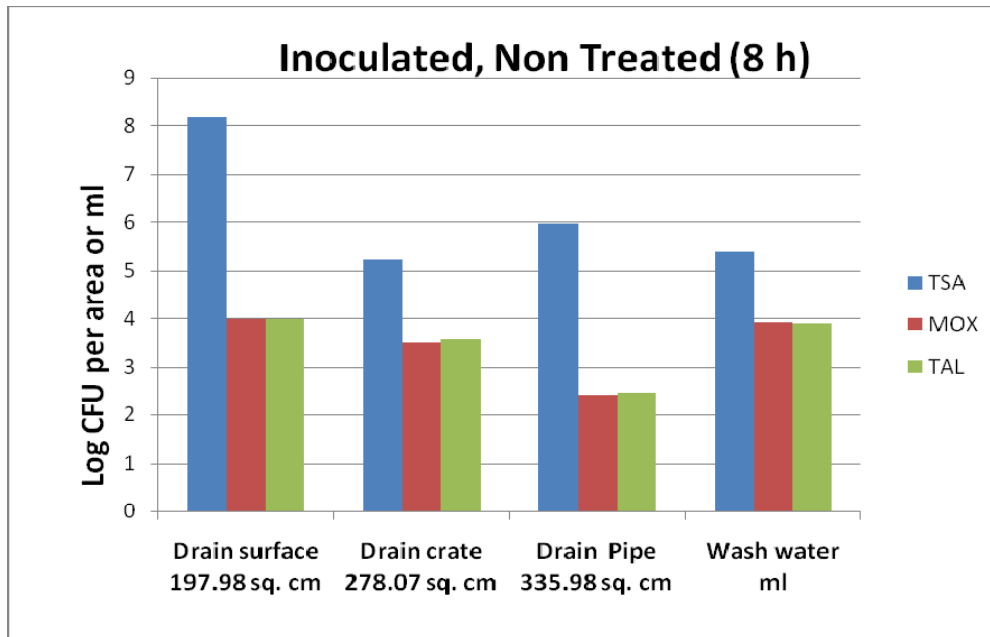


Figure 5 shows the average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe and wash water from Inoculated, Non Treated (8 h) set. In this set the total aerobic counts varied from 5-8 log CFU/area or ml for the different sampling locations. The *Listeria* spp. counts recovered on both MOX and TAL/MOX ranged between 2-4 log CFU/area or ml. The drain surface had 3.9 log CFU/area, drain crate 3.5 log CFU/area, drain pipe 2.4 log CFU/area and wash water 3.9 log CFU/ml for both MOX and TAL/MOX.

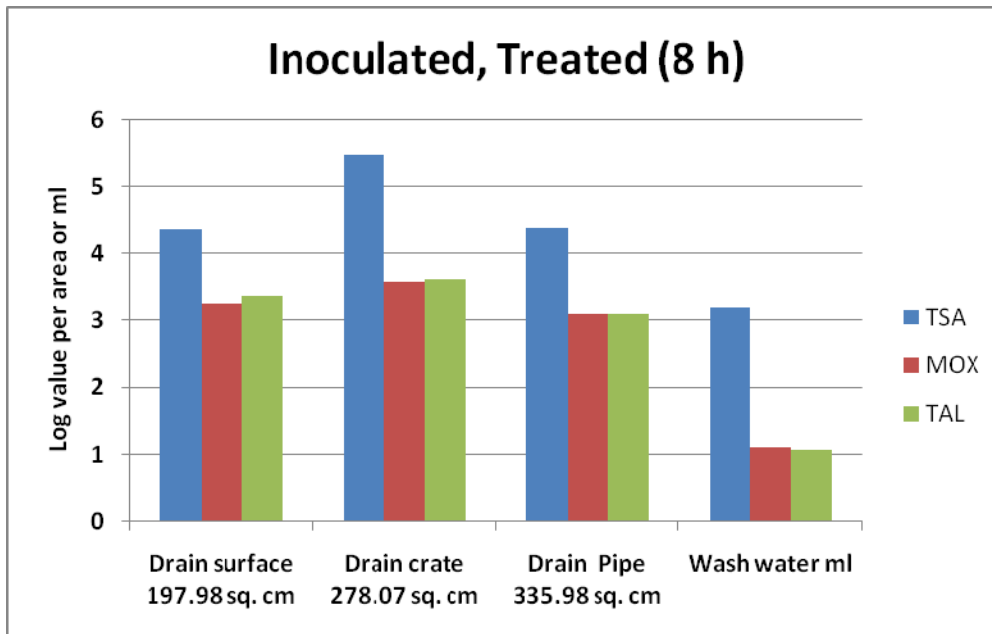
Similarly Figure 6 shows the average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe and wash water from Inoculated, Treated for the 8 h set. A comparison of the treated and the non treated set shows that the total aerobic counts recovered from the drain decreased by 3 log CFU/area or ml. The *Listeria* counts showed a smaller decrease. Statistically, there was only 0.5 log CFU/area or ml reduction in the bacterial counts between the treated and the non treated sets.



**Figure 5 Average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), and Thin Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe, and wash water from Inoculated, Non Treated (8h) set**



**Figure 6 Average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe, and wash water from Inoculated, Treated (8h) set**

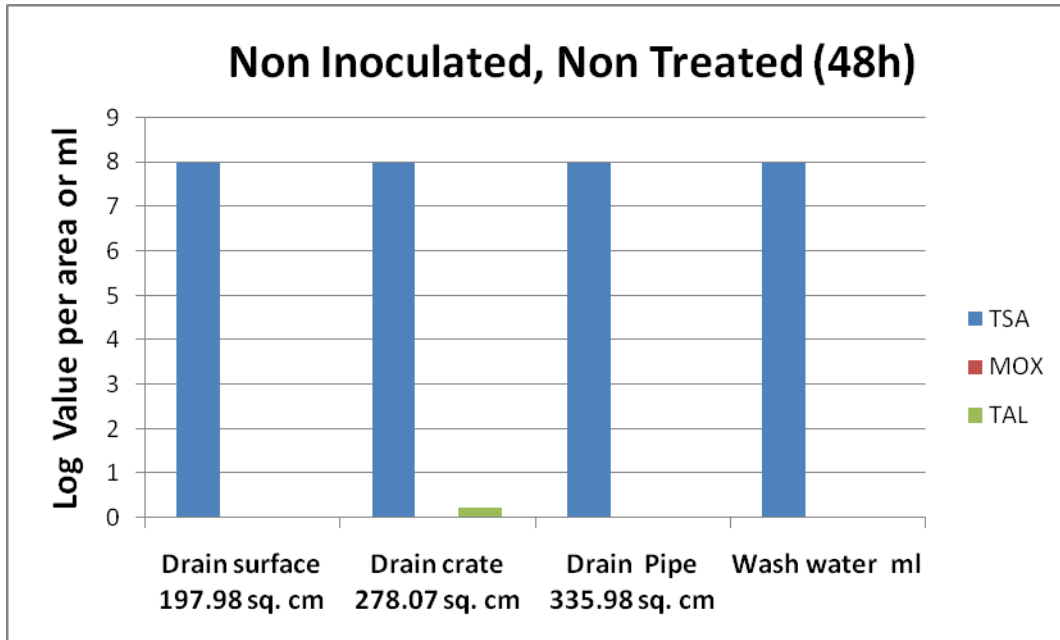


Figures 7 and 8 show average counts obtained on TSA, MOX and TAL/MOX for the non inoculated, treated and non inoculated, non treated sets for the 48 hour study period where the effectiveness of the given chemicals was tested against biofilms of *Listeria* spp. As the drain surface was not inoculated with *Listeria innocua*, no bacterial cells were recovered on MOX and TAL/MOX. However there was an estimated count of 0.23 log CFU/area of injured cells recovered on TAL/MOX was seen in the drain crate from the non inoculated, non treated set. The total aerobic counts recovered on TSA remained up to 8 log CFU/area or ml for both the sets for 48 h time period.

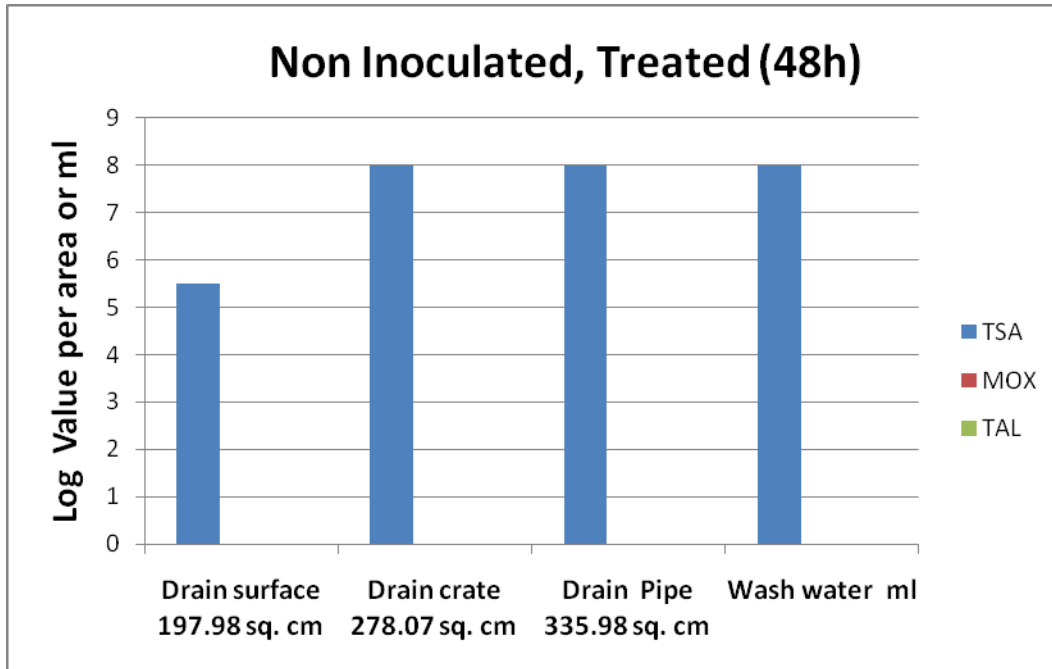
Similarly Figures 9 and 10 show average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe and wash water from inoculated, non treated and inoculated , treated for 48 h. The total aerobic counts ranged between 6-8 log CFU/area or ml. For the *Listeria* spp. a reduction of 4 log CFU/ml was seen for the wash water on comparing the treated and the non treated sets.

The analysis shows that the treatment effect is highly significant ( $p < 0.05$ ). However, the treatment did not interact with the time. The results obtained were similar for 8h and 48 h of study. The location had no effect on the log reduction of bacterial cells while media and hour showed significant effect on the reduction in log values ( $p < 0.05$ ). The treatment effect was same for both the log reductions obtained as the result treatment and reductions as compared to the inoculum used due to the treatment.

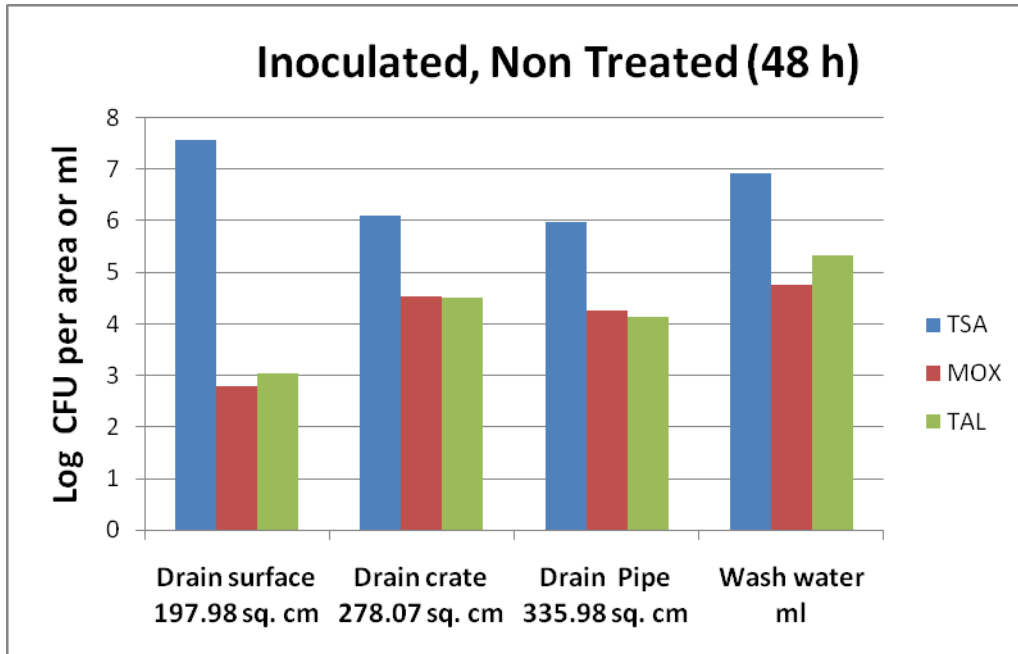
**Figure 7 Average counts on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe, and wash water from Non Inoculated, Non Treated (48h) set**



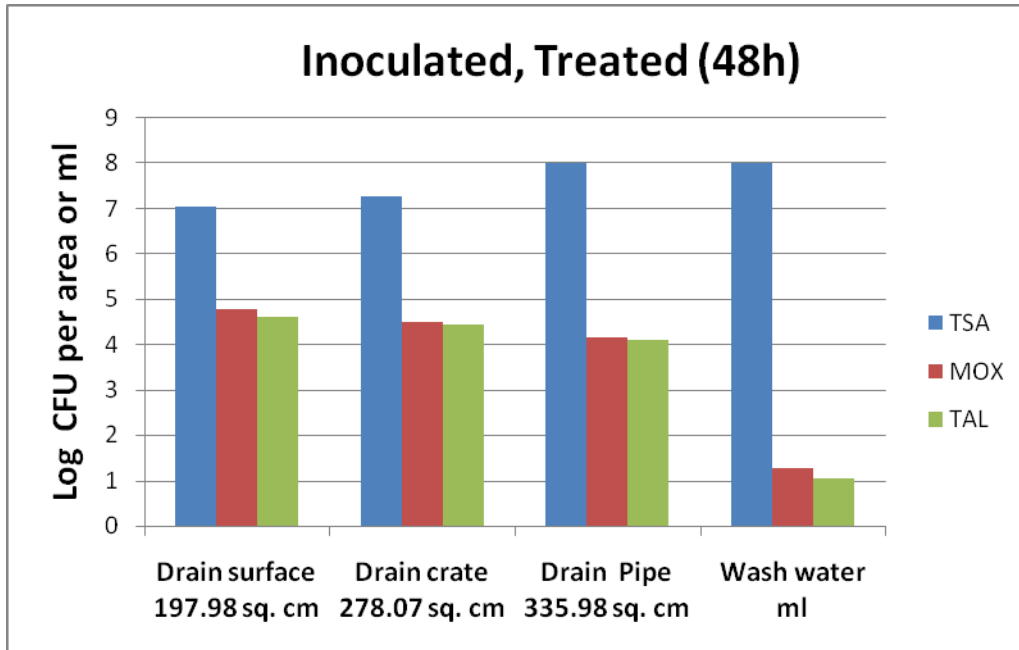
**Figure 8 Average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX) and Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe, and wash water from Non Inoculated, Non Treated (48h) set**



**Figure 9 Average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), and Then Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe, and wash water from Inoculated, Non Treated (48h) set**



**Figure 10 Average counts obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), Thin Agar Layer MOX (TAL/MOX) from drain surface, drain crate, drain pipe, and wash water from Inoculated, Treated (48h) set**



Figures 11, 12, and 13 compare the average counts for Non Inoculated, Non Treated and Non Inoculated, Treated sets for the 8 h duration obtained on Tryptic Soy Agar (TSA), Modified Oxford Medium (MOX), and Thin Agar Layer Modified Oxford Medium (TAL/MOX) respectively on sampling drain surface, drain crate, drain pipe and wash water.

Figure 11 clearly shows a reduction in total aerobic bacterial log counts obtained on TSA between the treated and the non treated set. Highest reduction of was seen in case of wash water. Counts reduced from 8 log CFU/area or ml in the non treated set to 0.23 log CFU/area or ml for the treated set. A reduction of 3 log CFU/area was seen for the drain pipe while drain surface and drain crate showed 0.81 log CFU/area and 0.07 log CFU/area respectively.

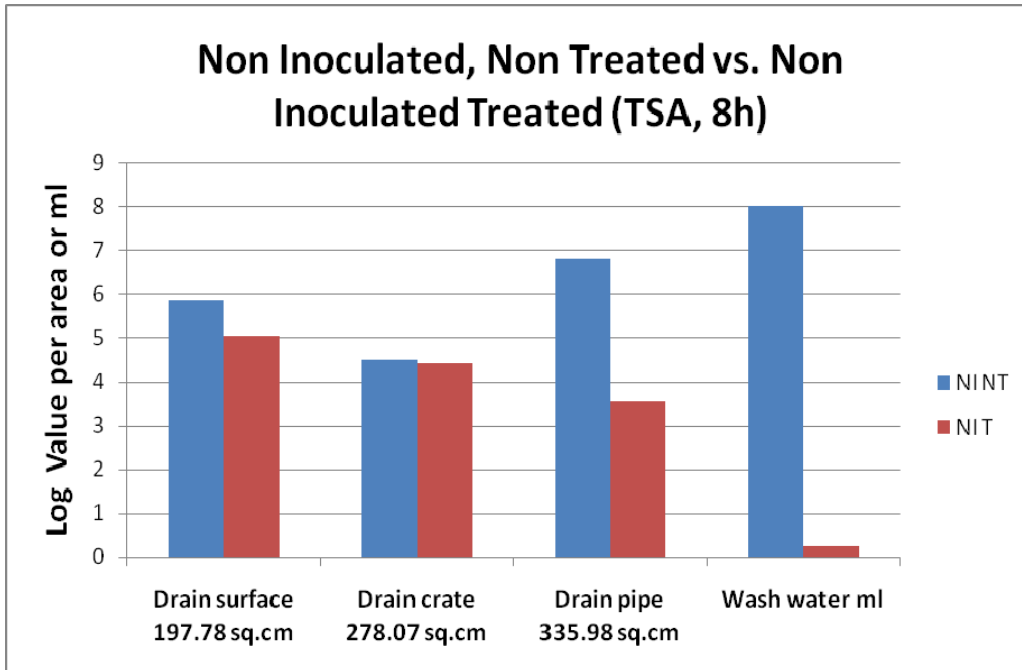
For Modified Oxford Medium (MOX), as shown in figure 10, no bacterial cells were recovered from the drain crate and wash water. An overall low counts < 2 log CFU/area or ml were observed for both the non inoculated, non treated and non inoculated treated sets.

Thin Agar Layer Modified Oxford Medium (TAL/MOX) showed similar results as MOX for the same experimental sets, shown in figure 13. Sampling of drain crate and wash water resulted in zero recovery of bacterial cells while for drain surface and drain pipe the counts remained below 2 log CFU/area or ml.

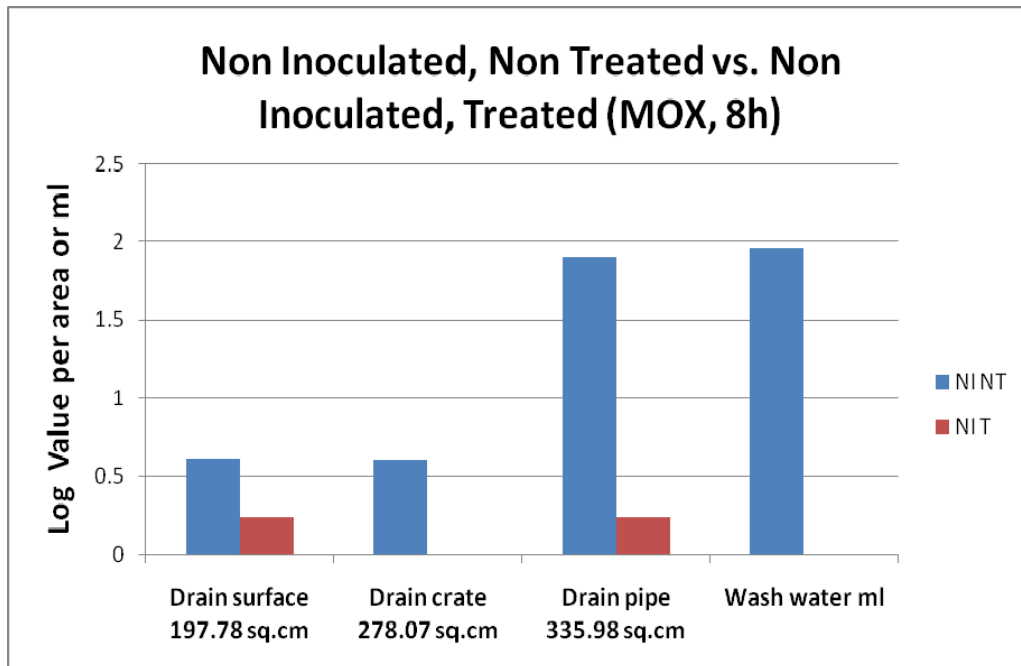
Figures 14, 15 and 16 compare the bacterial counts recovered on TSA, MOX, and TAL/MOX respectively, on sampling drain surface, drain crate, drain pipe and wash water in inoculated, non treated and inoculated treated 8 h set Greater log reductions up to 4 log CFU/ml were seen in case of wash water. Higher counts in the drain crate and drain pipe in the treated set as compared to the non treated could be due lack of mechanical cleaning and scrubbing during the cleaning of drain. The cells tend to colonize more in the screw threads where the pipe is attached to the drain. Also, the differences may be due to the experimental sets run on separate days.



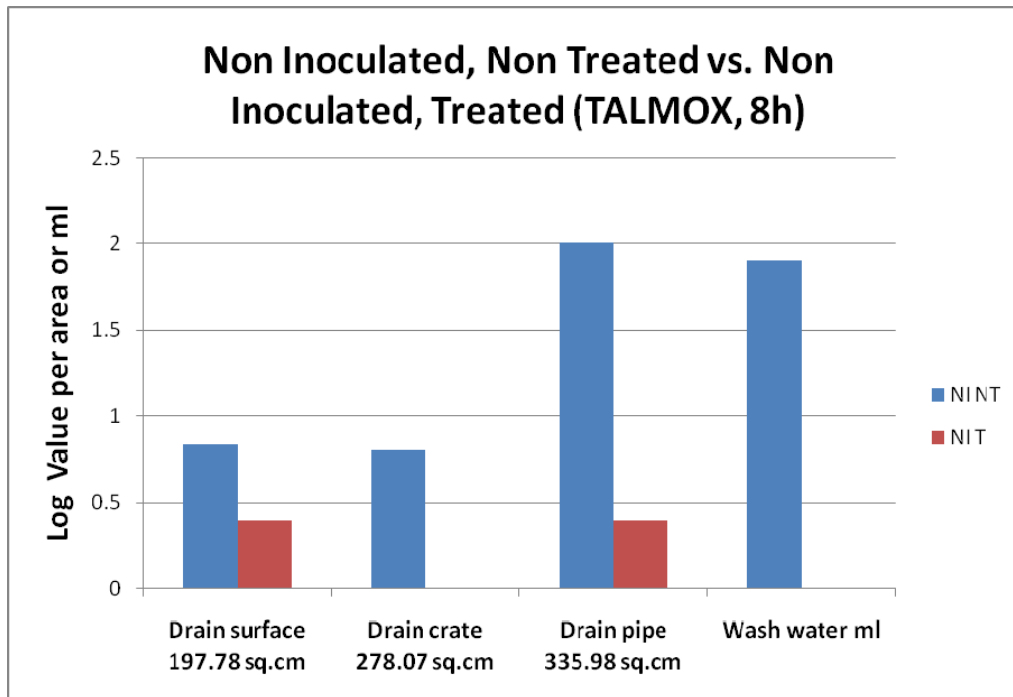
**Figure 11 Comparison of average counts obtained on Tryptic Soy Agar (TSA) for Non Inoculated, Non Treated and Non Inoculated Treated sets for 8h period**



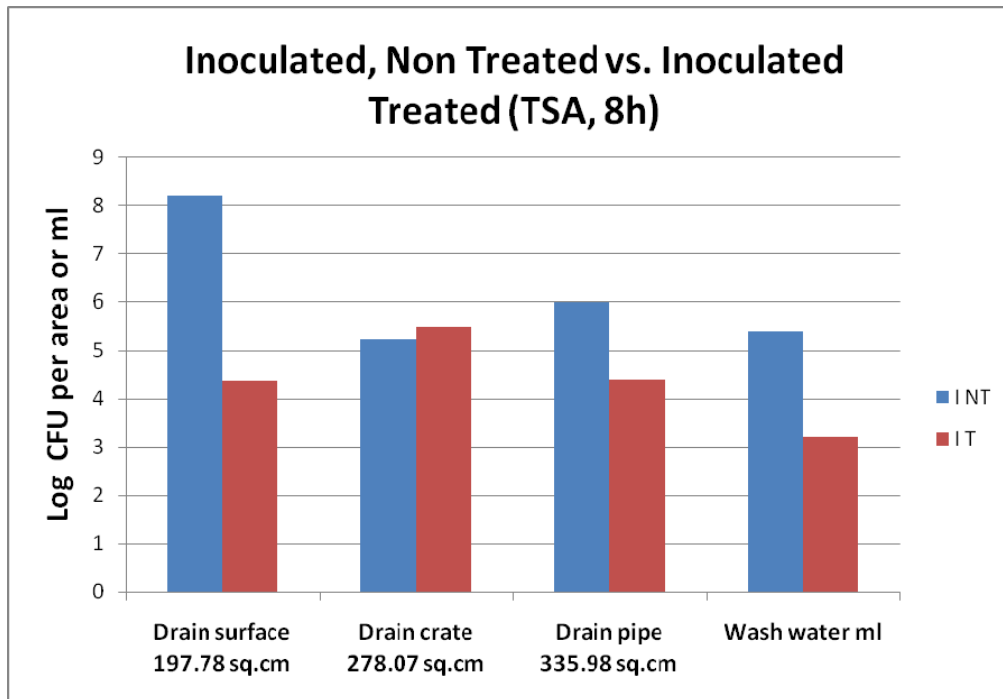
**Figure 12 Comparison of average counts obtained on Modified Oxford Medium (MOX) for Non Inoculated, Non Treated and Non Inoculated, Treated sets for 8h period**



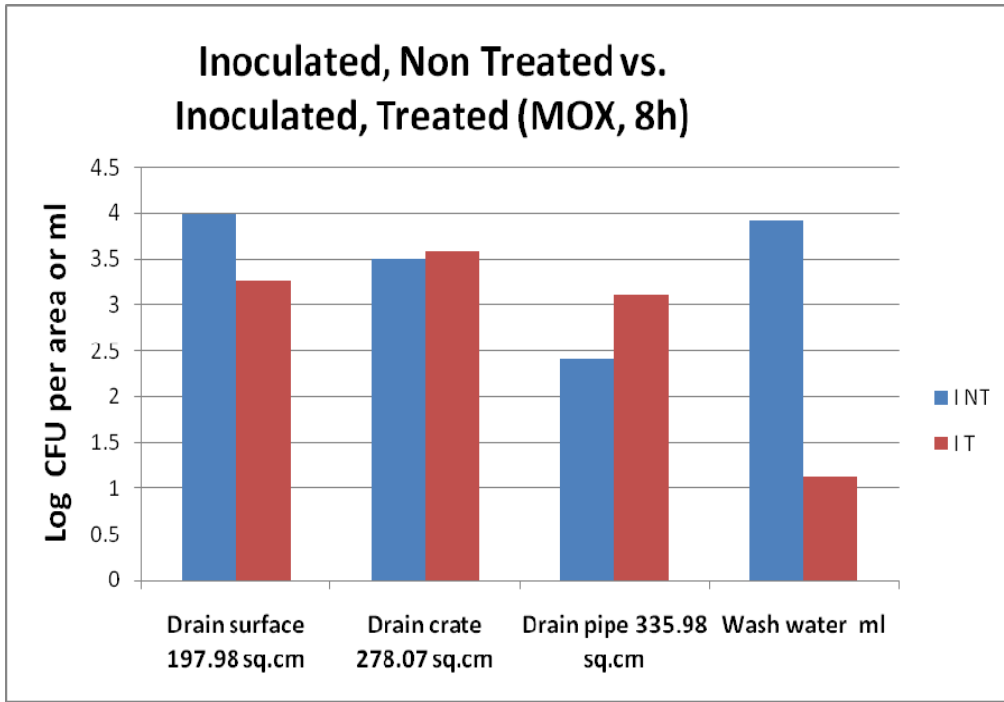
**Figure 13 Comparison of average counts obtained on Thin Agar Layer MOX (TAL/MOX) for Non Inoculated, Non Treated and Non Inoculated, Treated sets for 8h period**



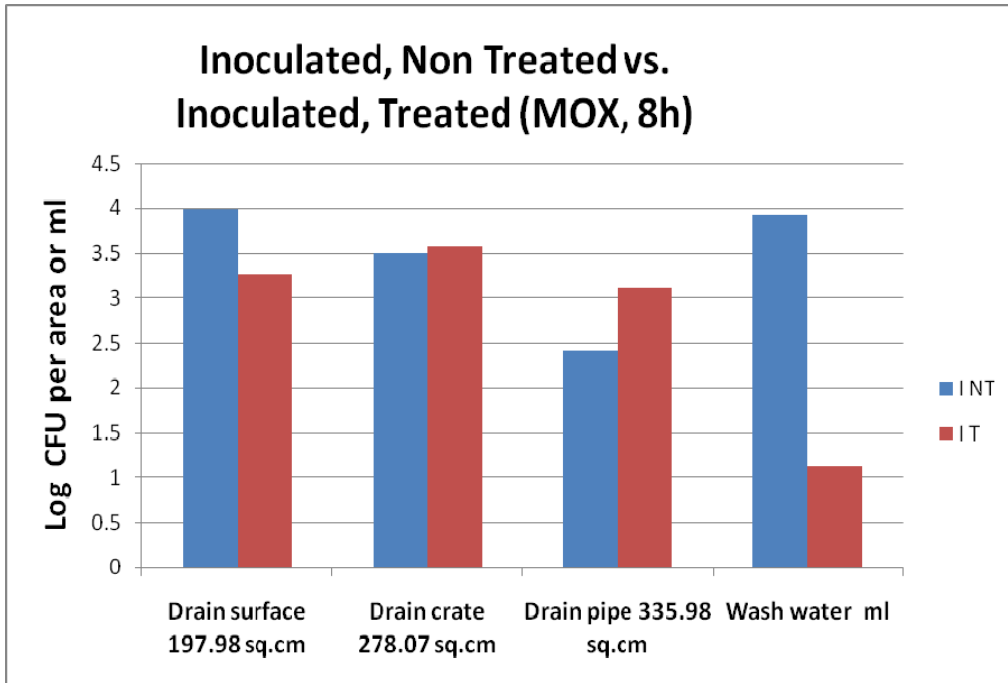
**Figure 14 Comparison of average counts obtained on Tryptic Soy Agar (TSA) for Inoculated, Non Treated and Inoculated, Treated sets for 8h period**



**Figure 15 Comparison of average counts obtained on Modified Oxford Medium (MOX) for Inoculated, Non Treated and Inoculated, Treated sets for 8h period**



**Figure 16 Comparison of average counts obtained on Thin Agar Layer MOX (TAL/MOX) for Inoculated, Non Treated and Inoculated, Treated sets for 8h period**



The different experimental sets were analyzed for their significance in reduction of bacterial cell numbers by using the GLM procedure in SAS. Comparison of the non inoculated, non treated and non inoculated, treated sets for both 8h and 48 h study periods show significant differences ( $p < 0.05$ ). This means the use of given cleaner and sanitizer did help in reducing the bacterial counts in the drain for these experimental sets.

Analysis of the inoculated, non treated set versus the inoculated, treated set for both 8 and 48 h indicated a significant difference ( $p = 0.05$ ) as well. This implies that when *Listeria* cells were intentionally added as a part of the validation study, the Johnson Diversey ‘Eliminex’ foaming drain cleaner and ‘Final Step’ 512 sanitizer were effective in reducing the bacterial counts.

Inoculated, non treated and non inoculated non treated sets for 8 h and 48 h were also compared and it showed that inoculation i.e. addition of bacterial cells to the drain had a significant effect ( $p < 0.05$ ) on the number of bacterial cells recovered at the end of sampling for the experimental sets where the commercial cleaner and sanitizer were not used.

Furthermore, comparison of inoculated, treated and non inoculated treated sets was also found to be statistically significant ( $p < 0.05$ ).

Each of the experimental sets were also analyzed based on the media, TSA, MOX, and TAL/MOX and sampling locations, drain surface, drain crate, drain pipe, and wash water and duration of the study, 8h and 48 h.

#### ***4.1.1 Based on the Media***

##### ***Tryptic Soy Agar (TSA)***

No significant difference ( $p > 0.05$ ) between the log counts recovered on the Tryptic Soy Agar, after sampling the different locations of the drain, was seen for the non inoculated, non treated in comparison to non inoculated treated set, inoculated, non treated in comparison to inoculated treated, and inoculated, treated in comparison to non inoculated treated for both 8h and 48 h. Total aerobic counts as high as 7-9 log CFU/area or ml were obtained for inoculated sets while in non inoculated sets 5-6 log CFU/area or ml were obtained on TSA. However, inoculated, treated set when compared against non inoculated, treated set showed a significant difference ( $p < 0.05$ ) between the log counts obtained after sampling. This shows the inoculated

set had higher total aerobic counts as opposed to non inoculated set irrespective of the treatment i.e. use of the cleaner and the sanitizer. This is because TSA enumerates the total aerobic bacteria, bacteria in meat slurry as well as the *L. innocua* cocktail that was added to the drain.

### ***Modified Oxford Medium (MOX)***

Sampling of the non inoculated, non treated and non inoculated, treated, and inoculated, non treated and inoculated, treated indicated no significant differences ( $p > 0.05$ ). In case of these sets, similar recoveries of bacterial cells i.e. *Listeria* spp. were made irrespective of the inoculation and the treatment. However, when inoculated, non treated and non inoculated, treated experimental sets were compared, the differences in the log reductions as recovered on the modified oxford medium (MOX) were found to be significant ( $p < 0.05$ ). This means greater reduction in the bacterial counts was seen between inoculated sets and their non inoculated controls.

### ***Thin Agar Layer Modified Oxford Medium (TAL/MOX)***

TAL/MOX showed similar results as MOX. Comparison of non inoculated, non treated and non inoculated, treated, and inoculated, non treated and inoculated, treated indicated no significant difference ( $p > 0.05$ ) while difference in log reductions were found to be significant ( $p < 0.05$ ) in inoculated, non treated and non inoculated, treated experimental sets.

## ***4.1.2 Based on Sampling Location***

### ***Drain Surface and Drain Crate***

Statistical analysis indicated similar results for drain surface and drain crate. Log reductions as recovered on all three media, TSA, MOX, and TAL/MOX were significant ( $p < 0.05$ ) in experimental sets inoculated, non treated and inoculated, treated when compared against non inoculated, non treated and non inoculated, treated respectively. No significant differences ( $p > 0.05$ ) in non inoculated, non treated set in comparison to non inoculated treated set and inoculated, non treated set in comparison to inoculated, treated set were seen on these sampling locations. This clearly shows that when the drain was inoculated with *Listeria* spp. and the 'Eliminex' foaming drain cleaner and 'Final Step' 512 sanitizer were applied to the drain during cleaning and washing, sampling of the drain surface area of 197.98 cm<sup>2</sup> and drain crate area of



278.07 cm<sup>2</sup> indicated significant reduction in log counts up to 5 log CFU/area or 2 log CFU/area in drain crease and drain surface respectively.

### ***Drain Pipe***

No significant differences were seen between the treated and the non treated sets in case of the drain pipe. Slightly higher counts up to 0.7 log CFU/area indicate the importance of mechanical action during cleaning and washing activities undertaken in food processing facilities for environmental surfaces. However when inoculated, treated set was compared to non inoculated, treated set, the reductions in bacterial counts on the drain pipe were found to be significant ( $p < 0.05$ ).

### ***Wash Water***

Sampling of the wash water showed significant reduction ( $p < 0.05$ ) in bacterial counts up to 5 log CFU/ml when recovered on to TSA, MOX and TAL/MOX from treated set in comparison to the non treated sets each of which were inoculated with the *Listeria* spp for both 8 and 48 h study period indicating that the treatment i.e the application of cleaner and sanitizer to the drain was found to effective in reducing the bacterial populations present in the drain.

## ***4.1.3 Based on the time***

### ***8 hour and 48 hour***

Comparison of the different experimental sets for the 8 h study period indicated significant differences ( $p < 0.05$ ). In case of 48 hour, only inoculated, non treated versus non inoculated, treated and inoculated, treated versus non inoculated treated were found to show a significant difference ( $p < 0.05$ ) in reduction in bacterial populations after the treatment. Repeated inoculation of bacterial cells during the 48 hours of study had a significant effect on the reductions that were seen as a result of treatment used. *Listeria* spp. formed the biofilm when it was allowed to grow in the drain for 48 h period. Cell numbers increased significantly during this time and the treatment of drain with the given cleaner and sanitiser resulted in significant reduction in log counts of *Listeria* spp. attached to the drain by forming a biofilm.

## 4.2 Part 2

Tables 5 and 6 show the percentage positive samples obtained for the different experimental sets, non inoculated, non treated; non inoculated, treated; inoculated, non treated; inoculated, treated for 8h and 48 h, respectively, where inoculation refers to the use of *Listeria innocua* and treatment refers to the use to commercial cleaning and sanitizing compounds, the Johnson Diversey ‘Eliminex’ Foaming drain cleaner and ‘Final Step’ 512 sanitizer.

**Table 5 Percentage positive samples (coupons) for *Listeria* spp. ue to translocation from the drain to the surrounding environment**

Experimental Set	Height (feet)	Percent Positive
Non Inoculated, Non Treated (8 h)	1	0 (0/36)
	3	0 (0/36)
	5	0 (0/36)
Non Inoculated, Treated (8 h)	1	0 (0/36)
	3	0 (0/36)
	5	0 (0/36)
Inoculated, Non Treated (8 h)	1	16.6(6/36)
	3	11.1(4/36)
	5	2.7(1/36)
Inoculated, Treated (8 h)	1	13.8(5/36)
	3	5.5(2/36)
	5	0 (0/36)

In the 8 hour set, translocation of bacterial cells was seen at all three heights, 1, 3, and 5 feet. The percentage positive samples ranged from 2-17% based on the sample size. Higher translocation was seen at 1 foot, followed by 3 feet and 5 feet indicating the closer the proximity from the drain, the greater the number of bacterial cells that transfer from the drain to the surrounding surfaces.

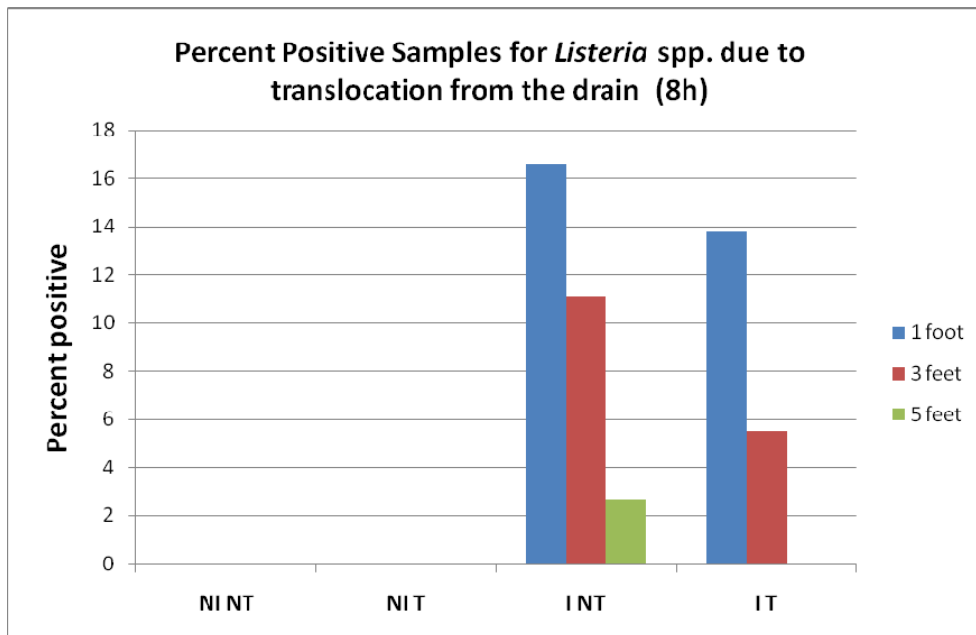
The translocation at 1 foot for the inoculated, non treated set was 16.6% while in the inoculated, treated set was 13.8%. These percentage figures based on the experimental set further

indicates that if a treatment was applied to control or eliminate the bacterial cells in the drain, the number of cells that translocate are lesser as compared to when the drain remains untreated.

The translocation at 3 feet for the inoculated, non treated set was 11.1%, while in the inoculated, treated set was 5.5%. At 5 feet, the translocation for the inoculated, non treated set was 2.7% while for the inoculated, treated was 0%. These percentage figures further reinforce that the need for cleaning and sanitizing treatments to floor drains as number of cells translocated from the non treated drain is higher than the treated one.

Figure 17 shows the percent positive samples of stainless steel coupons for *Listeria* spp. due to translocation from the drain at three different heights, 1, 3, and 5 feet during the 8 hour study for four experimental sets, Non Inoculated , Non Treated (NI NT), Non Inoculated, Treated (NI T), Inoculated, Non Treated (I NT), and Inoculated, Treated (I T).

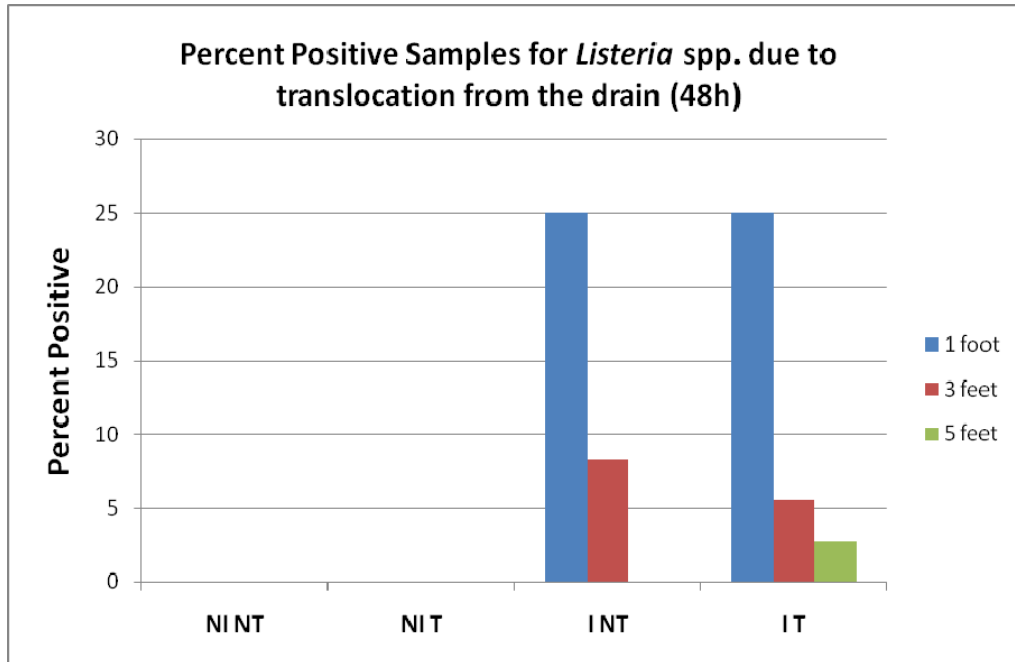
**Figure 17 Percent positive samples (coupons) for *Listeria* spp. due to translocation from the drain at three different heights 1, 3, and 5 feet during 8h study for four experimental sets**



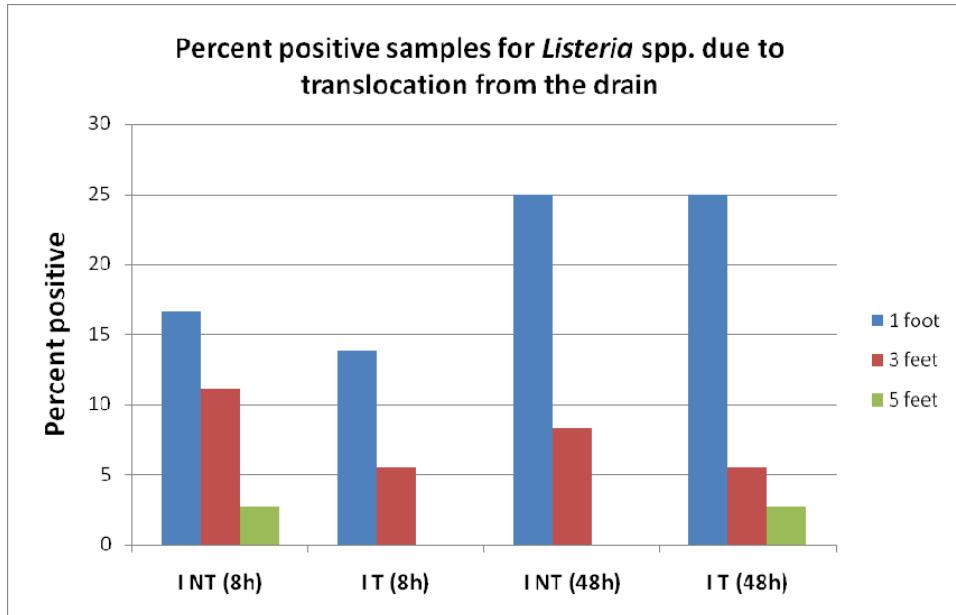
**Table 6 Percentage positive samples (coupons) for *Listeria* spp. due to translocation from drain to the surrounding environment**

<b>Experimental Set</b>	<b>Height (feet)</b>	<b>Percent Positive</b>
Non Inoculated, Non Treated (48 h)	1	0 (0/36)
	3	0 (0/36)
	5	0 (0/36)
Non Inoculated, Treated (48 h)	1	0 (0/36)
	3	0 (0/36)
	5	0 (0/36)
Inoculated, Non Treated (48 h)	1	25(9/36)
	3	8.3(3/36)
	5	0(0/36)
Inoculated, Treated (48 h)	1	25(9/36)
	3	5.5(2/36)
	5	2.7 (1/36)

**Figure 18 Percent positive samples (coupons) for *Listeria* spp. due to translocation from drain at the three different heights, 1, 3, and 5 feet during 48h study for four experimental sets**



**Figure 19 Comparison of percent positive samples (coupons) for *Listeria spp. due* translocation from drain at three different heights 1, 3, and 5 feet during 8h and 48 h study**



In the 48 h set, the coupons were found positive for the translocation at 1, 3, and 5 feet. The range of percentage positives in this case was higher as compared to the 8 h set, 2-25%. This may be attributed to the longer time available for the bacterial cells to grow and proliferate in the drain and also for m a biofilm as a protection against environmental stress. The average translocation at 1 foot was the highest, which was 25%, as compared to 6.9% at 3 feet and 1.8% at 5 feet height.

At 1 foot height, the percentage translocation for both inoculated, non treated and inoculated, treated sets was found to be 25%. At 3 feet height, 8.3% positive coupons were obtained from the inoculated, non treated set while 5.5% were seen in inoculated, treated set. At 5 feet, 2.7% positive samples were seen in the inoculated, treated set. Fig 18 shows the percent positive samples of stainless steel coupons for *Listeria spp.* due to translocation from the drain at three different heights, 1, 3, and 5 feet during the 48 hour study for four experimental sets, Non Inoculated , Non Treated (NI NT), Non Inoculated, Treated (NI T), Inoculated, Non Treated (I NT), and Inoculated, Treated (I T).

Data in the tables 5 and 6 indicate that if there was no contamination in the drain to begin with, as indicated by non inoculated sets for both 8 h and 48 h, there was no translocation of bacterial cells from the drain on to the coupons and the surrounding environment.

In the Fig 19 shows a comparison of percent positive samples of stainless steel coupons for *Listeria* spp. due to translocation from the drain at three different heights, 1, 3, and 5 feet during the 8 hour and 48 hour study for Inoculated, Non Treated (I NT), and Inoculated, Treated (I T) sets has been shown.

The aerosols generated as a result of cleaning and washing activities directly contacted the walls of the chamber in which the drain was placed. Any bacterial cells present in the drain can transfer to these walls or any surface of contact and may become a cause for contaminated food product along the line in a processing facility. Analysis of the samples obtained from the walls indicate percentage positives up to 100% further emphasizing that the bacterial cells are translocated via the aerosols generated due to use of high water pressure during cleaning and washing activities.

#### ***4.2.1 Statistical Analysis***

The analysis was done to get the probability for positive test coupons obtained as a result of translocation of bacterial cells from the drain to the stainless steel coupons. The experimental sets, inoculated, treated and inoculated, non treated for both 8 and 48 h period were observed to fit adequately in to the model. The height at which the coupons were hung inside the chamber showed significant effect ( $p < 0.05$ ) on the number of positive coupons obtained due to cells translocated from drain to the coupons. 1 foot height showed greater translocation as compared to 3 feet and 5 feet respectively.

## CHAPTER 5 - Conclusion

The study in the simulated environment setting of food processing facilities showed that the Johnson Diversey 'Eliminex' Foaming Drain Cleaner and 'Final Step' 512 sanitizer being tested were found to be effective in controlling or inhibiting *Listeria monocytogenes* in drain. The results obtained in the second part of the study clearly indicate that bacterial cells present in the drain are being translocated in to the surrounding environment and the food contact surfaces which result in contaminated product along the production and processing line even though the food is adequately processed.

The study also suggests the scope for increasing importance of the cleaning phase in terms of removal of attached bacteria. Optimization is required in terms of efficacy of removal and limitation of the generation of viable aerosols. Higher degree of mechanical action, scrubbing and the use of detergents may play a role in the reduction in the spread of contamination by aerosols.

Because of the ubiquitous nature of *L. monocytogenes* in general environment, in order to minimize its presence throughout the food production and processing environments from which the presence of the organism presents a hazard to the products, effective and reliable personnel hygiene and personnel practices are required in addition to the application of effective cleaning procedures to the manufacturing equipment and the food processing environment itself.



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