EVALUATION OF FOUR DIFFERENT SURFACE SAMPLING TECHNIQUES FOR MICROBES ON THREE DIFFERENT FOOD PREPARATION SURFACES

by

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B.S., Kansas State University, 2007

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2009

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Abstract

There are many different environmental sampling methods that are currently used in the industry. They include swab, sponge, flocked swab, direct agar contact, and M-Vac. Several studies have been conducted to determine the benefits and drawbacks of each method. Sampling methods utilized in this study were the swab, flocked swab, and M-Vac.

Three surfaces were utilized in this study: ultra high density polypropylene, 304 stainless steel with a 2B finish, and 304 stainless steel with a 2B finish and a buffed surface. Surfaces sampled were 100 cm^2. Prior to inoculation, surfaces were autoclaved for 15 min at 121 °C for sterilization.

Surfaces were inoculated by either *Listeria monocytogenes* or *Escherichia coli* O157:H7 at a concentration of 9 $\log_{10}$ CFU/ml by painting the inoculum onto the surface with a sterilized paintbrush. Brushes were dipped in inoculum for 2 sec before painting from left to right once and then from up to down once. Brushes were redipped for 2 sec and the painting step was repeated. The same brush was used for all *E. coli* O157:H7 samples and a different brush was used for all *L. monocytogenes* samples. Then, the surfaces were allowed to dry for 30 min before sampling took place.

Listeria monocytogenes samples were appropriately diluted and plated in duplicate onto Tryptic Soy Agar (TSA) and Modified Oxford Media (MOX). *Escherichia coli* O157:H7 samples were properly diluted and plated in duplicate onto TSA and MacConkey Sorbital Agar (MSA).

After plating, dry surfaces were stained using LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit. The Zeiss LSM 5 Pascal confocal laser scanning electron microscope was used for

microscopy images and photographs. Six 1 mm by 1 mm random and representative images were taken of each surface.

Viable cell count results show that the sponge sampling method, in general, recovered a higher number of microorganisms. The swab was normally shown to recover the least number of microorganisms.

When examining the microscopy images it can be concluded that biofilms are more easily formed with *L. monocytogenes* than *E. coli* O157:H7. Imaging also allowed for a visual representation of the remaining organisms that made it appear as if there was actually more bacteria recovery when the M-Vac sampling method was employed than when the sponge method was utilized.

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Acknowledgements

There are many people who helped make this project possible. I would like to extend my gratitude to my major professor, Dr. Daniel Y.C. Fung. His support and guidance has been immeasurable. Also, Dr. Beth Ann Crozier-Dodson, thank you for all of your direction and encouragement. It's been a great experience and without you both it wouldn't have been the same! I would like to express thanks also to Dr. Curtis Kastner and Dr. James Marsden for serving on my committee.

Dr. Dan Boyle, an immense thank you to you! I knew so little about microscopy and yet you took the time to make sure I learned. I know I took up more of your time than you probably had planned, thank you.

Several companies provided financial or material contributions for this research. Microbial Vac Systems®, Inc. of Bluffdale, Utah not only provided the M-Vac and supplies, but also the stains needed for the confocal laser scanning microscope. Hardy Diagnostics supplied the flocked swabs that were used. The steel was specially ordered and cut by Zephyr Products, Inc of Leavenworth, Kansas. Thank you to Randy Reinhardt for answering my many questions about steel!

A special thanks to everyone in our lab who helped from making media and washing dishes to helping with the plating of this project. The little things DO matter and did not go unnoticed.

Dr. Melvin C. Hunt and Dr. Karen Schmidt, thanks for having such an open door policy and truly showing interest in my well-being. I've greatly enjoyed all of our little (or long) talks.

To my friends, without your continued support and encouragement I would have given up long ago. Thanks for always being there.

Dedication

I would like to dedicate this thesis to my family. Their never-ending support and love is remarkable. Mom and Dad, I am so grateful that God chose you to be my parents – not everyone can be so lucky.

Introduction

Environmental/surface sampling is important in any food processing plant. There are many different environmental sampling methods that are currently used in the industry. Two main reasons to maintain a clean environment for processing foods are to maintain shelf life and to ensure that the food does not become contaminated (Moore and Griffith, 2002). Common methods include sterile swabs and sponges. Other methods now available are the M-Vac and flocked swabs. Several studies have been conducted to determine the benefits and drawbacks of each method, but no such study has looked at all four methods.

This research was to compare the four sampling methods of swab, sponge, flocked swab, and M-Vac on the basis of bacterial recovery. The surfaces investigated were ultra high density polyethylene, 304 stainless steel with a 2B finish, and 304 stainless steel with a 2B finish with a buffed surface. The objective of this research was to compare the four methods to determine which is best in recovering microorganisms. The sampling surfaces were then viewed and photographed using a laser scanning confocal microscope to determine where microbes remain. *Listeria monocytogenes* and *Escherichia coli* O157:H7 were the pathogens of concern in this study.

CHAPTER 1 - Literature Review

1.1 Environmental/Surface Sampling Methods

Environmental/surface sampling is important in any food processing plant. There are many different environmental sampling methods that are currently used in the industry which include swab, sponge, flocked swab (Puritan Medical Products Company, LLC, Guilford, Maine), direct agar contact, and M-Vac (Microbial Vac Systems®, Inc., Bluffdale, Utah). Surface sampling of food products can also be done by excising a portion of tissue. Several studies have been conducted to determine the benefits and drawbacks of using each method to test for cleanliness of food plants.

Two main reasons to maintain a clean environment for processing foods are to maintain shelf life and to ensure that the food does not become contaminated or re-contaminated (Moore and Griffith, 2002). One of the most common incidences of contamination in production facilities occurs when feces of harvested animals contaminate the carcass's surface. The surface then contaminates the processing surfaces and other carcasses (Niskanen and Pohja, 1977). If microorganisms have attached themselves to a surface, anything that comes into contact with that surface is at potential risk for contamination (Mafu et al., 1990).

The traditional swab method relies on two important points for true detection and enumeration of microbial contaminants. First, the swab must pick up the microbes from the surface being sampled. Then, the swab must release the bacteria into the diluent fluid for plating (Moore and Griffith, 2002). This same logic can be applied to the sponge method. However, it is thought that the sponge tends to retain higher microbial

populations due to its more porous composition (Daley et al., 1995). The flocked swab was designed to help eliminate the number of microorganisms remaining on the swab as it is composed of out reaching nylon fibers instead of the traditional weave of either cotton or polydacron.

In one study, rinsed, excised samples yielded more bacterial recovery than the moist swab contact method. However, more bacteria were recovered when identical samples were blended (composite sampling) after being excised as opposed to just being swabbed and/or rinsed (Lazarus et al., 1976). While in another study, the sponge method was found to recover more bacteria than the swab method (Moore and Griffith, 2002). It has also been discovered that Replicate Organism Detection and Counting (RODAC) plate direct contact sampling was superior to the swabbing technique when a sterile cotton swab was the swab of choice (Foschino et al., 2003). A further study showed that using a calcium alginate swab was superior to the contact plate method (Niskanen and Pohja, 1976). The difference in these two studies was the swab chosen to evaluate in the study. This shows what a difference material can make in a sampling method. Difco's HYcheck™ contact slide has been found to have the same bacterial recovery rate as the swab method. Moreover, two different media can be used on the HYcheck™ for enumeration of two bacterial groups at once (Restaino et al., 1994). Swab and direct agar contact methods have also been evaluated for their effectiveness on carcasses.

When pork carcasses were sampled at four different areas (the ham, loin, shoulder, and inside the thoracic cavity) a significant difference was found between the moist-swab and direct agar contact methods, as well as, the moist-swab and mylar adhesive tape methods. The mylar adhesive tape method was performed by pressing the

adhesive tape to the sampling location for 1 min and then transferring this tape to a petri dish with pre-poured Plate Count Agar (PCA, Difco). After a contact time of 1 min the tape was removed from the agar. In each case the swab method detected more bacterial contamination. However, no difference was discovered between the direct agar contact and mylar methods (Cordray and Huffman, 1985). In a similar study, meat samples were evaluated using a double swab, an excision, and an agar sausage technique (direct agar contact method). This analysis established that the excision technique provided the highest recovery rate of bacteria (Nortje et al., 1982). Excision was also proven to be a better sampling method than sponge swabbing for turkey carcass bacterial recovery (McEvoy et al., 2005). Beef carcass surface tissue samples showed that the sponge method provided less bacterial recovery than the excision method. The samples were taken at three different steps on the processing line and it was found that the difference between the sponge method and the excision method was statistically the same (0.3 to 0.5 $\log_{10}$ CFU/100cm^2) at each of these three points. Higher levels of total aerobic bacteria were recovered after the 24 h in the chill cooler and low levels of *Escherichia coli* and coliforms were recovered utilizing both the sponge and excision methods (Dorsa et al., 1997).

It has been found that when comparing excision sampling and wet-dry swab sampling with a control sample both the wet-dry swabbing counts were always lower than the controls. Nevertheless, the same study found that there was no difference in the microbial results from the controls and the excision samples when the meat sections were inoculated with bacterial solutions at a concentration of 10^3 CFU/ml (Cenci-Goga et al., 2007).

While removing an excised portion of a meat surface and then stomaching that portion recovered the most bacteria; the efficiency of this method is relative because of the small amount of the total microbes that are recovered. A new method with an increased ability to recover more total bacteria is needed as suggested by Fliss et al. (1991). The M-Vac was designed by Microbial-Vac Systems®, Inc (Bluffdale, Utah) to accomplish this goal. The vacuum suction and solution method that the M-Vac uses was developed to eliminate the need for bacteria to be released from the sampling device such as the swab or sponge. The M-Vac employs the vacuum method that Lee and Fung (1986) reported having a consistent excess of 80% particle removal efficiency.

When comparing the sponge sampling method with 3M™ Quick swabs there was no difference in the ability to recover un-injured *Listeria monocytogenes*. However, the M-Vac was significantly less effective than both the sponge method and 3M™ Quick swabs in recovering *L. monocytogenes* from all four surfaces tested (brick, stainless steel, dairy board, and epoxy resin). The 3M™ Quick swabs and sponges were similar in all tested cases. These data imply that the M-Vac is not a good alternative to the conventional sponge method or the 3M™ Quick swab method when testing these surfaces. When the *L. monocytogenes* cells were injured, similar results were shown in each of the test cases (Nyachuba and Donnelly, 2007).

There are advantages and disadvantages to any environmental surface sampling method. Both the swab and sponge methods are very simple to perform. It does not take a trained microbiologist to swab or sponge a given surface area. Both methods are also very cost effective. All materials for both of these samples can be purchased pre-made and ready for use. Each of the two methods is designed for small to large surface areas

depending on a sampler's individual needs. The amount of bacteria present on a sample is not a main concern for either the swab or sponge method. Dilutions can be made after each type of sample is taken. The largest disadvantage is that while both methods are approved for use in food plants the materials that comprise these methods are designed to hold microorganisms, not to release them. The contact plate method is also widely used in the industry. Like the swab and sponge methods, it is very simple to perform and cost effective as the entire sampling and plating procedures are carried out by one device. Disadvantages of the contact plate method are that it is designed for smaller surface areas. If too many bacteria are present the contact surface becomes crowded and difficult to accurately count (Capita et al., 2004).

Choosing a sampling method is impacted by the type and nature of the surface to be sampled, levels and type of contamination, and the objective of the test (Fliss et al., 1991). Other factors that affect the type of surface sampling method chosen include the presence or absence of a bactericide. It may also be necessary to use multiple sampling methods to ensure the greatest bacterial recovery (Favero et al., 1968). It is impossible to recover and enumerate 100% of bacteria from a contaminated surface (Niskanen and Pohja, 1976). Regardless of how well a sampling method performs, there will always be bacteria that stick in crevices, or stay adhered to the sampling surfaces.

1.2 *Escherichia coli* O157:H7

Escherichia coli is a Gram-negative short rod. It is found in the intestines of all animals including humans. There are four classes of the enterovirulent *E. coli* group. They are enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), and enterohemorrhagic (EHEC). ETEC is the cause of traveler's diarrhea and illness. This

illness presents itself with little or no fever. It is commonly found in water and soft cheese. The toxins produced closely resemble the cholera toxin. EPEC is commonly found in contaminated drinking water and/or some meat products. This infection results in profuse, watery, diarrheal disease and in developing countries is the leading cause of infantile diarrhea. EIEC are atypical to most *E. coli*. They do not decarboxylate lysine or ferment lactose and are non-motile. EIEC mimics *Shigella* causing a persistent, dysenteric form of diarrhea. The source of these bacteria is infected humans (Feng, 2002).

Escherichia coli O157:H7 is part of the EHEC classification. EHEC bacteria cause hemorrhagic colitis. This illness results in bloody diarrhea and severe abdominal pain. Rarely vomiting and a low-grade fever will occur. Foods associated with *E. coli* O157:H7 include raw or undercooked ground beef, alfalfa sprouts, dry-cure salami, game meat, cheese curds, unpasteurized fruit juices, and raw milk (Walderhaug, 2001). About 70,000 infections caused by *E. coli* O157:H7 occur each year in the United States according to experts at the Centers for Disease Control and Prevention (CDC). This number is only an estimate because of the unknown number of cases that go unreported each year (Centers for Disease Control and Prevention, March 2008). The infective dose of *E. coli* O157:H7 is estimated to be 10 – 100 cells (Hitchens, 2006). 5-10% of diagnosed individuals will develop hemolytic uremic syndrome (HUS). Asymptomatic infections may also occur with *E. coli* O157:H7 infections (Centers for Disease Control and Prevention, 2008, EHEC). This life-threatening illness will result in hospitalization. Some patients will experience kidney failure or other further complications. Many will recover in a few weeks, while others may suffer permanent damage or die (Centers for

Disease Control and Prevention, 2008, EHEC). Case classifications are divided into suspect cases, probable cases, and confirmed cases (Centers for Disease Control and Prevention, 2008, STEC; Centers for Disease Control and Prevention, 2008, EHEC). Suspect cases are postdiarrheal HUS or thrombotic thrombocytopenic purpura (TTP) cases. Probable cases include cases with isolation of *E. coli* O157 from a clinical sample waiting on confirmation of H7 or Shiga toxin production, clinically compatible cases that are linked to confirmed cases, identification of Shiga toxin in a sample from a clinically compatible case, or absolute evidence of an elevated antibody titer to a known enterohemorrhagic (EHEC) serotype from a clinically compatible case (Centers for Disease Control and Prevention, 2008, EHEC). Antibiotics should not be used to treat this infection since they have not proven to be effective (Centers for Disease Control and Prevention, March 2008). Antibiotics are not effective because *E. coli* O157:H7 produces a verotoxin that is released into the body after the microorganism is killed. Infection can be prevented by properly washing hands, cooking meats thoroughly, avoiding unpasteurized milk, dairy, and juices as well as swallowing water in lakes, ponds, steams, and swimming pools. Cross contamination should also be prevented by thoroughly washing hands, counters, cutting boards, and utensils after they have been in contact with raw meat or poultry (Centers for Disease Control and Prevention, March 2008).

Several outbreaks have occurred in the last two decades. Most severe outbreaks noted by the CDC were from 1993 to 2000. In 1994 an outbreak was traced back to hamburger from a local Californian grocery store. Three cases were confirmed. An outbreak in 1995 involved hamburgers from a fast food restaurant which affected communities in both Georgia and Tennessee. Cases began appearing in Georgia, and

because of the short distance from Tennessee, Georgia officials notified Tennessee officials and two more cases were recorded. A lake swimming beach was closed in Illinois after lake water was determined as the source of an *E. coli* O157:H7 outbreak that affected five children in 1995. Unpasteurized apple cider or juice was determined to be the cause of a 1996 outbreak that affected both the Western United States and the Northeast United States. Wisconsin Department of Health and Family Services was informed of 12 *E. coli* O157:H7 infections when west-central Wisconsin residents became ill due to fresh cheese curds from a dairy plant in 1998. At least 10 children were hospitialized with *E. coli* O157:H7 infection after attending the Washington County Fair near Albany, New York in 1999. Also in 1999, teenagers at a cheerleading camp in Texas contracted *E. coli* O157:H7. Two of the group were hospitalized with HUS while two more endured appendectomies (Walderhaug, 2001).

One of the largest recalls resulting from the contamination of ground beef with *E. coli* O157:H7 was the Hudson Foods recall in 1997. It started with just 20,000 lbs and three days later was expanded to include 1.2 million pounds of ground beef (Food Safety Inspection Service, 1997; Knight, 1997). A zero-tolerance for *E. coli* O157:H7 in ground beef has been the result of linking the enterohemorrhagic serotypes with products of cattle origin (Karmali, 1989; Nataro and Kaper, 1998). In 1993, *E. coli* O157:H7 was classified as an adulterant in ground beef in the United States.

Reporting *E. coli* cases has a very specific timeline. Incubation time for *E. coli* O157:H7 is usually three to four days. Following incubation, time for treatment could take up to five days. The time for diagnosis is generally from one to three days; after diagnosis the sample must be shipped to the state public health authorities for “DNA

fingerprinting". In all, this takes from one to eleven days. Total confirmation time is about two weeks (Centers for Disease Control and Prevention, September 2006).

1.3 *Listeria monocytogenes*

Listeria is a Gram-positive, motile, nonsporeforming, facultative anaerobic rod bacterium normally found in fruits, vegetables, and ready-to-eat products. *Listeria* can grow from -0.4 to 50 °C (Farber and Peterkin, 1991) and in a wide pH range of 5 to 9 (Herald and Zottola, 1988). There are six different species of *Listeria.* They include: *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*. While both *L. ivanovii* and *L. monocytogenes* are known pathogens for animals, only *L. monocytogenes* is associated with human listeriosis (Hitchins, 2002).

Foods that are thought to be at a higher risk for *Listeria monocytogenes* contamination include hog dogs, luncheon meat, smoked seafood, unpasteurized milk, or store made salads (Centers for Disease Control and Prevention, 2006). Populations declared at risk for listeriosis are pregnant women, immunocompromised individuals, cancer patients, and the elderly. Perhaps more interesting, 1-10% of humans as well as at least 37 other mammalian species, 17 bird species, and some fish and shellfish species are thought to be intestinal carriers of *L. monocytogenes* (Centers for Disease Control and Prevention, June 2006).

Listeriosis is a serious infection that is caused by *Listeria monocytogenes*. Common symptoms are fever, muscle aches, and nausea or diarrhea. In more severe cases, headaches, confusion, loss of balance, and convulsions can occur. In pregnant woman listeriosis can lead to stillbirths or premature delivery (Centers for Disease

Control and Prevention, 2005). *L. monocytogenes* has a high fatality rate of 20 to 30% (Tiwari and Alenrath, 1990; Ryser and Marth, 1999).

Listeria spp. is easy to isolate in domestic environments. When 213 Netherlands' households were tested for *Listeria* spp. it was isolated in 47.4% of the households. Moreover, in the same study, 21.1% of households were found to be harboring *L. monocytogenes*, the only known species identified as a human pathogen (Beumer et al., 1996).

Finding *Listeria* spp. in different areas along the processing line is not a difficult task. One such study showed that 1.6% of farm bulk raw milk samples contained *L. monocytogenes*, and this number was higher (26.6%) in raw milk from diary plant silo tanks. In raw ground meat samples the number increased to 63.6%; however, only 14.9% of the processed packaged meat samples studied were contaminated with *L. monocytogenes* (Dorsa et al., 1997). A ten year study of *L. monocytogenes* prevalence shows that *L. monocytogenes* is in sliced ham and luncheon meat 5.16%, cooked corn beef 3.09%, salads, spreads, and pates 3.03%, small-diameter cooked sausages 3.56%, cooked, uncured poultry products 2.12%, large-diameter cooked sausages 1.31%, and jerky 0.52% of the time on average (Levine et al., 2001).

There are many different strains of *L. monocytogenes*. The Scott A strain has been widely studied. However, in comparison to the Scott A strain it was found that 21 of the 36 tested strains resulted in the same number of adsorbed cells; although, all of the strains had the same approximate level of surface adsorption (Klamokoff et al., 2001).

Listeria monocytogenes is usually not due to poor heat processing but because of post-processing contamination which explains why common products associated with *L.*

monocytogenes are now high hydrostatic pressure pasteurized post-processing and packaging (Beumer et al., 1996). These products include lunch meat, hot dogs, and summer sausages, etc. High hydrostatic pressure pasteurization allows for the product to remain chilled and generally lasts four minutes.

Drains, wash water, standing water, residues and food contact surfaces have all been found to harbor *L. monocytogenes* in food manufacturing plants. Contamination found in drains is especially easy to spread throughout the processing area. In one study *Listeria* spp. was found in 15-53% of all samples taken; *L. monocytogenes* accounted for 5-20% (Cox et al., 1989). While there are many ways for *L. monocytogenes* to contaminate foods one of the main concerns is the ability of this microorganism to attach to both food-contact and nonfood-contact surfaces throughout the processing plant (Mai and Conner, 2007).

As of 2004, *L. monocytogenes* was the leading cause of Class I microbiologically related recalls with over 80 recalls implicating more than 130 million pounds of product (Levine et al., 2001). There were two major outbreaks involving post-process contamination of ready-to-eat products (Centers for Disease Control and Prevention, 2000; Vorst et al., 2004). The apparent cause of both major outbreaks was deli turkey meat.

Many studies have been conducted to determine how *Listeria* spp. react to a variety of different conditions. One such study found that nitrogen flushing or the use of antimicrobial dips in addition to 8 ºC storage improved the resilience and growth of *Listeria* populations on shredded lettuce (Francis and O' Beirne, 1997). Another study

found that at pressures of 200 MPa *E. coli* and *L. monocytogenes* could be inactivated at similar levels (Jordan et al., 2001).

The percentage of attached cells is not significantly different between cheese industry isolates of *L. monocytogenes* between polystyrene and stainless steel. In the cheese industry greater attachment was found for the environmental samples than the cheese isolates (Tresse et al., 2007).

1.4 Biofilm Formation

Bacteria, such as *Listeria*, stick by forming a mass of polysaccharides that reach out from the bacterial surface and form a glycocalyx surrounding a cell or colony of cells. This glycocalyx is vital to the biological growth of most bacteria in varied natural environments (Costerton et al., 1978).

Several factors affect the way and speed that bacteria attach to solid surfaces. These factors include the bacterial species, cell density, and surface properties. Furthermore, environmental conditions such as pH and contact time must also be considered (Notermans et al., 1975; Rosenberg et al., 1977; Arnold and Shimkets, 1988). Other issues to consider when trying to determine how a biofilm will form are cell surface hydrophobicity and surface charges that may be present (Mafu et al., 1991; Carson and Allsop, 1983).

Microorganisms can attach to equipment surfaces by being trapped in channels or crevices in surfaces. It has been shown that immediately after inoculation, extracellular material can be produced on the test surface. This extracellular material enhances attachment (Speersdf et al., 1983). Water has been proven to be a very important factor in holding a biofilm to stainless steel. As the *L. monocytogenes* biofilms dried, the cell-to-

cell adhesion forces were weakened. This biofilm detachment and propagation ensures bacterial survival and thus the possible spread throughout the processing plant (Rodriguez et al., 2007).

Pseudomonas spp. has also been shown to grow on both glass and stainless steel surfaces, but not rubber (Speers et al., 1983). *Escherichia coli* was found to be adequately detached by cleaning stainless steel with both distilled water and an alkaline solution. The type of finish on the stainless steel did not affect the release of the *E. coli* (Foschino et al., 2003).

Listeria monocytogenes has been found to form biofilms on surfaces commonly used in food processing plants such as stainless steel, polypropylene, rubber, and glass (Blackman and Frank, 1996). *Listeria monocytogenes* is capable of attaching to stainless steel, glass, polypropylene, and rubber surfaces at both 20 °C and 4 °C after contact times as short as 20 minutes or 1 hour respectively (Mafu et al., 1990). *L. monocytogenes*' ability to attach to stainless steel may be enhanced by the motility and polymer surrounding the cells (Herald amd Zottola, 1988).

The type of surface did not affect the number of attached *Listeria innocua* cells that were observed. A single culture of *L. innocua* increased very rapidly until the second day of the study and then more slowly after the third day. Surfaces observed were Teflon, stainless steel, and rubber. It was also seen that the population of several strains of *L. innocua* in a biofilm was able to flourish more readily and for a longer period of time (5 days) than the single culture biofilm (Bourion and Cerf, 1996).

It has been shown that as relative humidity decreases, the transfer of *L. monocytogenes* from bologna and hard salami to stainless steel increases. Similarly, as

relative humidity increases the transfer of *L. monocytogenes* from stainless steel to bologna and hard salami decreases (Rodriguez et al., 2007).

1.5 Surfaces Sampled

A common stainless steel type used in the food industry is 304 (Reinhardt, 2009). This type of steel has been used in many different studies. *Listeria monocytogenes* will attach to stainless steel and polypropylene after 20 minutes contact time (Mafu et al., 1990). There are several ways to treat finished 304 stainless steel. When comparing untreated stainless steel type 304 with a 2B mill finish to the same steel that had been treated three different ways, it was found that untreated stainless steel allowed for the most bacterial attachment followed by sandblasted treatment, sanding, and electropolished finish respectively (Arnold and Bailey, 2000). In addition, the number of *L. monocytogenes* cells that attach to stainless steel surfaces was greatest at 30 to 37 °C (Mai and Conner, 2007). In a study comparing domestic food contact surfaces it was found that regardless of holding time or application method *Salmonella* recovery was greater from Formica and stainless steel than from polypropylene or wood (Moore et al., 2007).

Imaging of these samples is best taken by laser scanning confocal microscopy. Laser scanning confocal microscopy is able to make thin optical sections of fluorescent specimens. This series is accumulated by coordinating incremental changes in the fine focus device with imaging at each step. The principle behind the laser scanning confocal microscopy is that the light is emitted by the laser system and passed through the pinhole aperture. After this, the laser is mirrored by a dichromatic mirror and scanned across the specimen in the defined focal plane. The fluorescence this creates is produced by the

specimen and sent back through the dichromatic mirror and then focused as a confocal point at the detector pinhole aperture (Claxton et al., 2006).

When comparing surfaces in a poultry processing facilities it was found that bacteria attached to polyethylene, stainless steel, and belting equally well, however; the picker finger rubber (de-feathering equipment) seemed to act as an inhibitor for bacterial growth (Arnold and Silvers, 1999).

1.6 Research Objectives

This research was to compare the four sampling methods of swab, sponge, flocked swab, and M-Vac on the basis of bacterial recovery. The surfaces investigated were ultra high density polyethylene, 304 stainless steel with a 2B finish, and 304 stainless steel with a 2B finish that was buffed to a finer grade 'A' medium final finish. These three surfaces simulate food contact surfaces found in the food industry. The ultra-high density polypropylene is a common plastic surface used on the production line as are both of the stainless steel surfaces. The objective of this research was to compare the four methods to determine which works best in recovering microorganisms from three processing surfaces. After sampling, the surfaces were then viewed and photographed using a laser scanning confocal microscope to determine where microbes remain. *Listeria monocytogenes* and *E. coli* O157:H7 were the pathogens of concern in this study. Five replications of this study were performed, and statistical analyses were performed using Statistical Analysis Software (SAS, 2003).

CHAPTER 2 - Materials and Methods

2.1 Media

Media used in this study include Tryptic Soy Broth (TSB, Difco Laboratories, Sparks, MD), Tryptic Soy Agar (TSA, Difco Laboratories, Sparks, MD), peptone (Difco Laboratories, Sparks, MD), Butterfield's Buffer Solution (Microbial-Vac Systems®, Inc., Bluffdale, Utah), Oxford medium base (MOX, Difco Laboratories, Sparks, MD), and MacConkey Sorbital Agar (MSA, Difco Laboratories, Sparks, MD). TSB is a nutrient broth that the cultures were grown in and is a general purpose liquid nutrient medium that allows for growth of both fastidious and non-fastidious microorganisms. TSA is a nutrient agar medium for both fastidious and non-fastidious bacteria. This agar allows for the growth and enumeration of the bacteria grown aerobically in this research. Peptone and Butterfield's Buffer Solution, both liquids, are used as a diluent for enumeration purposes. MOX, an agar, is a selective medium for *Listeria monocytogenes*. MOX agar was used with the added Modified Oxford Antimicrobic Supplement for further specificity for *L. monocytogenes* colonies which appear black on MOX. MSA is a selective agar for enteric microorganisms, and was used to enumerate *Escherichia coli* O157:H7 in this study. No other supplements were added to this medium. *Escherichia coli* O157:H7 colonies appear colorless after incubation due to their inability to ferment sorbitol.

2.2 Bacterial Cultures and Inoculum Preparation

Four strains of *L. monocytogenes* (American Type Culture Collection (ATCC) 19115, ATCC 19112, ATCC 13932, and Cornell University SLR2249) and five strains of

E. coli O157:H7 (ATCC 43895, ATCC 43890, ATCC 43889, ATCC 35150, ATCC 43894) were used in this study. All ATCC *L. monocytogenes* cultures were derived from human sources and the Cornell University culture source is unknown. *E. coli* O157:H7 culture ATCC 43895 was taken from raw hamburger meat that was previously implicated in a hemorrhagic colitis outbreak. All other *E. coli* O157:H7 cultures originated from human feces. Cultured microorganisms were individually transferred to 9 mL sterile TSB, vortexed, and incubated at 35 °C for 24 h. These 7-8 log CFU/ml culture suspensions were used for inoculation. The pure cultures of *L. monocytogenes* and *E. coli* O157:H7 were serially diluted and plated in duplicate on TSA and MOX and TSA and MSA, respectively, to determine cell density. MOX plates were enumerated after incubating at 35 °C for 48 h while TSA and MSA plates were counted after incubating at 35 °C for 24 h.

2.3 Preparation and Inoculation of Sampling Surfaces

Three surfaces were utilized in this study: ultra high density polypropylene (Wal-Mart, Manhattan, Kansas; Figure 2.1a), 304 stainless steel with a 2B finish (Zephyr Products, Inc., Leavenworth, Kansas; Figure 2.1b), and 304 stainless steel with a 2B finish and a buffed surface (Zephyr Products, Inc., Leavenworth, Kansas; Figure 2.1c). Both stainless steel surfaces were cut from the same 11 gauge sheet. The make-up of the required components of the stainless steel in percent were as follows: 0.0330 Carbon, 18.0385 Chromium, 0.3847 Copper, 1.831 Manganese, 0.3267 Molybdenum, 0.0742 Nitrogen, 8.0781 Nickel, 0.0323 Phosphorus, 0.0002 Sulfur, and 0.4567 Silicon. The buffed stainless steel was buffed using an ‘A’ Med grade surface conditioning disc (3M

Abrasive Systems, St. Paul, Minnesota). Surfaces sampled were 100 cm^2. Prior to inoculation, surfaces were autoclaved for 15 min at 121 °C for sterilization.

Surfaces were inoculated by either *L. monocytogenes* or *E. coli* O157:H7 by painting the inoculum onto the surface with a sterilized paintbrush (Home Depot, Manhattan, Kansas; Figure 2.1d). The paint brushes were 63 mm wide. Brush bristles were 80 mm long and made of 100% natural white China bristle. The paint brushes were autoclaved wrapped in foil for 45 min prior to use. Brushes were dipped in inoculum for 2 sec before painting from left to right once and then from up to down once. Brushes were re-dipped for 2 sec and the painting step was repeated. The same brush was used for all *E. coli* O157:H7 samples and a different brush was used for all *L. monocytogenes* samples. Then the surfaces were allowed to dry for 30 min before sampling.

2.4 Sampling Methods

Four different sampling methods were employed during this study. Each method was used according to the manufacturer's instructions as described below.

2.4.1 Swab Method

Sterile cotton swabs (Fisher Scientific, Fairlawn, New Jersey; Figure 2.2a) were used in this study. Samples designated for the swab method were sampled by moistening the cotton swab tip in 9 ml of 0.1% peptone diluent. The excess peptone was removed from the swab by gently pressing the tip of the swab against the side of the test tube. The swab was moved from left to right, up and down, and across one diagonal as shown in Figure 2.2b. The tip of the swab was then broken off into the 9 ml peptone diluent for further dilutions.

2.4.2 Flocked Swab Method

Samples denoted for the nylon flocked swab (Puritan Medical Products Company, LLC, Guilford, Maine; Figure 2.3a) method were sampled by moistening the tip of the swab in 9 ml 0.1% peptone diluent. The excess peptone was removed from the swab by gently pressing the tip of the swab against the side of the test tube. The swab was moved from left to right, up and down, and across one diagonal as shown in Figure 2.3b. The tip of the swab was then broken off into the 9 ml peptone diluent for further dilutions.

2.4.3 Sponge Method

Sponges (Whirlpack, Fort Atkinson, Wisconsin; Figure 2.4a) were used by adding 25 ml of 0.1% peptone diluent to the sponge bags. The excess peptone was gently squeezed out of the sponge in the bag. The sponge was moved left to right, up and down, and across one diagonal as shown in Figure 2.4b then returned to the bag for subsequent dilutions.

2.4.4 M-Vac Method

The M-Vac (Microbial-Vac Systems®, Inc., Bluffdale, Utah; Figure 2.5a) samples were sampled with a sterile sampling head by moving the head in an up and down motion as shown in Figure 2.5b. 25 ml of Butterfield's Buffer Solution was pulled by vacuum force through each sample. This solution was then used for further dilutions.

2.5 Staining and Microscopy

After plating, dry surfaces were stained using LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit (Invitrogen, Chicago, Illinois). Equal amounts (75 µL) of Component A (SYTO 9 dye in solution with dimethyl sulfoxide) and Component B (propidium iodide in solution with dimethyl sulfoxide) were mixed thoroughly. The

Component A and B mixture was combined with 2 mL of sterile deionized (DI) water. Each surface was stained with 1 ml of this mixture. Stained surfaces were then incubated at room temperature in the absence of light for 15 minutes. Excess stain was removed and samples were allowed to dry completely overnight in the absence of light before being viewed under the confocal laser scanning microscope (CLSM).

The Zeiss LSM 5 Pascal (Carl Zeiss Microbiology, Inc., Thornwood, NY) microscope was used. Six 1 mm by 1 mm random and representative images were taken of each sample.

2.6 Procedure

For determining which sampling method recovers the most bacteria from each surface, a set of eleven experimental combinations was designed:

1. Uninoculated Unsampled
2. Inoculated *L. monocytogenes* Unsampled
3. Inoculated *L. monocytogenes* Swab Sampled
4. Inoculated *L. monocytogenes* Flocked Swab Sampled
5. Inoculated *L. monocytogenes* Sponge Sampled
6. Inoculated *L. monocytogenes* M-Vac Sampled
7. Inoculated *E. coli* O157:H7 Unsampled
8. Inoculated *E. coli* O157:H7 Swab Sampled
9. Inoculated *E. coli* O157:H7 Flocked Swab Sampled
10. Inoculated *E. coli* O157:H7 Sponge Sampled
11. Inoculated *E. coli* O157:H7 M-Vac Sampled

Each of these scenarios for each bacterium was performed during the same sampling period. Each of the 11 sample sets also included one of each of the surfaces sampled (ultra high density polypropylene, 304 stainless steel with a 2B finish, and 304 stainless steel with a 2B finish and a buffed surface).

Samples were inoculated as described previously. The uninoculated surfaces were used as an initial sample for the CLSM. Once the inoculated samples were dry, sampling took place as described earlier. *L. monocytogenes* samples were appropriately diluted and plated in duplicate onto TSA and MOX. Meanwhile, *E. coli* O157:H7 samples were properly diluted and plated in duplicate onto TSA and MSA. After plating, all surfaces were stained and viewed under the laser scanning confocal microscope as formerly described.

2.7 Statistical Analysis

Three surfaces and four surface sampling methods were studied in the experiment. Each of the three surfaces was sampled with each of the four sampling methods for a total of twelve different combinations. Thus, the treatment structure was a 3 × 4 factorial with three surfaces (ultra high density polypropylene, 304 stainless steel with a 2B finish, and 304 stainless steel with a 2B finish and a buffed surface) and four surface sampling methods (swab, sponge, flocked swab, and M-Vac). The mixed procedure of SAS (2003) was used to perform type-3 tests of fixed effects for all variables. Least squares means for protected F-tests ($P < 0.05$) were separated by using least significant differences (LSD, $P < 0.05$). Denominator degrees of freedom were estimated by using the default contain method as there was no missing data.

Figure 2.1 Surfaces sampled and paintbrush used.

Figure 2.1a – Ultra High Density Polypropylene (10 × 10 cm^2).

Figure 2.1b – 304 Stainless Steel; 2B Finish (10 × 10 cm^2).

Figure 2.1c – 304 Stainless Steel; 2B Finish; Buffed (10 × 10 cm^2).

Figure 2.1d – Paintbrush (63 mm).

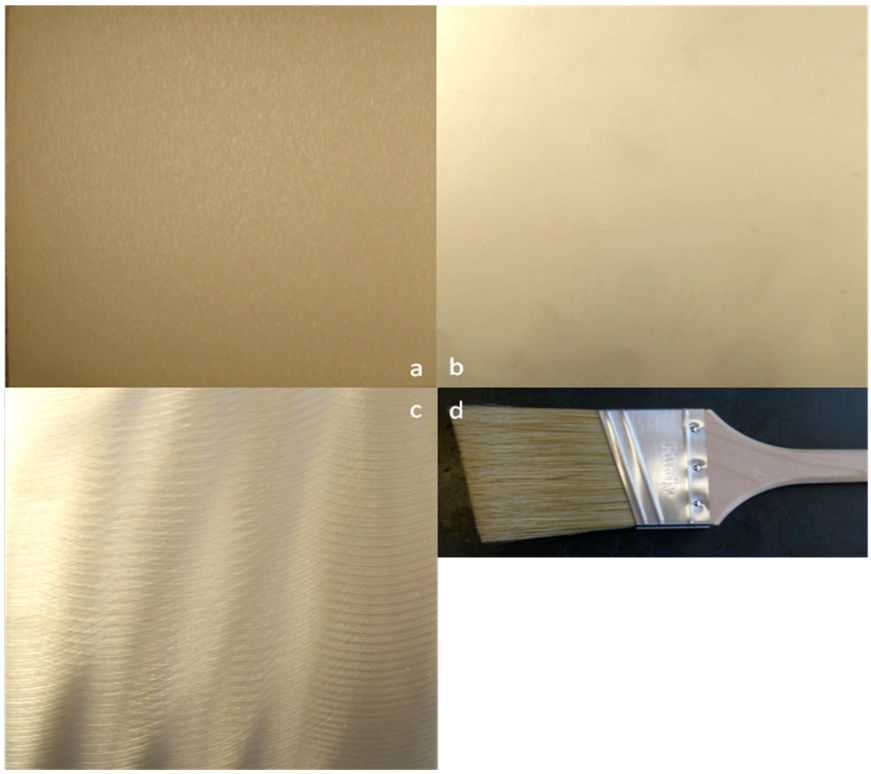
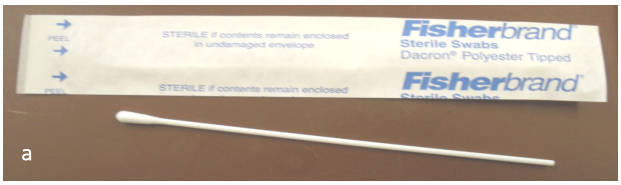


Figure 2.2 Swab sampling methods used.

Figure 2.2a – Swab.

Figure 2.2b – Swab Directional Motions (10 × 10 cm^2).



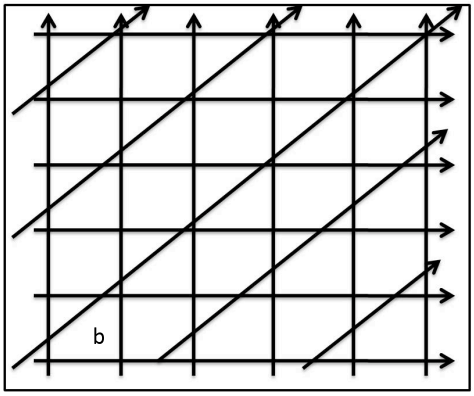


Figure 2.3 Flocked swab sampling methods used.

Figure 2.3a – Flocked Swab.

Figure 2.3b – Flocked Swab Directional Motions (10 × 10 cm^2).

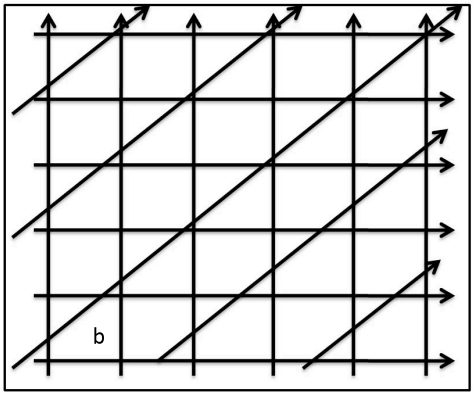


Figure 2.4 Sponge sampling methods used.

Figure 2.4a – Sponge.

Figure 2.4b – Sponge Directional Motions (10 × 10 cm^2).

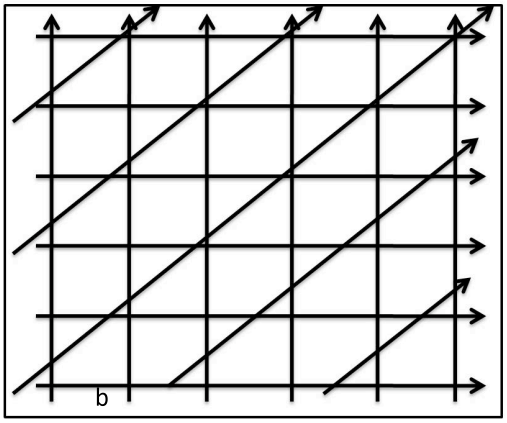
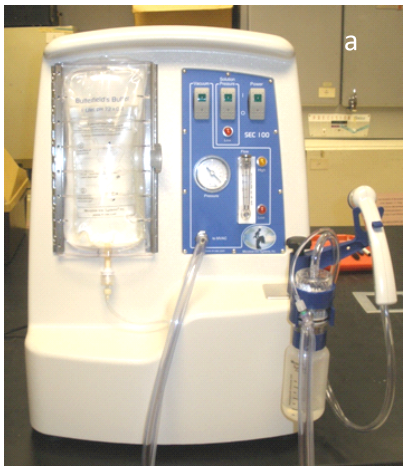
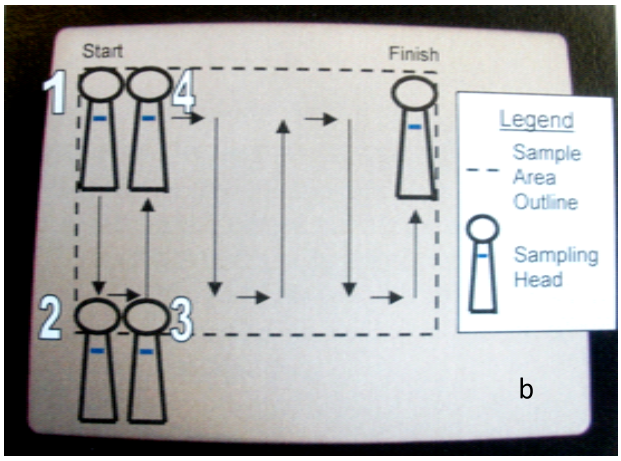


Figure 2.5 M-Vac sampling methods used.

Figure 2.5a – M-Vac.

Figure 2.5b – M-Vac Directional Motions (10 × 10 cm^2).





CHAPTER 3 - Results and Discussion

3.1 *Escherichia coli* O157:H7

When looking at *E. coli* O157:H7 and the sampling methods of swab, flocked swab, sponge, and M-Vac the swab method recovered statistically fewer ($P < 0.05$) microorganisms than the flocked swab, sponge, and M-Vac. The later three methods each recovered statistically similar ($P > 0.05$) numbers of bacteria. The sponge recovered, in general, greater numbers of bacteria from all surfaces while the swab recovered the least.

3.1.1 Ultra High Density Polypropylene

Sampling methods reflected similar results for samples plated on Tryptic Soy Agar (TSA) and MacConkey Sorbitol Agar (MSA). In both cases, the sponge (5.70 and 5.62 $\log_{10}$ CFU/cm^2, respectively), M-Vac (4.95 and 4.92 $\log_{10}$ CFU/cm^2, respectively), and flocked swab (5.03 and 4.91 $\log_{10}$ CFU/cm^2, respectively) showed similar results ($P > 0.05$). In addition, the swab (4.05 and 3.97 $\log_{10}$ CFU/cm^2, respectively), flocked swab, and M-Vac were similar ($P > 0.05$). However, the sponge and the swab differed ($P < 0.05$). These similarities and differences are shown in Figure 3.1. When samples were plated onto MSA versus TSA there were more recovered bacteria from the non-selective TSA than from the selective MSA as was expected. This indicates injured microorganisms.

3.1.2 304 Stainless Steel with a 2B Finish

Figure 3.2 shows the sponge (4.85 – TSA and 4.62 – MSA $\log_{10}$ CFU/cm^2) and flocked swab (4.77 – TSA and 4.39 – MSA $\log_{10}$ CFU/cm^2) were the best methods to

recover bacteria from the stainless steel used in this study. There was no significant difference ($P > 0.05$) among the sponge, flocked swab, and M-Vac (4.29 – TSA and 4.22 – MSA $\log_{10}$ CFU/cm^2) or between the M-Vac and the swab (3.28 – TSA and 3.07 – MSA $\log_{10}$ CFU/cm^2). There was a significant difference ($P < 0.05$) between the sponge and swab and the flocked swab and swab. As was seen before, counts were higher when samples were plated onto TSA rather than MSA; again, indicating injured cells.

3.1.3 304 Stainless Steel with a 2B Finish and a Buffed Surface

There were no significant differences ($P > 0.05$) among any of the samples when plated onto TSA. Significant differences ($P < 0.05$) were shown between the sponge method (4.93 $\log_{10}$ CFU/cm^2) and the swab (3.60 $\log_{10}$ CFU/cm^2) method when plated onto MSA. Each method except for the flocked swab (4.70 $\log_{10}$ CFU/cm^2 versus 4.97 $\log_{10}$ CFU/cm^2) had a higher bacterial recovery rate when plated onto TSA. The flocked swab average recovery rate was the same (4.5 $\log_{10}$ CFU/cm^2) when plated on MSA or TSA. These similarities and differences can be seen in Figure 3.3.

3.2 *Listeria monocytogenes*

The sponge method was the most successful in removing *L. monocytogenes* microorganisms from the surfaces testing. While, like *E. coli* O157:H7, the swab recovered the least amount. The flocked swab and the M-Vac both recovered statistically the same amount ($P > 0.05$) of bacteria regardless of the surface investigated.

3.2.1 Ultra High Density Polypropylene

There were no statistical differences ($P > 0.05$) between any of the methods when the surface tested was the ultra high density polypropylene. There was less than a log difference between the sponge, which recovered the most, and the swab, which recovered

the least number of organisms. These results can be observed in Figure 3.4. There were few injured cells as the counts on the TSA were similar to that on the MOX.

3.2.2 304 Stainless Steel with a 2B Finish

Both the M-Vac (3.60 – TSA and 3.41 – MOX $\log_{10}$ CFU/cm^2) and the swab (2.91 – TSA and 2.57 – MOX $\log_{10}$ CFU/cm^2) recovered significantly ($P < 0.05$) fewer colony forming units than the sponge (5.08 – TSA and 4.92 – MOX $\log_{10}$ CFU/cm^2). The flocked swab (4.53 – TSA and 3.81 – MOX $\log_{10}$ CFU/cm^2) was similar to each of the other three methods. The 304 stainless steel with a 2B finish surface seemed to injure the *L. monocytogenes* as there was a higher recovery rate from the TSA than that of the MOX. These data can be seen in Figure 3.5.

3.2.3 304 Stainless Steel with a 2B Finish and a Buffed Surface

When plated on TSA, the M-Vac (3.67 $\log_{10}$ CFU/cm^2) and swab (3.02 $\log_{10}$ CFU/cm^2) significantly differed ($P < 0.05$) from the sponge (5.06 $\log_{10}$ CFU/cm^2). The flocked swab (4.06 $\log_{10}$ CFU/cm^2) was similar ($P > 0.05$) to the other three sampling methods. However, when plated on MOX, the only significant difference ($P < 0.05$) seen was between the sponge (5.09 $\log_{10}$ CFU/cm^2) and the swab (3.15 $\log_{10}$ CFU/cm^2) methods. The 304 stainless steel with a 2B finish and a buffed surface appeared to have been very conducive to forming biofilms as the counts from the TSA and MOX suggest that the *L. monocytogenes* was not injured. This can be compared in Figure 3.6.

3.3 Microscopy

Observing the amount of bacteria remaining on the surfaces proved to be very valuable information. The green bacteria were live and the red bacteria were dead. Any

other colors indicate cells absorbed both the green and red dyes and were considered injured.

Control images of *E. coli* O157:H7 and *L. monocytogenes* were taken of inoculated surfaces samples that had not been sampled by any of the four tested methods (swab, flocked swab, sponge, or M-Vac). These inoculated controls are seen in Figures 3.7, 3.8, 3.9, 3.10, 3.11, and 3.12. A set of uninoculated surface samples were also viewed and can be inspected in Figures 3.13, 3.14, and 3.15.

It is clear that the bacteria settled into the buffed surface areas on the 304 stainless steel with a 2B finished and buffed surface. It is evident that the surface of the ultra high density polypropylene was a more textured surface that allowed for a unique pattern of microorganisms to form. The 304 stainless steel with a 2B finish was by far the easiest for both microorganisms to form a biofilm on of the three surfaces tested. This was also found in the study done by Mafu et al. in 1990.

3.3.1 Microscopy – Escherichia coli O157:H7

The imagery confirmed that the swab left the most bacteria on each of the three surfaces. These images can be reviewed in Figures 3.16, 3.17, and 3.18. While it appears that the *E. coli* O157:H7 formed a biofilm, this film does not appear to have lived – thus the red coloring. There are several striation marks on each of the images that seem as if those are the paths that the swab took across the surface samples.

In Figures 3.19, 3.20, and 3.21 the sponge method was corroborated in leaving very few microorganisms on the surfaces. These remaining organisms were also unviable when stained. There was no apparent pattern to the remaining microorganisms.

The flocked swab images more closely resemble that of the swab than the sponge. There are also patterns of a presumptive sampling path across the surfaces. A biofilm can be observed in Figures 3.22, 3.23, and 3.24.

There are differences, however; seen in the viable cell counts and the imaging of the M-Vac samples. While the viable cell counts imply that the imaging of these samples should more closely resemble that of the flocked swab, they in fact are more closely identifiable with those of the sponge. This would indicate that more microorganisms were being collected for plating purposes than were actually being recovered through standard plating methods. These images can be seen in Figures 3.25, 3.26, and 3.27. Lee and Fung (1986) found that about 80% of bacteria were recovered with the vacuum method. These images confirm this.

3.3.2 Microscopy – Listeria monocytogenes

As with the *E. coli* O157:H7, observing the amount of bacteria remaining on the surfaces was very beneficial information. Again, the green bacteria were live and the red bacteria were dead. Any other colors show cells that absorbed both the green and red dyes and were considered injured.

The swab surfaces have the most unrecovered bacteria remaining on each of the surfaces. There are small streaks across the surface samples that seem as if that is where the swab sampled the surface. The organisms are mostly injured given the staining color. These images can be inspected in Figures 3.28, 3.29, and 3.30. A biofilm was clearly formed on these surfaces.

Figures 3.31, 3.32, and 3.33 show images taken of the sponge samples. The sponge samples show far fewer organisms still present corresponding with the viable cell

counts. These organisms are both live and dead and show no clear biofilm formation on the ultra high density polypropylene or 304 stainless steel with a 2B finish surfaces. However, with so few microorganisms this is to be expected. There is a clear biofilm on the 304 stainless steel with a 2B finish and a buffed surface.

Clear biofilm formation is shown in the pictures captured from the flocked swab surface samples. These results closely resemble that of the swab surface samples. *Listeria monocytogenes* can be seen in the pits of the ultra high density polypropylene. The 304 stainless steel with a 2B finish and a buffed surface does not seem to retain as many bacteria. The biofilm formation is very clear on the 304 stainless steel with a 2B finish and is further supported by the mix of live and dead bacteria. These results can be observed in Figures 3.34, 3.35, and 3.36.

As with the *E. coli* O157:H7 results the most dissimilar results are seen between the M-Vac viable cell count and the images retained of the sampled surfaces (Figures 3.37, 3.38, and 3.39). While the viable cell counts would indicate that the remaining *L. monocytogenes* should appear more closely to the swab surfaces test, they in fact show fewer remaining organisms than the sponge. These unusual results were also seen in the *E. coli* O157:H7 images.

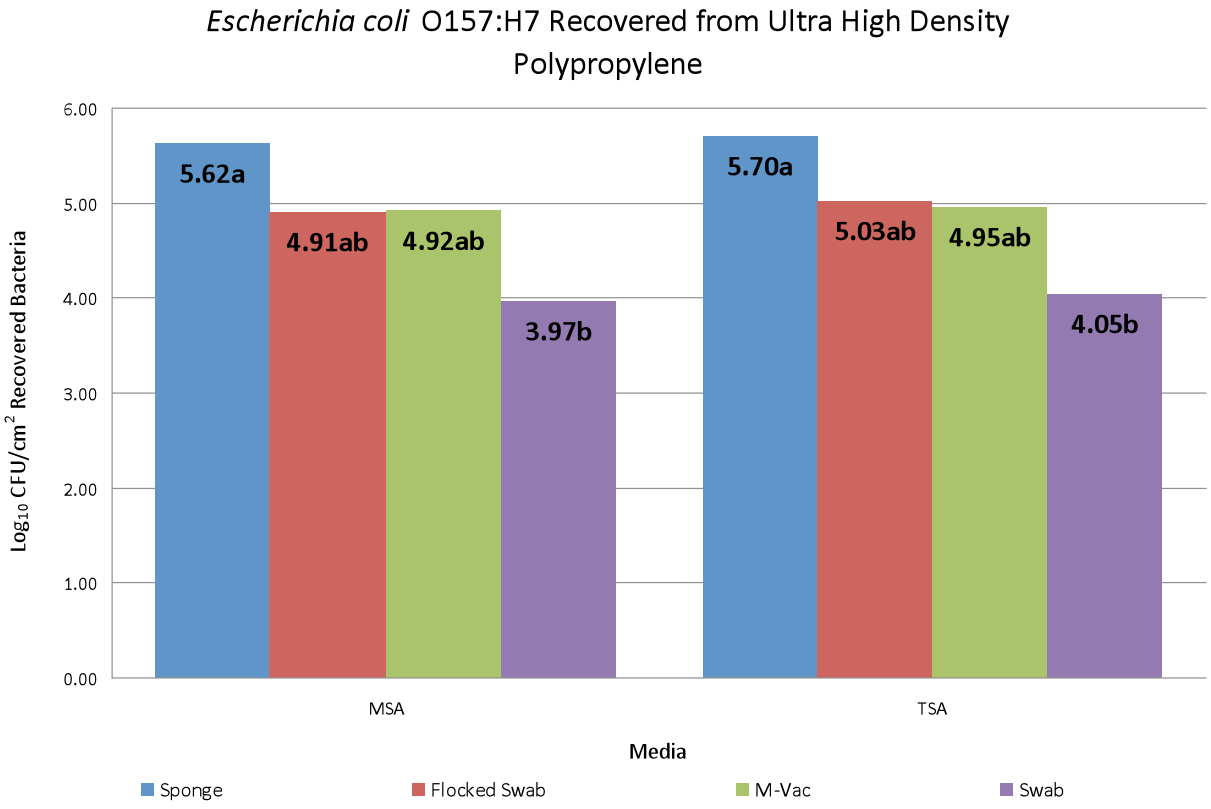
3.3.3 Additional Research

After observing these unusual results, two replications were conducted sampling the using the four initial sampling methods; however, this time the M-Vac sampling head and tubing was also included. To accomplish this, after sampling with the M-Vac, the head and tubing (aseptically cut into 1½ inch pieces) was placed into a sterile stomacher bag (Spiral Biotech, Norwood, MA) with 100 ml of peptone. It was all then pulsified with

the Pulsifier® (Microbiology International, Frederick, MD) for 1 min. This solution was diluted and plated. Only *L. monocytogenes* was tested in this manner so only TSA and MOX were used. Statistical analysis was done the same way as described previously. The average count (log_{10} CFU/cm^2) of the two replications that were done was used as the count for the three replications of the M-Vac tubing samples that were not done. Given more time and resources more replications of both organisms would have been done for better results.

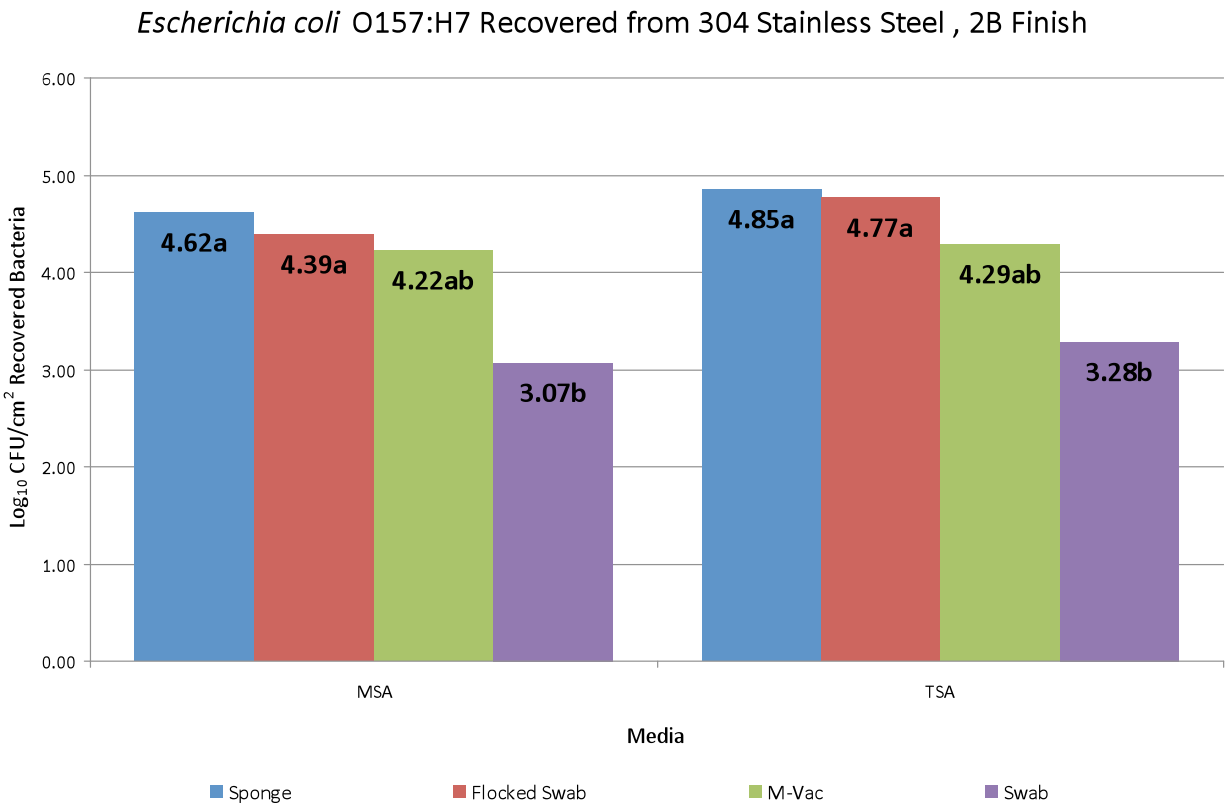
The results of the combined original M-Vac plating with that of the tubing and head show that this is a far superior method in almost every case. The exception is the 304 stainless steel with a 2B finish surface plated onto MOX. There seems to have been an outlier in the data. More replications would help determine this possibility. Comparisons from this extra data can be found in Figures 3.40, 3.41, and 3.42.

Figure 3.1 *Escherichia coli* O157:H7 Sampled from Ultra High Density Polypropylene.



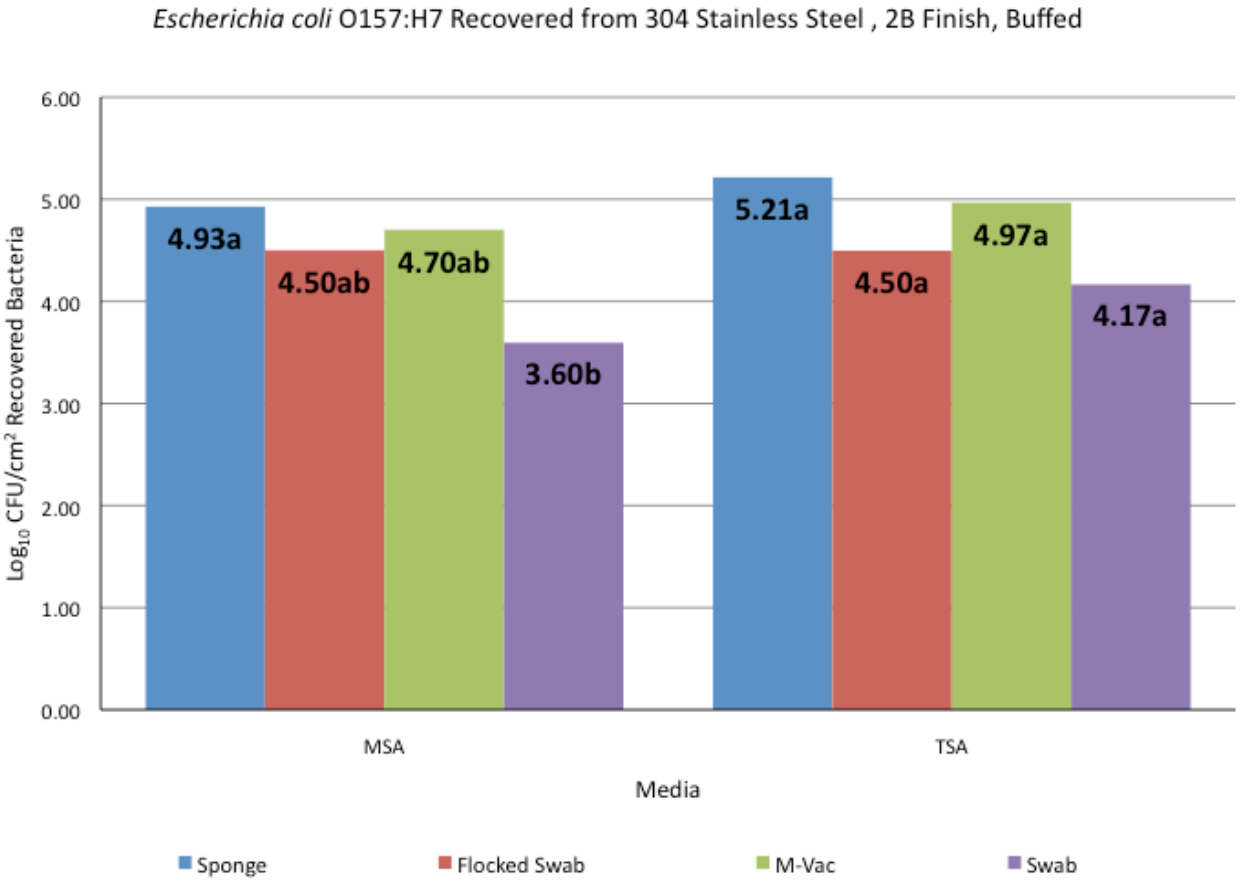
[ab] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.2 *Escherichia coli* O157:H7 Sampled from 304 Stainless Steel with a 2B Finish.



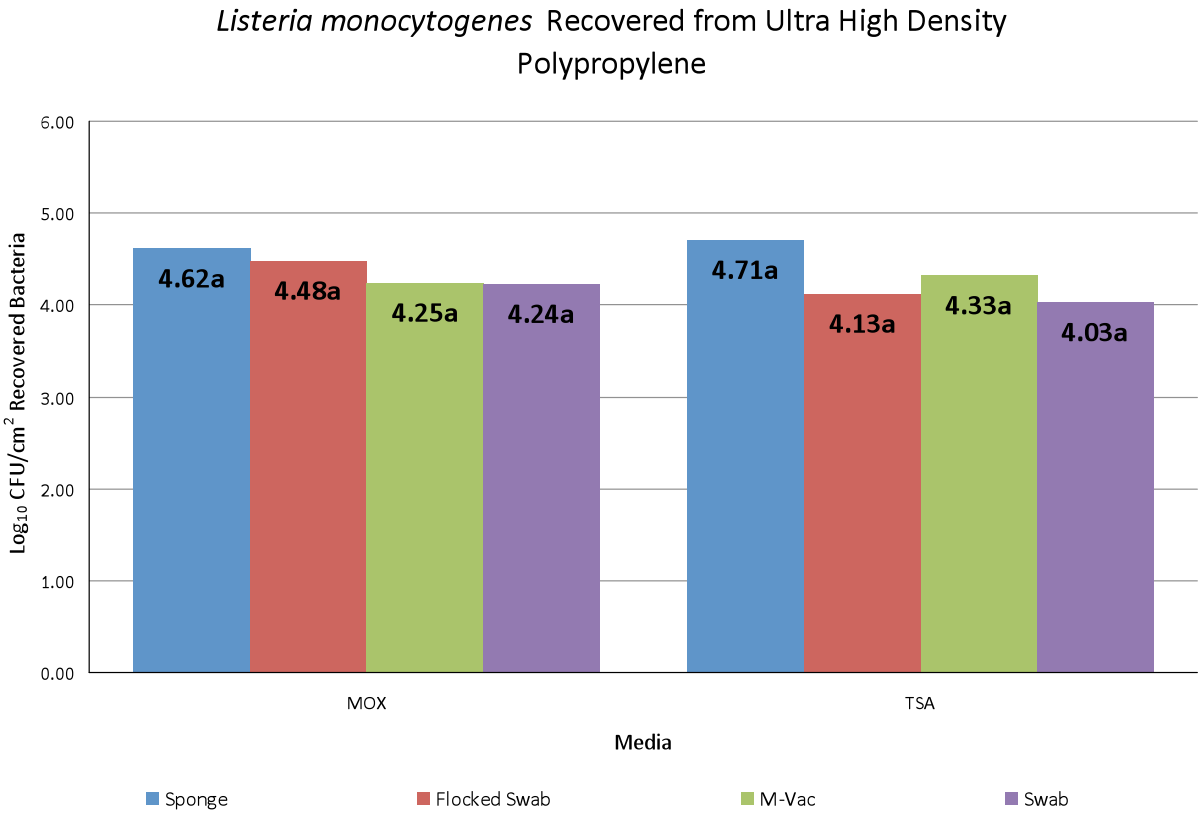
[ab] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.3 *Escherichia coli* O157:H7 Sampled from 304 Stainless Steel with a 2B Finish and a Buffed Surface.



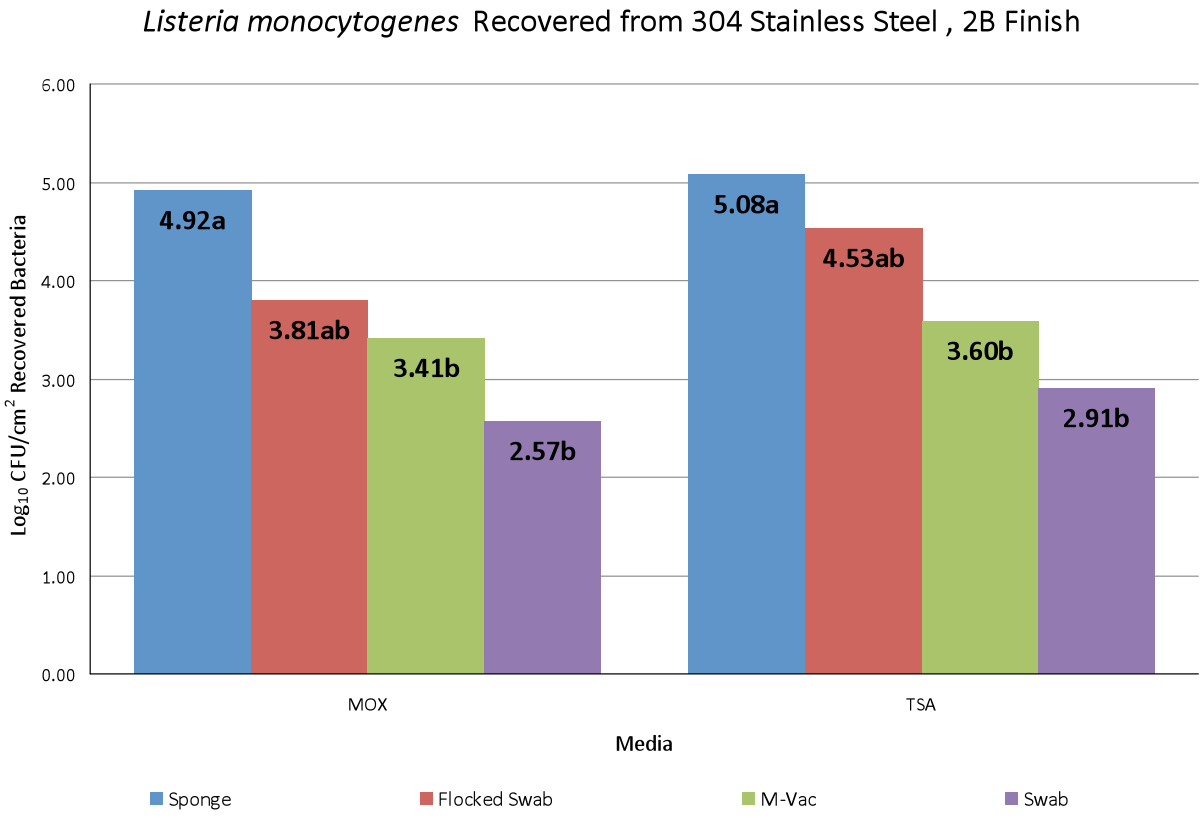
[ab] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.4 *Listeria monocytogenes* Sampled from Ultra High Density Polypropylene.



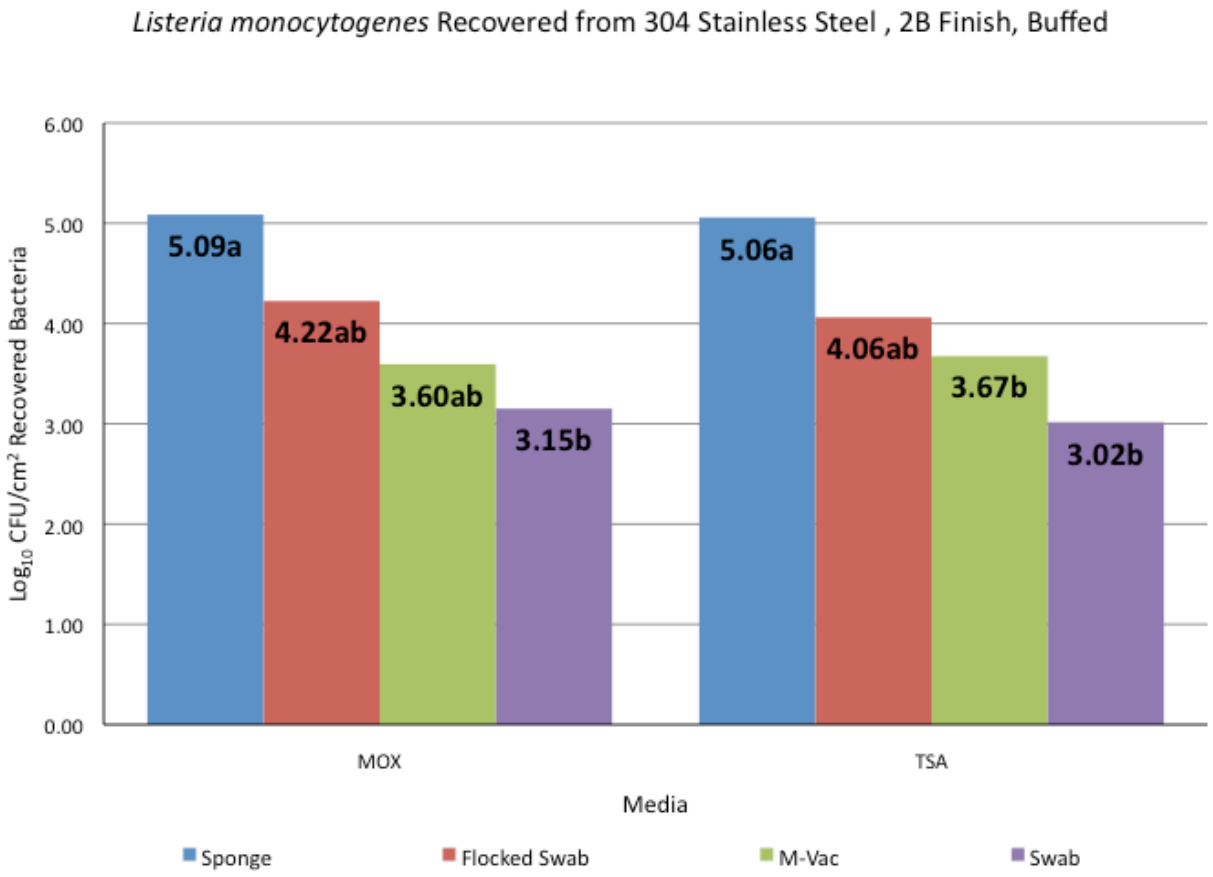
[a] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.5 *Listeria monocytogenes* Sampled from 304 Stainless Steel with a 2B Finish.



[ab] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.6 *Listeria monocytogenes* Sampled from 304 Stainless Steel with a 2B Finish and a Buffed Surface.



[ab] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.7 *Escherichia coli* O157:H7 Inoculated and Unsampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

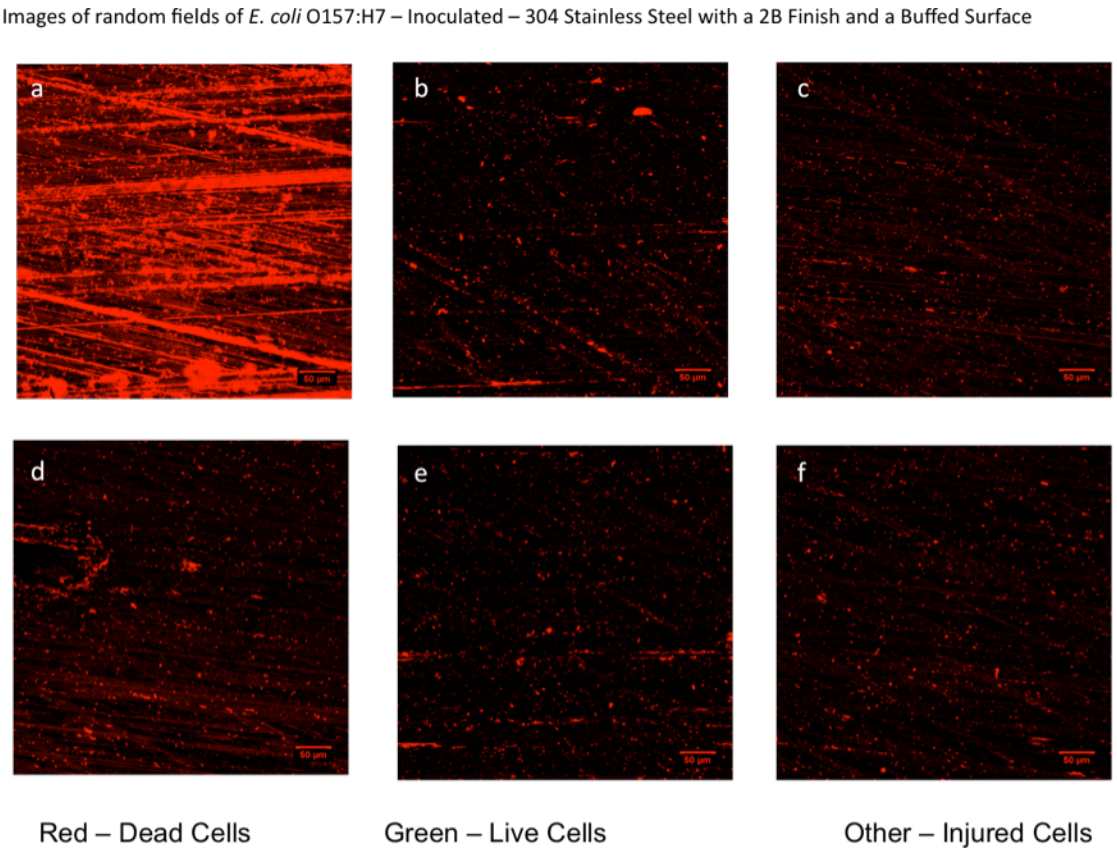


Figure 3.8 *Escherichia coli* O157:H7 Inoculated and Unsampled on Ultra High Density Polypropylene.

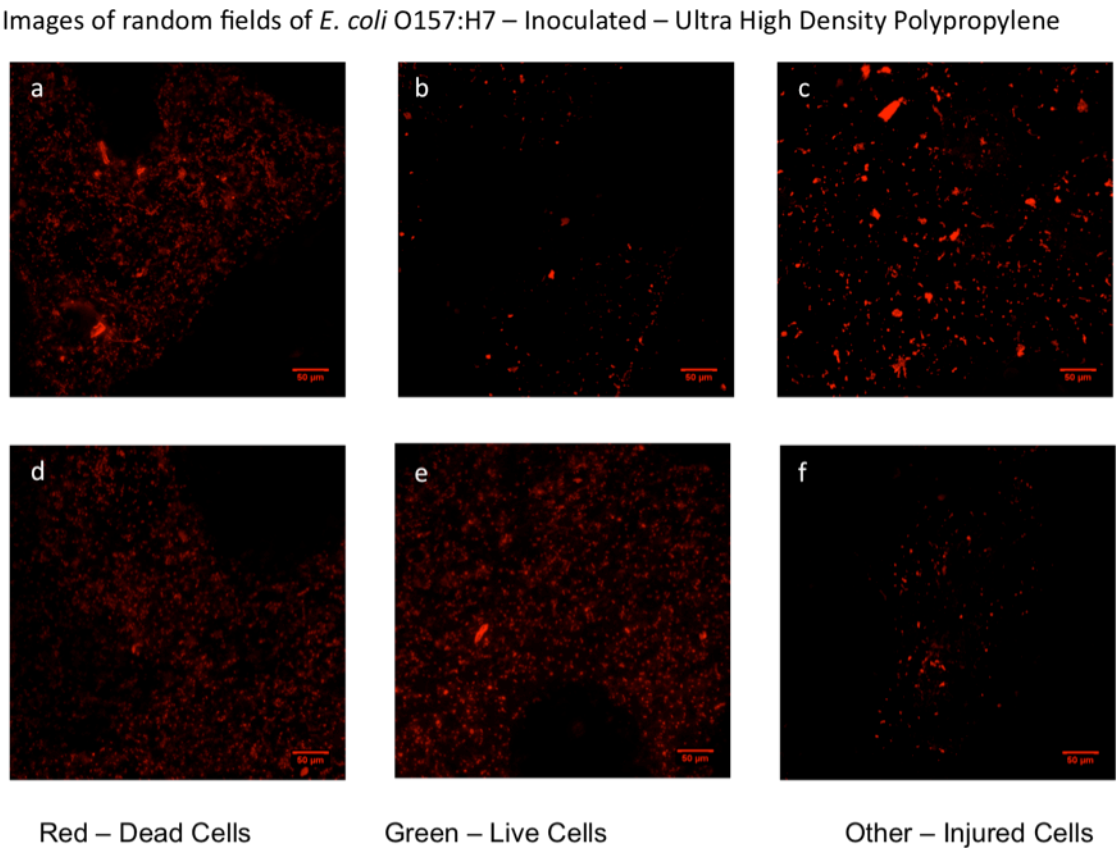


Figure 3.9 *Escherichia coli* O157:H7 Inoculated and Unsampled on 304 Stainless Steel with a 2B Finish.

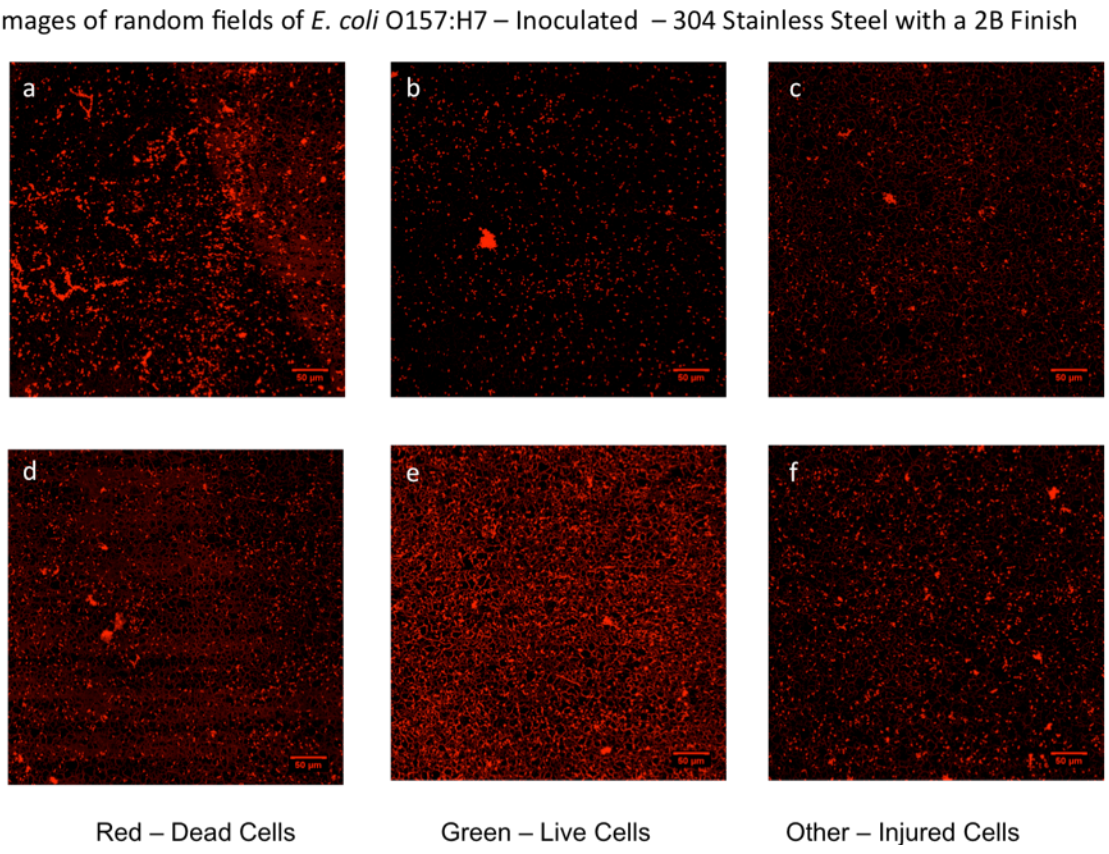
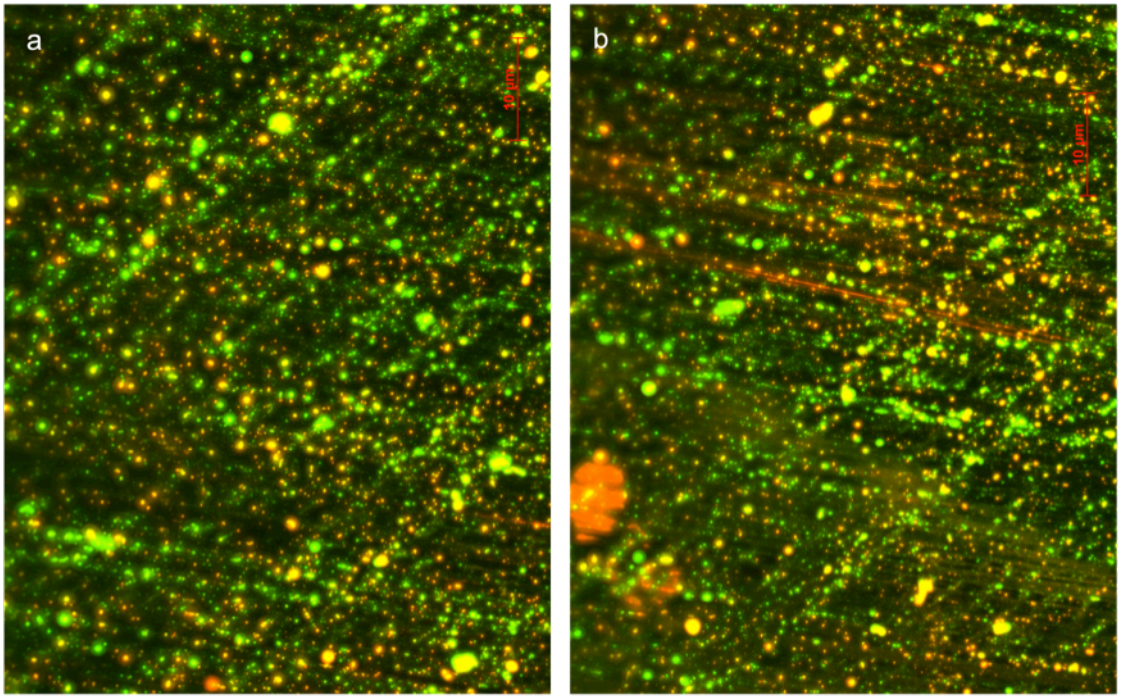


Figure 3.10 *Listeria monocytogenes* Inoculated and Unsampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

Images of random fields of *L. monocytogenes* – Inoculated – 304 Stainless Steel with a 2B Finish and a Buffed Surface



Red – Dead Cells Green – Live Cells Other – Injured Cells

Figure 3.11 *Listeria monocytogenes* Inoculated and Unsampled on Ultra High Density Polypropylene.

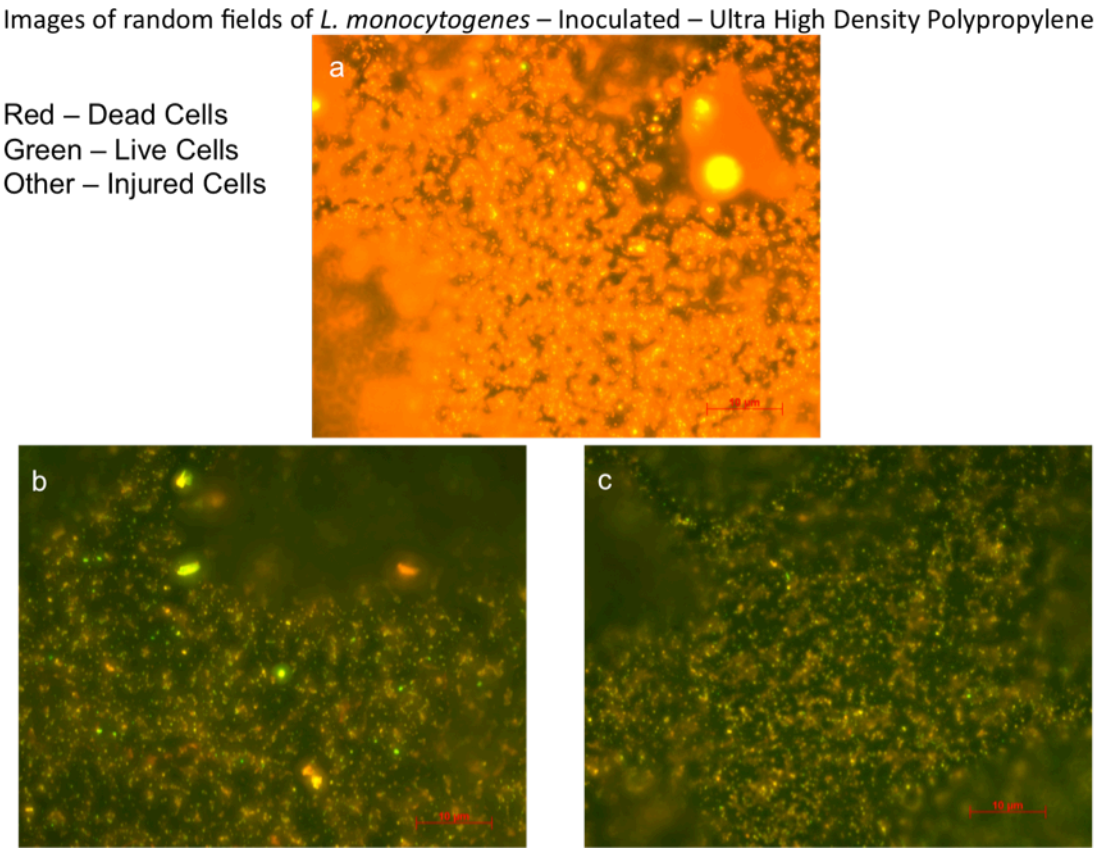


Figure 3.12 *Listeria monocytogenes* Inoculated and Unsampled on 304 Stainless Steel with a 2B Finish.

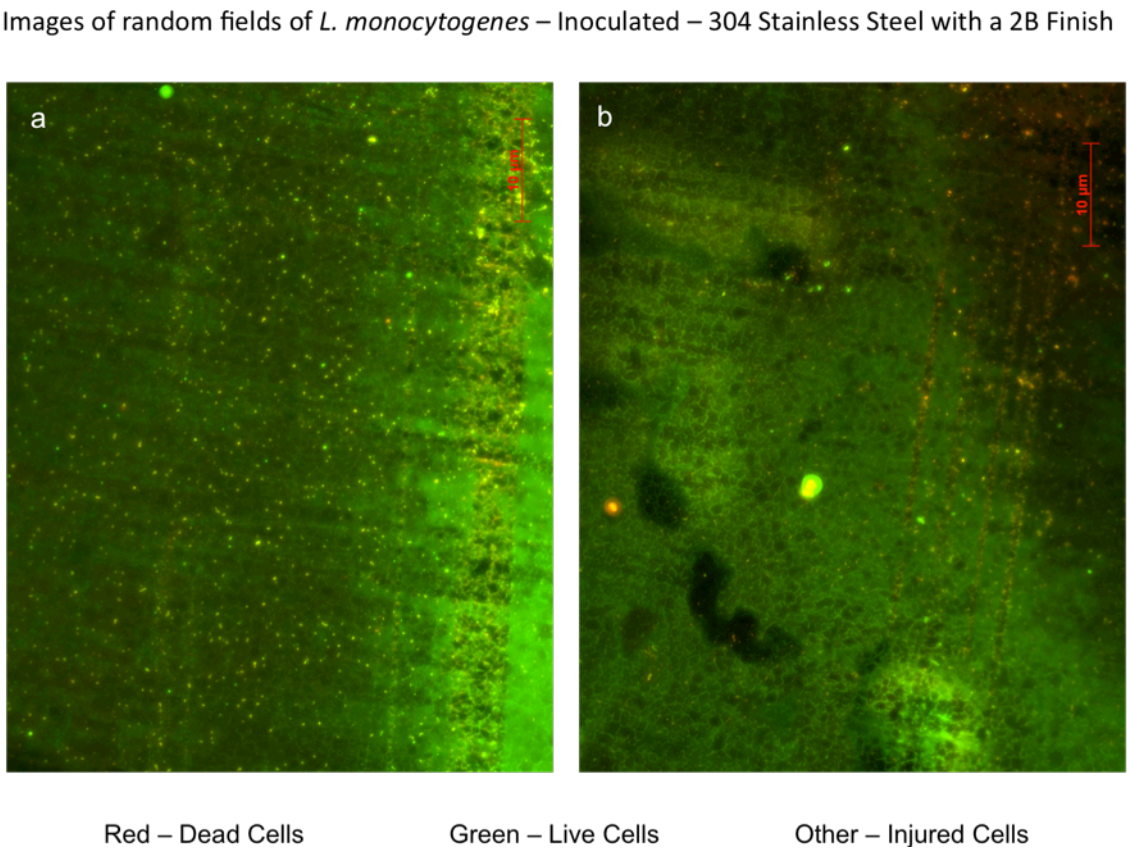


Figure 3.13 Uninoculated and Unsampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

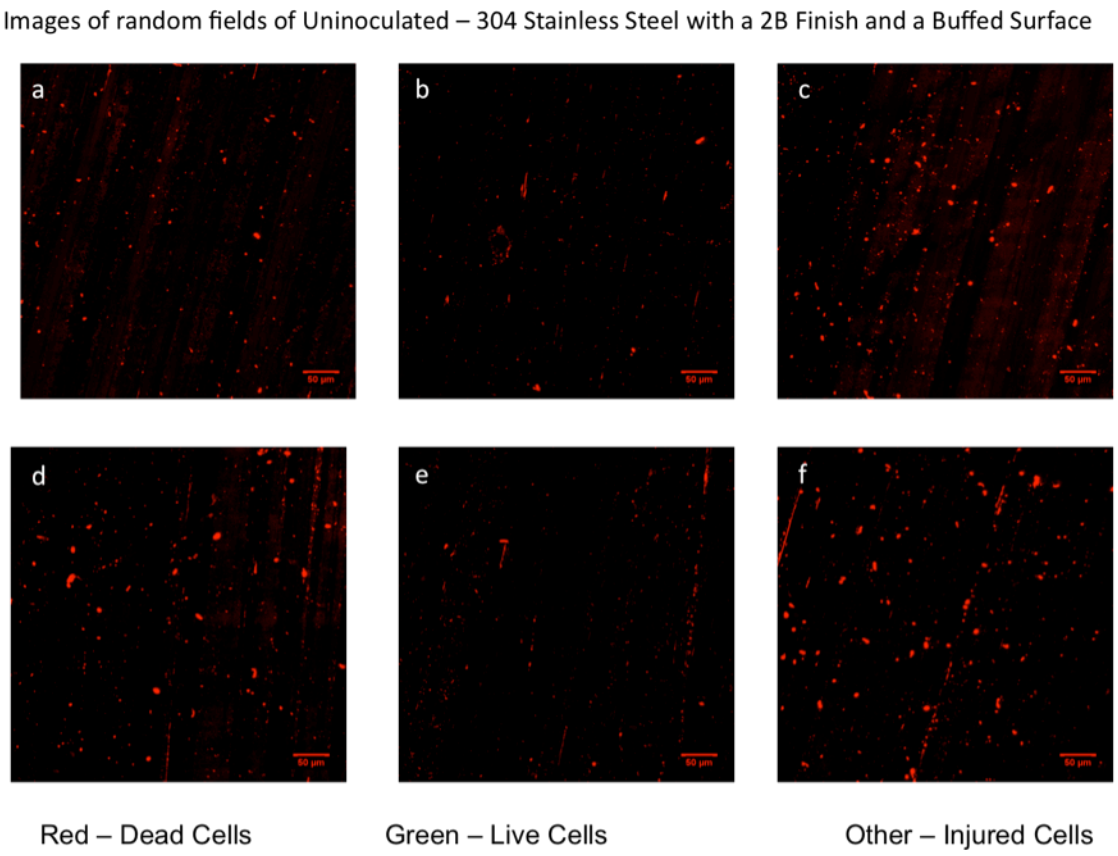


Figure 3.14 Uninoculated and Unsampled on Ultra High Density Polypropylene.

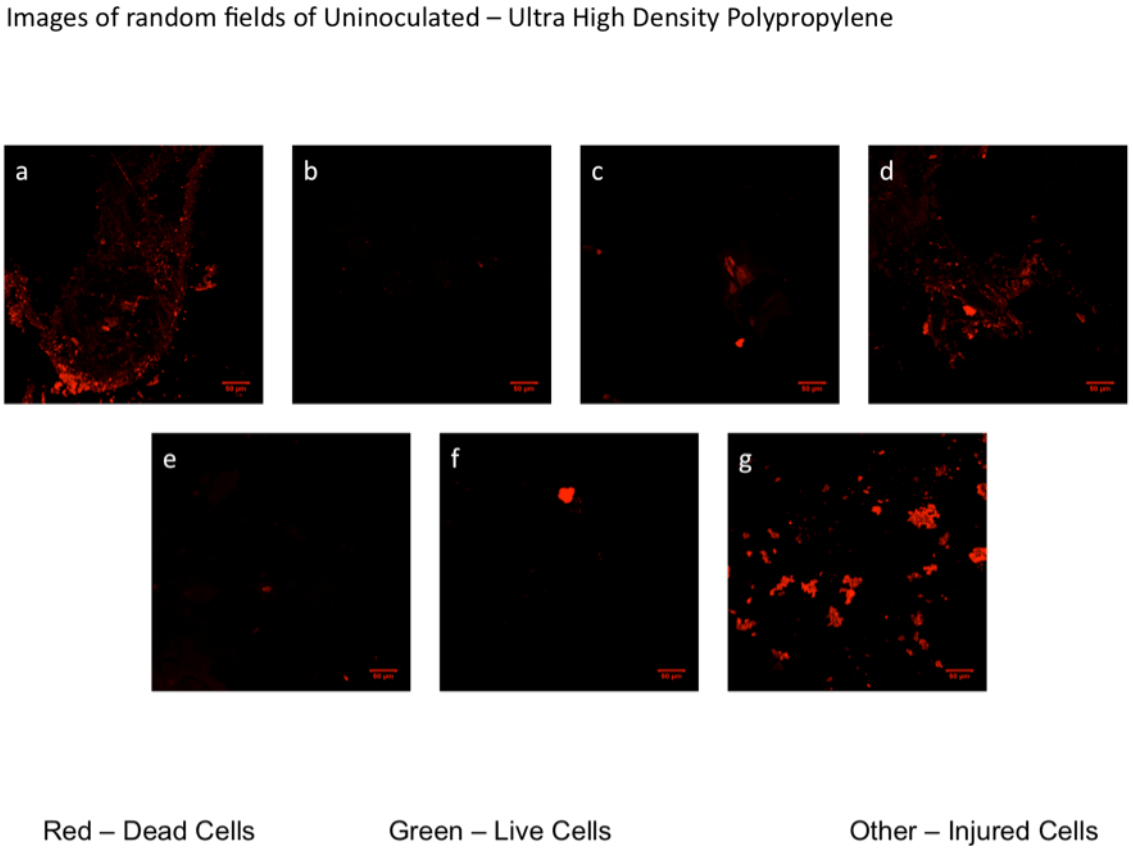


Figure 3.15 Uninoculated and Unsampled on 304 Stainless Steel with a 2B Finish.

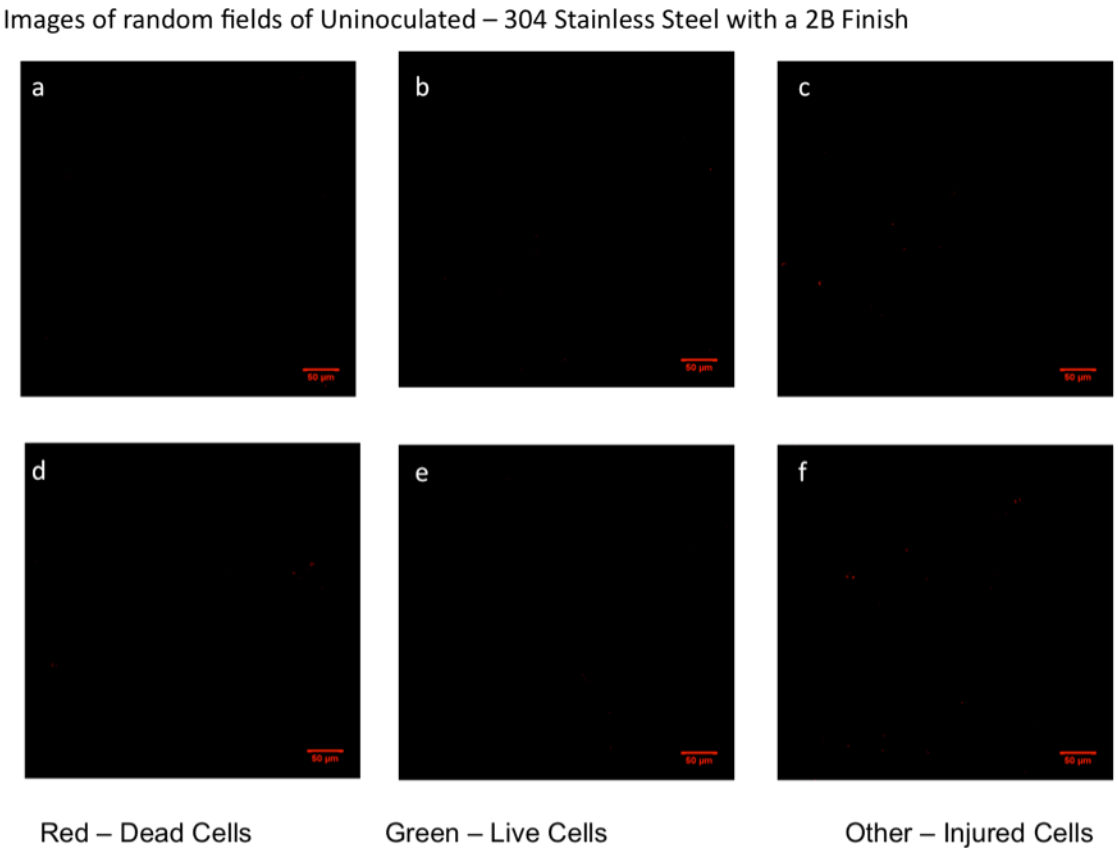


Figure 3.16 *Escherichia coli* O157:H7 Inoculated and Swab Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

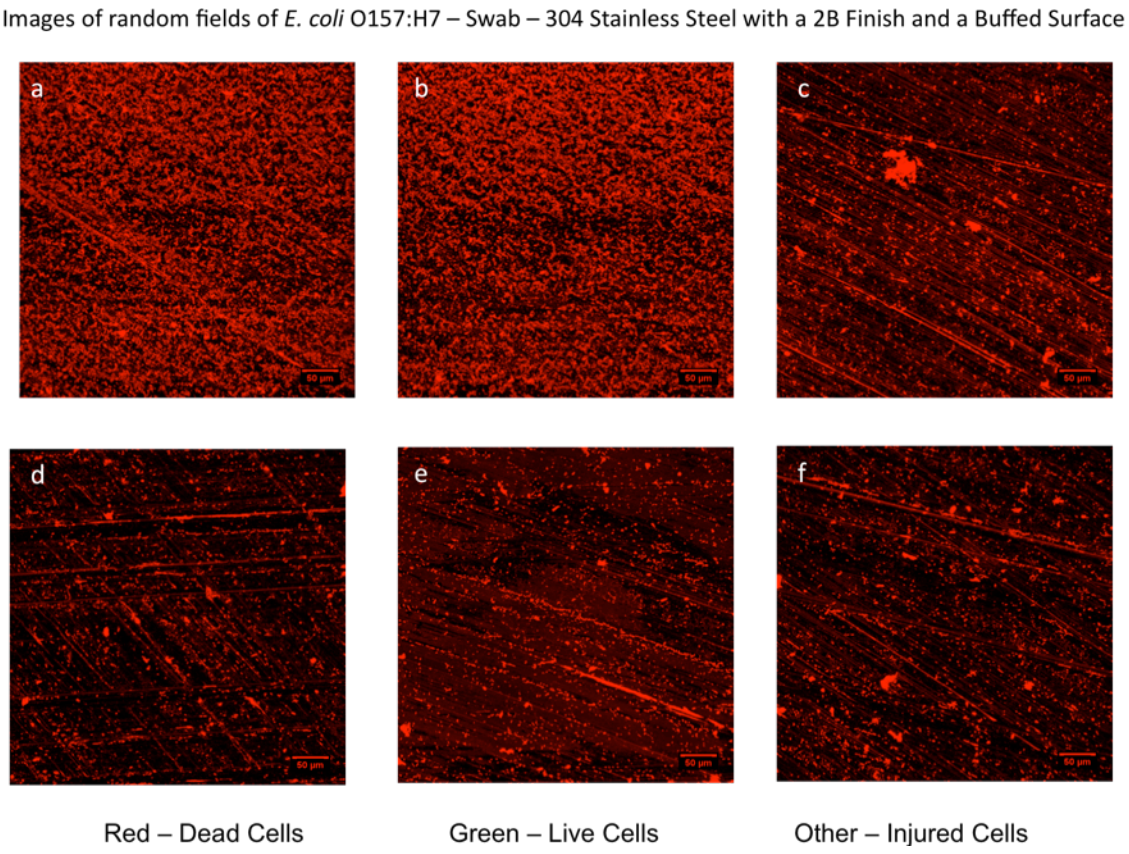


Figure 3.17 *Escherichia coli* O157:H7 Inoculated and Swab Sampled on Ultra High Density Polypropylene.

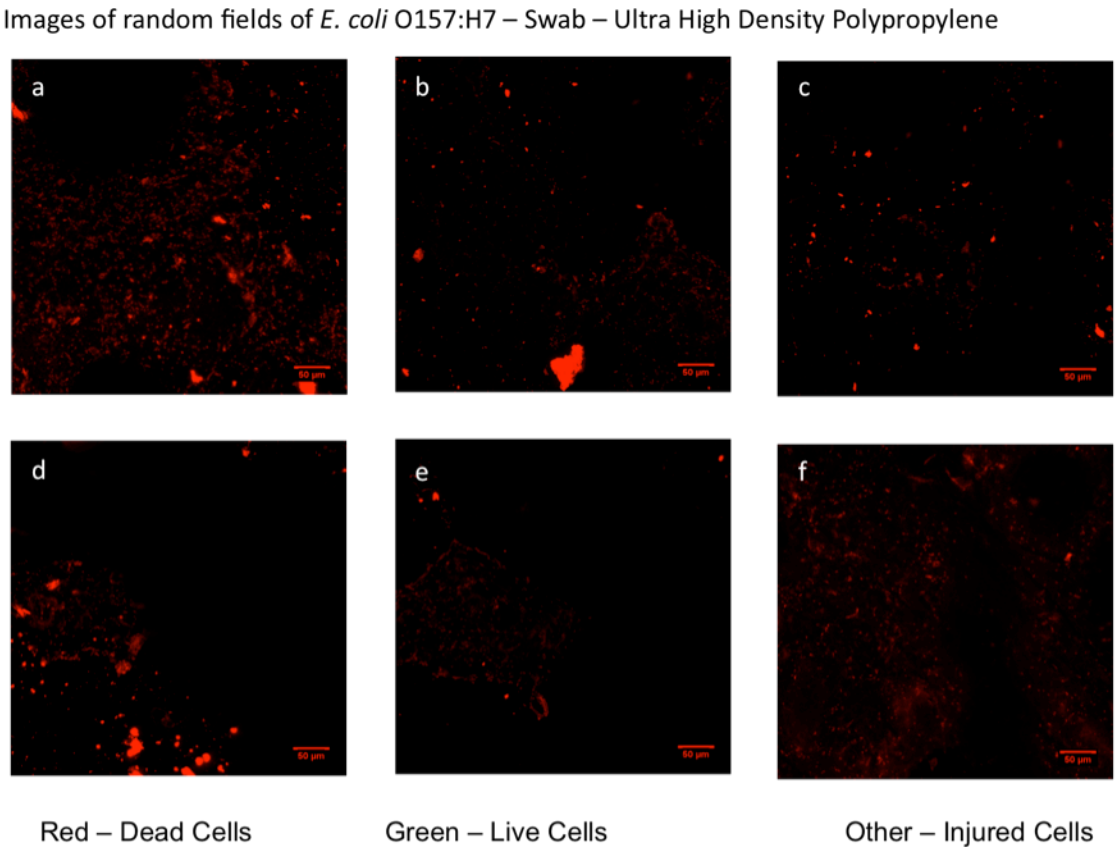


Figure 3.18 *Escherichia coli* O157:H7 Inoculated and Swab Sampled on 304 Stainless Steel with a 2B Finish.

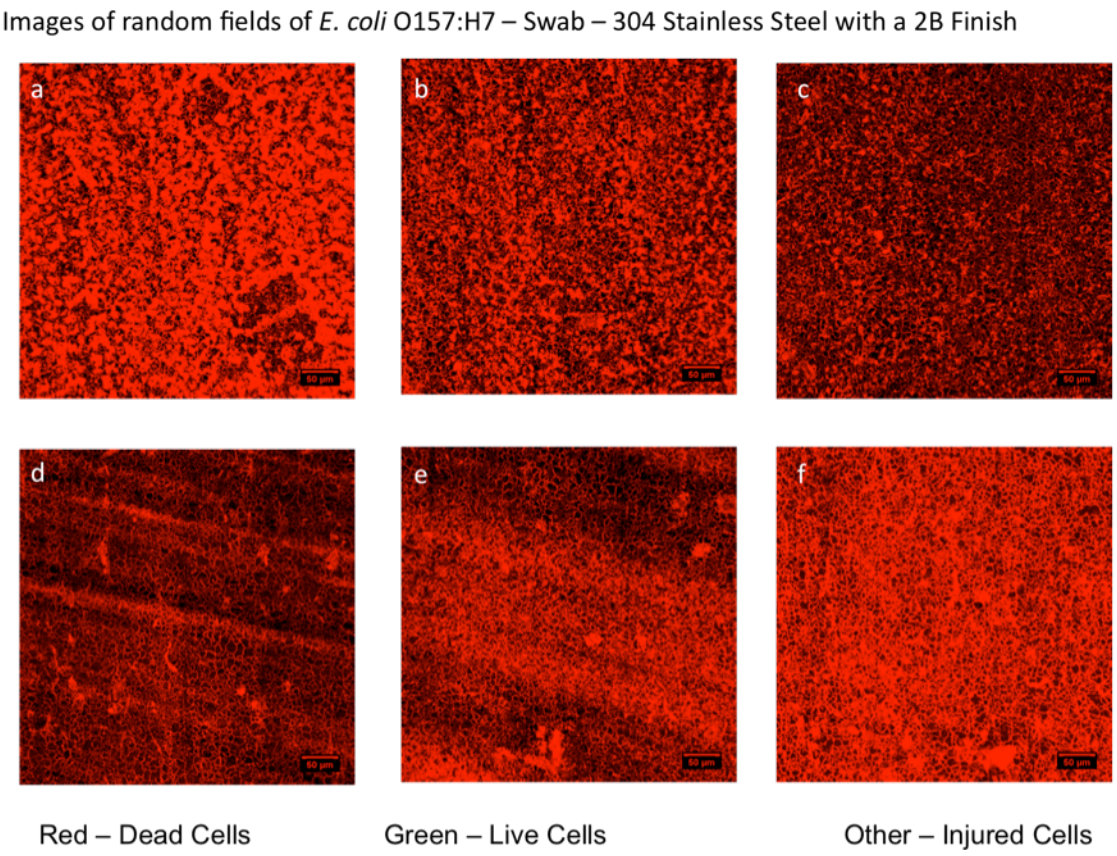


Figure 3.19 *Escherichia coli* O157:H7 Inoculated and Sponge Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

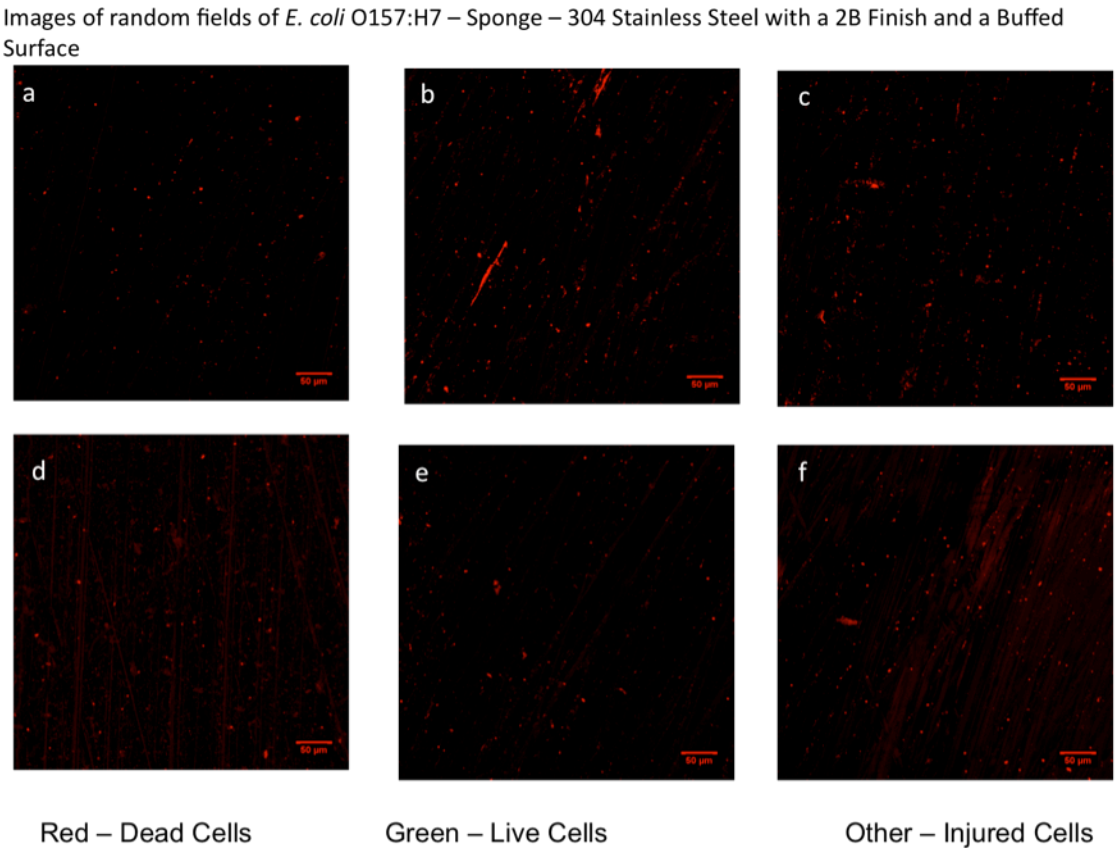


Figure 3.20 *Escherichia coli* O157:H7 Inoculated and Sponge Sampled on Ultra High Density Polypropylene.

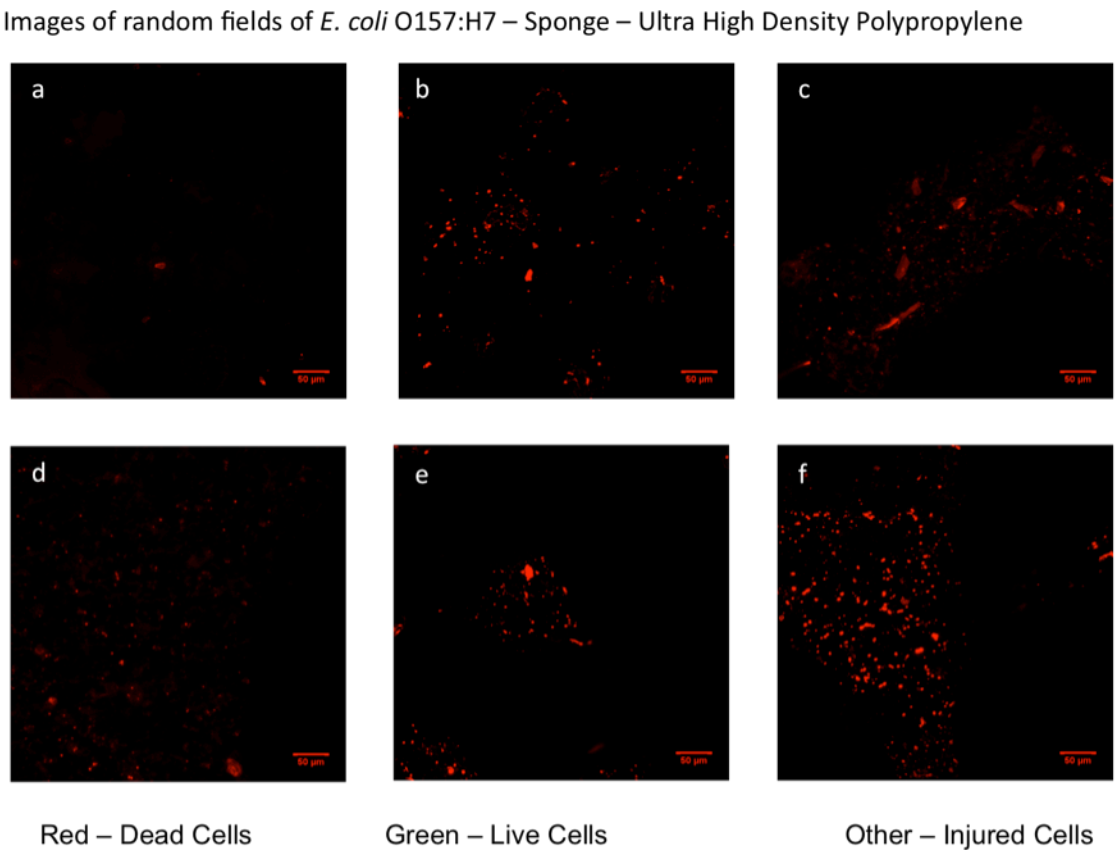


Figure 3.21 *Escherichia coli* O157:H7 Inoculated and Sponge Sampled on 304 Stainless Steel with a 2B Finish.

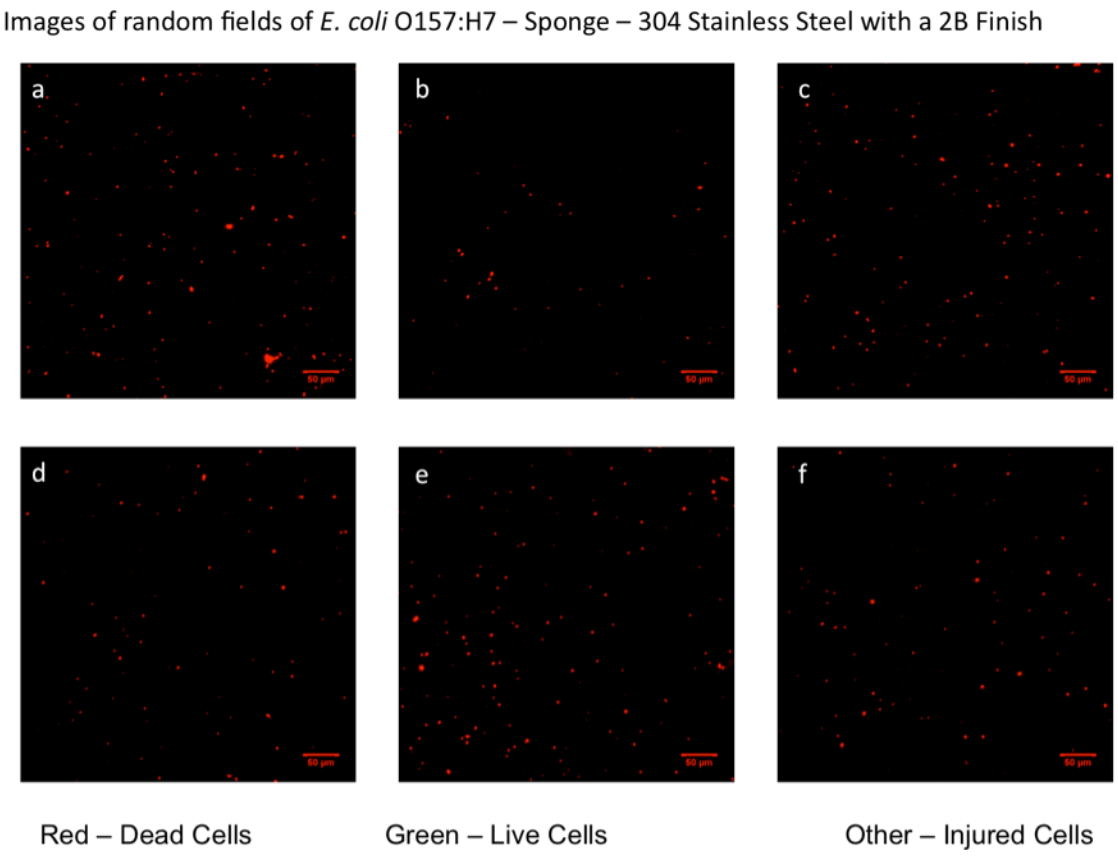


Figure 3.22 *Escherichia coli* O157:H7 Inoculated and Flocked Swab Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

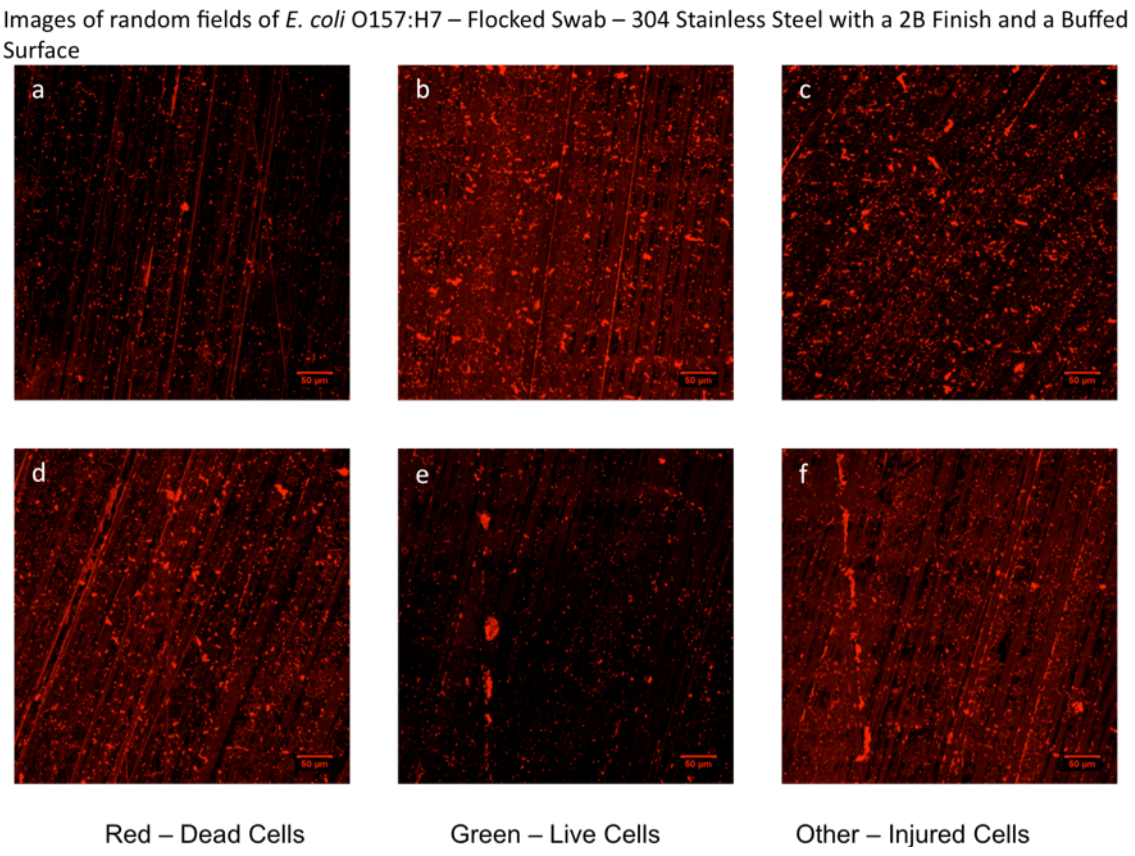


Figure 3.23 *Escherichia coli* O157:H7 Inoculated and Flocked Swab Sampled on Ultra High Density Polypropylene.

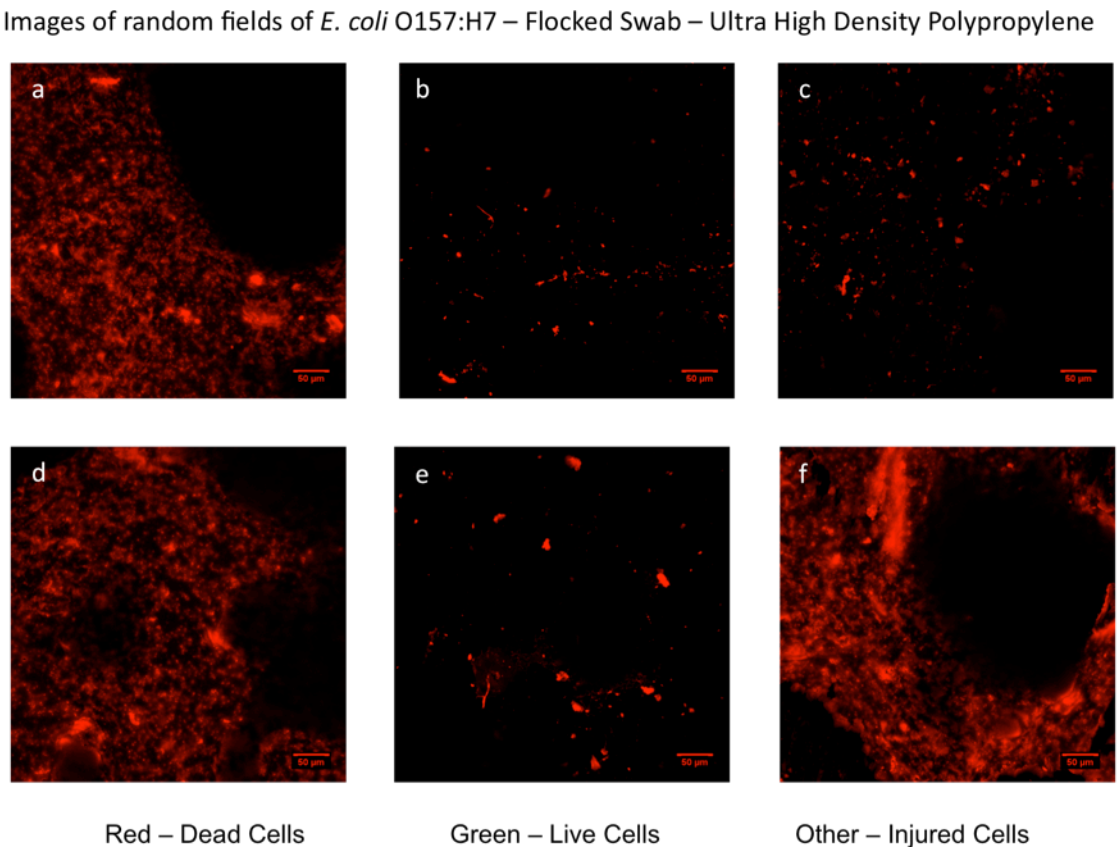


Figure 3.24 *Escherichia coli* O157:H7 Inoculated and Flocked Swab Sampled on 304 Stainless Steel with a 2B Finish.

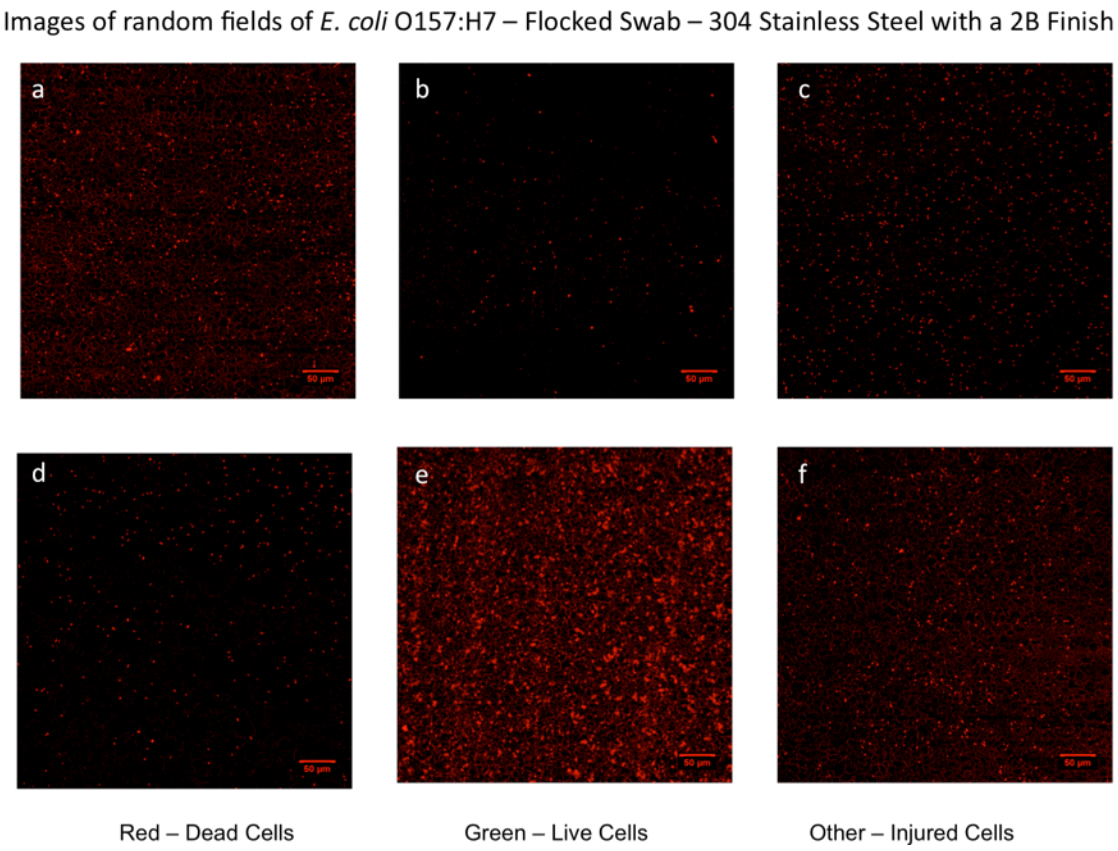


Figure 3.25 *Escherichia coli* O157:H7 Inoculated and M-Vac Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

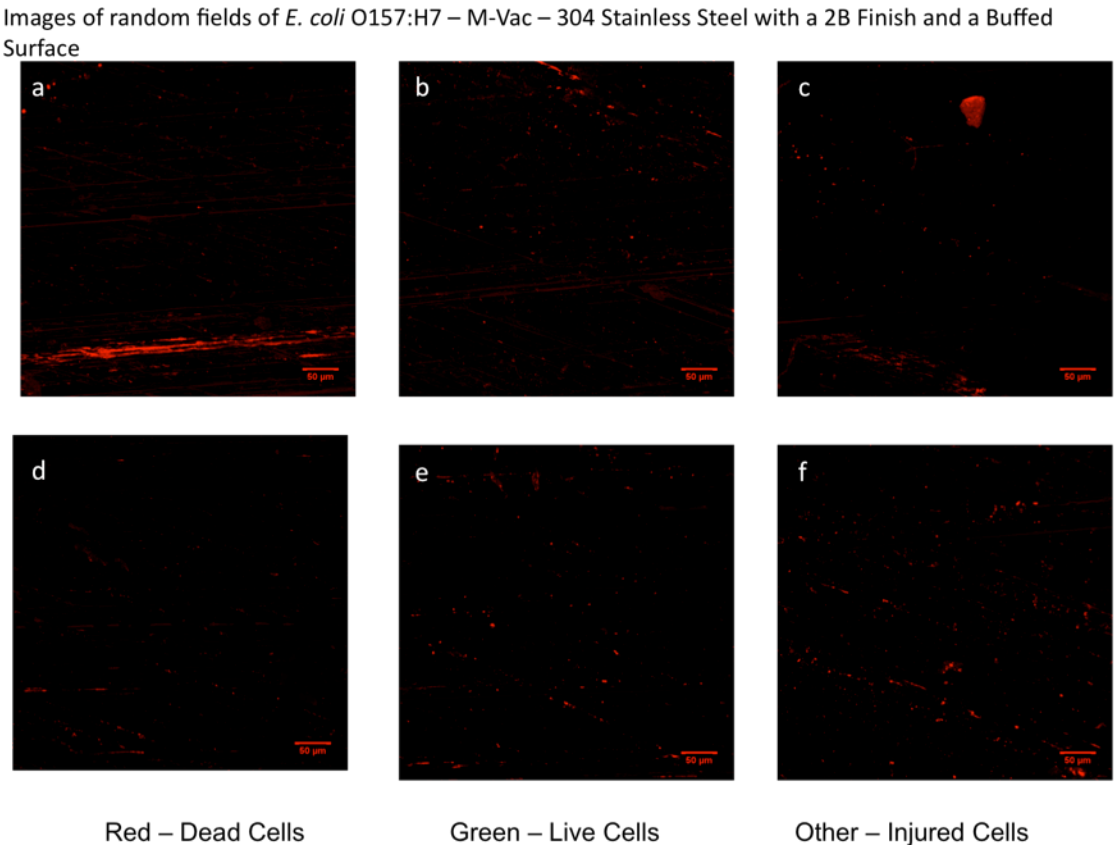


Figure 3.26 *Escherichia coli* O157:H7 Inoculated and M-Vac Sampled on Ultra High Density Polypropylene.

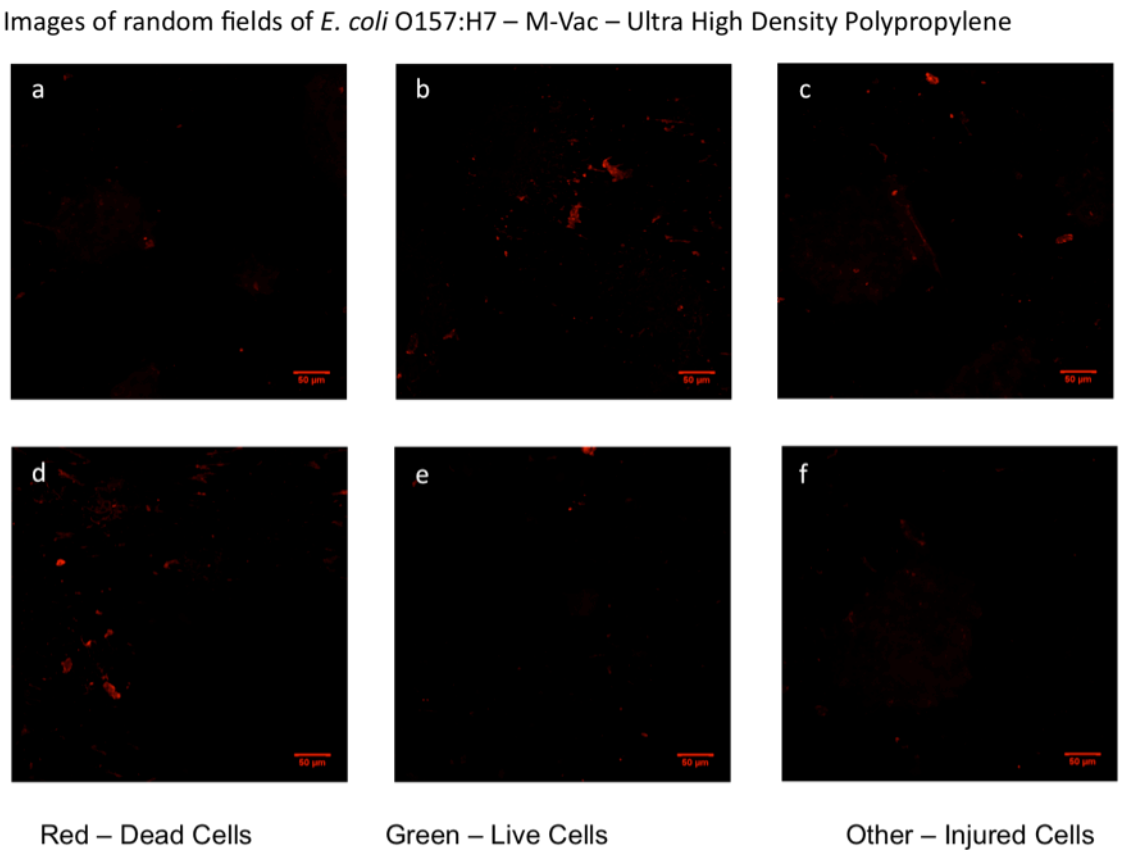


Figure 3.27 *Escherichia coli* O157:H7 Inoculated and M-Vac Sampled on 304 Stainless Steel with a 2B Finish.

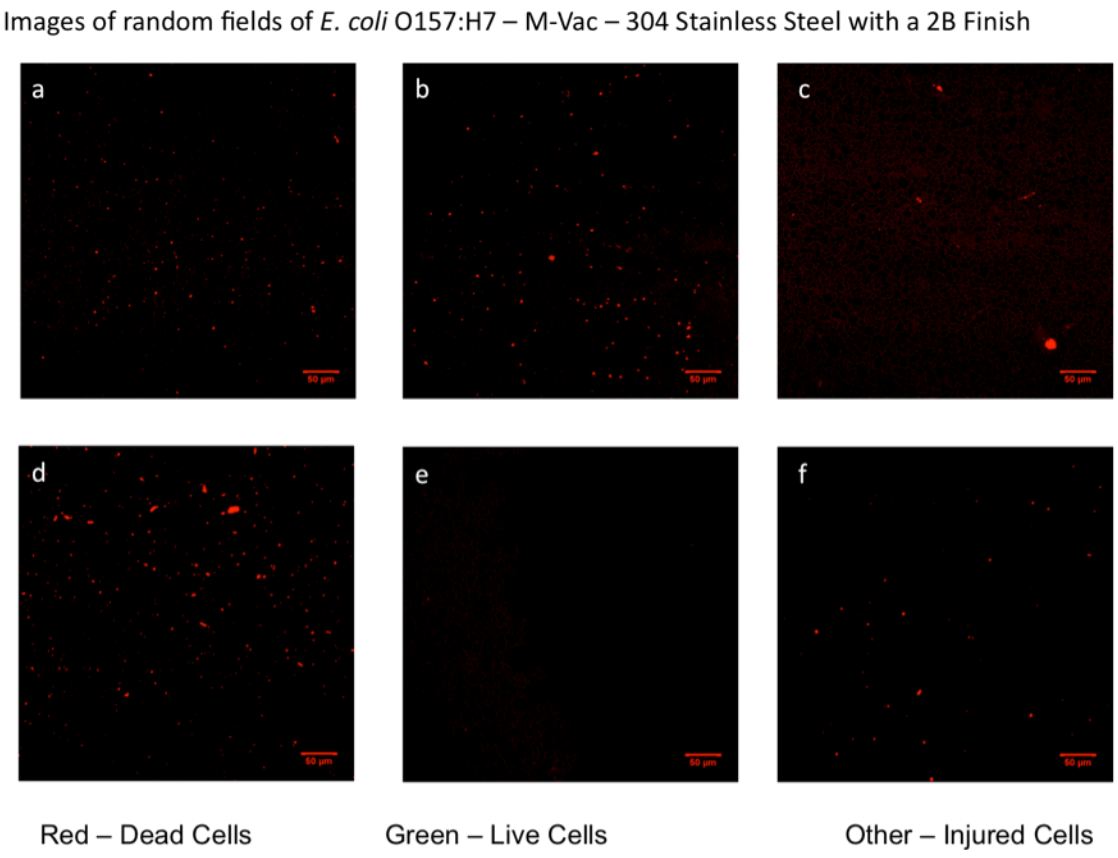


Figure 3.28 *Listeria monocytogenes* Inoculated and Swab Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

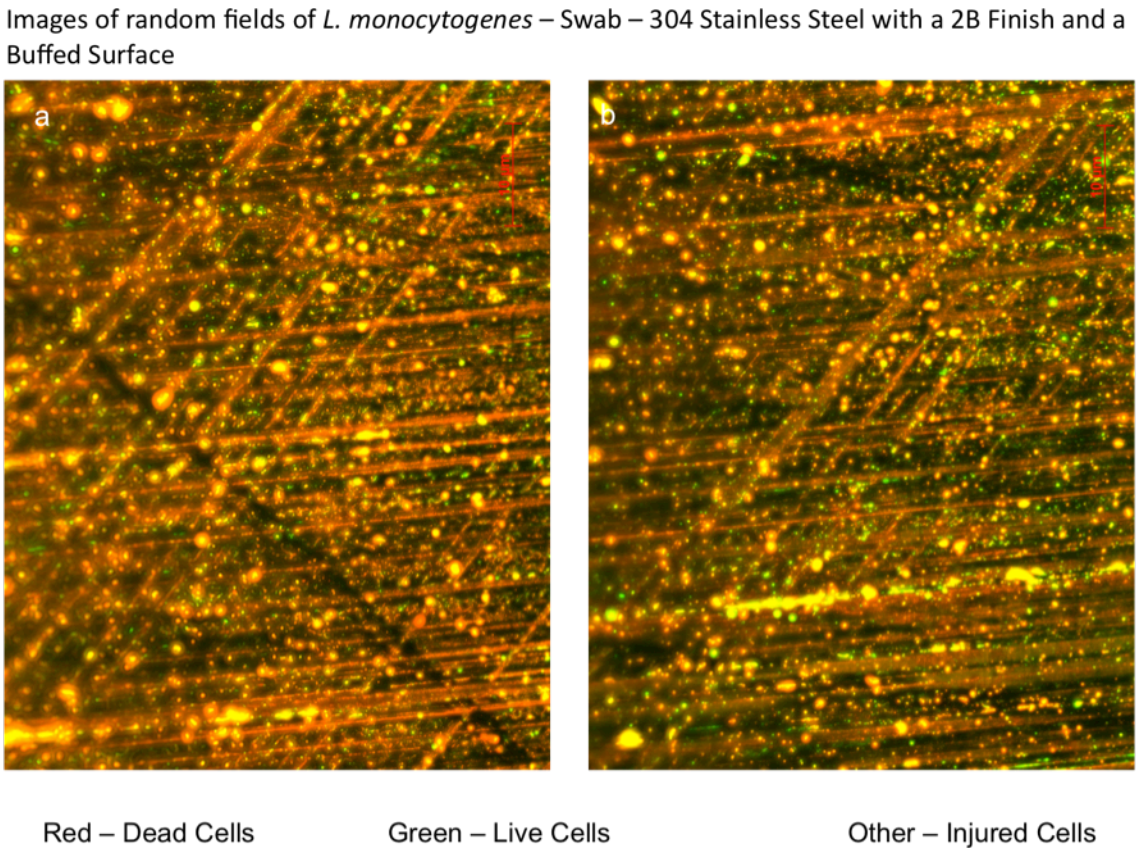
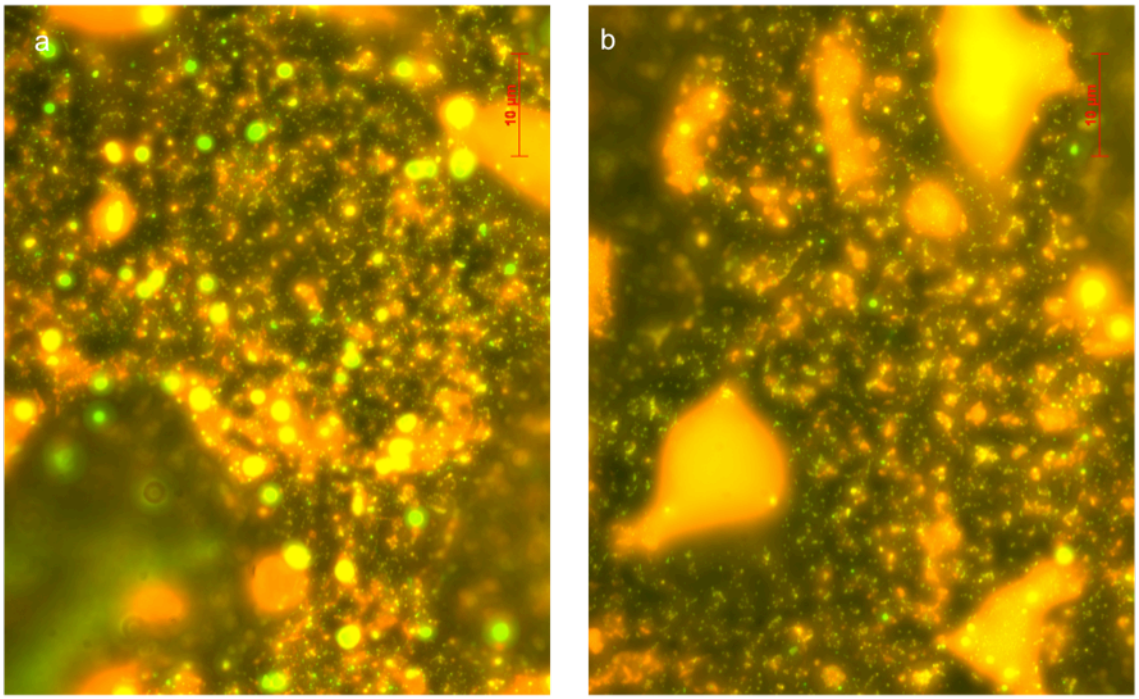


Figure 3.29 *Listeria monocytogenes* Inoculated and Swab Sampled on Ultra High Density Polypropylene.

Images of random fields of *L. monocytogenes* – Swab – Ultra High Density Polypropylene



Red – Dead Cells Green – Live Cells Other – Injured Cells

Figure 3.30 *Listeria monocytogenes* Inoculated and Swab Sampled on 304 Stainless Steel with a 2B Finish.

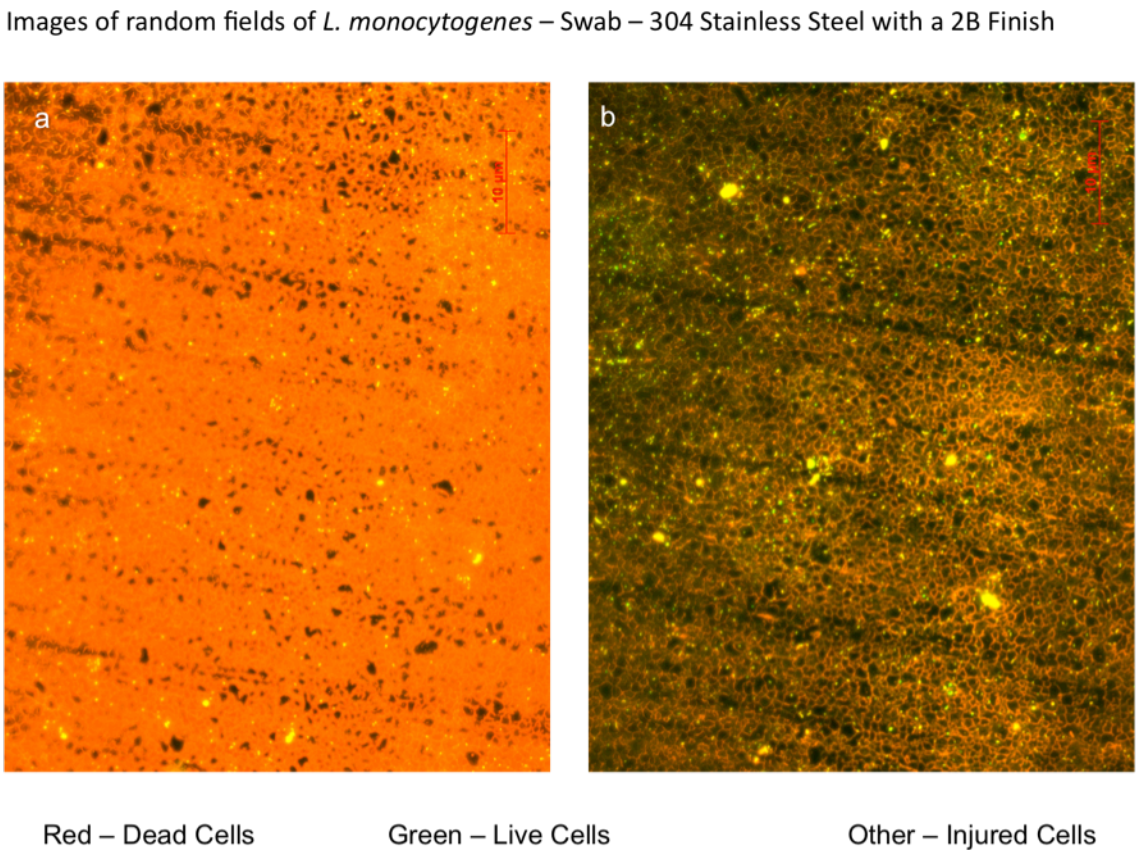


Figure 3.31 *Listeria monocytogenes* Inoculated and Sponge Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

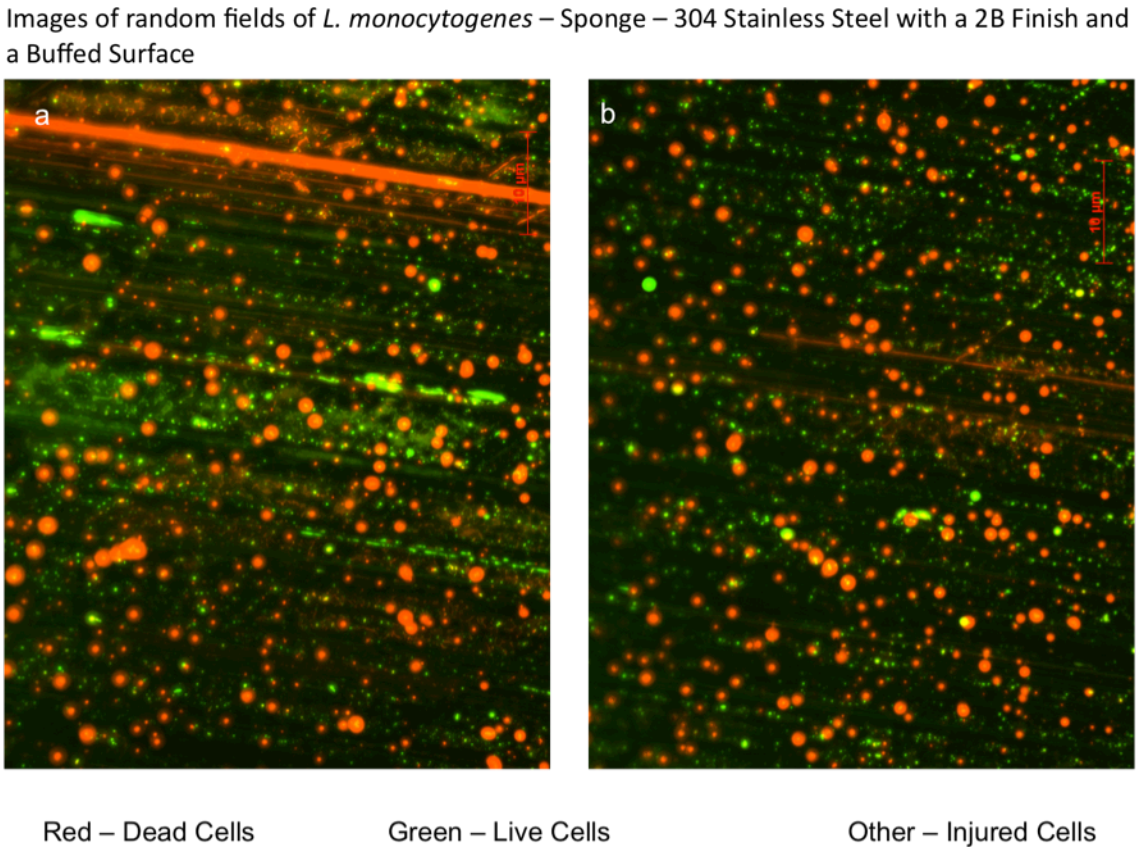


Figure 3.32 *Listeria monocytogenes* Inoculated and Sponge Sampled on Ultra High Density Polypropylene.

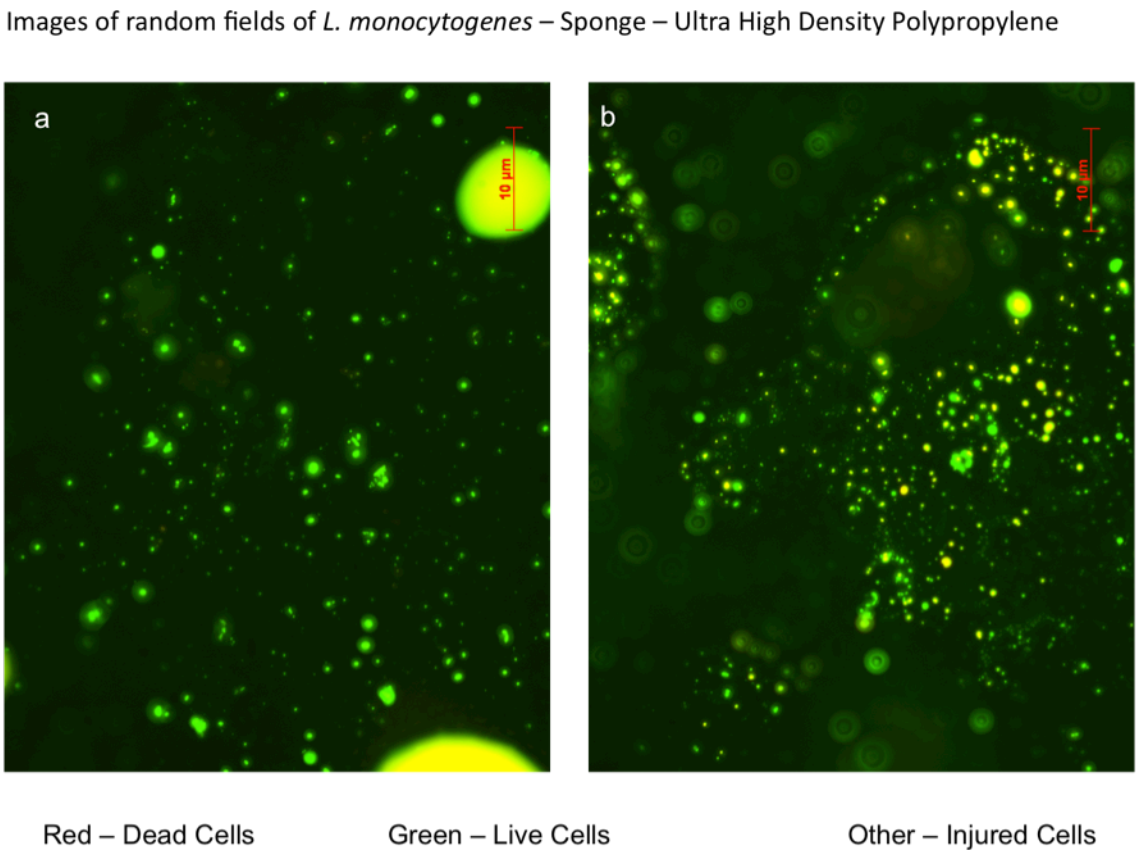


Figure 3.33 *Listeria monocytogenes* Inoculated and Sponge Sampled on 304 Stainless Steel with a 2B Finish.

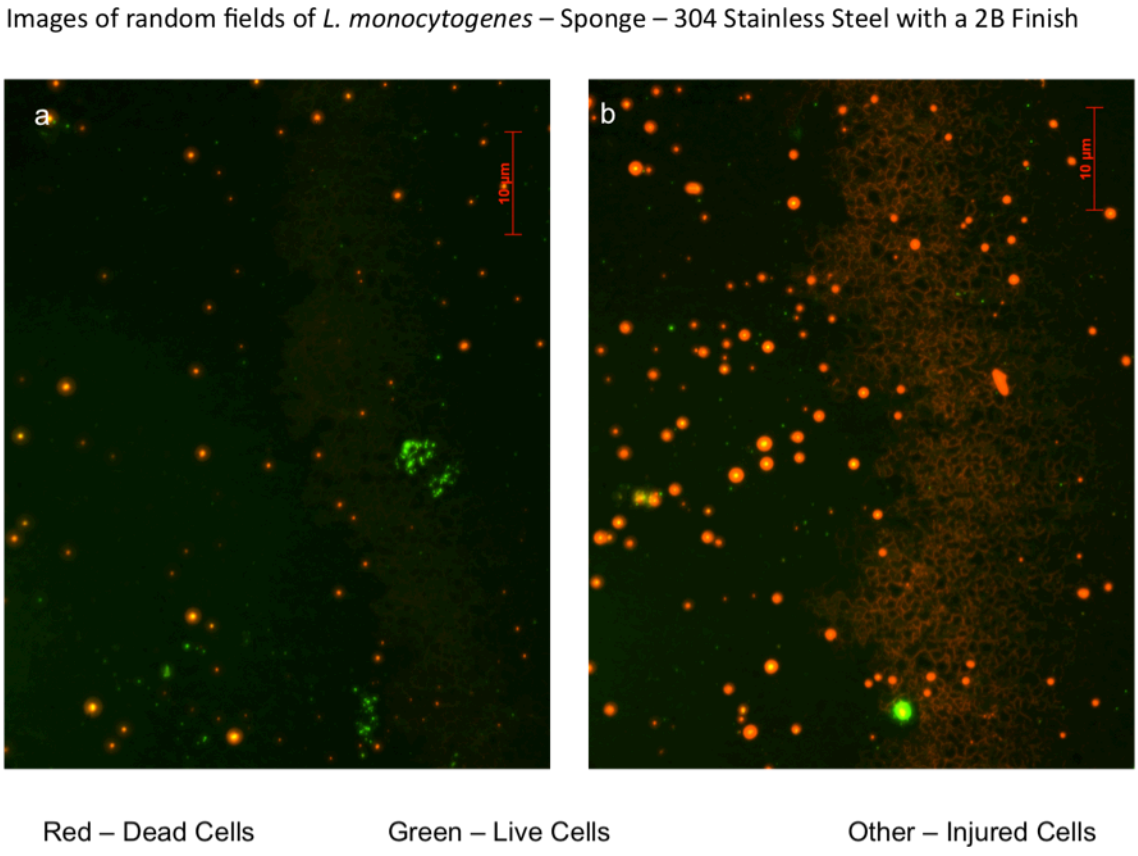
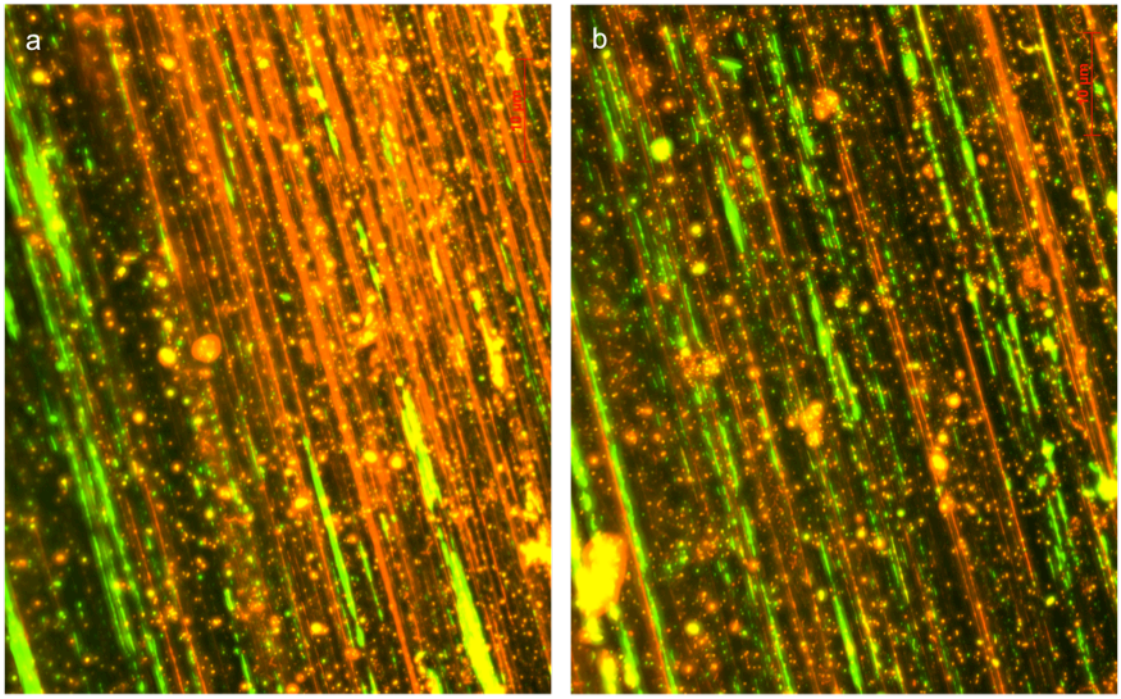


Figure 3.34 *Listeria monocytogenes* Inoculated and Flocked Swab Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

Images of random fields of *L. monocytogenes* – Flocked Swab – 304 Stainless Steel with a 2B Finish and a Buffed Surface



Red – Dead Cells Green – Live Cells Other – Injured Cells

Figure 3.35 *Listeria monocytogenes* Inoculated and Flocked Swab Sampled on Ultra High Density Polypropylene.

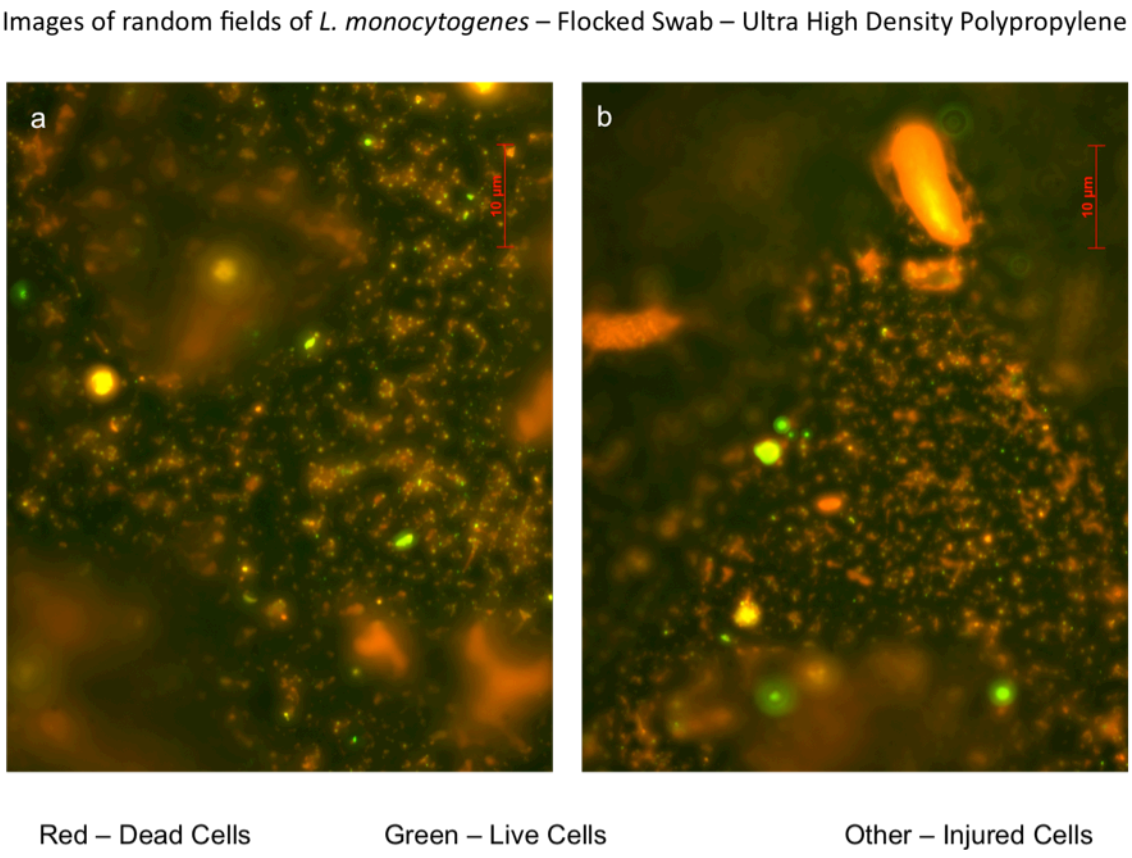


Figure 3.36 *Listeria monocytogenes* Inoculated and Flocked Swab Sampled on 304 Stainless Steel with a 2B Finish.

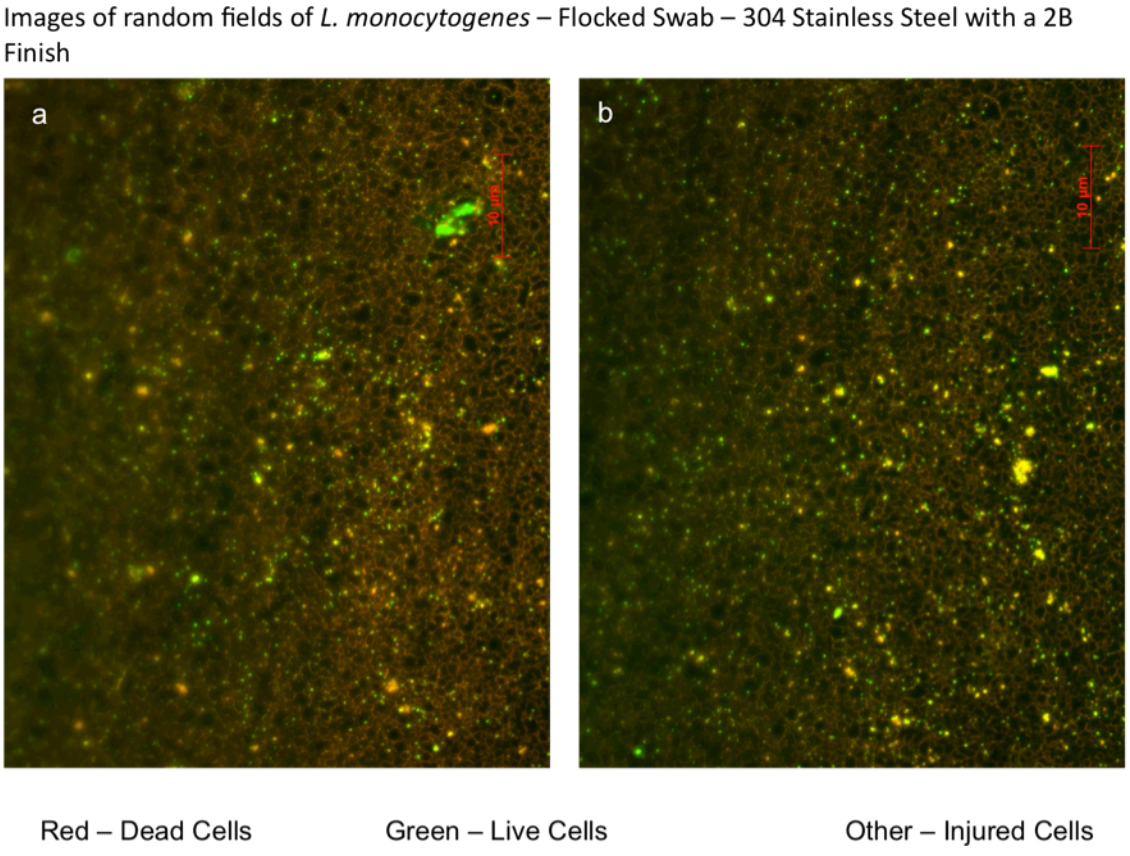
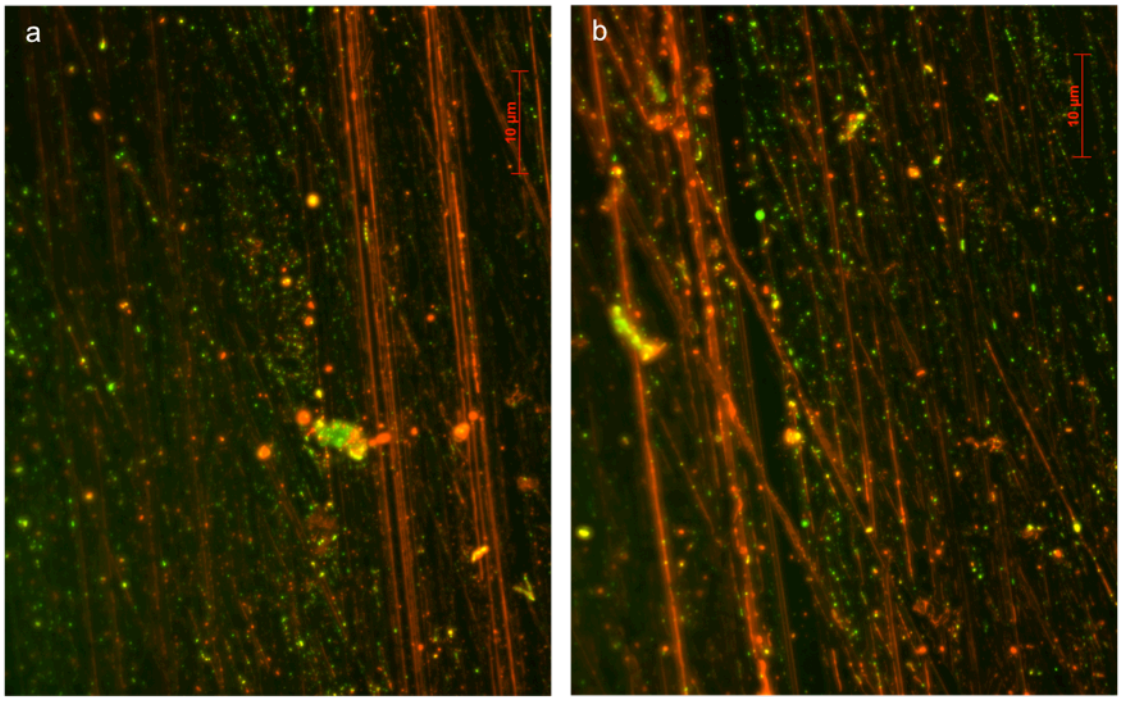


Figure 3.37 *Listeria monocytogenes* Inoculated and M-Vac Sampled on 304 Stainless Steel with a 2B Finish and a Buffed Surface.

Images of random fields of *L. monocytogenes* – M-Vac – 304 Stainless Steel with a 2B Finish and a Buffed Surface



Red – Dead Cells Green – Live Cells Other – Injured Cells

Figure 3.38 *Listeria monocytogenes* Inoculated and M-Vac Sampled on Ultra high Density Polypropylene.

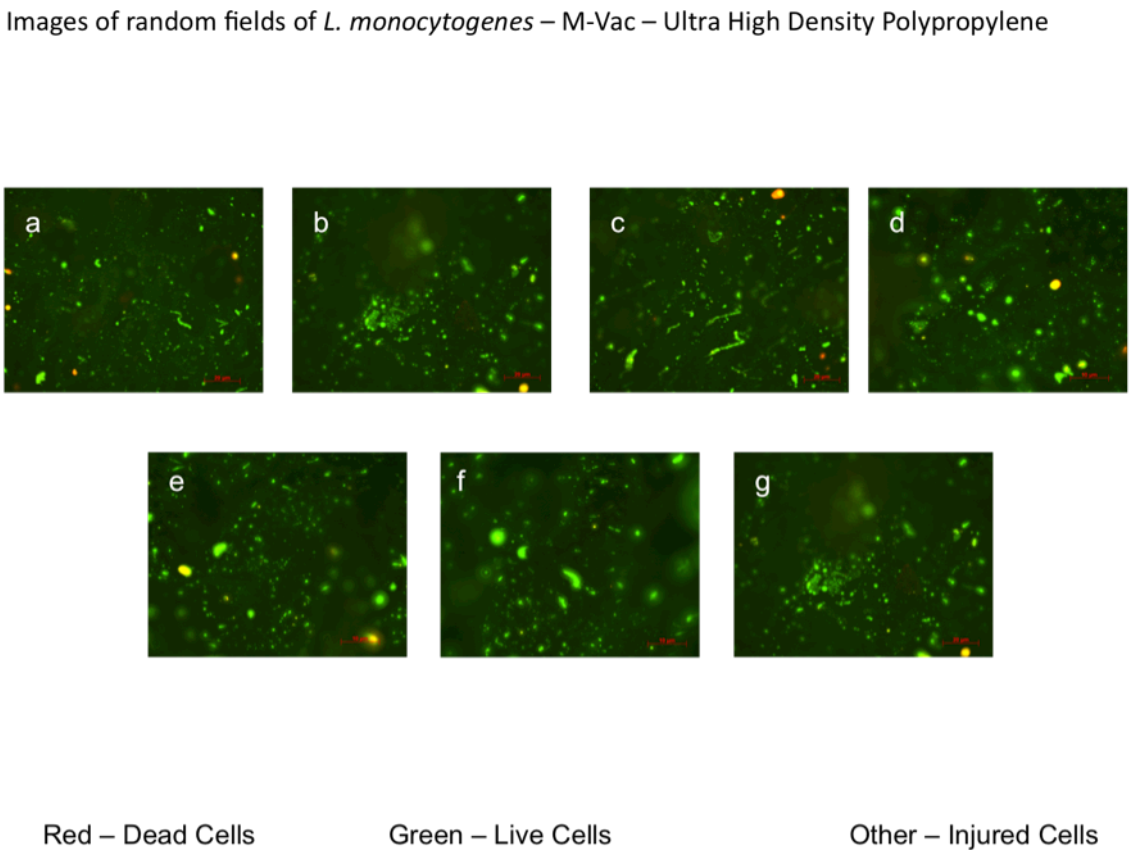


Figure 3.39 *Listeria monocytogenes* Inoculated and M-Vac Sampled on 304 Stainless Steel with a 2B Finish.

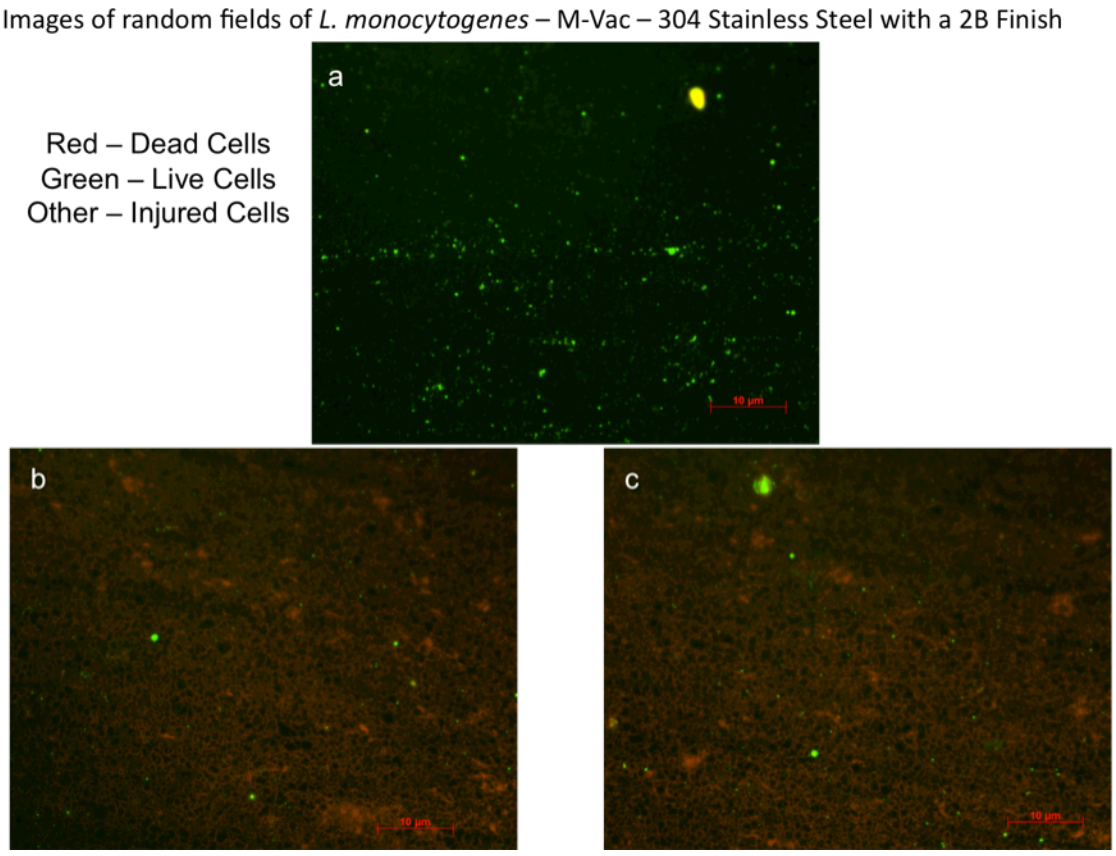
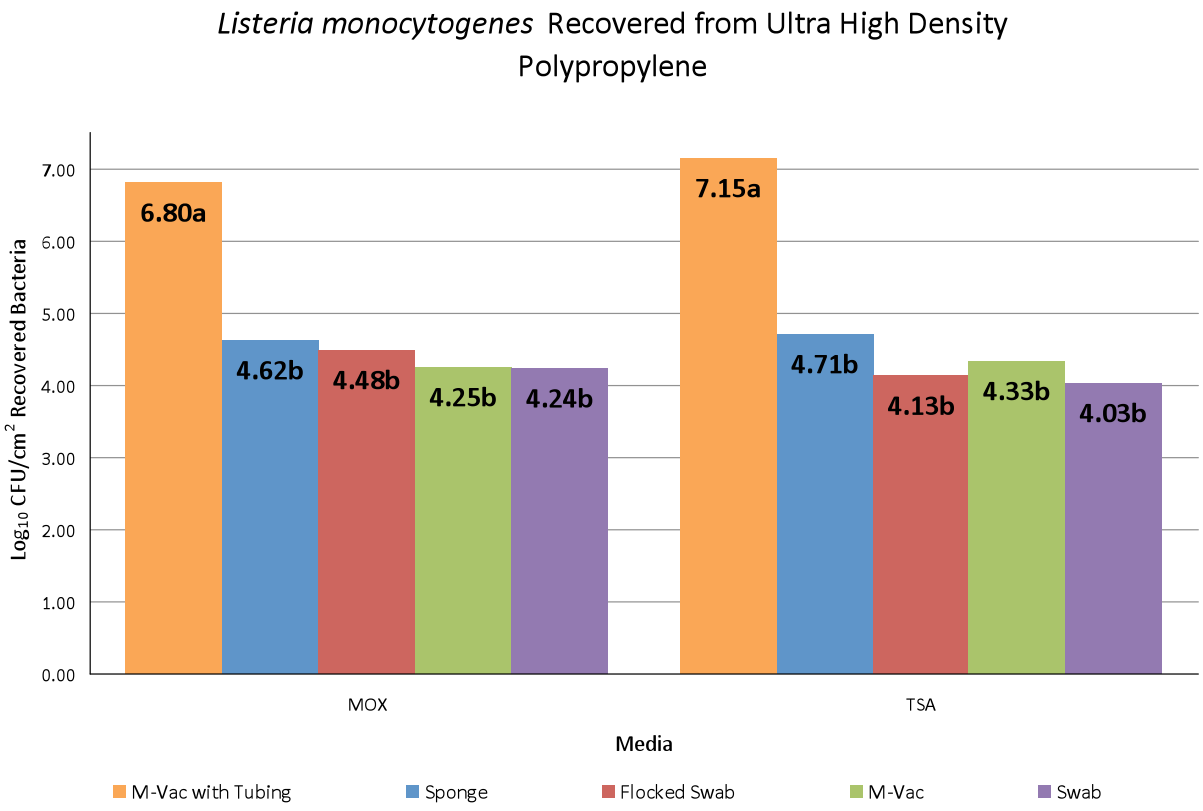
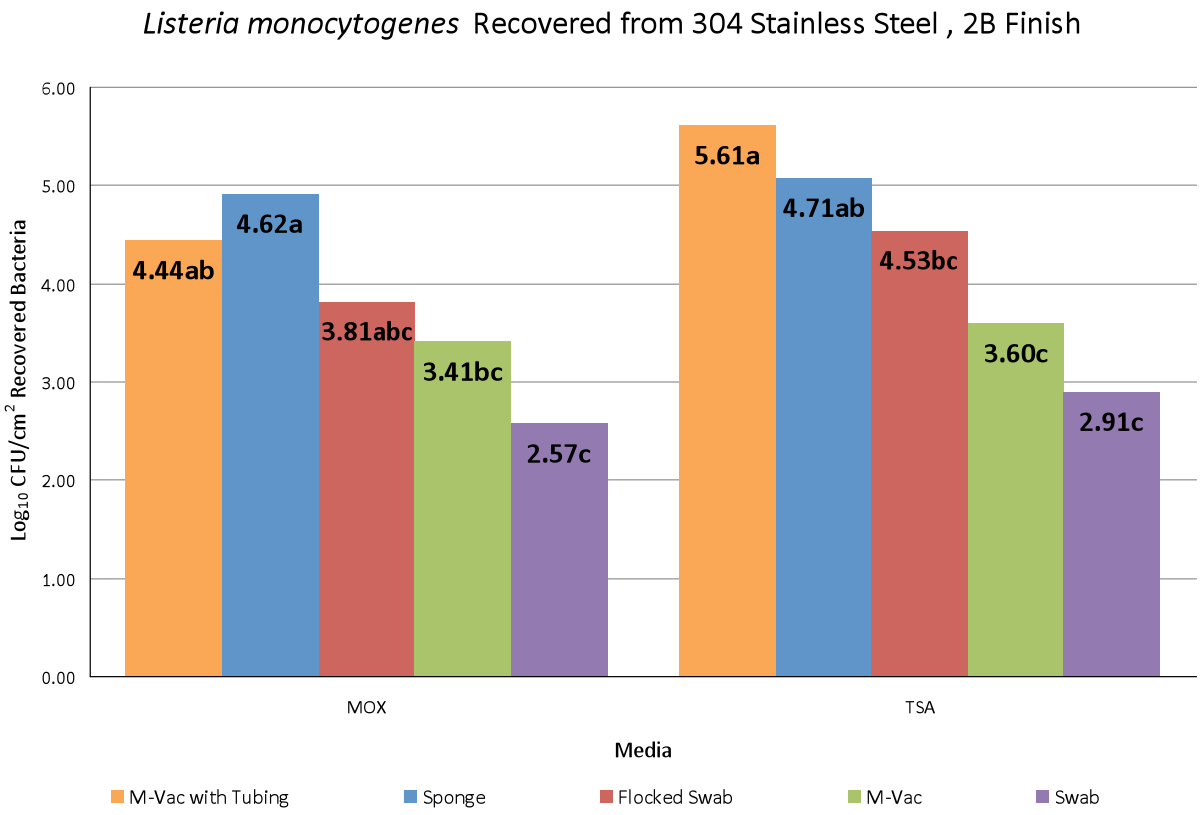


Figure 3.40 *Listeria monocytogenes* Sampled from Ultra High Density Polypropylene.



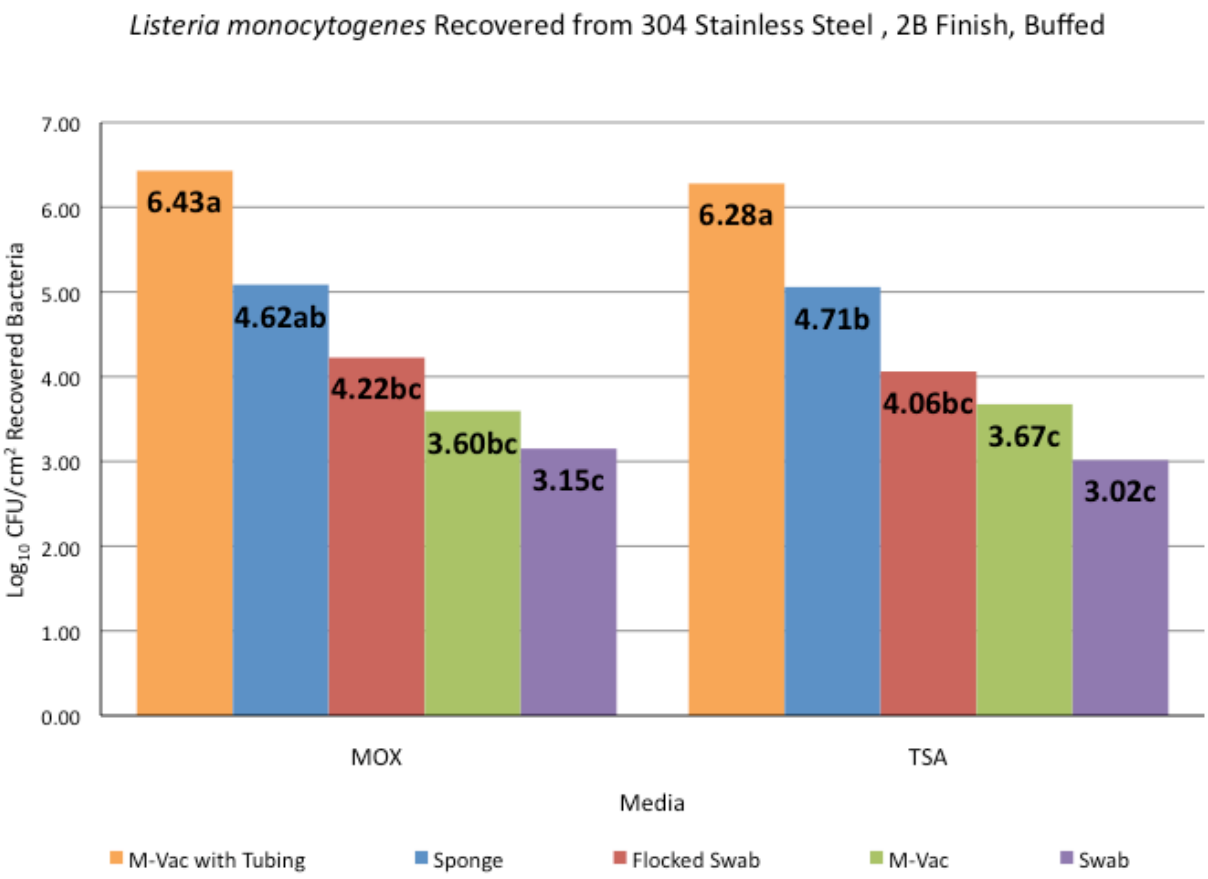
[ab] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.41 *Listeria monocytogenes* Sampled from 304 Stainless Steel with a 2B Finish.



[abc] Means in a row with a different superscript letter differ ($P < 0.05$).

Figure 3.42 *Listeria monocytogenes* Sampled from 304 Stainless Steel , 2B Finish and a Buffed Surface.



[abc] Means in a row with a different superscript letter differ ($P < 0.05$).

CHAPTER 4 - Conclusions

Based on viable cell counts, the sponge sampling method recovers the greatest ($P < 0.05$) number of bacteria from all surface types (ultra high density polypropylene, 304 stainless steel with a 2B finish, and 304 stainless steel with a 2B finish and a buffed surface) than any other sampling method. The swab sampling method was the least effective ($P < 0.05$) in recovering microorganism. In most cases, the flocked swab and the M-Vac were similar ($P > 0.05$) in the number of organisms recovered, falling between the sponge method and the swab method.

When examining the microscopy images it can be concluded that biofilms are more easily formed with *Listeria monocytogenes* than *Escherichia coli* O157:H7. Imaging also allowed for a visual representation of the remaining organisms that made it appear as if there was actually more bacteria recovery when the M-Vac sampling method was employed than when the sponge method was utilized. Further studies should be conducted to determine where these organisms end up. Further research shows that the missing organisms are left in the tubing of the instrument.

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