Enumeration of Escherichia coli O157:H7 from ground beef using a re-circulating immuno-magnetic bead separation

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Enumeration of *Escherichia coli* O157:H7 from ground beef using a re-circulating immuno-magnetic bead separation

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Program of Study Committee:
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Graduate College
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This is to certify that the master’s thesis of

Armitra Lavette Jackson

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
This work is dedicated to the loving memory of my beautiful Aunt Marie Doss who was one of my biggest cheerleaders. I love and miss you dearly. You will always serve as a source of inspiration.
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CHAPTER 1. GENERAL INTRODUCTION

Introduction

Since its emergence over 20 years ago as a human pathogen, *Escherichia coli* O157:H7 (hereafter referred to as *E. coli O157:H7*) has been implicated in many foodborne outbreaks involving meat. Of all *E. coli O157:H7* foodborne outbreaks, ground beef has been the most identifiable vehicle of transmission. There have been many instances in which companies have recalled products because of known contamination or the likelihood of contamination. This, in turn, has had a tremendous effect on the economy and on consumer confidence in the safety of ground beef. In addition to cases involving meat, there have also been many reported incidents of individuals becoming ill after consuming other kinds of food products such as milk and apple cider. Individuals who have been exposed to *E. coli O157:H7* have developed diseases such as hemorrhagic colitis, thrombotic thrombocytopenic purpura (TTP), and hemolytic uremic syndrome (HUS). These diseases are extremely serious and can ultimately result in death. From this, one can clearly understand the importance of controlling *E. coli O157:H7*.

According to the Food Safety and Inspection Service, *E. coli O157:H7* is considered an adulterant if found in raw ground beef. Most of the meat industry has imposed a test-and-hold system. This system requires that all microbiological results be received before the products are released for distribution. This ensures that contaminated meat does not reach consumers. Because ground beef is known to be an extremely perishable product, it is essential that methods used to detect and enumerate possible *E. coli O157:H7* be as rapid as possible. While there are currently many enumeration and detection methods, their sensitivity and rapid detection capabilities need to be explored.
Enumeration is extremely important because, unlike detection, it can give one a better understanding of the scope of a particular problem. Because enumeration methods face sensitivity issues that can result in some E. coli O157:H7 cells not being counted, there is a need for a system that can better account for all E. coli O157:H7 cells in food matrices. In order to meet the demand for a system that could detect and purify pathogens from different food matrices in a rapid manner, MATRIX MicroScience developed the Pathatrix™. This system is currently used in industry for the detection (not enumeration) of foodborne pathogens. The research outlined in the thesis was conducted in an effort to determine the ability of the Pathatrix™ to enumerate E. coli O157:H7 in raw ground beef. This research was also conducted to determine the limitations of the Pathatrix™.

**Thesis Organization**

This thesis is organized into three chapters. The first chapter, which includes the literature review, is intended to give the reader a clear understanding of E. coli O157:H7 in ground beef and other food products and the methods currently used for the detection and enumeration of this foodborne pathogen. The second chapter describes the enumeration of E. coli O157:H7 in ground beef using the Pathatrix™ detection system. The third chapter of this work will give general conclusions of the research conducted and recommendations for future research.

**Literature Review**

First recognized as a human pathogen in the United States in 1982, E. coli O157:H7 has been the cause of many foodborne disease outbreaks. A few of the modes of transmission have included raw milk (Keene et al. 1997), water (Swerdlow et al. 1992), and ground beef (CDC 2002). These foodborne outbreaks, along with others, have resulted in many
hospitalizations and deaths. In the United States, there have been reports of approximately 73,480 cases of illness involving *E. coli O157:H7*. Of those cases, 62,458 were foodborne (Mead et al. 1999). However, through the research of Mead et al. (1999), we now know that the numbers were actually not that large. Because the currently used enumeration methods are not always sensitive enough to detect all of the *E. coli O157:H7* present, there is a need for a system that can better account for all *E. coli O157:H7* cells. For example, background microflora, or non-specific bacteria, can inhibit the proper enumeration of *E. coli O157:H7*. From this, one can understand the importance of a system that has the ability to purify the target bacteria, which in this case is *E. coli O157:H7*. Because *E. coli O157:H7* is known to be present in the microflora of cattle, there is currently work being done to monitor this foodborne pathogen on beef carcasses (Erdmann et al. 2002).

**An Overview of *Escherichia coli* spp.**

According to Garmendia et al. (2005), *E. coli* is the most abundant gram-negative facultative anaerobic bacterium of the intestinal microflora. Although *E. coli* is known to be a natural part of the microflora in the human intestinal tract, there are a few strains that are known to be pathogenic (Padhye et al. 1992). The five main categories include enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC), enterotoxigenic *E. coli* (ETEC), and enterohemorrhagic *E. coli* (EHEC).

EIEC, in which humans are a major reservoir, is known for its ability to cause non-bloody diarrhea and dysentery closely related to that of *Shigella* spp (Doyle et al. 2001). Prior to the 1970's this group of strains was known as “paracolons”. As a mode of action, the bacteria will localize in the colon and begin to invade and multiply in the epithelial cells (Garmendia et al. 2005; Doyle et al. 2001). The serogroups that are most affiliated with
illness include O28ac, O29, O112, O124, O136, O143, O144, O152, O164, and O167 (Doyle et al. 2001).

EPEC has been thought to be virulent for humans since the 1940’s (Donnenberg et al. 1992). The major O serogroups associated with EPEC are O55, O86, O111ab, O119, O125ac, O127, O128ab, and O142 (Doyle et al. 2001). This group is the first type of *E. coli* to be linked to human disease and is one of the more common causes of diarrhea in infants (Garmendia et al. 2005). Although these strains can cause diarrhea, they generally do not produce enterotoxins (Jay et al. 2005). EPEC is known to cause lesions in the intestine that are somewhat similar to that of *E. coli* O157:H7 (ICMSF 1996).

The hallmark clinical feature of EAggEC is the persistence of diarrhea in children that can last >14 days (Jay 2000). This group’s ability to produce a pattern of aggregative attachment on HEp-2 cells makes them unique (Doyle et al. 2001). Whether or not this group is considered to be foodborne pathogens remains unclear (Jay 2000). Future research of this group will give the scientific world a clearer understanding of the taxonomy of this group.

ETEC accounts for a large number of diarrheal disease in developing countries, causing approximately 12.9 million deaths among children under the age of five years in 1990 alone (WHO 1994). The ETEC group, in which humans are primary reservoirs, is known to adhere to and colonize the small intestine (Jay et al. 2005). These strains are known to cause traveler’s and infant diarrhea. The most common serogroups include O6, O8, O15, O20, O25, O27, O63, O78, O85, O115, O128ac, O148, O159, and O167 (Doyle et al. 2001).
EHEC is considered an emerging zoonotic pathogen (Garmendia et al. 2005). Although other serogroups have been associated with foodborne outbreaks, the principal serotype in this group is *E. coli O157:H7*. The O157 serotype, which was first isolated in the feces of swine, was named in 1972. The H7 was isolated in 1944 from a human specimen (Jay 2000). The *E. coli O157:H7* strain is characterized by two antigens that are present on the surface of the bacterium and of the locomotive appendage called the flagella (bookrags.com). Shiga-like toxins are associated with EHEC strains. The two prototypes of the toxin are known as Stx1 (formally known as SLT-I) and Stx2 (formally known as SLT-II (Calderwood et al. 1996). Hemolytic uremic syndrome and hemorrhagic colitis are common diseases caused by this group. Hemorrhagic colitis was first clinically recognized as being associated with foodborne illness in 1982 when individuals consumed sandwiches that were not cooked properly. The bloody stool, which links its etiological agent in the colon, is the hallmark symptom for this disease. In 1999, the Tarrant County Health Department reported that a group of individuals attending a cheerleading camp became ill and showed symptoms that included bloody diarrhea, vomiting, and nausea. Stool samples from the ill individuals were sent to the Centers for Disease Control and Prevention and the Texas Department of Heath for analysis. As a result, *E. coli O111:H8* was isolated. Two of the individuals developed hemolytic uremic syndrome while two other individuals underwent appendectomies (CDC 2000).

**An Overview of *Escherichia coli O157:H7***

*E. coli O157:H7* was initially isolated in Argentina in 1977 (bookrags.com). In 1975, Enterohemorrhagic *E. coli O-157* was first isolated in the United States from a woman who had consumed undercooked ground beef (Ota 1999). Following this, *E. coli O157:H7* was
first recognized as a human pathogen in response to an outbreak in the United States where contaminated ground beef was implicated (Riley et al. 1983). For this pathogen, isolates are differentiated by three major surface antigens: the H (flagella), O (somatic) and K (capsule). The different surface antigens are what allows serotyping (Doyle et al. 2001).

**Reservoirs of E. coli O157:H7**

Some studies argue that E. coli O157:H7 is known to exist naturally in the microbial flora of sheep (Kudva et al. 1996) and cattle (Armstrong et al. 1996), while others argue over whether or not E. coli O157:H7 is colonized in the alimentary tract of the ruminant or if its continued occurrence is the result of its unceasing reintroduction from the environment (LeJeune et al. 2001). Nevertheless, research has shown that cattle fecal excretion of E. coli O157 is only temporal, lasting on average only 3 to 4 weeks (Shere et al. 1998). On the contrary, E. coli O157 has been extracted from the environment on farms for periods lasting many years (Rice et al. 1999). In the 1992 and 1993 western United States outbreak, a traceback found that the cattle were initially colonized with E. coli O157:H7, and contamination occurred during the slaughter process. This caused the surface of the carcass to be contaminated. The contaminated meat was then combined with meat from other sources, resulting in a large volume of contaminated ground beef patties (Tuttle et al. 1999). E. coli O157:H7 is not a normal inhabitant of the human intestinal tract. When E. coli O157:H7 invades the human intestinal tract via contaminated food, water or person-to-person transmission, life-threatening diseases can occur.

**Characteristics of E. coli O157:H7**

Unlike many foodborne pathogens, E. coli O157:H7 can survive extremely acidic conditions (Conner et al. 1995). E. coli O157:H7 outbreaks have been linked to products
such as apple cider that are known to have a low pH (Besser et al. 1993). Studies that
sprayed beef with substances that had citric, acetic or lactic acid concentrations as high as
1.5% showed that *E. coli* O157:H7 populations were not significantly affected by the
treatments (Brackett et al. 1994). Their ability to survive extremely acidic conditions allows
*E. coli* O157:H7 populations to survive in the stomach.

**Toxic Infective Dose of *E. coli* O157:H7**

One of the reasons why *E. coli* O157:H7 is a pathogen of concern is because of its
low infective dose. For example, 0.3 to 0.4 *E. coli* O157:H7 cells per gram were later
identified in many intact packages of dry-cured salami in outbreaks that occurred in
Washington and California (Tilden et al. 1996). In the 1992 and 1993 western United States
outbreak, 1.5 cells per gram or 67.5 cells per patty was enough to cause illness (Tuttle et al.
1999). The ability of *E. coli* O157:H7 to be transmitted from person-to-person is evidence of
its low infective dose (Doyle et al. 2001).

**Diseases Associated with *E. coli* O157:H7**

There are three diseases that are associated with *E. coli* O157:H7. They are
hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic
purpura (TTP).

The symptoms associated with hemorrhagic colitis include bloody diarrhea and
severe abdominal cramps. In outbreaks that occur in community settings, the incubation
period can range from 1 to 9 days (Riley et al. 1983). In outbreaks that occur in institutional
settings, the incubation period can range from 1 to 14 days (Ryan et al. 1986).

Although HUS has been reported in adults, this disease is most commonly found in
young children after a period of bloody diarrhea brought on by *E. coli* O157:H7 (Grabowski
2002). Several children at a day care center developed HUS that was associated with *E. coli O157:H7*. *E. coli* *O157:H7* was detected in some of their stool specimens (Spika et al. 1986). According to Rangel et al. (2005), of the 325 outbreaks that occurred between 1982 and 2002, 40 deaths were reported. Of those 40 deaths, 25 were in individuals who had HUS.

Thrombotic thrombocytopenic purpura is a disease that affects adults more often than children. TTP consists of neurologic symptoms, thrombocytopenia, microangiopathic hemolytic anemia, fever and renal failure (Chinyu et al. 1995). Individuals infected with TTP have clinical features that closely correspond to that of HUS (CDC 1986). Although TTP is a rare syndrome of *E. coli O157:H7* infection, this disease is characterized by blood clots in the brain that ultimately lead to neurological abnormalities (Doyle et al. 2001).

**Foodborne Disease Outbreaks Associated with *Escherichia coli O157:H7***

Foodborne diseases are a major problem in the United States. Each year, foodborne diseases are the cause of many illnesses, hospitalizations and deaths (Mead et al. 1999). It has been estimated that approximately 73, 480 cases of *E. coli O157:H7* occur each year (Mead et al. 1999). As a result, *E. coli O157:H7* is responsible for 3.1% of all foodborne outbreaks and 27.6% of all fatalities related to foodborne outbreaks in the United States (CDC 2000). From 1982 to 2002, *E. coli O157* accounted for 8,598 cases. These cases resulted in 1,493 hospitalizations, 354 cases of HUS and 40 deaths. Among the 350 reported outbreaks, 183 were found to be foodborne (Rangel et al. 2005). The Economic Research Service (ERS) of the USDA estimated that in the United States, *E. coli O157:H7* disease costs society approximately $659.1 million each year (ERS 2000).
The Food Diseases Active Surveillance Network (FoodNet) is an extension of the Centers for Disease Control whose objectives are to determine the frequency of foodborne outbreaks, monitor these outbreaks over time, and determine the association of common foodborne diseases with eating specific foods (CDC 2005). According to FoodNet, there has been a 43% decrease in the incidence of infections caused by \( E. coli \) \( O157 \) in comparison to the baseline data from the period of 1996-1998 and 2003. This decrease has surpassed the Healthy People 2010 objectives. This decrease is most likely due to the enhancement of food safety interventions and the reassessment of Hazard Analysis and Critical Control Points (CDC 2005). Although there has been a decrease in the incidences of \( E. coli \) \( O157:H7 \) infections, there have still been many reported incidences of foodborne outbreaks involving this pathogen. Of all \( E. coli \) \( O157:H7 \) foodborne outbreaks, ground beef was the most identifiable vehicle of transmission (Rangel et al. 2005).

**Multistate \( E. coli \) \( O157:H7 \) Outbreaks Associated With Meat**

The most recent case of an \( E. coli \) \( O157:H7 \) outbreak occurred in 2002 in Colorado where the Colorado Department of Public Health and Environment reported an outbreak in which ground beef products were implicated. This outbreak involved residents in Colorado and six other states. As of June 2002, seven patients were hospitalized and five developed HUS. As a result, ConAgra Beef Company voluntarily recalled 354,000 pounds of ground beef products (CDC 2002).

In Washington and California in 1994, an \( E. coli \) \( O157:H7 \) outbreak was linked to commercially distributed dry-cured salami. Three patients were hospitalized and a two-year-old child developed HUS. It was later discovered that all of the individuals involved had eaten salami from the delicatessen counters from the local grocery chain (CDC 1995). In
response to this outbreak, the United States Department of Agriculture developed regulations that would guarantee the safety of fermented sausages that were shelf stable (USDA 2001).

In Washington State in 1992 and 1993, ground beef was implicated in an outbreak of *E. coli O157:H7*. This outbreak, which occurred at a fast-food chain, resulted in more than 700 infections of *E. coli O157:H7* and four deaths (Tuttle et al. 1999). As a result, 151 individuals were hospitalized and 45 developed HUS. The outbreak resulted from errors in meat processing and the failure to cook the meat to the proper temperature. This was the largest *E. coli O157:H7* outbreak involving ground beef (Bell et al. 1994).

**Other *E. coli O157:H7* Outbreaks Associated with Meat**

In 2004, the Okinawa Prefectural Chubu Health Center and the Okinawa Prefectural Institute of Health and Environment reported an *E. coli O157:H7* infection associated with ground beef from a United States Military Installation in Okinawa, Japan. This incident resulted in the hospitalization of a child. *E. coli O157:H7* was isolated from the remaining ground beef possessed by the family. As a result of this, 90,000 lbs. of frozen ground beef in the United States and the Far East were recalled (CDC 2005).

In 1997, the Colorado Department of Public Health and Environment reported an outbreak of *E. coli O157:H7* infections associated with eating a nationally distributed commercial brand of frozen ground beef patties and burgers. In this case, five patients were hospitalized, but none developed HUS. Hudson Food beef burgers collected from the freezers of 2 of the 15 patients both confirmed the presence of *E. coli O157:H7* when cultured at the USDA and FSIS Laboratories in Athens, Georgia. Preliminary findings suggested that six potentially contaminated lots could have been distributed to at least 48
contiguous states. As a result, Hudson Foods recalled potentially contaminated products (CDC 1997).

In 1995, members of an Oregon community became ill after consuming jerky made from deer meat that was contaminated with *E. coli O157:H7* (Keene et al. 1997). In 1984, an outbreak of *E. coli O157:H7* occurred at a nursing home in the United States. This incident marked the first recognized outbreak since the two 1982 outbreaks that led to this organism being known as a pathogen. Thirty-four of the nursing home residents developed diarrheal illnesses while 14 others were hospitalized with symptoms that included bloody diarrhea, and abdominal pain. As a result of this outbreak, four individuals died. The investigation showed that hamburgers were the mode of transmission (Ryan et al. 1986).

**E. coli O157:H7 Outbreaks Associated with Produce**

In addition to meat products, other foods have also been implicated in outbreaks of *E. coli O157:H7*. Recently, certain Dole prepackaged salads were connected to an outbreak of *E. coli O157:H7*. In the Dole case, at least 11 individuals became sick and two individuals were hospitalized. According to the FDA, a recall of the potentially contaminated products was issued (Associated Press 2005). In July of 1995, approximately 40 Montana residents were affected by an *E. coli O157:H7* outbreak which involved lettuce. Thirteen individuals were hospitalized and one individual developed HUS (Ackers 1998).

In southern Massachusetts, there was a reported outbreak of *E. coli O157:H7* in one particular brand of apple cider. An investigation found that the apples used for the cider were not washed, the cider was not pasteurized, and no preservatives were added (Besser et al. 1993). In the western United States and British Columbia, Canada, unpasteurized commercial apple juice was the vehicle of transmission for an outbreak of *E. coli O157:H7*. 
In this incident, 25 individuals were hospitalized, 14 developed HUS, and one died. The source of contamination was thought to be incoming apples contaminated by deer that frequented the lots where the apples where grown (Cody et al. 1999). As a result of this outbreak, apple juice must be either pasteurized or, if in raw form, the product must be labeled to inform the consumer of the risks involved with consuming raw apple juice (FDA 1998).

This overview was an attempt to give the reader an idea of the outbreaks associated with *E. coli O157:H7*. However, one must remember that outbreak surveillance is extremely difficult and has many limitations. For example, many outbreaks are from unknown sources, while others are not reported by public health officials (Cieslak et al. 1997).

**Traditional Methods Used for the Detection and Enumeration of *E. coli O157:H7***

**Detection of *E. coli O157:H7***

There are currently many methods used in the detection of *E. coli O157:H7*. An immunoassay is one that uses antibodies for the determination of sample components (Hage 1999). Immunomagnetic separation (IMS) is defined as the capture of target bacteria by immunomagnetic particles (or beads) (Varshney et al. 2005). Immunomagnetic separation methods are beneficial in that they are able to capture target bacteria, thus avoiding the capture of non-specific background microflora. The reagents used in an immunoassay are important in determining the sensitivity, selectivity and limits of detection. Of the reagents, the antibodies used are the most important component (Hage 1999). Although these methods are beneficial, there is one main disadvantage. If there is a food matrix present, there is a chance that it will interfere with the capture of the magnetic beads, thus resulting in low
capture efficiency (Liu et al. 2003). As a result of this, Liu et al. (2003) found that the chemiluminescence biosensor in combination with IMS was successful in detecting *E. coli O157:H7* in ground beef, vegetable samples and chicken carcasses. Bioluminescence (which involves fluorescence and phosphorescence) has also been studied (Steinberg et al. 1995). Because this method yields indirect measurements, it is not considered completely accurate (Park et al. 2001).

Polymerase Chain Reaction (PCR) based detection methods are also common (Arthur et al. 2005; Grant 2003; Kawasaki 2005; Li et al. 2005; Li et al. 2003; Miyamoto et al. 2002; Moon et al. 2004; McKillip et al. 2002; Ogunjimi et al. 1999; Shima et al. 2004; Taylor et al. 2005). Sharma (2002) used Real-Time Polymerase Chain Reaction (R-PCR) to simultaneously enumerate and detect EHEC O157, O111, and O26 in beef and bovine feces via the presence of stx and eae genes. Another technique used is the aminopeptidase method (Castro et al. 1998). This technique is rapid in that it can provide results within three hours. However, this method only yields an estimate of the bacterial load.

**Enumeration of *Escherichia coli O157:H7***

There are currently many methods that are used for the detection and enumeration of *E. coli O157:H7*. These methods include the most probable number (MPN) technique, the colony count method and the direct microscopic count. These methods have been used extensively for the enumeration of *E. coli* food products (Bredie et al. 1992). The MPN method, which was introduced by McCrady in 1915 (Jay 2000), can be achieved via the multiple tube count and the hydrophobic grid membrane filter (HGMF). For the multiple tube count method, serial dilutions of the food sample are prepared. Tubes of culture media are then inoculated. After the tubes are incubated, positive tubes for each dilution are
counted. The pattern of positive tubes is then checked using the most probable numbers table. Because this method is based on ranges, this technique does not yield precise measurements (Swanson et al. 1992).

The HGMF was advanced by Sharpe and Michaud in 1974 (Sharpe et al. 1974). In this procedure, 1 ml of a 1:10 homogenate is filtered through a filter membrane grid. After this, the filter membrane is placed on the appropriate agar medium. To allow the formation of colony forming units, the membrane is left in the agar to incubate over night. The grids in which the colonies are formed are enumerated and the most probable number is calculated using a given formula (Jay 2000). Through this method, the maximum precision is achieved only when half of the cells of the grid yield positive results (Harrigan 1998). This means that the researcher would spend countless hours counting the grids. This is extremely laborious and the researcher will fatigue. This, in turn, will decrease the precision of the results.

The E. coli count via the MPN method is a time consuming process that can take up to six days for results (Bloch et al. 1996). This six-day period would make it impossible to be in compliance with the meat industry’s test-and-hold system which serves as a way to prevent contaminated products from being released for distribution (Brabban et al. 2004). E. coli O157:H7 is an organism that is extremely challenging to isolate (Ronner et al. 1990). As a result, Szabo et al. (1986) found that the MPN technique could not, at an acceptable level, isolate hemorrhagic colitis strains from food.

The colony count is another traditional method used for enumeration. This method can be achieved through the pour-plate method and the spiral plater. The pour plate method involves the preparation of dilutions and plates. After the preparation occurs, the plates are poured and allowed to set and incubate. Following incubation, the plates are counted. The
pour plate method enables the researcher to count colony forming units. The spiral plate
maker was developed by the United States Food and Drug Administration in the 1970’s
(Gilchrist et al. 1973). This method involves the even distribution of dilutions on solidified
agar plates. All these procedures are based on the assumption that each cell will be
successful in forming a visible colony on the medium. This does not always happen.
Because microbial growth requirements for cells may be different, some cells may not grow
under conditions provided by the colony count method. As a result, some microorganisms
may fail to form visible colonies on the medium. Also, the researcher’s ability to see distinct
colonies may be impaired. There is certainly room for error. Because of these problems, the
precision of the results is decreased.

Another technique used involves fluorescence microscopy. This technique was
originally designed for the enumeration of bacteria in raw milk (Pettipher et al. 1980), but
was later utilized in meat and other foods (Pettipher et al. 1982; Tortorello et al. 1994). The
fluorescence microscopy technique can be achieved via the Direct Epifluorescent Filter
Technique (DEFT). This technique involves the collecting of microorganisms on membrane
filters, staining them with fluorescent dyes, and counting them with an epifluorescence
microscope (Armesto et al. 1993). In a study conducted by Pettipher et al. (1982), it was
reported that this technique took less than 30 minutes to perform and had a lower sensitivity
of <60,000 microorganisms per gram for all products that were examined.

Petrifilm is another method that is used in the enumeration of *E. coli O157:H7*. The
advantages to this method include lower costs, less space requirement and simplicity
(Dawkins et al. 2005). However, Blackburn et al. (1996) found that some organisms will
tend to liquefy the gel, causing the colonies of other organisms to be obscured. To support
this conclusion even more, Dawkins et al. (2005) reported that 17.4% of the samples tested resulted in liquefaction of the Petrifilm plates. Of the 17.4% of samples tested, 16.0% were dairy products and 1.4% were meat products. On the other hand, Park et al. (2001) found that the petrifilm plate method could replace the conventional methods in the analysis of microorganism contamination in various meat products without compromising the accuracy of the results.

**PATHATRIX™**

The Pathatrix™ was developed by MATRIX MicroScience. This immuno-capture system was developed in response to the demand for a rapid method to detect and purify foodborne pathogens and spoilage organisms in different food matrices. The Pathatrix™ is unique because it uses heat in the system. Providing heat to the system allows for an environment that is conducive for the growth of the target bacteria. This, in turn, allows the microorganism to be cultured simultaneously.

Unlike the traditional methods, the system is also unique in that it is able to analyze the sample by re-circulating the entire sample through a capture phase where the antibody coated magnetic beads are immobilized. The magnetic beads are coated with antibodies that are specific to the target bacteria. *E. coli O157* is usually present in low numbers in ground beef, while non-O157 is present at high numbers. As a result, the non-O157 will compete with the *E. coli O157*. Immuno-magnetic separation will not only increase the sensitivity of the rapid detection of *E. coli O157*, but also prevent the effect of competing non-*E. coli O157* (Wan et al. 2000). According to Arthur et al. (2005), the Pathatrix™ was able to detect a greater number of *E. coli O157:H7* positive samples and the coupling of Pathatrix™ with PCR yielded the fastest results. Traditional methods are only capable of analyzing a “sub-
sample". This can be a disadvantage when only low populations per gram are present. In this case, the chances of all cells not being accounted for are high. Because the Pathatrix™ re-circulates the sample, there are several opportunities for the target bacteria to be captured. Re-circulating the sample will yield results that are more representative of the total product, thus providing more accurate results.

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CHAPTER 2. THE ENUMERATION OF *Escherichia coli* O157:H7 FROM GROUND BEEF USING A RE-CIRCULATING IMMUNO-MAGNETIC BEAD SEPARATION

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ABSTRACT

The ability of magnetic immunocapture to enumerate *Escherichia coli* O157:H7 in ground beef was investigated. Ground beef was inoculated with a known quantity of a five strain mixed culture of *E. coli* O157:H7, and the population subsequently enumerated by re-circulating the ground beef sample with immuno-magnetic beads. Trypsin and Polyoxyethylene Sorbitan Monolaurate (tween 20) were added to the ground beef sample and allowed to incubate for one hour to achieve a higher recovery of the target bacteria. There was statistical difference (P<0.05) between the known population inoculated into the ground beef and the populations enumerated in the ground beef by the immunocapture method. The regression model showed that there is a linear relationship between the two groups. This model proves that the Pathatrix™ System can be used for the enumeration of *E. coli* O157:H7 in ground beef.
INTRODUCTION

First recognized as a human pathogen in the United States in 1982, *E. coli O157:H7* has been the cause of many foodborne disease outbreaks. It has been estimated that approximately 73,480 cases of *E. coli O157:H7* occur each year (Mead et al. 1999). As a result, *E. coli O157:H7* is responsible for 3.1% of all foodborne outbreaks and 27.6% of all fatalities related to foodborne outbreaks in the United States (CDC 2000). From 1982 to 2002, *E. coli O157* accounted for 8,598 cases. These cases resulted in 1,493 hospitalizations, 354 cases of HUS and 40 deaths. The Economic Research Service (ERS) of the USDA estimated that in the United States, *E. coli O157:H7* disease costs society approximately $659.1 million each year (ERS 2000).

A few of the modes of transmission have included raw milk (Keene et al. 1997), water (Swerdlow et al. 1992), and ground beef (CDC 2002). In the United States, there have been approximately 73,480 reported cases of illness involving *E. coli O157:H7*. Of those cases, 62,458 were foodborne (Mead et al. 1999). Because the currently used enumeration methods are not always sensitive enough to detect all of the *E. coli O157:H7* cells, there is a need for a system that can better account for all *E. coli O157:H7* cells. For example, background microflora, or non-specific bacteria, can inhibit the proper enumeration of *E. coli O157:H7*. From this, one can understand the importance of a system that has the ability to purify the target bacteria, which in this case is *E. coli O157:H7*. Because *E. coli O157:H7* is known to be present in the microflora of cattle, there is currently work being done to monitor this foodborne pathogen on beef carcasses (Erdmann et al. 2002).
MATERIALS AND METHODS

Bacterial strains and culture media. *E. coli O157:H7* strains (ATCC 43894, ATCC 48895, ATCC 35150, WS 3062 and WS 3331) were obtained from the Iowa State University Food Safety Research Laboratory. The strains were checked for purity using the Reveal microbial screening test (Neogen Corporation, Lansing, MI, USA). They were individually grown overnight at 37°C in tryptic soy broth (Difco, BD Diagnostic Systems, Sparks, MD, USA). The strains were then combined to form a mixed culture. Each culture was equally represented. Ten-fold serial dilutions were prepared in 0.1% sterile peptone water.

Preparation of meat samples. Ground beef samples were obtained from a local grocery in Ames, Iowa. The ground beef was then irradiated at 2 kGy at the Iowa State University Meat Laboratory. This was done to ensure that all *E. coli O157:H7* cells recovered were the cells that were inoculated. Twenty-five grams of ground beef were transferred to Whirl Pak Filter sample bags (Nasco, Fort Atkinson, WI, USA) and inoculated with 2.5 ml of the appropriate dilutions (which were $10^{-5}$, $10^{-6}$ and $10^{-7}$). Following this, 225 ml of buffered peptone water (Difco, BD Diagnostic Systems, Sparks, MD, USA) was pre-warmed to 37°C. The buffered peptone water contained 10 g of trypsin (Sigma Chemical Company, St. Louis, MO, USA) and 10 ml of Polyoxyethylene Sorbitan Monolaurate (tween 20) (Sigma Chemical Company, St. Louis, MO, USA). Trypsin and tween 20 were incorporated to aide in the recovery of the antibody coated beads (Lee 1977). This solution was added to the Whirl Pak Filter sample bags. These substances were added to aid in recovery of the organism. Each sample bag was stomached for 30 seconds. All samples were allowed to incubate at 37°C for one hour to achieve a higher recovery rate of the target pathogen. A non-inoculated sample served as the control for each replicate. Experiments were repeated three times.
**Pathatrix™ System.** The Pathatrix™ System (Matrix MicroScience Ltd) is an immuno-capture system that is able to analyze the sample by re-circulating the entire sample through a capture phase where the antibody coated magnetic beads are immobilized (Figures 1-4). Samples in the Whirl Pak Filter sample bags were placed in the system's incubation pots, which are independent of one another. The manufacturer's instructions were carefully followed. This included adding 50 µl of *E. coli O157* antibody coated beads into the connector, re-circulating the sample at 37°C for 30 minutes, washing and eluting the beads and allowing the beads with the captured bacteria to draw to the magnet. After this step, 0.1 ml from the wash vessels was plated on Cefixime Tellurite Sorbitol MacConkey agar (Oxid Ltd., Basingstoke, Hampshire, England). 100% of the beads were plated. Duplicates of each sample were plated. Bacterial colonies on agar plates were counted following incubation at 37°C for 18 hours.

**Statistical Analysis.** The results of the inoculated populations and the Pathatrix™ populations were compared using SAS statistical software version 8.2 (SAS Institute, Inc., Cary, N.C.). Linear Regression was performed using SigmaPlot 9.0 (Systat Software, Inc., Point Richmond, CA) to determine the relationship between the inoculated populations and the Pathatrix™ populations. Populations were converted to Log_{10}.

**RESULTS AND DISCUSSION**

There was statistical difference (P<0.05) between the known population of *E. coli O157:H7* inoculated into the ground beef and the population enumerated in the ground beef by the immunocapture method (Table 1). The known population counts were significantly higher than the counts from the Pathatrix™ at dilutions 10^{-5}, 10^{-6} and 10^{-7}. In an attempt to see if there was a linear trend in proportion due to dilution, the linear regression was used.
(Figure 5). There is a linear trend between the two variables. The model shows that one log Pathatrix™ population is equal to 1.13 log inoculated population. One explanation for the results could be that all the antibody coated beads containing the target bacteria were not all pulled from the ground beef. This, consequently, would affect the counts. This parallels with the results of Liu et al. (2003), who found that if there is a food matrix present, there is a chance that it will interfere with the capture of the magnetic beads, thus resulting in low capture efficiency.

**Figure 5**

![Graph showing linear trend between Pathatrix Population and Inoculated Population. The equation is Inoculated Population = 1.13(Pathatrix population) + 1.10 with R² = 0.99.](image)
Table 1

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Inoculated Population</th>
<th>Pathatrix™ Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-5}$</td>
<td>3.6</td>
<td>2.2</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>2.7</td>
<td>1.4</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>1.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

$\text{Log}_{10} \text{ cfu/g}$

CONCLUSION

From the model discussed, the Pathatrix™ can be used to enumerate *E. coli O157:H7* in ground beef. As a result, this system can be used as a rapid method for the enumeration of *E. coli O157:H7* in ground beef. This system would allow the meat industry to be in compliance with the test-and-hold system.

REFERENCES


Figure 1: Antibody coated beads capturing on surface

Figure 2: Capture of target in food

Used with permission of Matrix MicroScience, Inc.
Figure 3: Captured target bacteria after wash

Figure 4: Captured target bacteria are eluted

Used with permission of Matrix MicroScience, Inc.
CHAPTER 3. GENERAL CONCLUSIONS

General Discussion

Foodborne diseases have been responsible for many illnesses and deaths. The illnesses and deaths have been the result of consumers consuming products that are not microbiologically safe. *E. coli O157:H7* has been responsible for many of these incidences. Because of its low infective dose, there have been many measures taken to control this pathogen. Nevertheless, this pathogen is still a serious problem. Because of this, there is a need for a system that has the ability to quickly and accurately enumerate foodborne pathogens. Because the currently used enumeration methods are not always sensitive enough to detect all of the *E. coli O157:H7* cells, there is a need for a system that can better account for all *E. coli O157:H7* cells. For example, background microflora, or non-specific bacteria, can inhibit the proper enumeration of *E. coli O157:H7*. From this, one can understand the importance of a system that has the ability to purify the target bacteria, which in this case is *E. coli O157:H7*.

Enumeration is extremely important because, unlike detection, it can give one a better understanding of the scope of a particular problem. Enumeration also gives agencies such as the CDC a better understanding of infective dose. The Pathatrix™ was able to enumerate *E. coli O157:H7* from artificially inoculated ground beef using the model described. This is phenomenal in the fact that this system would allow companies to be in compliance with the meat industry’s test-and-hold system.
Recommendations for Future Work

From this work, the ability of the Pathatrix™ to enumerate E. coli O157:H7 in ground beef was defined. Knowing its ability to enumerate other pathogens in other food matrices (such as produce) would be useful, especially since E. coli O157:H7 has been implemented in products other than ground beef. This information would prove to be helpful to other food industries that perhaps face difficulties with their standard methods of enumeration.

Ready-to-eat (RTE) meats are popular among consumers. We enjoy the pleasure of consuming these products without any thermal processing. Unfortunately, research has shown that RTE meats can become contaminated with pathogens such as Listeria monocytogenes after thermal processing. Because RTE meats are typically not heated by the consumer, there is a zero tolerance for pathogens in these products. Knowing the ability of the Pathatrix™ to enumerate pathogens in RTE meats would be purposeful. In the event that contamination was to occur, knowing the ability of the Pathatrix™ to accurately enumerate the pathogen would be insightful. This information would also give the industry a better scope of the problem.

For this work, the ground beef was irradiated in an effort to ensure that the organism recovered was the organism that was inoculated. However, this situation is not realistic to what really happens. To portray more of a realistic situation, future work should include performing the experiment with and without irradiation. This would validate the ability of the Pathatrix™ to capture only the target bacteria (and not the non-specific bacteria).
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And to anyone who may read this and wonder whether or not this is possible for
them, BE ENCOURAGED! All things are possible through Jesus Christ our Savior!