EVALUATION OF THE APPROPRIATENESS OF PREVIOUSLY DEVELOPED *ESCHERICHIA COLI* BIOTYPE I SURROGATES AS PREDICTORS OF NON-O157:H7 SHIGA TOXIN-PRODUCING *E. COLI* IN BEEF PROCESSING

A Dissertation

by

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ABSTRACT

Non-O157 Shiga-toxin producing *Escherichia coli* (STECs) serovars O145:NM, O45:H2, O26:H11, O103:H11, O111 (organism was originally ordered from American Type Culture Collection (ATCC) under accession # BAA-2217, and has since then been reclassified as O147, see Appendix), O121:H19, and *E. coli* biotype I surrogates were individually cultured in tryptic soy broth (TSB) at 37°C for 18 hours. Rifampicin-resistant (rif-resistant) surrogates were compared to the parent strains listed above. Stationary phase and acid-adapted organisms were each transferred into phosphate buffer saline (PBS) acidified with L-lactic acid at pH 2.5, 3.0, and 3.5, and enumerated for survivors following a 2-hour exposure time. In order to construct a thermal destruction curve, organisms were transferred into a capillary tube, flame sealed, submerged in a water bath at 55, 60 and 65 ± 0.5°C, and enumerated for survivors. For freezing (-20 ± 0.5°C) and refrigerated (4 ± 0.5°C) storage, bacterial strains were enumerated on days 0, 7, 14, 21, 28, 60, and 90 to determine the response to freezing and refrigerated storage.

Growth curves of *E. coli* biotype I surrogates (parent and rif-resistant) were similar to those of STECs throughout the evaluation. For acid resistance, acid-adapted organisms at pH 3.0 showed initial log reductions (CFU/ml) ranging from 1.7-2.5, where organism O26:H11 had the greatest log reduction (2.5). After 2 hours exposure time, reductions ranged from 5.1-7.4 log CFU/ml. *D*-values were calculated for each organism

at 55, 60 and 65 ± 0.5°C. Acid-adapted organisms at 65°C had *D*-values ranging from 0.13-0.64 min, with a rif -resistant *E. coli* biotype I surrogate (BAA-1429 rif) having the highest *D*-value at this temperature. For the response to refrigeration and freezing temperatures, there were no notable trends or patterns observed, and no one single *E. coli* biotype I surrogate represented all of the non-O157 STECs.

Organisms were analyzed individually and in sets (surrogates, rif-resistant surrogates and STECs) to represent a mean. Analyzing the organisms in sets eliminated certain individual strain-to-strain variation, and showed fewer differences ($P < 0.05$). Surrogates analyzed as a mean of the five strains indicate they may be best utilized in combination to represent all six of the non-O157 STECs.

DEDICATION

I dedicate this dissertation to my Mom, Dad, Andrew, Carson, and Aunt Sylvia. Without their continued love and support throughout this entire process, this degree would not have been possible.

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1. INTRODUCTION

Meat and meat products may contain pathogens that can be associated with foodborne illness. *E. coli* O157:H7, a pathogen associated with foodborne illness is naturally found in the intestinal tract of beef cattle. Through the process of harvesting cattle, potential pathogens, including *E. coli* O157:H7, may contaminate the carcass surface through the necessary procedures required for the conversion of the live animal into meat. For example, contamination may occur during the removal of the hide, or at the evisceration stand. *E. coli* O157:H7 is a human health risk and is naturally occurring in low numbers. If the potential pathogen remains on the meat or meat product, the consumer may contract a foodborne illness. *E. coli* O157:H7 can cause severe illness including hemolytic uremic syndrome (HUS). In 1992, an outbreak of *E. coli* O157:H7 attributed to undercooked hamburgers caused hundreds of illnesses and four deaths. Following the outbreak, many government agencies, academic personnel, and industry representatives have made advancements into researching and improving the safety of meat and meat products.

The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) has declared *E. coli* O157:H7 an adulterant in raw ground beef. For this reason, the meat industry has implemented points in the process of producing meat and meat products that may reduce or eliminate potential pathogens. Most commonly, antimicrobial interventions including hot water, lactic acid, acidified sodium chloride, or

peroxyacetic acid can be applied to carcass surfaces or further processed products to reduce or eliminate potential pathogens that may be present.

E. coli O157:H7 is not the only potential pathogen that may be found in the intestinal tract of cattle. Other organisms, including the non-O157 STECs may also be present, and these can cause foodborne illness in humans. STECs are comprised of many pathogenic serotypes and *E. coli* O157:H7 is the most studied. Non-O157 STEC illnesses may not be as severe as *E. coli* O157:H7, and vary by serogroup, but the number of foodborne illnesses caused by these organisms is increasing in the United States and around the world. Six non-O157 STECs have been shown to cause the majority of the foodborne illness associated with these organisms (O26, O45, O103, O111, O121 and O145). The USDA-FSIS has declared these six non-O157 STECs, the "Big 6," as adulterants in raw, non-intact beef products, and raw, intact beef products intended for non-intact products.

Five non-pathogenic *E. coli* biotype I surrogates, identified previously from cattle hides have been shown to have similar acid resistance at pH 3.0, 4.0, and 5.0, and thermal resistance at 55 and 65°C when compared to *E. coli* O157:H7. For an organism to be appropriate for use as a surrogate, it is ideally isolated from the same environment, present when the target pathogen is present, correlated in population densities, and having the same performance characteristics, for example, growth rate and sensitivity to microbial intervention methods. In-plant validation studies are used to determine the effectiveness of a processing facility's interventions, and their ability to reduce or eliminate potential pathogens. Given that *E. coli* O157:H7 is an adulterant, it is not

approved for use in in-plant validation studies. The non-pathogenic surrogate organisms that represent *E. coli* O157:H7 can be taken into a processing facility to validate the processor's interventions with reference to *E. coli* O157:H7.

Since the six non-O157 STECs have been declared adulterants, there is a need to identify surrogate microorganisms that properly represent the characteristics of the non-O157 STECs to be used in plant validation studies. The purpose of this study was to test the appropriateness of the previously identified *E. coli* biotype I surrogates (used for *E. coli* O157:H7) to the six non-O157 STECs using growth characteristics, acid and thermal resistance, and response to refrigeration and freezing temperatures.

2. REVIEW OF LITERATURE

Foodborne illness as it relates to raw meat and meat products

It is estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States (U.S.) each year *(30)*. The causes of foodborne illness include viruses, bacteria, parasites, toxins, metals, and prions. More than two hundred diseases are transmitted through food *(30)*. The symptoms of foodborne illness range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes. An annual report from the Centers for Disease Control and Prevention (CDC) shows that foodborne infections continue to be an important public health risk in the U.S. *(14)*. Data from Foodborne Diseases Active Surveillance System (FoodNet), which monitors fifteen percent of the nation's population, provide the best measure of foodborne disease in the U.S., and these data compare rates for different time periods *(14)*. These numbers are an estimation based on the number of people in the U.S. and those sickened with foodborne infections that have been confirmed by laboratory tests *(14)*. Foodborne illness surveillance is complicated by several factors including the timeliness of the data collected, quality and quantity of data, and most importantly, underreporting. Another complication of foodborne illness surveillance is that many pathogens transmitted through food also can be transmitted through water or from person-to-person contact. Thus, the role of food as vehicle for illness may be obscured. Interestingly, pathogens or agents that have not yet been identified, and cannot therefore be diagnosed, cause some proportion of foodborne

illness *(30)*. Milder cases of foodborne illness are not often detected through routine surveillance; however, severe or even fatal cases are more likely detected. To survey foodborne illness, patients must go to a doctor's office, have a sample tested and confirmed to be diagnosed. Not all foodborne illnesses cause severe symptoms, some may cause digestive upset, diarrhea, fever, and vomiting, and a person may not go to a doctor's office to have a sample tested. If a person has a severe illness, or an illness causing death, a sample is more likely to be tested and confirmed to determine if there is a foodborne pathogen responsible for the illness or death. A highlight from the report is that *E. coli* infections continue to inch up and the progress noted from previous years has stalled, which is still about thirty percent lower than the FoodNet baseline year of 1996-1998 *(14)*.

In 1992, an outbreak of foodborne illness in several western states illustrated the seriousness of meat and poultry as important vehicles of pathogens associated with foodborne illness. The outbreak was attributed to undercooked hamburgers contaminated with *E. coli* O157:H7 that were served at a fast food chain restaurant, which caused several illnesses and four deaths. *E. coli* was described as a human pathogen in 1982, and this organism causes bloody diarrhea and hemolytic uremic syndrome (HUS) in some cases *(28)*. Cattle are common reservoirs of the organism, and therefore beef and beef products are a potential mode of infection.

Cattle are considered to be a definitive source of *E. coli* O157:H7, and through possible contamination of the carcass surface, a pathogen that may cause foodborne illness. In the U.S., individual animal prevalence is low and transient, but the natural

presence illustrates the complex relationship between the animal and its environment. Factors that may impact the animal and its' environment include weather, and livestock production practices. For example, it has been shown that the problem of *E. coli* O157:H7 increases during the summer and fall months, but the environmental factor that impacts this is unknown. Livestock production practices may include feed, and waste handling practices, along with insects and microbial interactions between the soil and water *(32)*.

Outbreaks of illness caused by STECs are related to contact with animals and consumption of meat and fresh produce *(26)*. Shiga toxin-producing *E. coli* can cause devastating illness, particularly in children, causing HUS leading to kidney failure. *E. coli* O157:H7 is the most notorious of the STEC strains causing approximately 73,500 cases in the U.S. each year *(26)*. The CDC estimates that non-O157:H7 STECs are responsible for about 37,000 cases of illness annually, but relatively fewer cases of virulent than *E. coli* O157:H7, a 2008 outbreak of STEC strain O111 in Oklahoma caused illness in at least 314 people, HUS in 17 cases, and one death *(31)*, and other non-O157 outbreaks in the U.S. have been traced to contaminated lake water, salad greens, and milk *(26)*.

Most foodborne illnesses can be prevented, but continued efforts are needed to understand how contamination occurs to develop and implement measures to reduce it in meat and meat products *(14)*. The nature of food and foodborne illness has drastically changed in the U.S. over the last century. Even with improved technology such as pasteurization and proper canning to eliminate some diseases, new causes of foodborne

illness continue to be identified *(30)*. The CDC is working with state health departments to develop and implement ways to detect and investigate outbreaks more quickly so that the foods that cause outbreaks are identified and illnesses can be prevented *(14)*. Enhanced measures are needed to control or eliminate pathogens in domestic and imported food, reduce or prevent contamination of food during growing, harvesting, and processing, and to continue the education of all food-handlers, including restaurant workers and consumers, about risks and prevention measures *(14)*.

Non-O157:H7 Shiga toxin-producing *Escherichia coli*

The CDC estimates that about one third of the STEC infections in the U.S. are caused by non-O157:H7 serotypes; however, due to the challenge in identifying the non-O157 strains, this may be an underestimate *(26)*. Many laboratories do not routinely screen for the shiga toxins, and may only attempt to isolate pathogens in the case of bloody diarrhea, or suspected outbreak *(26)*. Non-O157 STEC are generally associated with a less severe illness that *E. coli* O157:H7; however, this may change due to bacteria that are readily available to exchange genetic material, and are constantly gaining or losing genetic information such as virulence factors *(26)*. Non-O157 STEC infections can cause stomach cramps, diarrhea (sometimes bloody), and vomiting. If a fever is present, it is usually not very high and patients are better within 5-7 days. *E. coli* O157:H7 infections can cause bloody diarrhea, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and death. The symptoms of non-O157 STEC infections may be less severe than *E. coli* O157:H7 infections, but the ability of a bacterium to exchange genetic material, such as a virulence factor, may lend itself to the

non-O157 STEC infections potentially causing as severe foodborne illness as *E. coli* O157:H7. If the non-O157 STECs exchange genetic material with *E. coli* O157:H7 foodborne illnesses may become more severe and may lead to cause more deaths.

Numerous *E. coli* strains are capable of producing one or both Shiga toxins (*stx*1 and *stx*2) *(26)*. The STEC strains have been divided into five seropathotypes:

- A: including the O157 strains that are common causes of outbreaks and HUS in most countries;
- B: non-O157 strains that cause occasional outbreaks, but are fairly common isolates from sporadic cases and HUS (O26:H11, O103:H2, O111:NM, O121:H19, O145:NM);
- C: non-O157 strains associated only with sporadic cases;
- D: strains associated with diarrhea, not more severe symptoms; and E, strains not associated with human disease *(21, 25)*.

From outbreaks and sporadic cases of HUS and severe diarrhea, more than two hundred virulent non-O157 serotypes have been isolated in the U.S. and other countries. Among the most frequently detected serogroups are O111, O103, and O26 *(4)*. Because the standard stool culture methods routinely used in many clinical laboratories do not detect these bacteria, the true incidence of non-O157 STEC infections is more than likely underestimated.

Non-O157 STEC serotypes as with *E. coli* O157:H7, are often associated with cattle and other ruminants, as part of the natural gut micro flora. Cattle can often harbor multiple serotypes, some with which lack the virulence factors, and are therefore less of

a health risk. Microbes can; however, readily exchange genetic information, and the presence of any STEC in food production environments is of concern *(26)*. To minimize human infections with non-O157 STEC, it is necessary to understand which serotypes are most virulent, and the ways in which people are exposed to these pathogens. The epidemiology of infections caused by non-O157 STEC serotypes, with a more comprehensive understanding will lead to improved control methods to prevent illness and reduce economic loss to both food producers and processors.

Beef cattle slaughter operations and possible microbial contamination

Beef can be contaminated during slaughter and processing by exposure to feces or hides containing non-O157 STEC *(26)*. *E. coli* O157:H7 and non-O157:H7 STECs are a naturally occurring part of the gut microflora in cattle. During the slaughter process, close attention must be paid in order to avoid the contamination of what is otherwise sterile meat. Feces, ingesta, and milk are common vehicles for the transfer of pathogens to the meat. Beginning in the beef slaughter process, the use of dirty knives, dirty equipment, unsanitary conditions, and poor employee training must be avoided. The hide removal process must be done to ensure that feces or dirt containing feces does not drop back onto the carcass. During the evisceration process, it is important that the gut not be too full, in which case it may break, causing ingesta to come in contact with the carcass. If feces, ingesta, or milk come in contact with the carcass surface, potential pathogens may contaminate the meat or meat product further in the processing. A visual inspection of the carcass is completed to check for any signs of feces, milk or ingesta on the carcass. If any of the items are found, they must be trimmed away from the carcass.

The interior most portion of the muscle is considered sterile, so it is valuable to the food production process that no pathogens come in contact with the carcass that will eventually be broken down into subprimal, retail, and further processed products. Through the process of breaking down subprimals, any potential pathogen that may have been present on the exterior portion of the meat, may be transferred to the interior portion of the meat. Whether through a knife cut, blade-tenderization machine, or a vacuum tumble marinade, further processing meat and meat products could potentially contaminate all exterior and interior surfaces of the end product that is purchased by the consumer. The consumer has the responsibility of properly cooking, cooling, and preventing cross contamination of the product at home.

Hazard Analysis and Critical Control Point System (HACCP) in the beef slaughter process

HACCP is a preventive system that enables the production of safe meat and meat products, and provides the necessary tools to prove the product has been produced safely. There are seven principles within a HACCP system. Before beginning a HACCP plan, a production process must develop and identify pre-requisite programs, good manufacturing practices, and sanitation standard operating procedures. These items provide the necessary tools and background to maintain facilities, good employee hygiene, and proper and efficient cleaning procedures. The first step in a HACCP plan is to conduct a hazard analysis to identify any potential physical, chemical, or biological hazards that may be reasonably likely to occur *(23)*. Of the three different types of hazards that are reasonably likely to occur within a processing facility, biological,

chemical, or physical, a biological hazard is the hazard that impacts the most consumers. If a consumer finds a piece of plastic in their food, they are impacted by the hazard, but more often than not, it may simply be one consumer. If there is a product that has tested positive for *E. coli* O157:H7, that product may reach more consumers, and have a greater economical impact. With the characteristics of *E. coli*, elderly and young consumers have the highest risk of foodborne illness, should the adulterated product be consumed. The second step in a HACCP plan is to determine critical control points (CCPs), which are points in a process that may reduce or eliminate any potential hazard. Examples of CCPs may include a metal detector, cooking, cooling, or the use of an intervention such as an antimicrobial spray. After the CCPs have been identified, critical limits must be set. Critical limits may be a time or temperature, or the time/temperature relationship. The CCPs and critical limits have to be monitored to assure they are being met, which is the fourth step in a HACCP plan. The fifth step in a HACCP plan is to establish corrective actions, or determine what has to be done if a CCP is not met. The product may need to be rendered inedible, re-heated, re-sprayed, and the reason for the deviation must be identified and eliminated in the future. The sixth and seventh steps of a HACCP system include verification and record-keeping documentation and procedures. Verification must be done to assure that CCPs are working to control, reduce, or eliminate potential pathogens, and all records must be kept to be able to prove safe production of meat and meat products. In a HACCP system, with it being preventive, producers try to eliminate the *E. coli* before it may have a chance to contaminate a carcass.

Different antimicrobial interventions have been utilized as CCPs at different points in the slaughter and further processing processes to reduce or minimize meat contamination causing foodborne illness. During the slaughter process, bacterial contamination can be carried over from previous slaughter steps. The purpose of a CCP in the slaughter process is to prevent, remove, or reduce the bacterial contaminants. Physical or chemical procedures, such as antimicrobial interventions, reduce the pathogen load on the carcass surface.

Pathogen reduction on beef carcasses and intervention steps

Interventions may be used to reduce or eliminate the presence or growth of potential pathogens on the carcass. Most commonly, interventions are applied in the form of spraying; however, there are many intervention options beginning with pre-harvest interventions, processing interventions, and environmental interventions. Pre-harvest interventions may include dietary treatments such as feed, probiotics, bacteriophages, chlorate, drinking water, and vaccines.

Feeding a high-grain ration to feedlot cattle may cause starch from the grains to escape fermentation in the rumen, and pass to the hindgut affecting the survival of some bacteria. Bacteria that are naturally occurring in the intestinal tract of cattle include *E. coli* O157:H7 and non-O157 STECs, and if they survive, they may be more likely to contaminate carcass surfaces and cause foodborne illness if not properly eliminated. The addition of probiotics to feed has been suggested to prevent the growth of STEC in the ruminant of young animals. Probiotics may include live bacteria and yeasts that are healthy for gut production, and may, in a young animal, increase the effective bacteria,

and help to decrease the adverse bacteria. Concurrent with probiotic use is the use of bacteriophages, which are viruses that can kill bacteria. A bacteriophage can enter the bacteria, infect and replicate, and may decrease the incidence of non-O157 STECs. *E. coli* can metabolize chlorate, a toxic compound. Feeding chlorate to cattle, either in the feed or water, can significantly reduce the concentration of *E. coli* O157:H7 and other *E. coli* in feces *(1)*. If the organisms are reduced in the feces, feces that may contaminate the carcass surface through the slaughter process may be less likely to contain *E. coli* O157:H7 or non-O157 STECs. Drinking water can also be a source of STEC infection in cattle if it is contaminated with fecal matter. Chemical treatments may be added to the water, such as lactic acid or acidified calcium sulfate; however, the cattle may drink less water due to the taste of the chemicals, which is why they should not be used continuously.

One last pre-harvest intervention is the use of vaccines. Vaccines have proprietary considerations, and little information is often available, but if the vaccine is proven effective in cattle, it may offer protection against non-O157 strains if they induce antibodies to a common virulence factor *(26)*. Pre-harvest interventions are important because they may be useful in reducing the potential pathogens from entering the processing facility through fecal matter or ingesta.

The environment in which interventions are applied plays an important role in the effectiveness of the intervention to successfully reduce or eliminate potential pathogens. For example, the addition of a chemical to water in a feed trough may reduce the amount of water an animal drinks. Processing interventions involve the carcass

surfaces, and further processing of the carcass into subprimals, steaks, ground beef, or sausage for example. From the live animal to the steak on the grill, interventions are applied at many levels to produce a safe product.

Interventions applied during the processing of beef carcasses

Acid interventions are often applied to beef as antimicrobial treatments. The use of antimicrobial treatments lowers the carcass surface pH. L-lactic, acetic and peroxyacetic acids, acidified sodium chlorite and aqueous ozone solutions have demonstrated some reduction of the microbial populations on hot and chilled beef carcasses *(2, 10, 13, 15)*. L-lactic acid is one of the most effective and cost-efficient treatments for reducing microorganisms on the carcass surface. For this reason, in this study, L-lactic acid was used to acidify the PBS that was used to determine acid resistance of *E. coli* biotype I surrogates, and non-O157 STECs.

The efficacy of L-lactic acid can be increased when applied at 55°C, and when used in combination with other interventions such as a hot water wash *(10, 12, 17)*. The temperature at which lactic acid has an increased efficiency, 55°C, was one of the temperatures that was evaluated in this study. Two other temperatures above 55°C, (60°C and 65°C), were also evaluated to determine the resistance to heat of the *E. coli* biotype I surrogates and the non-O157 STECs in this study. Resistance to acid and heat are integral in determining the usefulness of an antimicrobial to reduce or eliminate potential pathogens.

Lactic acid has been demonstrated to effectively reduce *E. coli* O157:H7 and *Salmonella* populations on the surface of beef carcasses *(9, 11, 12, 16, 20, 34)*. *E. coli*

biotype I surrogate organisms have been shown to represent *E. coli* O157:H7 and *Salmonella*. To test the usefulness of these surrogate organisms for these pathogens, resistance to acid and heat were determined. It is important to test the resistance of acid and heat as it relates directly back to the use of antimicrobial interventions. Due to the increase in foodborne illness infections caused by non-O157 STECs, there is a need in the beef industry to determine potential surrogates for these organisms. Potential surrogates that have the same resistance to acid and heat as the non-O157 STECs could potentially be used to validate the effectiveness of a processing facilities' antimicrobial interventions, and their ability to reduce or eliminate potential pathogens that may cause foodborne illness.

The use of indicator and surrogate microorganisms

When evaluating the quality or safety of raw or processed food products and validating the effectiveness of microbial control measures, indicator or surrogate microorganisms are often used *(7)*. Indicator use is highly dependent upon the microbiological criteria that are set forth in the food product, and frequently used on an informal basis with a specified company. The use of indicator and surrogate microorganisms require specific attention to detail, including the specific food item, harvesting area, and other growth environmental conditions. It is important when choosing a surrogate or indicator microorganism to select the most significant microbial contaminant of concern, and the analytical methods to detect or enumerate the organism are also important *(7)*. Due to processing or microbial reduction treatments, special methods are needed to retrieve the indicator or surrogate or their metabolic or genetic

component if injured survivors or systems are in place to enumerate accurately *(7)*. A sampling plan that will assure statistically significant findings is an essential element to microbiological monitoring. Also to consider when choosing the appropriate surrogate or indicator microorganism is a scheduled review of the criteria for the surrogate or indicator to determine if they continue to be appropriate under the conditions are being evaluated *(7)*.

To be considered for use as an ideal surrogate, the organism would be a nonvirulent strain of the target or test pathogen. Ideally, the surrogate would carry all characteristics of the target pathogen except pathogenicity *(7)*. A surrogate organism may not be selected for use due to the possibility that the strain may return to pathogenicity. Another reason a surrogate may not be selected would be due to the possibility of detection leading to a false positive during routine testing. A false positive indicates a positive when in fact it is negative. For example if a test showed *E. coli* O157:H7 positive, but the sample did not in fact carry *E. coli* O157:H7. In the food industry, several issues may arise should a sample be deemed positive when in fact it is not. A surrogate is ideally picked out of a population of well-known organisms with a well-defined characteristic of being nonpathogenic. The most ideal characteristics of a surrogate are nonpathogenic, isolated from the same environment as the target pathogen, correlating in populations, and similar in growth characteristics, including population densities. Surrogate organisms must respond to processing parameters, such as antimicrobial interventions, in the same manner as the target pathogen *(7)*. Previously

published data have identified surrogate organisms that represent *E. coli* O157:H7 and *Salmonella*, whereas in this study the target pathogens were the non-O157 STECs.

Marshall et al. *(29)* sampled the hides of twenty beef cattle, obtaining 113 bacterial isolates. Growth and biochemical characteristics were evaluated, and thirteen of the isolates were selected for being similar to five clinical strains of *E. coli* O157:H7. *D*-values at 65°C for the thirteen isolates were not different from *E. coli* O157:H7 strains *(29)*. Pre-rigor lean and adipose tissue from beef carcasses were contaminated with a fecal inoculum (either five *E. coli* beef cattle isolates or a cocktail of five *E. coli* O157:H7 strains), and processed with antimicrobial interventions. Depending on the antimicrobial used (water, lactic acid, chlorine or trisodium phosphate), the appropriateness of the isolates for *E. coli* O157:H7 varied, but the mean log reductions of at least two *E. coli* isolates were not different ($P > 0.05$) from the mean log reduction of the *E. coli* O157:H7 cocktail *(29)*. Marshall et al. *(29)* demonstrated the use of potential surrogates that were isolated from the same environment as the target pathogen (beef cattle hides), and were shown to have similar characteristics (*D*-values), and effectiveness with antimicrobial interventions.

Cabrera-Diaz et al. *(8)* evaluated the effectiveness of the *E. coli* biotype I strains identified and isolated from cattle hides (previously discussed) to *E. coli* O157:H7 and *Salmonella*. In a collaborative study with Texas A&M University and Iowa State University, the potential surrogates were sent to the *E. coli* Reference Center at Penn State University, and tested negative for virulence attributes, and were considered

nonpathogenic. For beef processing, and use in a validation study, a nonpathogenic organism is key in assuring no potential contamination for the product or environment.

Throughout the beef industry, many antimicrobial interventions are used individually, in combination with water, steam or pressure, and in combination with other antimicrobial interventions. Due to variation among organisms, no one single organism, or isolate can realistically represent the possible effectiveness of all microbial interventions that may be used in a beef processing facility to reduce or eliminate potential pathogens. The combination of organisms may be required to adequately predict the effectiveness of microbial interventions that may be used in beef processing facilities and their effectiveness against *E. coli* O157:H7 or the non-O157 STECs.

To reduce enteric pathogens on beef carcasses, the efficacy of antimicrobial interventions implemented at the slaughter establishment must be validated under the commercial operating conditions. Surrogate organisms that are non-pathogenic present an ultimate opportunity to validate beef carcass interventions without the use of the enteric pathogen in the commercial facility. Cabrera-Diaz et al. *(8)* evaluated the appropriateness of *E. coli* biotype I surrogates to *E. coli* O157:H7 and *Salmonella* using growth curves, acid and thermal resistance, cell surface hydrophobicity, attachment to beef carcass surfaces and response to antimicrobial interventions on beef carcasses.

The process of identifying appropriate surrogate microorganisms for pathogenic organisms is demonstrated by first selecting the organisms from the appropriate environmental conditions, secondly, determining the characteristics of the organisms at a laboratory level; such as growth parameters, resistance to acid and thermal, and

resistance to freezing and refrigerated storage, and lastly, by conducting research using beef carcass surfaces, antimicrobial interventions and processing techniques. Organisms that best represent pathogenic strains have similar characteristics and perform similarly under processing conditions. Cabrera-Diaz et al. *(8)* found that growth parameters: growth rate, doubling time, maximum population density, and lag time, were not different among the potential surrogates and target pathogens ($P > 0.05$). The *E. coli* biotype I surrogates had similar acid resistance at pH 3.0, 4.0 and 5.0, and similar thermal resistance at 55 and 65°C when compared to the pathogenic *E. coli* O157:H7 strains *(8, 28)*. Following evaluation of the surrogate organisms in a laboratory setting, to be used efficiently as a surrogate, the exact processing conditions must be replicated. There are many factors that are involved in the processing of meat and meat products including the attachment of the organism to the carcass surface, and response to antimicrobial interventions.

Cabrera-Diaz et al. *(8)* evaluated cell surface hydrophobicity which is an attractive force that is correlated to microbial adhesion to surfaces, more specifically, carcass surfaces. This trait would be important in determining the ability of organisms to adhere to a beef carcass surface. Cell surface hydrophobicity was different ($P < 0.05$) among surrogates and some *E. coli* O157:H7 strains, but the strength of attachment to beef carcasses was not different ($P > 0.05$) among all of the organisms *(8)*.

Attachment to beef carcass surfaces is important in determining the efficacy of antimicrobial interventions. Bacterial cells may be loosely or strongly attached to a beef carcass surface, which may include muscle, fat, bone, and other tissue matter. A loosely

attached bacterial cell is freely located within the water film on the surface of tissues, and a strongly attached bacterial cell can be physically associated with the tissue surface. In this study, the numbers of loosely and strongly attached bacterial cells on the surfaces of hot-boned-outside rounds, briskets and clods were compared between the fluorescent protein-marked surrogate, *E. coli* O157:H7, and *Salmonella* strains *(8)*. Mean counts of loosely attached cells were not different ($P > 0.05$) among surrogate, *E. coli* O157:H7 and *Salmonella* strains (regardless of carcass region), and strongly attached cells were not different ($P > 0.05$) between surrogates and target pathogens on the same carcass region *(8)*. Differences ($P < 0.05$) were noted for the strongly attached cells of *Salmonella* on the outside round when compared with attached cells of the surrogates and *E. coli* O157:H7 strains on the clod. These differences may be related to carcass topography, which is important in determining the differences in bacterial attachment. When conducting research to determine the strength of bacterial attachment, it is important to note and maintain consistency in the use of carcass topography to eliminate the variable as a possible reason for variation among organisms and method of attachment.

The process of inoculating beef carcass surfaces for the purpose of research may impact the results of the study. For example, it is more difficult to remove *E. coli* O157:H7 from a beef carcass surface when inoculated with bovine feces rather than a buffered solution. Dickson and Koohmaraie *(18)* failed to find a correlation between the cell surface hydrophobicity and attachment on lean beef tissue, which supports other research demonstrating a lack of correlation between cell surface hydrophobicity and

attachment between *Salmonella* Choleraesuis to either fat or lean beef *(3)*. Rivas et al. *(33)* demonstrated a lack of correlation between the cell surface hydrophobicity of Shiga toxin-producing *E. coli* strains and their adhesion. The lack of correlation between cell surface hydrophobicity and attachment has been demonstrated by previously published research and can be attributed to the complexity and heterogeneous nature of the beef carcass surface, or more importantly, meat surface tissues.

Hot water washes and 2% L-lactic acid sprays were applied to beef carcass surfaces to demonstrate the response of fluorescent protein-marked surrogates, *E. coli* O157:H7 and *Salmonella* strains. Log reductions (CFU per square centimeter) after application of the treatments were not different ($P > 0.05$) among the surrogates, *E. coli* O157:H7 and *Salmonella* strains *(8)*.

Ingham et al. *(24)* conducted a study to compare the survival of potential pathogen surrogates with the survival of *E. coli* O157:H7. The surrogates used in this study were meat hygiene indicators (non-*E. coli* coliforms, biotype I *E. coli*, and lactic acid started cultures). The study was conducted to evaluate interventions using beef brisket (adipose and lean), cod fat membrane, or neck tissue. Treatments were grouped by abattoir size and may have included a dry-aging period, acidic antimicrobial intervention, or hot water treatment. These data demonstrated that all three-surrogate inocula (non-*E. coli* coliforms, biotype I *E. coli*, and lactic acid bacteria) represented *E. coli* O157:H7 for dry-aging and acid-spray plus dry-aging treatment used by small abattoirs *(24)*. In large abattoirs, no one inoculum was suitable for *E. coli* O157:H7 across the intervention treatments used, but the effects seen on neck tissue were

representative of other tissues, and the low value on the neck supports its use as the location for evaluating the treatment efficacy in in-plant trials *(24)*.

Keeling et al. *(28)* demonstrated further research into the evaluation of the previously identified *E. coli* biotype I strains as surrogates *(29)* for *E. coli* O157:H7. Meat processing conditions, outside of the harvesting of the animal, further impact the presence or growth of potential pathogens on the beef carcass surface or meat tissue. Four meat processes that may reduce bacterial load (cooking, fermenting, refrigerating and freezing) were conducted to compare the biotype I surrogate strains with *E. coli* O157:H7.

Throughout the duration of the study *(28)*, with all four processes, at least one of the surrogate organisms was not statistically different when compared to *E. coli* O157:H7. This study demonstrated no differences ($P > 0.10$) between isolates BAA-1428, BAA-1430 and *E. coli* O157:H7 during the frozen shelf life study. The remaining three surrogates that were evaluated survived better during the frozen shelf life study indicating a level of safety representing *E. coli* O157:H7. Throughout the evaluation at refrigerated temperatures, none of the *E. coli* biotype I surrogates were statistically different ($P > 0.10$) when compared to *E. coli* O157:H7. Variations did exist between the organisms over time; however, none of the surrogate strains showed statistically lower populations that E. coli O157:H7. In the fermentation study, all of the surrogates, with the exception of BAA-1428 had smaller reductions in populations when compared to *E. coli* O157:H7, which may prove valuable in over predicting the reduction in or survival of *E. coli* O157:H7 during fermentation. BAA-1427 was the only isolate during

fermentation that was not statistically different ($P > 0.10$) from *E. coli* O157:H7. In evaluating all of the processes demonstrated in this study, isolates BAA-1427, BAA-1429 and BAA 1430 would be good surrogates for *E. coli* O157:H7. In the thermal activation study, these isolates resulted in smaller population reductions that *E. coli* O157:H7. Smaller population reductions may indicate that the surrogate organisms may over predict the population of *E. coli* O157:H7 in these processing conditions.

3. MATERIALS AND METHODS

Bacterial cultures

The biochemical profiles of *E. coli* biotype I surrogates and non-O157 STECs were determined using the Vitek 2, Compact 15 system (bioMèrieux, Inc., Durham, NC) to determine the identification of *E. coli*. The biotype I surrogate isolates have been deposited with the ATCC (Manassas, VA) under accession numbers BAA-1427, BAA-1428, BAA-1429, BAA-1430, and BAA-1431. The non-O157 STEC isolates that were used were under ATCC accession numbers BAA-2192 (O145:NM), BAA-2193 (O45:H2), BAA-2196 (O26:H11), BAA-2215 (O103:H11), BAA-2217 (O111) (organism was originally ordered from American Type Culture Collection (ATCC) under accession # BAA-2217, and has since then been reclassified as O147, see Appendix), and BAA-2219 (O121:H19). Rif-resistant organisms were derived from each *E. coli* biotype I surrogate. Rif-resistant organisms were isolated, following the method described by Kaspar and Tamplin *(27)*, by spread plating 1.0 ml of a suspension containing approximately 10^8 CFU/ml on tryptic soy agar (TSA, Becton Dickinson, Sparks, MD) containing 0.1 g of rifampicin (Sigma Aldrich Life Science, St. Louis, MO) dissolved in 5 ml of methanol per liter (L). Rifampicin was added following the autoclaving and tempering (approximately 50°C). Inoculated plates were incubated at 37°C for 24 h, and several of the resulting colonies were streaked on rif-TSA plates. The strains were streaked on consecutive days on rif-TSA to ensure rif-resistance *(27)*. Stock working cultures were prepared on TSA.

Growth parameters

Growth characteristics were compared to determine if the surrogates could be used to represent non-*E. coli* O157 STECs. Each strain (*E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs) was cultured individually in tryptic soy broth (TSB, Becton Dickinson) at 37°C for 18 h. Rif-resistant surrogates were used in comparison against the parent strain. Following incubation, 0.1 ml of the approximate 10^8 CFU/ml culture was transferred to 9 ml of 0.1% peptone water (PW, Becton Dickinson) to dilute the culture, and subsequent serial dilutions were made in 0.1% PW for each culture until approximately 5 log CFU/ml. After each final culture was diluted, 0.1 ml was transferred into a fresh tube containing 10 ml TSB. All tubes were incubated at 37°C in a constant temperature water bath (VWR International (89032-220), Radnor, PA) and over a 24-h period, one tube from each strain was removed at 0, 1, 2, 3, 6, 7, 8, 10, 12, and 24 h for enumeration. Appropriate decimal dilutions in 0.1% PW were plated on TSA and rif-TSA using the Whitley Automatic Spiral Plater (WASP) (Model #S00600, Microbiology International, Frederick, MD). Plates were incubated (Gravity Convection Incubator E191047, VWR International) at 37°C for 24 h before determining counts. Plates were counted using the grid provided by DW Scientific, and two opposite quadrants were determined and counted. After a count was achieved, the count was converted to scientific notation using the conversion chart provided by DW Scientific and after accounting for the appropriate decimal dilutions, a final log count was achieved. Each growth curve was completed in three replications. The General Linear Model (GLM) of SAS (SAS Institute, Inc., Cary, NC) was used for

data analysis. Least squares means were calculated and used to compare growth characteristics of the parent and rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs, both as individual strains, and sets of strains (parent and rif-resistant *E. coli* biotype I strains, and non-O157 STECs). Sets of strains were analyzed to eliminate individual strain-to-strain variation and represent what may occur should organisms be used as a cocktail.

Acid resistance

Throughout the process of harvesting beef cattle, antimicrobial interventions are often applied to reduce the pH of the carcass surface, further reducing or eliminating potential pathogens. Each individual strain was cultured in TSB at 37°C for 18 h. 0.1 ml aliquots of the fresh cultures were then transferred to 10 ml TSB and 10 ml TSB+G tubes (supplemented with 1% glucose (10 ml glucose/1L of TSB), (D-Glucose, Anhydrous, Granular, Macron Fine Chemicals, Center Valley, PA), to induce an acid tolerance response). Buchannan and Edelson *(5)* demonstrated that prior growth of an organism in a medium with a fermentable carbohydrate (TSB+G) induces an acid-tolerance response. Following incubation at 37°C for 18 h in either TSB or TSB+G, each culture was transferred to a 15 ml conical centrifuge tube (#89039-666, VWR International). The cultures were then harvested using a Heraeus Megafuge 16 Centrifuge (Thermo Fisher Scientific, Waltham, MA) at 1,620 × *g* for 15 min. After the cultures were centrifuged, the supernatant was poured off and the pellet re-suspended in 10 ml phosphate buffered saline (PBS, EMP Chemical Inc., San Diego, CA). The pH of the PBS was taken each time it was prepared and found to be approximately 7.4.

Following re-suspension of the pellet in 10 ml PBS, the cultures were centrifuged again (1,620 × *g* for 15 min), and the procedure repeated an additional two times, for a total of four centrifugation cycles. After the final pellets were re-suspended in 10 ml PBS, 0.1 ml aliquots were transferred into glass tubes (16 × 150 mm) containing 10 ml pre-warmed (37°C) PBS acidified with 88% L-lactic acid (Purac, Lenexa, KS). Three different pH values were evaluated in this experiment, pH 2.5, 3.0, and 3.5. All tubes were incubated in a constant temperature water bath (37°C), with the water level at least 1 cm above the level of the suspension. Immediately after inoculation of the acidified PBS with the culture, one tube was removed for enumeration of survivors. The remaining tubes remained in the constant temperature water bath and were removed (one tube of each strain) at 0.5, 1.0, 1.5, and 2.0 h for enumeration of survivors. Survivors were serially diluted using 0.1% PW and using the WASP, plated on TSA or rif-TSA. Plates were incubated at 37°C for 24 h and survivors were counted using the method as previously described above. Each experiment was conducted in three replications, and log reductions (CFU/ml) were calculated by subtracting the log count (CFU/ml) of each strain after each exposure time (0.5, 1.0, 1.5, and 2.0 h) from the initial log count calculated following the initial inoculation. The GLM of SAS was used to calculate least squares means to determine significant differences between the individual strains, and sets of parent and rif-resistant *E. coli* biotype I surrogates, and the non-O157 STECs.

Thermal resistance

In order to determine and compare the decimal reduction time (*D*-value) or the time required at a given temperature to kill 90% of the exposed microorganisms, thermal resistance of the parent and rif-resistant *E. coli* biotype I surrogates, and the non-O157 STECs were calculated at three different temperatures. After calculating *D*-values, *z*-values were also calculated by plotting the $\log_{10}$ of the *D*-values across temperature. *Z*-values are calculated to determine the relative resistance of an organism to different temperatures, and to show the degrees (F or C) required for the thermal destruction curve to traverse 1 log cycle (the change in temperature that changes the *D*-value by a factor of 10).

Individual strains were cultured in TSB at 37°C for 18 h. Aliquots of 0.1 ml of each culture were transferred to individual 10 ml TSB and 10 ml TSB+G tubes for a second incubation at 37°C for 18 h. TSB+G was used in this experiment to determine if there was a cross-adaptation between acid-adapted organisms and temperature. For centrifugation, each culture was transferred to a 15 ml conical centrifuge tube, and harvested at 1,620 × *g* for 15 min. After centrifugation, the supernatant was poured off, and the pellet was re-suspended in 10 ml PBS. The pH of the PBS was 7.4. The cell suspension was centrifuged again, and the procedure of pouring of the supernatant and re-suspending the pellet in 10 ml PBS was followed. This procedure was performed two more times. The cells were washed 4 times. After the fourth and final re-suspension, 50 μL of each individual culture were inoculated into a capillary tube (1.5-1.8 mm, Chemglass Life Science, Vineland, NJ.). The capillary tubes were flame sealed on each

end, and submerged in a beaker (400 ml), covered with a watch glass to maintain temperature and prevent possible contamination, in a constant temperature water bath at 55, 60, and 65 ± 0.5°C. Depending on the temperature, pre-determined time intervals were calculated, and at each time point, one capillary tube of each strain was removed from the constant temperature water bath, dipped in pre-chilled 70% ethanol for 30 s, and rinsed in pre-chilled sterile water in an ice bath. Using sterile forceps, each capillary tube was then transferred to a conical centrifuge tube containing 10 ml PBS. The capillary tubes were crushed using a sterile glass rod. The suspension was serially diluted in 0.1% PW and plated using the WASP on TSA and rif-TSA. Enumeration was performed after incubation at 37°C for 24 h. Survivors were counted using the procedure previously described. Each experiment was conducted in three replications. Survivor data (log CFU/ml) was plotted as a function of time as a scatter plot (a mathematical diagram used to identify and display the type of relationship between two quantitative variables) in Microsoft Excel. Using at least three of the most linear points with the shallowest slope, a linear regression analysis was conducted to determine the slope of the line. At least three of the most linear points were used to predict the relationship between times and log value counts. *D*-values were calculated using the reciprocal of the calculated slope. After calculating the *D*-values, log *D*-values were calculated and plotted as a function of temperature as a scatter plot in Microsoft Excel. A linear regression analysis was conducted to determine the slope of the line, and the *z*-value was demonstrated by using the reciprocal of the calculated slope. The GLM of SAS was used

to determine least squares means to demonstrate significant differences between the parent and rif-resistant *E. coli* biotype I surrogates, and the non-O157 STECs.

Response to refrigeration and freezing temperatures

Throughout the processing of meat and meat products, items may be processed or stored in refrigeration or freezing temperatures. It is important to understand the organisms' response to these temperatures, to represent a scenario where the organisms may be present on the meat or meat product. If the organism is present and stored at refrigeration or freezing temperature, the characteristics of the organism must be identified. In using cold storage as a Critical Control Point (CCP), it must be documented if the organism is present whether it will grow, or be reduced.

To test the organism's resistance to refrigerated and frozen storage, each individual strain was cultured in TSB at 37°C for 18 h. A second transfer was then made to either 10 ml TSB or 10 ml TSB+G and incubated at 37°C for 18 h. As in thermal resistance, acid-adapted cultures (TSB+G) were also used to test resistance to refrigeration and frozen storage to determine if there was a cross-adaptation to the cold temperatures with the cells. Each individual culture was then transferred to a 15 ml conical centrifuge tube, and centrifuged at 1,620 × *g* for 15 min. After centrifugation, the supernatant was poured off, and the pellet was re-suspended in 10 ml PBS (pH 7.4). After the pellet was re-suspended, the cells were centrifuged again, the supernatant was discarded, and the pellet was re-suspended in 10 ml PBS. This process was repeated for a total of four centrifugation cycles. The final pellet was re-suspended in 100 ml PBS, and 1 ml of the bacterial strains was transferred into individual 1.5 ml sterile Safe-lock

tubes (micro-centrifuge tubes, Safe-lock, Eppendorf, Hauppauge, NY). Fifty % of the tubes (n = 672) were stored at 4 ± 0.5°C (refrigeration) and the remaining 50% (n = 672) at -20 ± 0.5°C (freezing). Initially, and at 7, 14, 21, 28, 60, and 90 day of storage, one tube of each culture was removed from the refrigerated or frozen storage. Frozen cultures were defrosted in an ice water bath for approximately 30 min before enumeration. Enumeration was determined using the WASP on TSA and rif-TSA plates, following serial dilutions in 0.1% PW, and incubation at 37°C for 24 h. Survivors were counted using the grid (DW Scientific) and counting two opposite quadrants, converted to scientific notation after accounting for the serial dilutions and converted to a log count. Each experiment was conducted in three replications. Mean log counts were compared using the GLM of SAS to determine significant differences between the parent and rif-resistant *E. coli* biotype I surrogates, and the non-O157 STECs.

4. RESULTS AND DISCUSSION

Growth parameters

For the *E. coli* biotype I surrogate microorganisms to be useful in predicting the behavior of pathogens during processing, the growth of these surrogates would ideally be equivalent or slightly greater, but not less than that of the target pathogens (six non-O157 STECs) *(7)*. The characteristics of a surrogate organism include having similar growth characteristics, including population densities. A surrogate organism would be equivalent or slightly greater in growth than the target pathogen to represent the greatest potential growth that may be achieved by the target pathogen. Growth curves of *E. coli* biotype I surrogates, both the parent strains and rif-resistant strains, were similar to those of all non-O157 STECs throughout the evaluation (Table 1), differing by 0.7 log or less at each 1-h evaluation during the 24-h growth period. There were differences between individual organisms ($P < 0.05$) throughout the evaluation, which supports previously published research *(19, 29)* that single strains are not able to mimic the behavior of all pathogenic strains tested. When utilized as a set, the non-pathogenic *E. coli* biotype I surrogates may provide a range of growth responses that may show them as appropriate surrogates for the non-O157 STECs.

To replicate the use of the individual organisms as a set, the five surrogate organisms, the five rif-resistant surrogate organisms, and the six non-O157 STECs were combined and averaged to create means separation based on the three individual replications. The organisms were averaged to create one number to represent the five or

six individual organisms with the same characteristics. The individual organisms in the study were collectively run as sets to get a mean. The individual organisms were not combined and analyzed prior to data collection to truly represent a cocktail. The collection of the individual organisms was analyzed to remove strain-to-strain variation, and to look at how the organisms may grow as a set. There may be instances in where the use of the organisms as a cocktail would not be similar to collectively analyzing the individual means due to potential background effects on the organisms. Growth curves for sets of *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs are shown in Figure 1. As shown in Table 2, there were no differences ($P > 0.05$) between the initial population of the *E. coli* biotype I surrogates and the non-O157 STECs. Throughout the evaluation, from 1-h to 10-h, the non-O157 STECs were not different ($P > 0.05$) from either the *E. coli* biotype I surrogates, both parent strains and rif-resistant strains, but not necessarily both at any given time point. The non-O157 STECs were significantly different ($P < 0.05$) from the parent and rif-resistant *E. coli* biotype I surrogates by having a lower population at each given time point. A population of the non-O157 STECs that is lower than the *E. coli* biotype I surrogates is ideal for the organisms to properly represent the target pathogens for use as surrogates. By hour 12, all three groups of organisms, *E. coli* biotype I surrogates, *E. coli* biotype I rif-resistant surrogates, and the non-O157 STECs were not different ($P > 0.05$) through the completion of the 24-hour growth curve evaluation. While some statistical differences ($P < 0.05$) were detected at intervals during growth from an initial population of approximately log 3.0 to log 9.0 over twenty-four hours, trends were clearly within the

scope for use as surrogates. Within the scope for use of surrogates refers to the definition of a surrogate in having growth that is equivalent or greater than the growth of the target pathogens, which is supported by the data represented.

Previously published research from Cabrera-Diaz et al. *(8)* evaluated growth parameters: growth rate, doubling time, maximum population density, and lag time. These data found few differences ($P < 0.05$) between the *E. coli* biotype I surrogates and the pathogenic *E. coli* O157:H7, which supports the data represented here that nonpathogenic surrogates might best represent pathogenic *E. coli,* specifically not O157, but the non-O157 STECs. In the Cabrera-Diaz et al. *(8)* study, the surrogates were determined to be appropriate for use to represent *E. coli* O157:H7, in that the populations were equivalent or greater in growth than the pathogen. In the data that is shown here, the surrogates were determined to be appropriate for use to represent the non-O157:H7 STECs, but there was not one surrogate that represented all of the non-O157 STECs appropriately, which is why the surrogates may be best utilized in a set versus individual organisms.

Table 1. Least squares means ± standard error of the mean for organisms comparing *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains in tryptic soy broth for growth at 37°C

	Mean[a] ± SEM[b]									
	Time (h)									
Organism	0	1	2	3	6	7	8	10	12	24
BAA 1427	3.1 ± 0.0 $_{BCDEF}$[c]	3.8 ± 0.1 $_{A}$	4.5 ± 0.1 $_{ABC}$	5.3 ± 0.1 $_{BCD}$	8.0 ± 0.2 $_{AB}$	8.2 ± 0.1 $_{BCDE}$	8.7 ± 0.1 $_{ABCD}$	8.8 ± 0.0 $_{ABC}$	8.8 ± 0.2 $_{A}$	8.8 ± 0.0 $_{CDEF}$
BAA 1428	3.3 ± 0.0 $_{AB}$	3.7 ± 0.1 $_{AB}$	4.5 ± 0.1 $_{ABC}$	5.5 ± 0.1 $_{AB}$	8.0 ± 0.2 $_{A}$	8.3 ± 0.1 $_{BCD}$	8.7 ± 0.1 $_{ABCD}$	8.8 ± 0.0 $_{ABCD}$	7.8 ± 0.2 $_{B}$	8.9 ± 0.0 $_{ABCDE}$
BAA 1429	3.3 ± 0.0 $_{A}$	3.7 ± 0.1 $_{ABC}$	4.4 ± 0.1 $_{ABC}$	5.4 ± 0.1 $_{ABC}$	7.9 ± 0.2 $_{ABC}$	8.3 ± 0.1 $_{BCD}$	8.8 ± 0.1 $_{ABC}$	8.8 ± 0.0 $_{ABCD}$	8.8 ± 0.2 $_{A}$	8.9 ± 0.0 $_{ABCDE}$
BAA 1430	3.3 ± 0.0 $_{AB}$	3.8 ± 0.1 $_{A}$	4.5 ± 0.1 $_{ABC}$	5.4 ± 0.1 $_{ABC}$	8.1 ± 0.2 $_{A}$	8.6 ± 0.1 $_{A}$	8.9 ± 0.1 $_{A}$	8.8 ± 0.0 $_{ABC}$	8.8 ± 0.2 $_{A}$	8.9 ± 0.0 $_{ABC}$
BAA 1431	3.1 ± 0.0 $_{CDEF}$	3.7 ± 0.1 $_{AB}$	4.5 ± 0.1 $_{ABC}$	5.5 ± 0.1 $_{A}$	8.1 ± 0.2 $_{A}$	8.5 ± 0.1 $_{AB}$	8.9 ± 0.1 $_{AB}$	8.8 ± 0.0 $_{ABCD}$	8.7 ± 0.2 $_{A}$	8.7 ± 0.0 $_{FG}$
BAA 1427 rif[d]	3.1 ± 0.0 $_{DEF}$	3.7 ± 0.1 $_{ABC}$	4.5 ± 0.1 $_{AB}$	5.2 ± 0.1 $_{DEF}$	7.4 ± 0.2 $_{DE}$	7.9 ± 0.1 $_{EF}$	8.5 ± 0.1 $_{DE}$	8.8 ± 0.0 $_{ABCD}$	8.7 ± 0.2 $_{A}$	9.0 ± 0.0 $_{AB}$
BAA 1428 rif	3.2 ± 0.0 $_{ABCD}$	3.6 ± 0.1 $_{ABC}$	4.4 ± 0.1 $_{BC}$	5.3 ± 0.1 $_{CDE}$	7.5 ± 0.2 $_{BCDE}$	8.0 ± 0.1 $_{DEF}$	8.4 ± 0.1 $_{EF}$	8.7 ± 0.0 $_{D}$	8.7 ± 0.2 $_{A}$	8.9 ± 0.0 $_{ABCDE}$
BAA 1429 rif	3.1 ± 0.0 $_{EF}$	3.5 ± 0.1 $_{ABC}$	4.4 ± 0.1 $_{ABC}$	5.1 ± 0.1 $_{FG}$	7.5 ± 0.2 $_{BCDE}$	8.1 ± 0.1 $_{CDE}$	8.7 ± 0.1 $_{BCD}$	8.7 ± 0.0 $_{BCD}$	8.7 ± 0.2 $_{A}$	8.8 ± 0.0 $_{CDEF}$
BAA 1430 rif	3.1 ± 0.0 $_{ABCDE}$	3.5 ± 0.1 $_{ABC}$	4.3 ± 0.1 $_{BC}$	5.2 ± 0.1 $_{EF}$	7.4 ± 0.2 $_{CDE}$	8.1 ± 0.1 $_{DE}$	8.6 ± 0.1 $_{CD}$	8.7 ± 0.0 $_{ABCD}$	8.8 ± 0.2 $_{A}$	9.0 ± 0.0 $_{A}$
BAA 1431 rif	3.0 ± 0.0 $_{F}$	3.5 ± 0.1 $_{ABC}$	4.6 ± 0.1 $_{A}$	5.4 ± 0.1 $_{ABC}$	7.8 ± 0.2 $_{ABCDE}$	8.4 ± 0.1 $_{ABC}$	8.8 ± 0.1 $_{ABC}$	8.7 ± 0.0 $_{CD}$	8.7 ± 0.2 $_{A}$	8.7 ± 0.0 $_{G}$
BAA-2219[e]	3.2 ± 0.0 $_{ABC}$	3.6 ± 0.1 $_{ABC}$	4.5 ± 0.1 $_{ABC}$	5.4 ± 0.1 $_{ABC}$	7.7 ± 0.2 $_{ABCDE}$	8.4 ± 0.1 $_{ABC}$	8.8 ± 0.1 $_{ABC}$	8.8 ± 0.0 $_{ABCD}$	8.8 ± 0.2 $_{A}$	8.8 ± 0.0 $_{EFG}$
BAA-2217	3.2 ± 0.0 $_{ABCDE}$	3.5 ± 0.1 $_{ABC}$	4.4 ± 0.1 $_{ABC}$	5.4 ± 0.1 $_{ABC}$	8.0 ± 0.2 $_{AB}$	8.5 ± 0.1 $_{AB}$	8.9 ± 0.1 $_{AB}$	8.8 ± 0.0 $_{ABCD}$	8.8 ± 0.2 $_{A}$	8.9 ± 0.0 $_{BCDE}$
BAA-2215	3.3 ± 0.0 $_{AB}$	3.4 ± 0.1 $_{C}$	4.3 ± 0.1 $_{BC}$	5.1 ± 0.1 $_{FG}$	7.3 ± 0.2 $_{E}$	8.0 ± 0.1 $_{DEF}$	8.6 ± 0.1 $_{CD}$	8.8 ± 0.0 $_{AB}$	8.7 ± 0.2 $_{A}$	8.8 ± 0.0 $_{DEFG}$
BAA-2196	3.2 ± 0.0 $_{ABCDE}$	3.4 ± 0.1 $_{BC}$	4.1 ± 0.1 $_{D}$	4.9 ± 0.1 $_{G}$	7.5 ± 0.2 $_{BCDE}$	7.7 ± 0.1 $_{F}$	8.3 ± 0.1 $_{F}$	8.7 ± 0.0 $_{ABCD}$	8.8 ± 0.2 $_{A}$	8.9 ± 0.0 $_{ABC}$
BAA-2193	3.2 ± 0.0 $_{ABCDE}$	3.5 ± 0.1 $_{ABC}$	4.3 ± 0.1 $_{CD}$	5.2 ± 0.1 $_{EF}$	7.7 ± 0.2 $_{ABCDE}$	8.4 ± 0.1 $_{ABC}$	8.8 ± 0.1 $_{ABC}$	8.8 ± 0.0 $_{A}$	8.8 ± 0.2 $_{A}$	8.9 ± 0.0 $_{ABCD}$
BAA-2192	3.2 ± 0.0 $_{ABCDE}$	3.6 ± 0.1 $_{ABC}$	4.5 ± 0.1 $_{ABC}$	5.3 ± 0.1 $_{BCD}$	7.8 ± 0.2 $_{ABCD}$	8.5 ± 0.1 $_{AB}$	8.8 ± 0.1 $_{ABC}$	8.7 ± 0.0 $_{ABCD}$	8.8 ± 0.2 $_{A}$	8.8 ± 0.0 $_{CDEF}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 2. Least squares means ± standard error of the mean for organisms comparing *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains in sets for growth in tryptic soy broth at 37°C

	Mean[a] ± SEM[b]									
	Time (h)									
Organism	0	1	2	3	6	7	8	10	12	24
E. coli surrogates[d]	3.2 ± 0.0 $_{A}$[c]	3.7 ± 0.0 $_{A}$	4.5 ± 0.0 $_{A}$	5.4 ± 0.0 $_{A}$	8.0 ± 0.1 $_{A}$	8.4 ± 0.1 $_{A}$	8.8 ± 0.1 $_{A}$	8.8 ± 0.0 $_{A}$	8.6 ± 0.1 $_{A}$	8.9 ± 0.0 $_{A}$
Rif-resistant[e] *E. coli* surrogates	3.1 ± 0.0 $_{B}$	3.6 ± 0.0 $_{B}$	4.5 ± 0.0 $_{AB}$	5.2 ± 0.0 $_{B}$	7.5 ± 0.1 $_{B}$	8.1 ± 0.1 $_{B}$	8.6 ± 0.1 $_{B}$	8.7 ± 0.0 $_{B}$	8.7 ± 0.1 $_{A}$	8.9 ± 0.0 $_{A}$
Non-O157:H7 STECs[f]	3.2 ± 0.0 $_{A}$	3.5 ± 0.0 $_{B}$	4.4 ± 0.0 $_{B}$	5.2 ± 0.0 $_{B}$	7.7 ± 0.1 $_{B}$	8.2 ± 0.1 $_{AB}$	8.7 ± 0.0 $_{AB}$	8.8 ± 0.0 $_{A}$	8.8 ± 0.1 $_{A}$	8.8 ± 0.0 $_{A}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] *E. coli* surrogates include BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431 strains
[e] Rif-resistant *E. coli* surrogates include rif-resistant strains BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431
[f] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

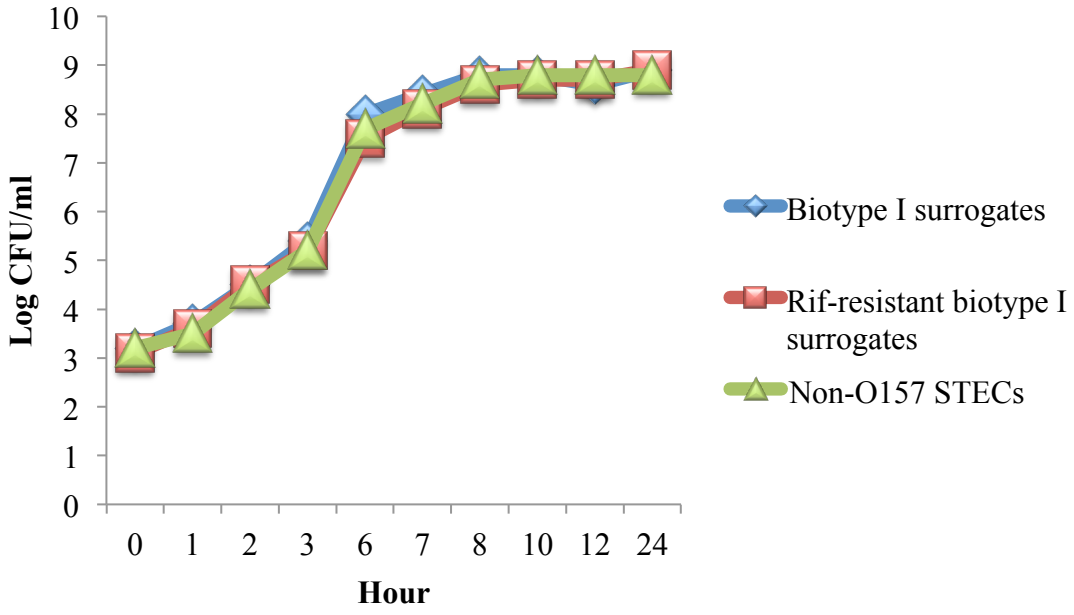

Figure 1. Growth curves for sets of *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains in tryptic soy broth at 37°C

Acid resistance

The acid resistance of *E. coli* biotype I surrogate microorganisms, and the non-O157 STECs were compared in PBS acidified with L-lactic acid at pH values 2.5, 3.0, and 3.5. This pH range was selected based on the expected carcass surface pH after application of 2% L-lactic acid *(22)*. To be used in the validation of lactic acid interventions, surrogate organisms would ideally have resistance to lactic acid similar to that of the target pathogens (six non-O157 STECs). All cultures were grown to stationary phase in TSB and in TSB+G to induce their acid tolerance. It has been demonstrated that *E. coli* cultures grown in the presence of glucose reduces the pH in the medium, and induces a pH-dependent acid tolerance response *(5)*. It has been reported that culturing enterohemorrhagic *E. coli* in the presence of glucose reduced the pH in the medium during the growth of the microorganisms making the cells more resistant to subsequent exposure to acidic conditions *(5, 6)*. In the intestinal tract of cattle, enteric pathogens, such as *E. coli* O157:H7 and *Salmonella* are exposed to a reduced pH condition due to the presence of volatile fatty acids. The exposure to mild acidic conditions has been demonstrated to increase their acid resistance.

Log reductions (CFU/ml) after initial, 0.5, 1.0 1.5, and 2.0 h of exposure to acidic conditions at pH 2.5, 3.0, and 3.5 were calculated and compared among the sixteen organisms included in this study, the five *E. coli* biotype I surrogates, the five *E. coli* rif-resistant biotype I surrogates, and the six non-O157 STECs. For acid resistance, most *E. coli* biotype I microorganisms had similar ($P > 0.05$) reductions to the non-O157 STECs; however, there were some instances of greater reductions ($P < 0.05$). For example, the

stationary phase organisms at pH 3.0, at the initial time point, organism BAA-1428 showed the greatest reduction at 3.1 log CFU/ml compared to the other fifteen organisms showing reductions from 2.2-2.9. For the stationary phase organisms at pH 3.5, the 0.5-h, organism BAA-1430 showed the greatest reduction (3.2 vs. 0.9-2.6) log CFU/ml, and at the 1-h time point, BAA-1430 reduced 3.9 CFU/ml compared to 1.3-3.8. Acid-adapted organisms at pH 2.5, organism BAA-1429 showed the greatest initial reduction at 4.3 compared to 3.6-4.2 log CFU/ml. Acid-adapted organisms at pH 3.0, following the 2-h exposure time, organism BAA-1430 showed the greatest reduction at 7.4 compared to 5.1-7.0 log CFU/ml. Acid-adapted organisms at pH 3.5, following the 0.5-h time point, organism 1428 showed the greatest reduction at 2.6 log CFU/ml compared to 1.9-2.4, and at 1-h, organisms BAA-1427 showed the greatest reduction at 2.6 compared to 2.1-2.5 log CFU/ml. These data support previously published data stating that the surrogate organisms (log reductions CFU/ml) were not different ($P > 0.05$) from *E. coli* O157:H7 *(8)*.

Stationary phase organisms at pH 2.5 were not compared using a least squares means separation due to missing data points at the initial level, which did not allow for subsequent analysis. The initial reduction ranged from 3.9-4.5 log CFU/ml, and the final log reduction following exposure to L-lactic acid acidified to pH 2.5 ranged from 6.7-7.6 log CFU/ml. Table 3 shows the stationary phase organisms at pH 3.0. Initially, there were differences ($P < 0.05$) between the sixteen individual organisms; however, following the 2.0-h exposure time, the differences ($P < 0.05$) were less variable, and the six non-O157 STECs were comparable with at least one *E. coli* biotype I surrogate or

one *E. coli* rif-resistant biotype I surrogate, if not more. Table 4 shows the stationary phase organisms at pH 3.5. Initially the log reductions (CFU/ml) ranged from 0.4-2.2. All organisms were not different ($P > 0.05$) except for organism BAA-1430 rif at the initial log reduction (CFU/ml) time point. At the one hour time point, BAA-1430 rif was different ($P < 0.05$) from the other fifteen organisms at a lower reduction of only 1.3 log CFU/ml compared to 2.5-3.8 log CFU/ml reduction. After 2 h exposure to L-lactic acid at pH 3.5 the log reductions (CFU/ml) varied from 2.3-5.1 with organism BAA-2192 showing the greatest reduction at 5.1 log CFU/ml.

The acid-adapted organisms (TSB+G) acidified with L-lactic acid at pH 2.5 are shown in Table 5. Initially, log reductions (CFU/ml) ranged from 3.6-4.3, and after 2 h exposure time to L-lactic acid at pH 2.5, the log reductions (CFU/ml) ranged from 5.7-7.5. At the 1-h time interval, BAA-1427 rif was the only organism that was different ($P < 0.05$) from the other fifteen organisms at 5.4 log reduction CFU/ml compared to 6.6-7.5 log reduction CFU/ml. Organism BAA-1427 rif, at the 1-h time interval showed more resistance to the pH, and did not have as great of a reduction as the other fifteen organisms. More research may be needed to understand the possible mechanism between the rif-resistant, and potential adaptations to pH. Further data collection may show that a resistance to an antibiotic may allow for the organism to have a greater resistance to pH or temperature. Table 6 shows all sixteen organisms, acid-adapted, and exposed to L-lactic acid at pH 3.0. Initially, the log reductions (CFU/ml) ranged from 1.7-2.5; however, organism BAA-2196 was different ($P < 0.05$) from the other fifteen organisms, showing the greatest initial log reduction (CFU/ml) at 2.5. Following exposure for 2 h

reductions ranged from 5.1-7.4 log CFU/ml at pH 3.0. Data shown in Table 7 represent all sixteen organisms, acid-adapted, and exposed to L-lactic acid at pH 3.5. Initially, log reductions (CFU/ml) ranged from 1.8-2.1, and final log reductions (CFU/ml) ranged from 2.7-3.7. Organism BAA-1431 showed a total reduction after 2-h exposure at pH 3.0 (stationary phase) of 4.1 log CFU/ml, 1.2 log at pH 3.5 (stationary phase), 2.9 log at pH 2.5 (acid-adapted), 5.2 log at pH 3.0 (acid-adapted), and 1.6 log at pH 3.5 (acid-adapted). Organism BAA-1431 showed greater reductions at a higher pH compared to the lower pH value. Organisms respond differently to stress. It is interesting to note that strain-to-strain variation does occur. Since each processing condition is unique, how the organisms are expected to react may not actually be the way they react to each individual experiment.

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Table 3. Mean reductions (log CFU/ml) for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.0 at 37°C

	Mean reduction[a] ± SEM[b]				
	Exposure time (h)				
Organism	0	0.5	1	1.5	2
BAA 1427	2.3 ± 0.2 $_{E}$[c]	5.1 ± 0.4 $_{BCDE}$	5.8 ± 0.6 $_{ABC}$	5.6 ± 0.6 $_{B}$	6.3 ± 0.5 $_{AB}$
BAA 1428	3.1 ± 0.2 $_{A}$	5.5 ± 0.4 $_{ABCD}$	6.4 ± 0.6 $_{ABC}$	6.5 ± 0.6 $_{AB}$	7.3 ± 0.5 $_{AB}$
BAA 1429	2.3 ± 0.2 $_{DE}$	5.9 ± 0.4 $_{ABC}$	6.4 ± 0.6 $_{ABC}$	6.3 ± 0.6 $_{AB}$	6.8 ± 0.5 $_{AB}$
BAA 1430	2.9 ± 0.2 $_{AB}$	5.3 ± 0.4 $_{BCD}$	6.0 ± 0.6 $_{ABC}$	6.4 ± 0.6 $_{AB}$	7.2 ± 0.5 $_{AB}$
BAA 1431	2.3 ± 0.2 $_{CDE}$	4.9 ± 0.4 $_{CDE}$	5.3 ± 0.6 $_{BC}$	6.5 ± 0.6 $_{AB}$	6.4 ± 0.5 $_{AB}$
BAA 1427 rif[d]	2.3 ± 0.2 $_{E}$	4.4 ± 0.4 $_{DE}$	5.1 ± 0.6 $_{BC}$	5.9 ± 0.6 $_{AB}$	7.0 ± 0.5 $_{AB}$
BAA 1428 rif	2.2 ± 0.2 $_{E}$	4.5 ± 0.4 $_{DE}$	5.9 ± 0.6 $_{ABC}$	5.5 ± 0.6 $_{B}$	6.6 ± 0.5 $_{AB}$
BAA 1429 rif	2.3 ± 0.2 $_{CDE}$	4.0 ± 0.4 $_{E}$	6.1 ± 0.6 $_{ABC}$	5.3 ± 0.6 $_{B}$	6.6 ± 0.5 $_{AB}$
BAA 1430 rif	2.2 ± 0.2 $_{E}$	4.3 ± 0.4 $_{DE}$	5.3 ± 0.6 $_{BC}$	5.5 ± 0.6 $_{B}$	7.2 ± 0.5 $_{AB}$
BAA 1431 rif	2.4 ± 0.2 $_{BCDE}$	4.3 ± 0.4 $_{DE}$	4.7 ± 0.6 $_{C}$	5.3 ± 0.6 $_{B}$	7.2 ± 0.5 $_{AB}$
BAA-2219[e]	2.8 ± 0.2 $_{ABCDE}$	6.0 ± 0.4 $_{ABC}$	6.7 ± 0.6 $_{AB}$	6.5 ± 0.6 $_{AB}$	7.6 ± 0.5 $_{A}$
BAA-2217	2.8 ± 0.2 $_{AB}$	4.9 ± 0.4 $_{BCDE}$	6.3 ± 0.6 $_{ABC}$	7.5 ± 0.6 $_{A}$	7.6 ± 0.5 $_{A}$
BAA-2215	2.5 ± 0.2 $_{BCDE}$	5.9 ± 0.4 $_{ABC}$	5.5 ± 0.6 $_{ABC}$	6.6 ± 0.6 $_{AB}$	6.8 ± 0.5 $_{AB}$
BAA-2196	2.7 ± 0.2 $_{ABCDE}$	6.2 ± 0.4 $_{AB}$	6.2 ± 0.6 $_{ABC}$	6.6 ± 0.6 $_{AB}$	6.0 ± 0.5 $_{B}$
BAA-2193	2.7 ± 0.2 $_{ABCDE}$	5.4 ± 0.4 $_{ABCD}$	7.2 ± 0.6 $_{A}$	6.1 ± 0.6 $_{AB}$	7.2 ± 0.5 $_{AB}$
BAA-2192	2.8 ± 0.2 $_{ABC}$	6.6 ± 0.4 $_{A}$	6.5 ± 0.6 $_{ABC}$	5.8 ± 0.6 $_{AB}$	6.0 ± 0.5 $_{B}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 4. Mean reductions (log CFU/ml) for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.5 at 37°C

	Mean reduction[a] ± SEM[b]				
	Exposure time (h)				
Organism	0	0.5	1	1.5	2
BAA 1427	1.8 ± 0.3 $_{A}$[c]	2.5 ± 0.4 $_{A}$	2.8 ± 0.4 $_{ABC}$	2.8 ± 0.5 $_{CD}$	3.3 ± 0.4 $_{DEF}$
BAA 1428	2.1 ± 0.3 $_{A}$	2.2 ± 0.4 $_{AB}$	3.0 ± 0.4 $_{ABC}$	2.8 ± 0.5 $_{CD}$	3.3 ± 0.4 $_{DEF}$
BAA 1429	1.7 ± 0.3 $_{A}$	2.1 ± 0.4 $_{AB}$	2.6 ± 0.4 $_{C}$	2.7 ± 0.5 $_{CD}$	3.4 ± 0.4 $_{DEF}$
BAA 1430	1.7 ± 0.3 $_{A}$	3.2 ± 0.4 $_{A}$	3.9 ± 0.4 $_{A}$	3.9 ± 0.5 $_{ABC}$	3.9 ± 0.4 $_{BCDE}$
BAA 1431	1.8 ± 0.3 $_{A}$	2.3 ± 0.4 $_{A}$	2.5 ± 0.4 $_{C}$	2.6 ± 0.5 $_{CD}$	3.0 ± 0.4 $_{EF}$
BAA 1427 rif[d]	1.7 ± 0.3 $_{A}$	2.3 ± 0.4 $_{A}$	2.6 ± 0.4 $_{BC}$	2.6 ± 0.5 $_{CD}$	3.2 ± 0.4 $_{DEF}$
BAA 1428 rif	2.2 ± 0.3 $_{A}$	2.3 ± 0.4 $_{A}$	3.4 ± 0.4 $_{ABC}$	2.9 ± 0.5 $_{CD}$	3.3 ± 0.4 $_{DEF}$
BAA 1429 rif	2.2 ± 0.3 $_{A}$	2.3 ± 0.4 $_{A}$	2.9 ± 0.4 $_{ABC}$	2.9 ± 0.5 $_{CD}$	3.2 ± 0.4 $_{DEF}$
BAA 1430 rif	0.4 ± 0.3 $_{B}$	0.9 ± 0.4 $_{B}$	1.3 ± 0.4 $_{D}$	1.5 ± 0.5 $_{D}$	2.3 ± 0.4 $_{F}$
BAA 1431 rif	1.8 ± 0.3 $_{A}$	2.3 ± 0.4 $_{A}$	2.9 ± 0.4 $_{ABC}$	2.6 ± 0.5 $_{CD}$	3.6 ± 0.4 $_{CDE}$
BAA-2219[e]	1.9 ± 0.3 $_{A}$	2.3 ± 0.4 $_{A}$	3.0 ± 0.4 $_{ABC}$	3.2 ± 0.5 $_{BC}$	3.6 ± 0.4 $_{CDE}$
BAA-2217	1.8 ± 0.3 $_{A}$	2.6 ± 0.4 $_{A}$	3.7 ± 0.4 $_{ABC}$	4.7 ± 0.5 $_{A}$	5.3 ± 0.4 $_{A}$
BAA-2215	1.7 ± 0.3 $_{A}$	2.3 ± 0.4 $_{A}$	3.0 ± 0.4 $_{ABC}$	3.6 ± 0.5 $_{ABC}$	4.3 ± 0.4 $_{ABCD}$
BAA-2196	1.7 ± 0.3 $_{A}$	2.1 ± 0.4 $_{AB}$	2.7 ± 0.4 $_{ABC}$	3.0 ± 0.5 $_{BC}$	3.7 ± 0.4 $_{CDE}$
BAA-2193	1.7 ± 0.3 $_{A}$	2.6 ± 0.4 $_{A}$	3.8 ± 0.4 $_{ABC}$	4.0 ± 0.5 $_{ABC}$	4.7 ± 0.4 $_{ABC}$
BAA-2192	1.7 ± 0.3 $_{A}$	2.6 ± 0.4 $_{A}$	3.2 ± 0.4 $_{ABC}$	4.4 ± 0.5 $_{AB}$	5.1 ± 0.4 $_{AB}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 5. Mean reductions (log CFU/ml) for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 2.5 at 37°C

	Mean reduction[a] ± SEM[b]				
	Exposure time (h)				
Organism	0	0.5	1	1.5	2
BAA 1427	3.6 ± 0.2 $_{C}$[c]	7.2 ± 0.6 $_{AB}$	7.3 ± 0.4 $_{A}$	7.3 ± 0.3 $_{ABC}$	7.3 ± 0.4 $_{A}$
BAA 1428	4.1 ± 0.2 $_{ABC}$	6.9 ± 0.6 $_{AB}$	7.2 ± 0.4 $_{A}$	6.9 ± 0.3 $_{ABCD}$	6.9 ± 0.4 $_{A}$
BAA 1429	4.3 ± 0.2 $_{A}$	6.5 ± 0.6 $_{AB}$	6.6 ± 0.4 $_{A}$	6.7 ± 0.3 $_{ABCD}$	6.8 ± 0.4 $_{AB}$
BAA 1430	3.7 ± 0.2 $_{C}$	6.8 ± 0.6 $_{AB}$	6.7 ± 0.4 $_{A}$	6.5 ± 0.3 $_{BCD}$	6.6 ± 0.4 $_{AB}$
BAA 1431	4.1 ± 0.2 $_{ABC}$	6.8 ± 0.6 $_{AB}$	6.8 ± 0.4 $_{A}$	7.4 ± 0.3 $_{A}$	7.0 ± 0.4 $_{A}$
BAA 1427 rif[d]	3.8 ± 0.2 $_{ABC}$	5.8 ± 0.6 $_{B}$	5.4 ± 0.4 $_{B}$	7.2 ± 0.3 $_{ABCD}$	6.8 ± 0.4 $_{AB}$
BAA 1428 rif	3.6 ± 0.2 $_{C}$	7.4 ± 0.6 $_{AB}$	7.1 ± 0.4 $_{A}$	7.3 ± 0.3 $_{ABCD}$	7.2 ± 0.4 $_{A}$
BAA 1429 rif	3.7 ± 0.2 $_{C}$	6.7 ± 0.6 $_{AB}$	7.4 ± 0.4 $_{A}$	7.3 ± 0.3 $_{ABC}$	7.4 ± 0.4 $_{A}$
BAA 1430 rif	4.0 ± 0.2 $_{ABC}$	6.4 ± 0.6 $_{AB}$	7.4 ± 0.4 $_{A}$	6.5 ± 0.3 $_{BCD}$	7.4 ± 0.4 $_{A}$
BAA 1431 rif	3.6 ± 0.2 $_{C}$	6.7 ± 0.6 $_{AB}$	6.6 ± 0.4 $_{A}$	6.5 ± 0.3 $_{CD}$	7.3 ± 0.4 $_{A}$
BAA-2219[e]	4.0 ± 0.2 $_{ABC}$	6.5 ± 0.6 $_{AB}$	6.7 ± 0.4 $_{A}$	7.4 ± 0.3 $_{A}$	6.5 ± 0.4 $_{AB}$
BAA-2217	3.7 ± 0.2 $_{BC}$	7.4 ± 0.6 $_{A}$	7.5 ± 0.4 $_{A}$	7.4 ± 0.3 $_{AB}$	7.5 ± 0.4 $_{A}$
BAA-2215	3.8 ± 0.2 $_{BC}$	6.5 ± 0.6 $_{AB}$	7.4 ± 0.4 $_{A}$	6.4 ± 0.3 $_{D}$	7.0 ± 0.4 $_{A}$
BAA-2196	4.0 ± 0.2 $_{ABC}$	6.1 ± 0.6 $_{AB}$	6.9 ± 0.4 $_{A}$	7.1 ± 0.3 $_{ABCD}$	7.3 ± 0.4 $_{A}$
BAA-2193	3.8 ± 0.2 $_{BC}$	6.9 ± 0.6 $_{AB}$	7.4 ± 0.4 $_{A}$	7.4 ± 0.3 $_{AB}$	5.7 ± 0.4 $_{B}$
BAA-2192	4.2 ± 0.2 $_{AB}$	7.0 ± 0.6 $_{AB}$	7.3 ± 0.4 $_{A}$	7.4 ± 0.3 $_{A}$	7.4 ± 0.4 $_{A}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 6. Mean reductions (log CFU/ml) for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.0 at 37°C

	Mean reduction[a] ± SEM[b]				
	Exposure time (h)				
Organism	0	0.5	1	1.5	2
BAA 1427	1.8 ± 0.1 $_{CDE}$[c]	4.0 ± 0.2 $_{DEF}$	4.6 ± 0.3 $_{DEF}$	5.4 ± 0.3 $_{CD}$	5.1 ± 0.4 $_{C}$
BAA 1428	2.1 ± 0.1 $_{B}$	4.4 ± 0.2 $_{BCD}$	5.3 ± 0.3 $_{BCD}$	6.4 ± 0.3 $_{AB}$	6.4 ± 0.4 $_{ABC}$
BAA 1429	2.0 ± 0.1 $_{BCDE}$	4.7 ± 0.2 $_{BC}$	5.3 ± 0.3 $_{BCD}$	6.7 ± 0.3 $_{AB}$	7.0 ± 0.4 $_{AB}$
BAA 1430	1.9 ± 0.1 $_{BCDE}$	4.6 ± 0.2 $_{BC}$	5.6 ± 0.3 $_{ABC}$	6.6 ± 0.3 $_{AB}$	7.4 ± 0.4 $_{A}$
BAA 1431	1.8 ± 0.1 $_{DE}$	3.7 ± 0.2 $_{FGH}$	4.2 ± 0.3 $_{F}$	5.3 ± 0.3 $_{CD}$	7.0 ± 0.4 $_{AB}$
BAA 1427 rif[d]	1.9 ± 0.1 $_{BCDE}$	3.3 ± 0.2 $_{H}$	4.2 ± 0.3 $_{F}$	4.7 ± 0.3 $_{D}$	6.1 ± 0.4 $_{BC}$
BAA 1428 rif	2.0 ± 0.1 $_{BCD}$	3.9 ± 0.2 $_{EFG}$	4.6 ± 0.3 $_{DEF}$	5.4 ± 0.3 $_{CD}$	5.8 ± 0.4 $_{BC}$
BAA 1429 rif	2.0 ± 0.1 $_{BCDE}$	3.5 ± 0.2 $_{FGH}$	4.4 ± 0.3 $_{EF}$	5.2 ± 0.3 $_{D}$	5.4 ± 0.4 $_{C}$
BAA 1430 rif	1.7 ± 0.1 $_{E}$	3.5 ± 0.2 $_{GH}$	4.3 ± 0.3 $_{F}$	5.1 ± 0.3 $_{D}$	5.4 ± 0.4 $_{C}$
BAA 1431 rif	2.0 ± 0.1 $_{BCDE}$	3.8 ± 0.2 $_{FGH}$	4.2 ± 0.3 $_{F}$	4.8 ± 0.3 $_{D}$	5.4 ± 0.4 $_{C}$
BAA-2219[e]	1.9 ± 0.1 $_{BCDE}$	4.3 ± 0.2 $_{CDE}$	4.7 ± 0.3 $_{DEF}$	5.1 ± 0.3 $_{D}$	5.2 ± 0.4 $_{C}$
BAA-2217	1.9 ± 0.1 $_{BCDE}$	4.9 ± 0.2 $_{AB}$	6.0 ± 0.3 $_{AB}$	6.1 ± 0.3 $_{BC}$	6.1 ± 0.4 $_{BC}$
BAA-2215	2.1 ± 0.1 $_{BC}$	4.5 ± 0.2 $_{BCD}$	5.3± 0.3 $_{BCD}$	6.4 ± 0.3 $_{AB}$	5.9 ± 0.4 $_{BC}$
BAA-2196	2.5 ± 0.1 $_{A}$	5.3 ± 0.2 $_{A}$	6.1 ± 0.3 $_{AB}$	6.9 ± 0.3 $_{A}$	6.9 ± 0.4 $_{AB}$
BAA-2193	1.9 ± 0.1 $_{BCDE}$	4.5 ± 0.2 $_{BC}$	5.2 ± 0.3 $_{CDE}$	6.6 ± 0.3 $_{AB}$	7.0 ± 0.4 $_{AB}$
BAA-2192	1.9 ± 0.1 $_{BCDE}$	4.5 ± 0.2 $_{BC}$	6.4 ± 0.3 $_{A}$	6.8 ± 0.3 $_{AB}$	6.0 ± 0.4 $_{BC}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 7. Mean reductions (log CFU/ml) for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.5 at 37°C

	Mean reduction[a] ± SEM[b]				
	Exposure time (h)				
Organism	0	0.5	1	1.5	2
BAA 1427	1.9 ± 0.1 $_{ABC}$[c]	1.9 ± 0.2 $_{C}$	2.6 ± 0.2 $_{A}$	2.6 ± 0.2 $_{ABC}$	3.5 ± 0.3 $_{AB}$
BAA 1428	2.1 ± 0.1 $_{A}$	2.6 ± 0.2 $_{A}$	2.2 ± 0.2 $_{AB}$	2.5 ± 0.2 $_{BC}$	2.9 ± 0.3 $_{AB}$
BAA 1429	1.8 ± 0.1 $_{BC}$	1.9 ± 0.2 $_{C}$	2.1 ± 0.2 $_{B}$	2.2 ± 0.2 $_{C}$	3.5 ± 0.3 $_{AB}$
BAA 1430	1.9 ± 0.1 $_{ABC}$	2.4 ± 0.2 $_{AB}$	2.3 ± 0.2 $_{AB}$	2.8 ± 0.2 $_{ABC}$	3.3 ± 0.3 $_{AB}$
BAA 1431	2.0 ± 0.1 $_{ABC}$	2.0 ± 0.2 $_{BC}$	2.2 ± 0.2 $_{AB}$	2.4 ± 0.2 $_{BC}$	3.6 ± 0.3 $_{A}$
BAA 1427 rif[d]	1.9 ± 0.1 $_{ABC}$	2.1 ± 0.2 $_{BC}$	2.2 ± 0.2 $_{AB}$	2.5 ± 0.2 $_{BC}$	3.1 ± 0.3 $_{AB}$
BAA 1428 rif	2.1 ± 0.1 $_{A}$	1.9 ± 0.2 $_{BC}$	2.2 ± 0.2 $_{AB}$	2.7 ± 0.2 $_{ABC}$	2.7 ± 0.3 $_{B}$
BAA 1429 rif	2.1 ± 0.1 $_{ABC}$	2.3 ± 0.2 $_{ABC}$	2.3 ± 0.2 $_{AB}$	2.5 ± 0.2 $_{ABC}$	3.0 ± 0.3 $_{AB}$
BAA 1430 rif	1.9 ± 0.1 $_{ABC}$	2.0 ± 0.2 $_{BC}$	2.3 ± 0.2 $_{AB}$	2.5 ± 0.2 $_{BC}$	3.0 ± 0.3 $_{AB}$
BAA 1431 rif	2.0 ± 0.1 $_{ABC}$	2.0 ± 0.2 $_{BC}$	2.3 ± 0.2 $_{AB}$	2.5 ± 0.2 $_{BC}$	2.9 ± 0.3 $_{AB}$
BAA-2219[e]	2.1 ± 0.1 $_{A}$	2.1 ± 0.2 $_{BC}$	2.4 ± 0.2 $_{AB}$	2.5 ± 0.2 $_{BC}$	3.4 ± 0.3 $_{AB}$
BAA-2217	1.8 ± 0.1 $_{C}$	2.0 ± 0.2 $_{BC}$	2.4 ± 0.2 $_{AB}$	3.1 ± 0.2 $_{A}$	3.6 ± 0.3 $_{A}$
BAA-2215	2.1 ± 0.1 $_{A}$	2.2 ± 0.2 $_{ABC}$	2.5 ± 0.2 $_{AB}$	2.7 ± 0.2 $_{ABC}$	3.6 ± 0.3 $_{A}$
BAA-2196	2.1 ± 0.1 $_{AB}$	2.3 ± 0.2 $_{ABC}$	2.5 ± 0.2 $_{AB}$	2.7 ± 0.2 $_{ABC}$	3.3 ± 0.3 $_{AB}$
BAA-2193	2.0 ± 0.1 $_{ABC}$	2.1 ± 0.2 $_{BC}$	2.4 ± 0.2 $_{AB}$	2.9 ± 0.2 $_{AB}$	3.3 ± 0.3 $_{AB}$
BAA-2192	1.9 ± 0.1 $_{ABC}$	2.0 ± 0.2 $_{BC}$	2.3 ± 0.2 $_{AB}$	2.9 ± 0.2 $_{AB}$	3.7 ± 0.3 $_{A}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 8 shows the sets of organisms that were analyzed together to create a mean for the *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and the non-O157 STECs. These data were combined to eliminate individual strain-to-strain variation to determine the potential use of the surrogates as a cocktail to represent all of the non-O157 STECs. For the stationary phase organisms at pH 2.5, these data were omitted due to the majority of the log reduction counts (CFU/ml) falling below the level of detection. The stationary phase organisms at pH 3.0 (Figure 2) showed no differences ($P > 0.05$) between the biotype I surrogate organisms and the non-O157 STECs throughout the 2-hour evaluation. At hour 0, 0.5, and 1.5, the rif-resistant biotype I surrogate organisms were different ($P < 0.05$) from the biotype I surrogates and the non-O157 STECs by showing lower log reductions (CFU/ml). At hour 1.0, the biotype I surrogates, and the rif-resistant biotype I surrogates were not different ($P > 0.05$), and at the 2-h time point, there were no differences ($P > 0.05$) between the three groups of organisms evaluated. Though there were differences at the varying time points, by the end of the 2-h evaluation, all three groups of organisms were not different ($P > 0.05$), which may be useful in identifying the biotype I surrogates appropriate to represent the behavior of the non-O157 STECs. For the stationary phase organisms at pH 3.5 (Figure 3), at time point zero and 0.5-h, there were no differences ($P > 0.05$) between the biotype I surrogates, rif-resistant biotype I surrogates, and the non-O157 STECs. At 1-h, there were no differences ($P > 0.05$) between the biotype I surrogates and the non-O157 STECs, and the rif-resistant biotype I surrogates were not different ($P > 0.05$) from the biotype I surrogates. At hour 1.5 and two, there were no differences ($P > 0.05$) between

the biotype I surrogates and the rif-resistant biotype I surrogates; however, the non-O157 STECs were different ($P < 0.05$), representing a greater log reduction count (CFU/ml). After exposure of the organisms to L-lactic acid acidified to pH 3.5, biotype I surrogates may be reduced, but the non-O157 STECs may be reduced to a greater log reduction count (CFU/ml).

Acid-adapted organisms at pH 2.5 (Figure 4) showed no differences ($P > 0.05$) between the biotype I surrogates, the rif-resistant surrogates, and the non-O157 STECs throughout the entire 2-h evaluation. Initial log reduction counts were 3.7-4.0 CFU/ml, and after 2-h, log reduction counts ranged from 6.9-7.2 CFU/ml. Acid-adapted organisms at pH 3.0 (Figure 5) showed no differences ($P > 0.05$) between the biotype I surrogates, rif-resistant biotype I surrogates, and the non-O157 STECs at the initial time point (1.9-2.0 log reductions (CFU/ml)). At the 0.5-h, and 1-h time point, all three groups of organisms were different ($P < 0.05$); however, the non-O157 STECs showed a greater log reduction count (CFU/ml). The non-O157 STECs showed a greater log reduction, which may mean the organisms, have a lower resistance to pH 3.0. For use as a surrogate, the *E. coli* biotype I surrogates would ideally have a higher resistance to the pH, showing a lower reduction when compared to the non-O157 STECs. These data may support the use of the surrogates to represent the non-O157 STECs, as the surrogates show a lower reduction, indicating a higher resistance to the pH value. At the 1.5-h time point evaluation, the biotype I surrogates and the non-O157 STECs were not different ($P > 0.05$) but the rif-resistant biotype I surrogates were different ($P < 0.05$). At the final 2-h time point evaluation, the non-O157 STECs were not different ($P > 0.05$) from the

biotype I surrogates or the rif-resistant surrogates, but there was a difference ($P < 0.05$) between the biotype I surrogates and the rif-resistant surrogates. Acid-adapted organisms at pH 3.5 (Figure 6) showed no differences ($P > 0.05$) between the biotype I surrogates, rif-resistant biotype I surrogates, and non-O157 STECs at the initial, 0.5-h and 1-h time point. At the 1.5-hour time point, there were no differences ($P > 0.05$) between the biotype I surrogates and the rif-resistant biotype I surrogates, but there was a difference ($P < 0.05$) between the biotype I surrogates, rif-resistant surrogates, and the non-O157 STECs. The non-O157 STECs did have a greater log reduction count (CFU/ml) (2.5 vs. 2.8, respectively). At the 2-h time point, there was no difference ($P > 0.05$) between the biotype I surrogates and the non-O157 STECs; however, the rif-resistant surrogates were different ($P < 0.05$) (3.4, 3.5 and 2.9 log reduction CFU/ml, respectively).

Table 8. Mean reductions (log CFU/ml) for *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains in sets after exposure to phosphate buffered saline acidified with L-lactic acid at 37°C

	Mean reduction[a] ± SEM[b]				
	Exposure time (h)				
Organism	0	0.5	1.0	1.5	2.0
	Stationary/pH 3.0				
E. coli surrogates[d]	2.6 ± 0.1 $_{A}$[c]	5.3 ± 0.2 $_{A}$	6.0 ± 0.3 $_{AB}$	6.3 ± 0.3 $_{A}$	6.8 ± 0.2 $_{A}$
rif-resistant[e] *E. coli* surrogates	2.3 ± 0.1 $_{B}$	4.3 ± 0.2 $_{B}$	5.4 ± 0.3 $_{B}$	5.5 ± 0.3 $_{B}$	6.9 ± 0.2 $_{A}$
Non-O157:H7 STECs[f]	2.7 ± 0.1 $_{A}$	5.8 ± 0.2 $_{A}$	6.4 ± 0.2 $_{A}$	6.5 ± 0.2 $_{A}$	6.9 ± 0.2 $_{A}$
	Stationary/pH 3.5				
E. coli surrogates	1.8 ± 0.2 $_{A}$	2.5 ± 0.2 $_{A}$	2.9 ± 0.2 $_{AB}$	3.0 ± 0.2 $_{B}$	3.4 ± 0.2 $_{B}$
rif-resistant *E. coli* surrogates	1.7 ± 0.2 $_{A}$	2.0 ± 0.2 $_{A}$	2.6 ± 0.2 $_{B}$	2.5 ± 0.2 $_{B}$	3.1 ± 0.2 $_{B}$
Non-O157:H7 STECs	1.7 ± 0.2 $_{A}$	2.4 ± 0.2 $_{A}$	3.2 ± 0.2 $_{A}$	3.8 ± 0.2 $_{A}$	4.5 ± 0.2 $_{A}$
	Acid-adapted/pH 2.5				
E. coli surrogates	4.0 ± 0.1 $_{A}$	6.8 ± 0.2 $_{A}$	6.9 ± 0.2 $_{A}$	7.0 ± 0.2 $_{A}$	6.9 ± 0.2 $_{A}$
rif-resistant *E. coli* surrogates	3.7 ± 0.1 $_{A}$	6.6 ± 0.2 $_{A}$	6.8 ± 0.2 $_{A}$	7.0 ± 0.2 $_{A}$	7.2 ± 0.2 $_{A}$
Non-O157:H7 STECs	3.9 ± 0.1 $_{A}$	6.7 ± 0.2 $_{A}$	7.2 ± 0.2 $_{A}$	7.2 ± 0.1 $_{A}$	6.9 ± 0.2 $_{A}$
	Acid-adapted/pH 3.0				
E. coli surrogates	1.9 ± 0.1 $_{A}$	4.3 ± 0.1 $_{B}$	5.0 ± 0.2 $_{B}$	6.1 ± 0.2 $_{A}$	6.6 ± 0.2 $_{A}$
rif-resistant *E. coli* surrogates	1.9 ± 0.1 $_{A}$	3.6 ± 0.1 $_{C}$	4.4 ± 0.2 $_{C}$	5.0 ± 0.2 $_{B}$	5.6 ± 0.2 $_{B}$
Non-O157:H7 STECs	2.0 ± 0.1 $_{A}$	4.7 ± 0.1 $_{A}$	5.6 ± 0.2 $_{A}$	6.3 ± 0.2 $_{A}$	6.2 ± 0.2 $_{AB}$
	Acid adapted/pH 3.5				
E. coli surrogates	2.0 ± 0.0 $_{A}$	2.2 ± 0.1 $_{A}$	2.3 ± 0.1 $_{A}$	2.5 ± 0.1 $_{B}$	3.4 ± 0.1 $_{A}$
rif-resistant *E. coli* surrogates	2.0 ± 0.0 $_{A}$	2.1 ± 0.1 $_{A}$	2.2 ± 0.1 $_{A}$	2.5 ± 0.1 $_{B}$	2.9 ± 0.1 $_{B}$
Non-O157:H7 STECs	2.0 ± 0.0 $_{A}$	2.1 ± 0.1 $_{A}$	2.4 ± 0.1 $_{A}$	2.8 ± 0.1 $_{A}$	3.5 ± 0.1 $_{A}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] *E. coli* surrogates include BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431 strains
[e] Rif-resistant *E. coli* surrogates include rif-resistant strains BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431
[f] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

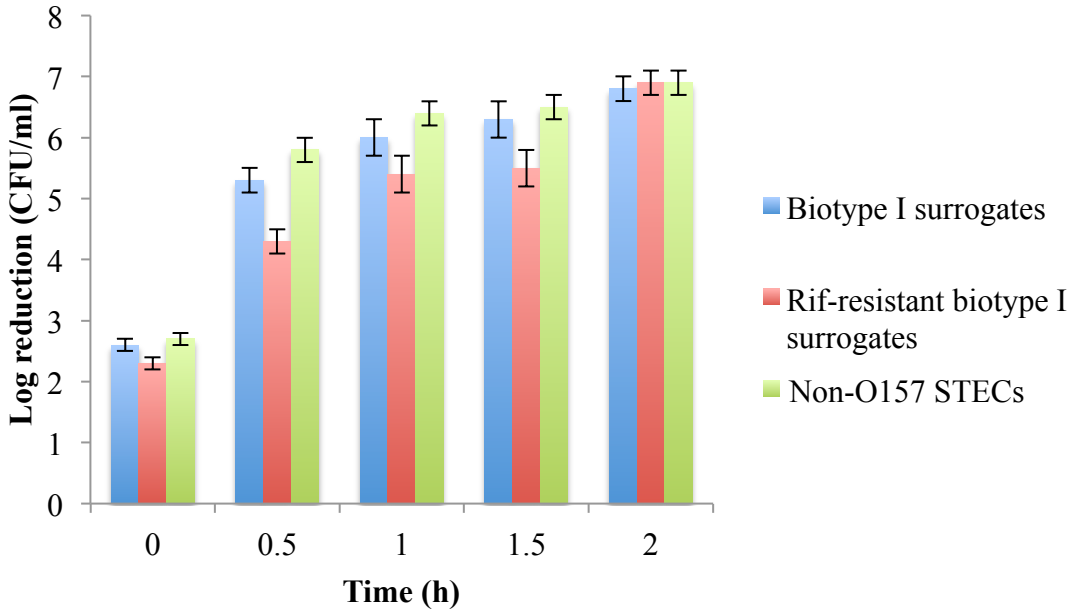

Figure 2. Mean log reductions (CFU/ml) for sets of stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.0 at 37°C

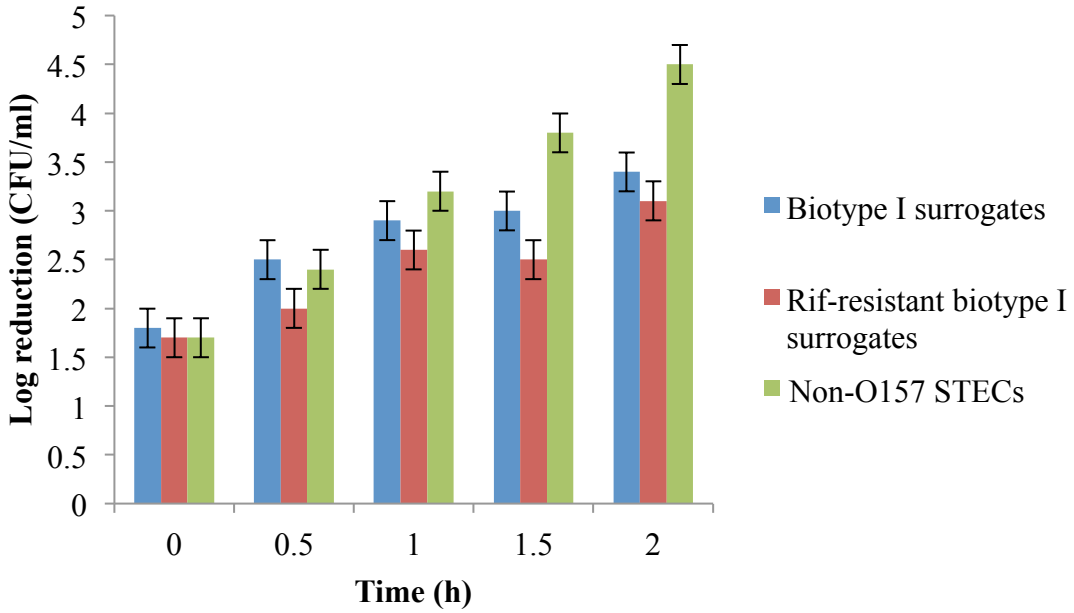

Figure 3. Mean log reductions (CFU/ml) for sets of stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.5 at 37°C

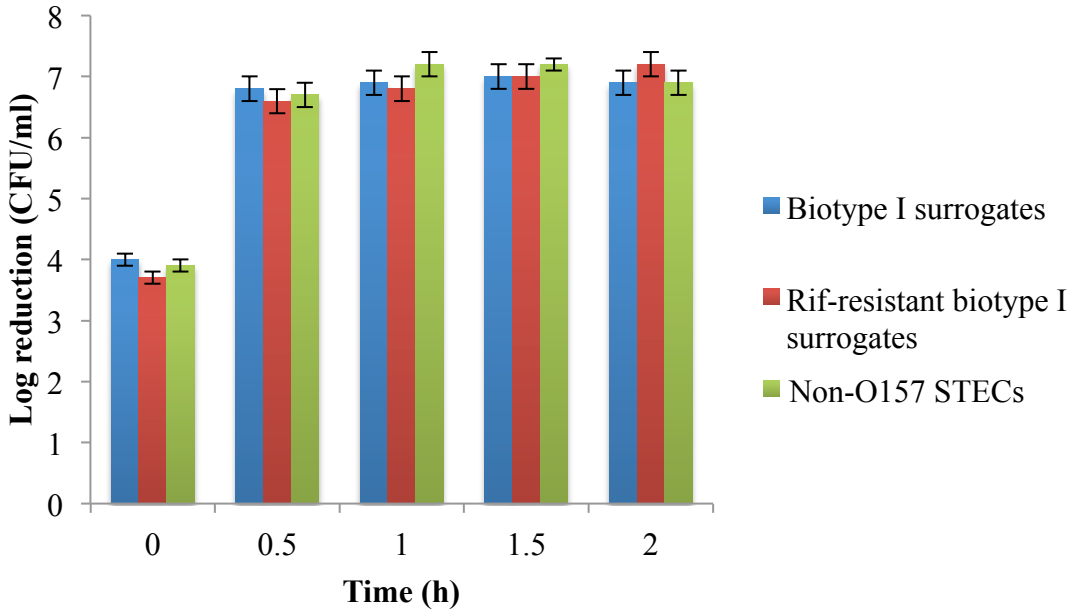

Figure 4. Mean log reductions (CFU/ml) for sets of acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 2.5 at 37°C

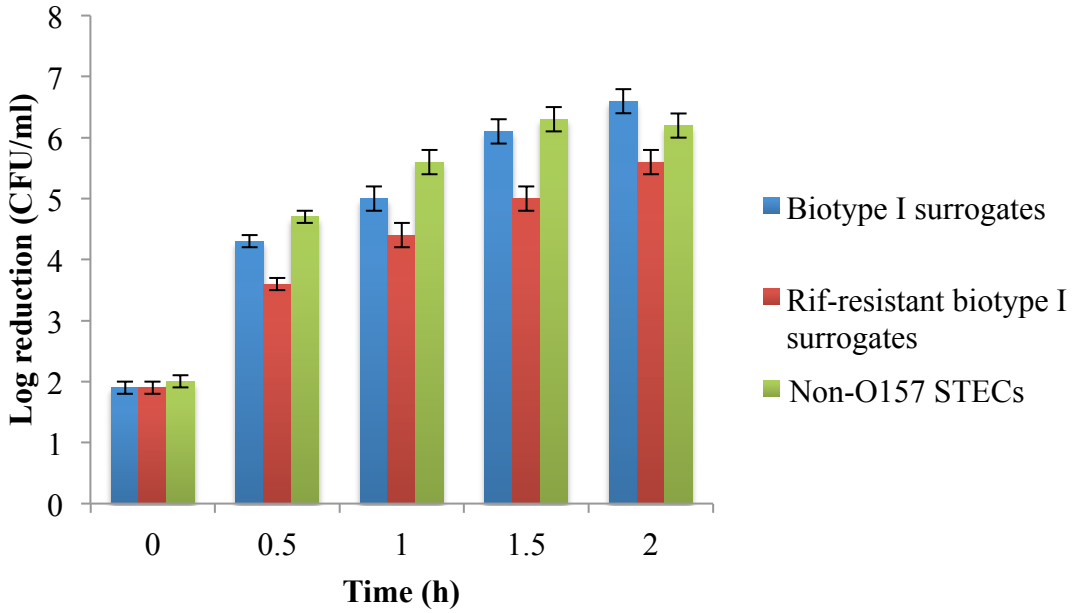

Figure 5. Mean log reductions (CFU/ml) for sets of acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.0 at 37°C

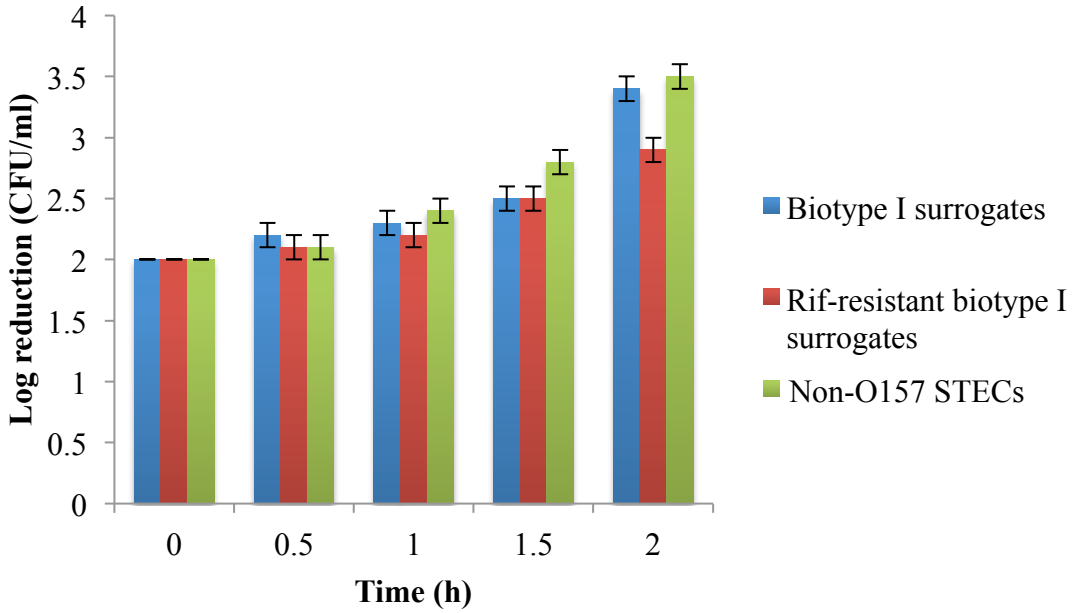

Figure 6. Mean log reductions (CFU/ml) for sets of acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at pH 3.5 at 37°C

Table 9 represents the sets of organisms that were analyzed together to create a mean for the *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and the non-O157 STECs, following 2-h exposure to the three individual pH values. The stationary phase organisms at pH value 2.5 were omitted due to the log reduction counts falling below the level of detection. For the first replication of the stationary phase organisms at pH 2.5, an initial count was not taken, and following the 0.5-h time point, organisms were below the level of detection. For the second and third replication of the stationary phase organisms at pH 2.5, an initial count was taken, and after the 0.5-h time point, organisms were below the level of detection. For the stationary phase organisms at pH 3.0 (Figure 7), there were no differences ($P > 0.05$) between the biotype I surrogates, rif-resistant biotype I surrogates, and the non-O157 STECs following a 2-h exposure to L-lactic acid. Stationary phase organisms at pH 3.5, showed no differences ($P > 0.05$) between the biotype I surrogates and the rif-resistant biotype I surrogates, but there was a difference ($P < 0.05$) with the non-O157 STECs, representing a greater log reduction count (CFU/ml) (3.4, 3.5 and 4.5 log reduction CFU/ml, respectively). Acid-adapted organisms at pH 2.5 (Figure 8) showed no differences ($P > 0.05$) between the biotype I surrogates, rif-resistant biotype I surrogates, and non-O157 STECs. Acid-adapted organisms at pH 3.0 showed no differences ($P > 0.05$) between the non-O157 STECs, and the biotype I surrogates and rif-resistant biotype I surrogates. At pH 3.5, acid-adapted biotype I surrogates were not different ($P > 0.05$) from the non-O157 STECs; however, the rif-resistant biotype I surrogates were different ($P < 0.05$).

Table 9. Mean reductions (log CFU/ml) for *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid at 37°C

	Mean reduction[a] ± SEM[b]		
	pH 2.5	pH 3.0	pH 3.5
Organism	Stationary		
E. coli surrogates[d]	.	6.8 ± 0.2 $_{A}$[c]	3.4 ± 0.2 $_{B}$
rif-resistant[e] *E. coli* surrogates	.	6.9 ± 0.2 $_{A}$	3.1 ± 0.2 $_{B}$
Non-O157:H7 STECs[f]	.	6.9 ± 0.2 $_{A}$	4.5 ± 0.2 $_{A}$
	Acid-adapted		
E. coli surrogates	6.9 ± 0.2 $_{A}$	6.6 ± 0.2 $_{A}$	3.4 ± 0.1 $_{A}$
rif-resistant *E. coli* surrogates	7.2 ± 0.2 $_{A}$	5.6 ± 0.2 $_{B}$	2.9 ± 0.1 $_{B}$
Non-O157:H7 STECs	6.9 ± 0.2 $_{A}$	6.2 ± 0.2 $_{AB}$	3.5 ± 0.1 $_{A}$

Overall mean log reductions after 0, 0.5, 1.0, 1.5 and 2.0 h exposure
[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] *E. coli* surrogates include BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431 strains
[e] Rif-resistant *E. coli* surrogates include rif-resistant strains BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431
[f] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

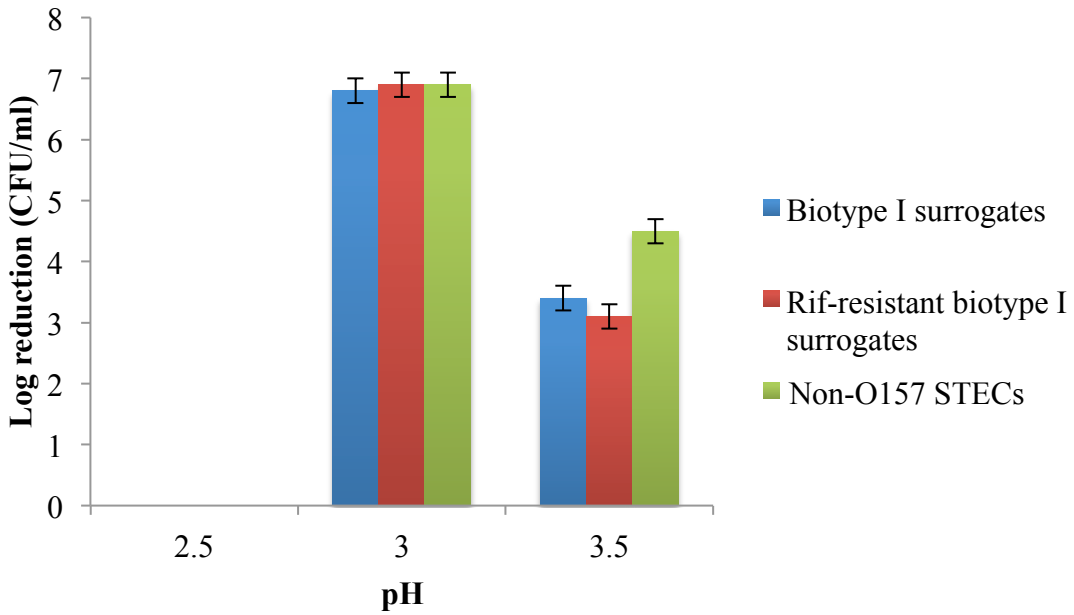

Figure 7. Mean log reductions (CFU/ml) for sets of stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid for 2-h at 37°C

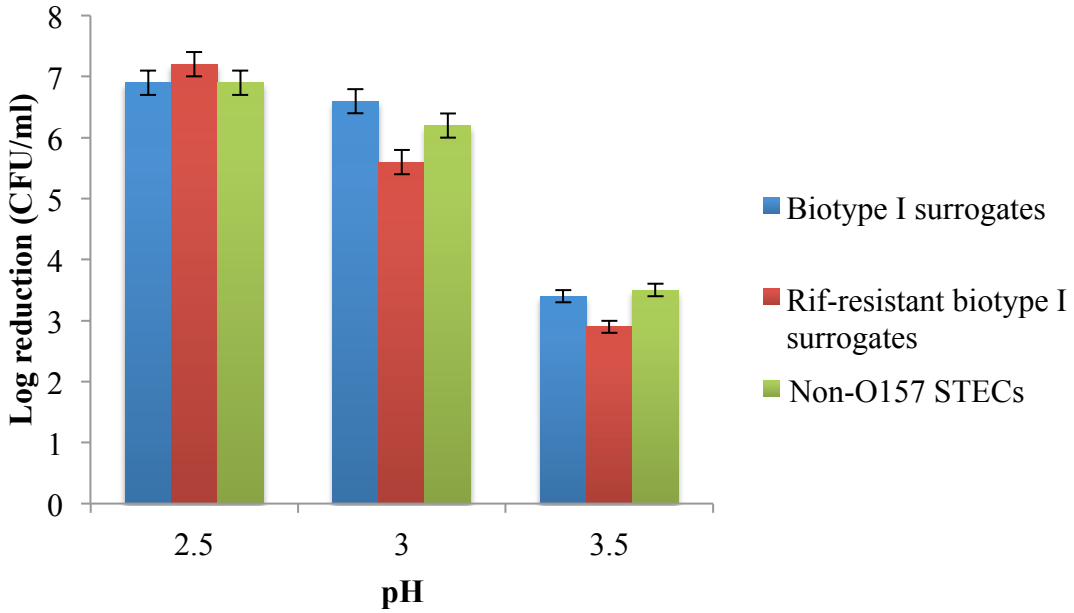

Figure 8. Mean log reductions (CFU/ml) for sets of acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline acidified with L-lactic acid for 2-h at 37°C

Thermal resistance

Decimal reduction time (*D*-value) refers to the time (in minutes) required at a given temperature to kill 90% or 1-log of the exposed microorganisms. For a surrogate to properly represent the non-O157 STECs, the surrogate would ideally have a higher *D*-value compared to the non-O157 STECs. It would ideally take longer to kill the surrogate, and therefore, process would be sufficient to kill the non-O157 STECs. Table 10 shows the *D*-values for the stationary phase organisms at three varying temperatures: 55°, 60°, and 65°C. At 55°C, the *D*-values for the surrogates ranged from 10.3-19.6 minutes, the rif-resistant surrogates ranged from 9.4-29.7 minutes, and the non-O157 STECs ranged from 5.6-11.2 minutes. There were varying differences ($P < 0.05$) between the individual 16 organisms, but each non-O157 STEC was not significantly different ($P > 0.05$) from at least one of the biotype I surrogates. This may indicate that at 55°C, if the biotype I surrogate organisms have been reduced, the non-O157 STECs have also been reduced, which is an ideal characteristic of a surrogate organism. These data supports previously published data demonstrating that *E. coli* biotype I surrogates had the same ($P > 0.05$) or higher ($P < 0.05$) *D*-values when compared to *E. coli* O157:H7 *(8)*.

At 60°C, there were fewer differences ($P < 0.05$) between the individual organisms, and the *D*-values ranged from 0.4-1.4 minutes for the stationary organisms. BAA-1431 was the individual organism that had the highest *D*-value at 60°C at 1.4 minutes for the biotype I surrogate. This would indicate that if organism BAA-1431 is reduced, the non-O157 STEC organism may also be reduced. Stationary organisms at

65°C had *D*-values ranging from 0.09-1.24 minutes. The low and high *D*-values both came from non-O157 STECs; therefore, indicating that at the higher temperature, following the reduction of the biotype I surrogate, the non-O157 STECs may not all be efficiently reduced. This may indicate that the biotype I surrogates may not represent the non-O157 STECs at 65°C.

Z-values were calculated by taking the log of the *D*-values and plotting them as a function of temperature (55, 60, and 65°C). A *z*-value represents the degrees in either F or C that are required for the thermal destruction curve to traverse 1 log cycle, the change in temperature that changes the *D*-value by a factor of 10. The *z*-value reflects the relative resistance of an organism to different temperatures. The *z*-values for the stationary phase organisms are reported in Table 10. The *z*-values ranged from 4.9 to 15.3. Organism BAA-2193 had the highest *z*-value (15.3), while BAA-1427 rif and BAA-1429 rif had the lowest *z*-value (4.9). Organism BAA-2193 was not different ($P > 0.05$) from one of the rif-resistant *E. coli* biotype I surrogates (BAA-1430 rif). These data may demonstrate that the *E. coli* biotype I surrogates may not represent all of the non-O157 STECs appropriately. It may require a higher temperature change to destroy the non-O157 STECs than what is required to change the *E. coli* biotype I surrogates.

Table 11 shows the *D*-values for the acid-adapted organisms at three varying temperatures: 55°, 60°, and 65°C. *D*-values at 55°C for all 16 organisms ranged from 7.1-22.2 minutes. At 55°C, BAA-2219 had the highest *D*-value at 22.2 minutes, and it was not different ($P > 0.05$) from at least one biotype I surrogate, and at least one rif-resistant surrogate. At 60°C, acid-adapted organisms' *D*-values ranged from 0.6-6.0

minutes, with BAA-2196 (5.3 minutes) and BAA-2217 (6.0 minutes) having the only different ($P < 0.05$) *D*-values from the remaining 14 organisms. This may indicate that by sufficiently reducing the biotype I surrogates at this temperature, all the non-O157 STECs may not be efficiently reduced. At 65°C, acid-adapted organisms had *D*-values ranging from 0.13-0.64 minutes. At this temperature, organism BAA-1429 rif had the highest *D*-value (0.64 minutes), and organism BAA-2217 and BAA-2192 had the lowest *D*-value (0.13 minutes). In this case, at this temperature, data may show that by reducing the rif-resistant biotype I surrogates, the non-O157 STECs may also be sufficiently reduced.

The *z*-values for the acid-adapted organisms are demonstrated in Table 11. The *z*-values ranged from 4.9 to 8.4. Organism BAA-2193 had the highest *z*-value (8.4) and BAA-2192 had the lowest (4.9). Both organisms (BAA-2193 and BAA-2192) were not different ($P > 0.05$) from at least one *E. coli* biotype I surrogate, both the parent and rif-resistant strains.

Keeling et al. *(28)* reported no difference ($P > 0.10$) in *D*-values between the biotype I surrogate microorganisms and *E. coli* O157:H7 at 60 or 71.1°C. The *D*-value for the pathogenic strain was numerically lower at 60°C than any surrogate, but the difference was not ($P > 0.10$) significant *(28)*. Keeling et al. *(28)* conducted thermal resistance evaluations in 80% lean ground beef. *D*-values have shown to differ between fat content among ground beef samples *(28)*. Data presented here show thermal resistance evaluations performed in a laboratory setting. Although the results may be similar, it is important to note that when using a surrogate organism in comparison for a

validation study, the exact processing conditions must be carried out. For example, in the data presented here, the organisms were in capillary tubes, not inculcated on a tissue surface. The results may vary when a tissue surface is inoculated due to differing characteristics of the surface and how the organism responds. For use in a validation study, the organism must be observed in the exact processing condition to be the most accurate, since different responses may occur due to the inoculation procedure.

Thermal death curves were constructed for sets of organisms (biotype I surrogates, rif-resistant biotype I surrogates, and non-O157 STECs) and fitted by linear regression (Figures 9-14). From the thermal death curves, *D*-values were calculated and compared among the sets of organisms (biotype I surrogates, rif-resistant biotype I surrogates, and non-O157 STECs). Table 12 represents the sets of organisms, including the biotype I surrogates, the rif-resistant biotype I surrogates, and the non-O157 STECs. Stationary phase organisms at 55°C showed a difference ($P < 0.05$) between the non-O157 STECs and the biotype I surrogates, and rif-resistant biotype I surrogates. The non-O157 STECs had a lower *D*-value (8.2 minutes) compared to the biotype I surrogates (14.6 minutes) and the rif-resistant biotype I surrogates (18.4 minutes). As shown by the individual organisms at this temperature, reducing the biotype I surrogates may also efficiently reduce the non-O157 STECs. Stationary phase organisms at 60°C showed no differences ($P > 0.05$) between the biotype I surrogates, rif-resistant biotype I surrogates, and the non-O157 STECs (0.6-0.9 minutes). Stationary phase organisms at 65°C reported no differences between ($P > 0.05$) the biotype I surrogates and the rif-resistant biotype I surrogates, or between the rif-resistant surrogates and the non-O157

STECs. It is important to note that at this temperature, the non-O157 STECs had the highest *D*-value (0.69 minutes) compared to the biotype I surrogates (0.21 minutes), and the rif-resistant biotype I surrogates (0.33 minutes), demonstrating that reducing the biotype I surrogate organisms may not result in a reduction of the non-O157 STECs. *Z*-values for the stationary phase organisms demonstrated no difference ($P > 0.05$) between the non-O157 STECs (8.9) and the rif-resistant *E. coli* biotype I surrogates (6.2). There was a difference ($P < 0.05$) between the *E. coli* biotype I surrogates (5.6) and the non-O157 STECs (8.9). It is important to note that the non-O157 STECs had the highest *z*-value compared to the *E. coli* biotype I surrogates, both parent and rif-resistant. These data may demonstrate that it may take a larger increase in the temperature to destroy the non-O157 STECs when compared to the *E. coli* biotype I surrogates, which may indicate that the *E. coli* biotype I surrogates may not represent the non-O157 STECs appropriately.

Acid-adapted organisms at 55°C showed no differences ($P > 0.05$) between the biotype I surrogates, rif-resistant biotype I surrogates, and the non-O157 STECs. The biotype I surrogates had the lowest *D*-value (9.6 minutes) compared to the rif-resistant biotype I surrogates (13.4 minutes) though the numbers are not different ($P > 0.05$). Acid-adapted organisms at 60°C showed a difference ($P < 0.05$) between the non-O157 STECs (3.0 minutes), biotype I surrogate (1.5 minutes) and rif-resistant biotype I surrogates (1.2 minutes). The non-O157 STECs had the highest *D*-value indicating that a reduction in biotype I surrogates may not result in a reduction of non-O157 STECs. Acid-adapted organisms at 65°C showed no differences ($P > 0.05$) between the biotype I

surrogates (0.27 minutes), and the rif-resistant biotype I surrogates (0.42 minutes). There was no difference ($P > 0.05$) between the biotype I surrogates and the non-O157 STECs (0.25 minutes). The non-O157 STECs had the lowest *D*-value at this temperature, indicating that reducing the biotype I surrogate may reduce the non-O157 STECs. *Z*-values for the acid-adapted organisms showed no differences ($P > 0.05$) between the *E. coli* biotype I surrogates (6.5), rif-resistant *E. coli* biotype I surrogates (6.4), and the non-O157 STECs (6.1).

Table 10. Decimal reduction time (*D*-value) for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains in phosphate buffered saline at different temperatures

	D-value (min)[a] ± SEM[b]			
Organism	55°C	60°C	65°C	*z*-value
BAA 1427	12.5 ± 4.9 $_{BC}$[c]	0.5 ± 0.3 $_{B}$	0.21 ± 0.37 $_{ABC}$	5.8 ± 2.5 $_{B}$
BAA 1428	19.6 ± 4.9 $_{ABC}$	0.5 ± 0.3 $_{B}$	0.31 ± 0.37 $_{ABC}$	5.4 ± 2.5 $_{B}$
BAA 1429	13.8 ± 4.9 $_{BC}$	0.7 ± 0.3 $_{AB}$	0.16 ± 0.37 $_{BC}$	5.3 ± 2.5 $_{B}$
BAA 1430	10.3 ± 4.9 $_{BC}$	1.2 ± 0.3 $_{AB}$	0.17 ± 0.37 $_{BC}$	6.0 ± 2.5 $_{B}$
BAA 1431	16.7 ± 4.9 $_{ABC}$	1.4 ± 0.3 $_{A}$	0.21 ± 0.37 $_{ABC}$	5.7 ± 2.5 $_{B}$
BAA 1427 rif[d]	29.7 ± 4.9 $_{A}$	0.7 ± 0.3 $_{AB}$	0.15 ± 0.37 $_{BC}$	4.9 ± 2.5 $_{B}$
BAA 1428 rif	22.6 ± 4.9 $_{AB}$	0.4 ± 0.3 $_{B}$	0.17 ± 0.37 $_{BC}$	5.3 ± 2.5 $_{B}$
BAA 1429 rif	18.9 ± 4.9 $_{ABC}$	0.5 ± 0.3 $_{B}$	0.15 ± 0.37 $_{BC}$	4.9 ± 2.5 $_{B}$
BAA 1430 rif	9.4 ± 4.9 $_{BC}$	0.4 ± 0.3 $_{B}$	0.83 ± 0.37 $_{ABC}$	9.3 ± 2.5 $_{AB}$
BAA 1431 rif	11.3 ± 4.9 $_{BC}$	0.8 ± 0.3 $_{AB}$	0.35 ± 0.37 $_{ABC}$	6.4 ± 2.5 $_{B}$
BAA-2219[e]	11.2 ± 4.9 $_{BC}$	0.6 ± 0.3 $_{B}$	1.21 ± 0.37 $_{AB}$	8.9 ± 2.5 $_{AB}$
BAA-2217	8.0 ± 4.9 $_{C}$	0.6 ± 0.3 $_{B}$	0.64 ± 0.37 $_{ABC}$	8.5 ± 2.5 $_{AB}$
BAA-2215	8.5 ± 4.9 $_{BC}$	0.7 ± 0.3 $_{AB}$	0.66 ± 0.37 $_{ABC}$	8.2 ± 2.5 $_{AB}$
BAA-2196	8.6 ± 4.9 $_{BC}$	0.4 ± 0.3 $_{B}$	0.09 ± 0.37 $_{C}$	5.1 ± 2.5 $_{B}$
BAA-2193	7.3 ± 4.9 $_{C}$	0.8 ± 0.3 $_{AB}$	1.24 ± 0.37 $_{A}$	15.3 ± 2.5 $_{A}$
BAA-2192	5.6 ± 4.9 $_{C}$	0.6 ± 0.3 $_{B}$	0.30 ± 0.37 $_{ABC}$	7.3 ± 2.5 $_{B}$

[a] Decimal reduction time (*D*-value) represents three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 11. Decimal reduction time (*D*-value) for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains in phosphate buffered saline at different temperatures

	D-value (min)[a] ± SEM[b]			
Organism	55°C	60°C	65°C	*z*-value
BAA 1427	7.9 ± 4.4 $_{BC}$[c]	1.4 ± 0.9 $_{B}$	0.15 ± 0.11 $_{E}$	5.9 ± 0.6 $_{BCD}$
BAA 1428	13.7 ± 4.4 $_{ABC}$	0.9 ± 0.9 $_{B}$	0.18 ± 0.11 $_{DE}$	5.4 ± 0.6 $_{CD}$
BAA 1429	7.1 ± 4.4 $_{C}$	1.7 ± 0.9 $_{B}$	0.18 ± 0.11 $_{DE}$	6.3 ± 0.6 $_{BCD}$
BAA 1430	9.9 ± 4.4 $_{ABC}$	1.9 ± 0.9 $_{B}$	0.50 ± 0.11 $_{ABCD}$	7.6 ± 0.6 $_{AB}$
BAA 1431	9.7 ± 4.4 $_{ABC}$	1.5 ± 0.9 $_{B}$	0.34 ± 0.11 $_{ABCDE}$	7.1 ± 0.6 $_{ABC}$
BAA 1427 rif[d]	10.4 ± 4.4 $_{ABC}$	1.2 ± 0.9 $_{B}$	0.53 ± 0.11 $_{ABC}$	6.1 ± 0.6 $_{BCD}$
BAA 1428 rif	8.5 ± 4.4 $_{BC}$	1.4 ± 0.9 $_{B}$	0.41 ± 0.11 $_{ABCDE}$	7.3 ± 0.6 $_{AB}$
BAA 1429 rif	19.8 ± 4.4 $_{AB}$	1.1 ± 0.9 $_{B}$	0.64 ± 0.11 $_{A}$	6.8 ± 0.6 $_{ABC}$
BAA 1430 rif	13.2 ± 4.4 $_{ABC}$	0.6 ± 0.9 $_{B}$	0.30 ± 0.11 $_{BCDE}$	6.1 ± 0.6 $_{BCD}$
BAA 1431 rif	15.0 ± 4.4 $_{ABC}$	1.6 ± 0.9 $_{B}$	0.21 ± 0.11 $_{CDE}$	5.6 ± 0.6 $_{CD}$
BAA-2219[e]	22.2 ± 4.4 $_{A}$	1.5 ± 0.9 $_{B}$	0.18 ± 0.11 $_{DE}$	5.4 ± 0.6 $_{CD}$
BAA-2217	10.8 ± 4.4 $_{ABC}$	6.0 ± 0.9 $_{A}$	0.13 ± 0.11 $_{E}$	5.5 ± 0.6 $_{CD}$
BAA-2215	10.1 ± 4.4 $_{ABC}$	1.5 ± 0.9 $_{B}$	0.29 ± 0.11 $_{BCDE}$	6.4 ± 0.6 $_{BCD}$
BAA-2196	9.5 ± 4.4 $_{BC}$	5.3 ± 0.9 $_{A}$	0.20 ± 0.11 $_{DE}$	5.9 ± 0.6 $_{BCD}$
BAA-2193	8.8 ± 4.4 $_{BC}$	2.3 ± 0.9 $_{B}$	0.58 ± 0.11 $_{AB}$	8.4 ± 0.6 $_{A}$
BAA-2192	16.6 ± 4.4 $_{ABC}$	1.2 ± 0.9 $_{B}$	0.13 ± 0.11 $_{E}$	4.9 ± 0.6 $_{D}$

[a] Decimal reduction time (*D*-value) represents three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

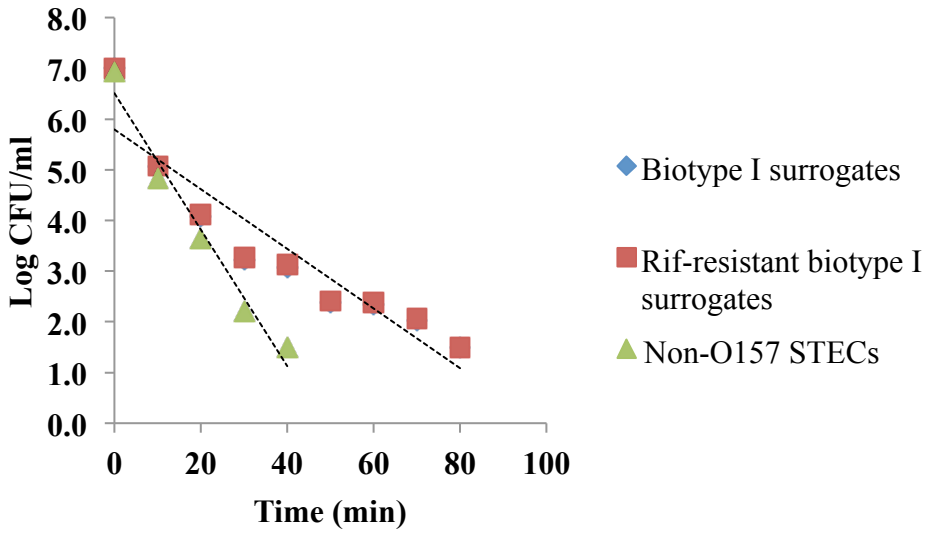

Figure 9. Thermal death curves for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 55°C. Each data point represents the mean count of three replicates of the strains. The dotted line represents the linear trendline of each survival curve.

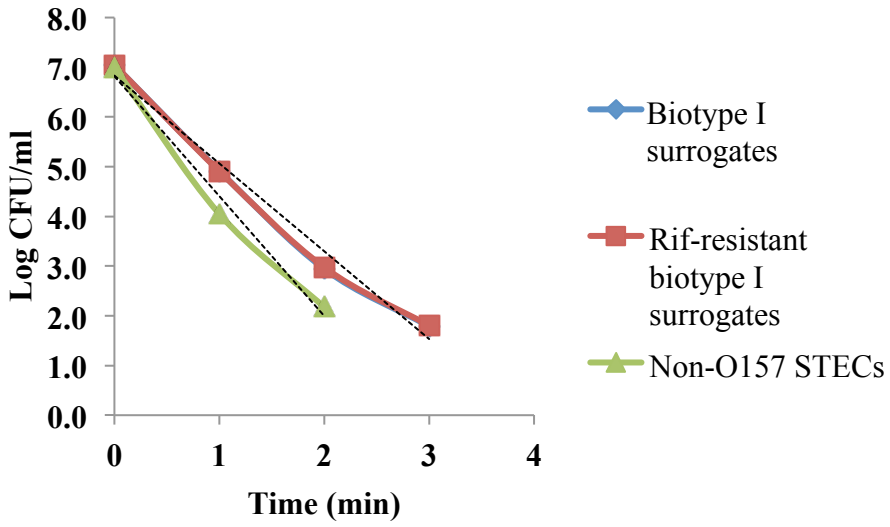

Figure 10. Thermal death curves for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 60°C. Each data point represents the mean count of three replicates of the strains. The dotted line represents the linear trendline of each survival curve.

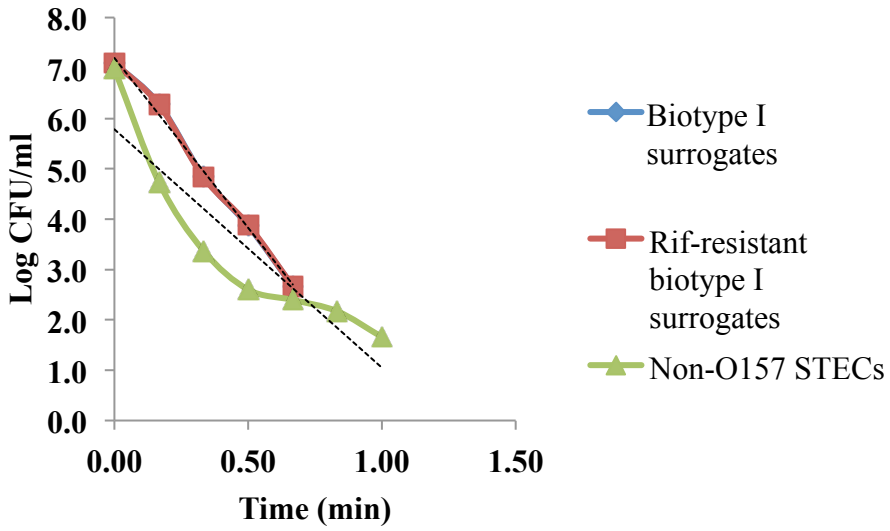

Figure 11. Thermal death curves for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 65°C. Each data point represents the mean count of three replicates of the strains. The dotted line represents the linear trendline of each survival curve.

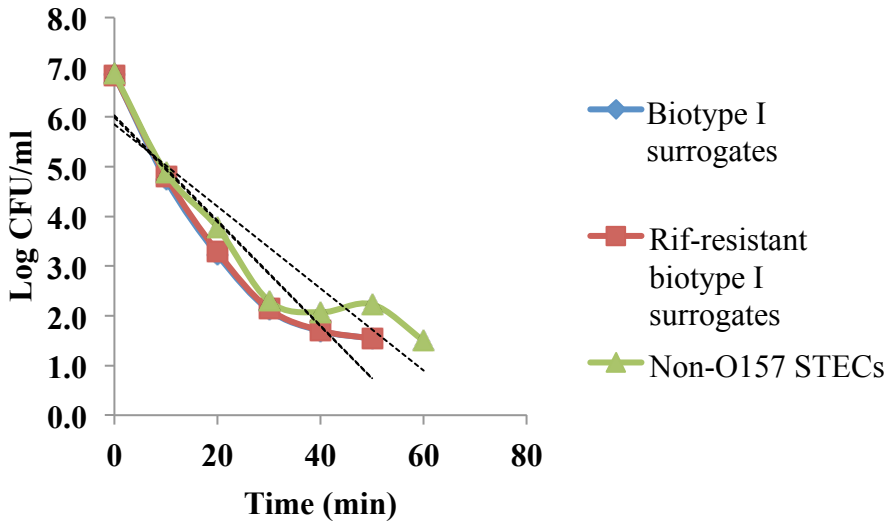

Figure 12. Thermal death curves for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 55°C. Each data point represents the mean count of three replicates of the strains. The dotted line represents the linear trendline of each survival curve.

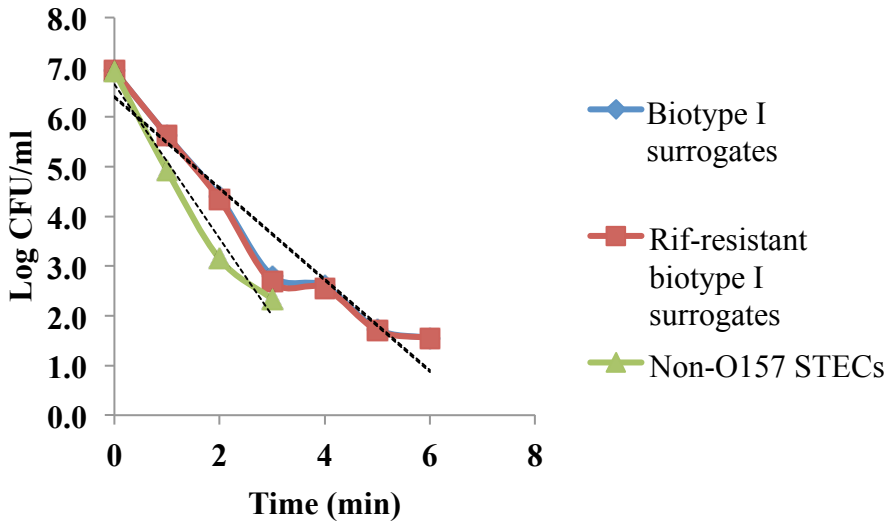

Figure 13. Thermal death curves for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 60°C. Each data point represents the mean count of three replicates of the strains. The dotted line represents the linear trendline of each survival curve.

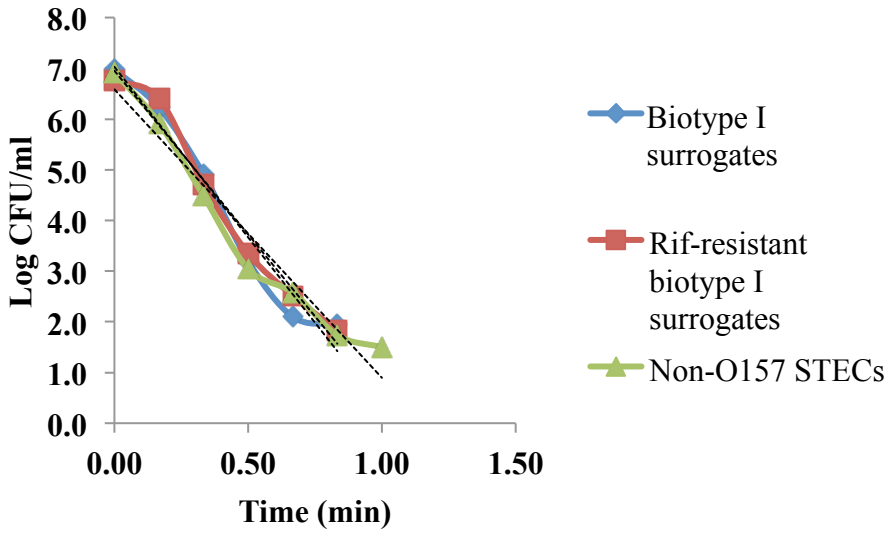

Figure 14. Thermal death curves for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 65°C. Each data point represents the mean count of three replicates of the strains. The dotted line represents the linear trendline of each survival curve.

Table 12. Decimal reduction time (*D*-value) for stationary phase and acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains in phosphate buffered saline at different temperatures

	D-value (min)[a] ± SEM[b]			
	55°C	60°C	65°C	*z*-value
Organism	Stationary			
E. coli surrogates[d]	14.6 ± 2.2 $_{A}$[c]	0.9 ± 0.1 $_{A}$	0.21 ± 0.16 $_{B}$	5.6 ± 1.1 $_{B}$
rif-resistant[e] *E. coli* surrogates	18.4 ± 2.2 $_{A}$	0.6 ± 0.1 $_{A}$	0.33 ± 0.16 $_{AB}$	6.2 ± 1.1 $_{AB}$
Non-O157:H7 STECs[f]	8.2 ± 2.0 $_{B}$	0.6 ± 0.1 $_{A}$	0.69 ± 0.15 $_{A}$	8.9 ± 1.0 $_{A}$
	Acid-adapted			
E. coli surrogates	9.6 ± 2.0 $_{A}$	1.5 ± 0.5 $_{B}$	0.27 ± 0.06 $_{AB}$	6.5 ± 0.3 $_{A}$
rif-resistant *E. coli* surrogates	13.4 ± 2.0 $_{A}$	1.2 ± 0.5 $_{B}$	0.42 ± 0.06 $_{A}$	6.4 ± 0.3 $_{A}$
Non-O157:H7 STECs	13.0 ± 1.8 $_{A}$	3.0 ± 0.4 $_{A}$	0.25 ± 0.05 $_{B}$	6.1 ± 0.3 $_{A}$

[a] Decimal reduction time (*D*-value) represents three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] *E. coli* surrogates include BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431 strains
[e] Rif-resistant *E. coli* surrogates include rif-resistant strains BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431
[f] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Response to refrigeration and freezing temperatures

One of the characteristics of an ideal surrogate organism is having similar characteristics, including growth, resistance to acid and thermal, and response to refrigeration and freezing temperatures. Storage at low temperatures is necessary for meat and meat products, both in processing and for preservation. If a surrogate organism were to be used to represent the non-O157 STECs, it would ideally be similar in response to refrigeration and freezing temperatures. The organisms were grown in and cultured in both TSB (stationary phase) and TSB+G (acid-adapted) to determine if there was a difference or cross protection between inducing an acid-adaptation response.

The mean counts (CFU/ml) for the *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates and non-O157 STECs, stationary phase and acid-adapted after storage at −20°C and 4°C during 0, 7, 14, 21, 28, 60, and 90 d, are presented in Tables 13-16. Based on the results obtained, no one single *E. coli* biotype I surrogate represented the pathogenic non-O157 STECs survival properties at −20°C and 4°C. Strain to strain reactions to stress vary from organism to organism, and is an inherent property. Both refrigerated and frozen storage resulted in random differences between counts of *E. coli* biotype I microorganisms and non-O157 STECs; however, there were no notable trends or patterns observed.

The ability to survive at low temperatures was also compared for the sets of organisms. The mean counts (CFU/ml) for the groups of *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates and non-O157 STECs, stationary phase and acid-adapted after storage at −20°C and 4°C during 0, 7, 14, 21, 28, 60, and 90 d, are

presented in Tables 17 (Figures 15-18). By the completion of the 90 d storage period in phosphate buffered saline, all three sets of organisms at both conditions (stationary phase and acid-adapted) and at both temperatures (−20°C and 4°C) showed no differences ($P > 0.05$). There were differences ($P < 0.05$) throughout the sampling period, but over time the organisms evened out and were not different ($P > 0.05$) following 90 d storage time at refrigerated and freezing temperatures.

Table 13. Mean counts (log CFU/ml) for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at −20°C

	Mean[a] ± SEM[b]						
	Time (days)						
Organism	0	7	14	21	28	60	90
BAA 1427	7.8 ± 0.1 $_{ABCD}$[c]	6.6 ± 0.2 $_{ABC}$	6.0 ± 0.2 $_{ABCD}$	5.4 ± 0.2 $_{DEF}$	5.1 ± 0.2 $_{D}$	4.9 ± 0.3 $_{CDE}$	4.4 ± 0.3 $_{BCD}$
BAA 1428	7.8 ± 0.1 $_{ABCD}$	6.4 ± 0.2 $_{BC}$	5.8 ± 0.2 $_{BCD}$	5.2 ± 0.2 $_{F}$	5.1 ± 0.2 $_{D}$	5.7 ± 0.3 $_{AB}$	5.0 ± 0.3 $_{ABC}$
BAA 1429	7.9 ± 0.1 $_{AB}$	7.1 ± 0.2 $_{A}$	6.4 ± 0.2 $_{AB}$	6.3 ± 0.2 $_{A}$	6.0 ± 0.2 $_{AB}$	6.0 ± 0.3 $_{A}$	5.4 ± 0.3 $_{A}$
BAA 1430	8.0 ± 0.1 $_{A}$	6.5 ± 0.2 $_{ABC}$	5.6 ± 0.2 $_{D}$	5.9 ± 0.2 $_{ABCDE}$	5.0 ± 0.2 $_{D}$	4.7 ± 0.3 $_{DE}$	4.0 ± 0.3 $_{D}$
BAA 1431	7.7 ± 0.1 $_{D}$	6.3 ± 0.2 $_{BC}$	5.9 ± 0.2 $_{BCD}$	5.4 ± 0.2 $_{DEF}$	5.2 ± 0.2 $_{CD}$	5.0 ± 0.3 $_{BCDE}$	4.7 ± 0.3 $_{ABCD}$
BAA 1427 rif[d]	7.8 ± 0.1 $_{BCD}$	6.9 ± 0.2 $_{AB}$	6.5 ± 0.2 $_{A}$	6.1 ± 0.2 $_{ABC}$	6.1 ± 0.2 $_{A}$	5.1 ± 0.3 $_{BCDE}$	4.9 ± 0.3 $_{ABCD}$
BAA 1428 rif	7.7 ± 0.1 $_{CD}$	6.5 ± 0.2 $_{ABC}$	5.8 ± 0.2 $_{BCD}$	5.6 ± 0.2 $_{BCDEF}$	5.5 ± 0.2 $_{BCD}$	5.6 ± 0.3 $_{AB}$	4.5 ± 0.3 $_{ABCD}$
BAA 1429 rif	7.9 ± 0.1 $_{AB}$	6.6 ± 0.2 $_{ABC}$	6.2 ± 0.2 $_{ABC}$	6.0 ± 0.2 $_{ABCD}$	5.6 ± 0.2 $_{ABC}$	5.0 ± 0.3 $_{BCDE}$	4.5 ± 0.3 $_{BCD}$
BAA 1430 rif	7.8 ± 0.1 $_{ABCD}$	6.5 ± 0.2 $_{ABC}$	6.2 ± 0.2 $_{ABC}$	5.5 ± 0.2 $_{BCDEF}$	5.2 ± 0.2 $_{CD}$	4.8 ± 0.3 $_{CDE}$	5.0 ± 0.3 $_{ABC}$
BAA 1431 rif	7.8 ± 0.1 $_{BCD}$	6.6 ± 0.2 $_{ABC}$	6.3 ± 0.2 $_{ABC}$	5.9 ± 0.2 $_{ABCDE}$	5.4 ± 0.2 $_{CD}$	5.5 ± 0.3 $_{ABC}$	5.0 ± 0.3 $_{ABC}$
BAA-2219[e]	7.9 ± 0.1 $_{ABC}$	6.6 ± 0.2 $_{ABC}$	6.0 ± 0.2 $_{ABCD}$	6.1 ± 0.2 $_{AB}$	5.2 ± 0.2 $_{CD}$	5.6 ± 0.3 $_{AB}$	5.3 ± 0.3 $_{AB}$
BAA-2217	7.9 ± 0.1 $_{AB}$	6.6 ± 0.2 $_{ABC}$	5.9 ± 0.2 $_{BCD}$	5.7 ± 0.2 $_{ABCDEF}$	5.1 ± 0.2 $_{CD}$	5.0 ± 0.3 $_{BCDE}$	4.3 ± 0.3 $_{CD}$
BAA-2215	7.7 ± 0.1 $_{CD}$	6.5 ± 0.2 $_{BC}$	5.9 ± 0.2 $_{ABCD}$	5.4 ± 0.2 $_{EF}$	5.3 ± 0.2 $_{CD}$	5.2 ± 0.3 $_{BCD}$	4.7 ± 0.3 $_{ABCD}$
BAA-2196	7.4 ± 0.1 $_{E}$	6.1 ± 0.2 $_{C}$	5.6 ± 0.2 $_{D}$	5.4 ± 0.2 $_{CDEF}$	5.1 ± 0.2 $_{CD}$	4.8 ± 0.3 $_{DE}$	4.4 ± 0.3 $_{BCD}$
BAA-2193	7.8 ± 0.1 $_{BCD}$	6.4 ± 0.2 $_{BC}$	5.5 ± 0.2 $_{D}$	5.2 ± 0.2 $_{F}$	5.2 ± 0.2 $_{CD}$	4.4 ± 0.3 $_{E}$	4.3 ± 0.3 $_{CD}$
BAA-2192	7.9 ± 0.1 $_{AB}$	6.1 ± 0.2 $_{C}$	5.7 ± 0.2 $_{CD}$	5.2 ± 0.2 $_{F}$	5.1 ± 0.2 $_{CD}$	4.6 ± 0.3 $_{DE}$	4.8 ± 0.3 $_{ABCD}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 14. Mean counts (log CFU/ml) for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 4°C

	Mean[a] ± SEM[b]						
	Time (days)						
Organism	0	7	14	21	28	60	90
BAA 1427	8.0 ± 0.1 $_{A}$[c]	7.7 ± 0.1 $_{ABC}$	7.7 ± 0.1 $_{AB}$	7.6 ± 0.1 $_{AB}$	7.6 ± 0.1 $_{BC}$	7.3 ± 0.1 $_{ABC}$	7.2 ± 0.1 $_{B}$
BAA 1428	7.8 ± 0.1 $_{ABC}$	7.8 ± 0.1 $_{A}$	7.6 ± 0.1 $_{AB}$	7.5 ± 0.1 $_{ABC}$	7.6 ± 0.1 $_{BC}$	7.2 ± 0.1 $_{CD}$	6.7 ± 0.1 $_{CD}$
BAA 1429	8.0 ± 0.1 $_{A}$	7.7 ± 0.1 $_{ABC}$	7.7 ± 0.1 $_{A}$	7.7 ± 0.1 $_{A}$	7.9 ± 0.1 $_{A}$	7.5 ± 0.1 $_{A}$	6.9 ± 0.1 $_{C}$
BAA 1430	8.0 ± 0.1 $_{A}$	7.7 ± 0.1 $_{ABCD}$	7.5 ± 0.1 $_{AB}$	7.5 ± 0.1 $_{ABC}$	7.4 ± 0.1 $_{BCD}$	7.1 ± 0.1 $_{CDE}$	6.7 ± 0.1 $_{CD}$
BAA 1431	7.6 ± 0.1 $_{DE}$	7.4 ± 0.1 $_{EF}$	7.2 ± 0.1 $_{D}$	7.1 ± 0.1 $_{DE}$	6.9 ± 0.1 $_{GH}$	6.7 ± 0.1 $_{FG}$	6.6 ± 0.1 $_{D}$
BAA 1427 rif[d]	7.9 ± 0.1 $_{AB}$	7.5 ± 0.1 $_{CDE}$	7.3 ± 0.1 $_{CD}$	7.2 ± 0.1 $_{CD}$	7.1 ± 0.1 $_{EFG}$	6.8 ± 0.1 $_{FG}$	6.7 ± 0.1 $_{CD}$
BAA 1428 rif	7.6 ± 0.1 $_{F}$	7.6 ± 0.1 $_{BCDE}$	7.5 ± 0.1 $_{AB}$	7.3 ± 0.1 $_{BCD}$	7.5 ± 0.1 $_{BCD}$	7.3 ± 0.1 $_{BCD}$	7.4 ± 0.1 $_{A}$
BAA 1429 rif	7.8 ± 0.1 $_{ABCD}$	7.8 ± 0.1 $_{AB}$	7.5 ± 0.1 $_{BC}$	7.4 ± 0.1 $_{BCD}$	7.2 ± 0.1 $_{DEF}$	6.8 ± 0.1 $_{FG}$	6.7 ± 0.1 $_{CD}$
BAA 1430 rif	7.8 ± 0.1 $_{BCD}$	7.4 ± 0.1 $_{DEF}$	7.2 ± 0.1 $_{D}$	7.1 ± 0.1 $_{DE}$	7.0 ± 0.1 $_{FGH}$	6.7 ± 0.1 $_{FG}$	6.6 ± 0.1 $_{D}$
BAA 1431 rif	7.8 ± 0.1 $_{ABCD}$	7.6 ± 0.1 $_{ABCDE}$	7.3 ± 0.1 $_{CD}$	7.2 ± 0.1 $_{D}$	7.1 ± 0.1 $_{EFGH}$	6.8 ± 0.1 $_{FG}$	6.6 ± 0.1 $_{D}$
BAA-2219[e]	7.8 ± 0.1 $_{ABCD}$	7.8 ± 0.1 $_{AB}$	7.7 ± 0.1 $_{AB}$	7.6 ± 0.1 $_{AB}$	7.5 ± 0.1 $_{BC}$	6.7 ± 0.1 $_{FG}$	6.6 ± 0.1 $_{D}$
BAA-2217	8.0 ± 0.1 $_{A}$	7.8 ± 0.1 $_{A}$	7.7 ± 0.1 $_{A}$	7.6 ± 0.1 $_{AB}$	7.7 ± 0.1 $_{AB}$	7.1 ± 0.1 $_{DE}$	6.6 ± 0.1 $_{D}$
BAA-2215	7.7 ± 0.1 $_{CDE}$	7.4 ± 0.1 $_{EF}$	7.3 ± 0.1 $_{CD}$	7.3 ± 0.1 $_{CD}$	7.3 ± 0.1 $_{CDE}$	6.9 ± 0.1 $_{EF}$	6.7 ± 0.1 $_{CD}$
BAA-2196	7.5 ± 0.1 $_{E}$	7.2 ± 0.1 $_{F}$	6.9 ± 0.1 $_{E}$	6.8 ± 0.1 $_{EF}$	6.8 ± 0.1 $_{HI}$	6.6 ± 0.1 $_{G}$	6.6 ± 0.1 $_{D}$
BAA-2193	7.9 ± 0.1 $_{AB}$	7.8 ± 0.1 $_{A}$	7.6 ± 0.1 $_{AB}$	7.7 ± 0.1 $_{A}$	7.6 ± 0.1 $_{ABC}$	7.5 ± 0.1 $_{AB}$	7.2 ± 0.1 $_{AB}$
BAA-2192	7.9 ± 0.1 $_{AB}$	7.4 ± 0.1 $_{EF}$	6.8 ± 0.1 $_{E}$	6.6 ± 0.1 $_{F}$	6.6 ± 0.1 $_{I}$	6.6 ± 0.1 $_{G}$	6.6 ± 0.1 $_{D}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 15. Mean counts (log CFU/ml) for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at −20°C

	Mean[a] ± SEM[b]						
	Time (days)						
Organism	0	7	14	21	28	60	90
BAA 1427	7.8 ± 0.1 $_{ABCD}$[c]	5.9 ± 0.2 $_{ABCDE}$	5.4 ± 0.2 $_{BCD}$	5.0 ± 0.1 $_{B}$	4.7 ± 0.2 $_{BCD}$	4.3 ± 0.3 $_{CDE}$	3.9 ± 0.4 $_{ABCD}$
BAA 1428	8.0 ± 0.1 $_{A}$	5.5 ± 0.2 $_{E}$	5.0 ± 0.2 $_{D}$	5.0 ± 0.1 $_{B}$	4.2 ± 0.2 $_{D}$	4.4 ± 0.3 $_{BCDE}$	3.5 ± 0.4 $_{CD}$
BAA 1429	7.9 ± 0.1 $_{ABC}$	6.3 ± 0.2 $_{AB}$	6.2 ± 0.2 $_{A}$	5.3 ± 0.1 $_{AB}$	4.8 ± 0.2 $_{ABCD}$	4.7 ± 0.3 $_{ABCDE}$	4.8 ± 0.4 $_{A}$
BAA 1430	7.9 ± 0.1 $_{AB}$	5.7 ± 0.2 $_{CDE}$	5.1 ± 0.2 $_{CD}$	5.0 ± 0.1 $_{B}$	4.1 ± 0.2 $_{D}$	4.6 ± 0.3 $_{BCDE}$	3.3 ± 0.4 $_{D}$
BAA 1431	7.4 ± 0.1 $_{DE}$	5.8 ± 0.2 $_{CDE}$	5.2 ± 0.2 $_{CD}$	5.0 ± 0.1 $_{B}$	4.4 ± 0.2 $_{CD}$	4.3 ± 0.3 $_{CDE}$	3.8 ± 0.4 $_{ABCD}$
BAA 1427 rif[d]	7.6 ± 0.1 $_{BCDE}$	5.9 ± 0.2 $_{BCDE}$	5.5 ± 0.2 $_{BCD}$	5.1 ± 0.1 $_{B}$	5.3 ± 0.2 $_{AB}$	4.6 ± 0.3 $_{BCDE}$	4.3 ± 0.4 $_{ABCD}$
BAA 1428 rif	7.8 ± 0.1 $_{ABCD}$	6.4 ± 0.2 $_{A}$	5.6 ± 0.2 $_{ABCD}$	5.4 ± 0.1 $_{AB}$	5.5 ± 0.2 $_{A}$	5.4 ± 0.3 $_{A}$	3.9 ± 0.4 $_{ABCD}$
BAA 1429 rif	7.7 ± 0.1 $_{ABCD}$	6.0 ± 0.2 $_{ABCD}$	5.2 ± 0.2 $_{CD}$	5.1 ± 0.1 $_{AB}$	4.8 ± 0.2 $_{ABCD}$	4.8 ± 0.3 $_{ABCD}$	3.7 ± 0.4 $_{BCD}$
BAA 1430 rif	7.5 ± 0.1 $_{DE}$	5.6 ± 0.2 $_{DE}$	5.4 ± 0.2 $_{BCD}$	5.0 ± 0.1 $_{B}$	4.3 ± 0.2 $_{CD}$	4.1 ± 0.3 $_{DE}$	3.6 ± 0.4 $_{BCD}$
BAA 1431 rif	7.5 ± 0.1 $_{CDE}$	5.9 ± 0.2 $_{ABCDE}$	5.4 ± 0.2 $_{BCD}$	5.0 ± 0.1 $_{B}$	4.5 ± 0.2 $_{CD}$	4.6 ± 0.3 $_{BCDE}$	3.5 ± 0.4 $_{CD}$
BAA-2219[e]	7.8 ± 0.1 $_{ABCD}$	6.2 ± 0.2 $_{ABC}$	5.5 ± 0.2 $_{BCD}$	5.0 ± 0.1 $_{B}$	4.6 ± 0.2 $_{BCD}$	5.1 ± 0.3 $_{AB}$	4.4 ± 0.4 $_{ABC}$
BAA-2217	7.8 ± 0.1 $_{ABCD}$	6.1 ± 0.2 $_{ABC}$	5.7 ± 0.2 $_{ABC}$	5.5 ± 0.1 $_{A}$	4.2 ± 0.2 $_{D}$	4.0 ± 0.3 $_{E}$	3.5 ± 0.4 $_{CD}$
BAA-2215	7.6 ± 0.1 $_{BCDE}$	6.0 ± 0.2 $_{ABCDE}$	5.2 ± 0.2 $_{CD}$	5.1 ± 0.1 $_{AB}$	4.8 ± 0.2 $_{BCD}$	4.9 ± 0.3 $_{ABC}$	4.4 ± 0.4 $_{ABC}$
BAA-2196	7.3 ± 0.1 $_{E}$	6.1 ± 0.2 $_{ABCD}$	5.2 ± 0.2 $_{CD}$	5.4 ± 0.1 $_{AB}$	4.9 ± 0.2 $_{ABC}$	4.8 ± 0.3 $_{ABC}$	4.6 ± 0.4 $_{AB}$
BAA-2193	7.8 ± 0.1 $_{ABCD}$	6.1 ± 0.2 $_{ABCD}$	5.9 ± 0.2 $_{AB}$	5.0 ± 0.1 $_{B}$	4.6 ± 0.2 $_{BCD}$	4.9 ± 0.3 $_{ABC}$	3.7 ± 0.4 $_{BCD}$
BAA-2192	7.8 ± 0.1 $_{ABCD}$	5.8 ± 0.2 $_{BCDE}$	5.5 ± 0.2 $_{BCD}$	5.1 ± 0.1 $_{B}$	4.6 ± 0.2 $_{BCD}$	4.0 ± 0.3 $_{E}$	3.6 ± 0.4 $_{BCD}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 16. Mean counts (log CFU/ml) for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline at 4°C

	Mean[a] ± SEM[b]						
	Time (days)						
Organism	0	7	14	21	28	60	90
BAA 1427	7.8 ± 0.1 $_{AB}$[c]	7.7 ± 0.1 $_{A}$	7.6 ± 0.1 $_{A}$	7.7 ± 0.1 $_{A}$	7.6 ± 0.1 $_{AB}$	7.6 ± 0.1 $_{A}$	7.5 ± 0.1 $_{A}$
BAA 1428	7.5 ± 0.1 $_{F}$	7.2 ± 0.1 $_{DE}$	6.8 ± 0.1 $_{EFG}$	6.7 ± 0.1 $_{EF}$	6.6 ± 0.1 $_{H}$	6.6 ± 0.1 $_{EF}$	6.4 ± 0.1 $_{CDEF}$
BAA 1429	7.9 ± 0.1 $_{A}$	7.4 ± 0.1 $_{BC}$	7.1 ± 0.1 $_{CD}$	7.4 ± 0.1 $_{C}$	7.2 ± 0.1 $_{DE}$	7.2 ± 0.1 $_{BCD}$	7.0 ± 0.1 $_{B}$
BAA 1430	7.8 ± 0.1 $_{AB}$	7.4 ± 0.1 $_{BC}$	7.0 ± 0.1 $_{DE}$	7.0 ± 0.1 $_{D}$	6.9 ± 0.1 $_{FG}$	6.9 ± 0.1 $_{DE}$	6.5 ± 0.1 $_{CDE}$
BAA 1431	7.6 ± 0.1 $_{EF}$	7.1 ± 0.1 $_{DE}$	6.7 ± 0.1 $_{FG}$	6.7 ± 0.1 $_{EF}$	6.7 ± 0.1 $_{H}$	6.2 ± 0.1 $_{G}$	6.1 ± 0.1 $_{F}$
BAA 1427 rif[d]	7.5 ± 0.1 $_{F}$	7.0 ± 0.1 $_{EF}$	6.9 ± 0.1 $_{DEF}$	6.8 ± 0.1 $_{DE}$	6.7 ± 0.1 $_{H}$	6.5 ± 0.1 $_{FG}$	6.1 ± 0.1 $_{F}$
BAA 1428 rif	7.7 ± 0.1 $_{CDEF}$	7.5 ± 0.1 $_{AB}$	7.6 ± 0.1 $_{A}$	7.5 ± 0.1 $_{C}$	7.5 ± 0.1 $_{ABC}$	7.5 ± 0.1 $_{AB}$	7.5 ± 0.1 $_{A}$
BAA 1429 rif	7.6 ± 0.1 $_{DEF}$	7.0 ± 0.1 $_{EF}$	6.7 ± 0.1 $_{FG}$	6.8 ± 0.1 $_{EF}$	6.6 ± 0.1 $_{H}$	6.6 ± 0.1 $_{EF}$	6.3 ± 0.1 $_{DEF}$
BAA 1430 rif	7.5 ± 0.1 $_{F}$	6.9 ± 0.1 $_{F}$	6.6 ± 0.1 $_{G}$	6.6 ± 0.1 $_{F}$	6.6 ± 0.1 $_{H}$	6.2 ± 0.1 $_{G}$	5.7 ± 0.1 $_{G}$
BAA 1431 rif	7.6 ± 0.1 $_{DEF}$	7.0 ± 0.1 $_{EF}$	6.6 ± 0.1 $_{FG}$	6.6 ± 0.1 $_{F}$	6.7 ± 0.1 $_{H}$	6.6 ± 0.1 $_{EF}$	6.5 ± 0.1 $_{CDE}$
BAA-2219[e]	7.8 ± 0.1 $_{ABC}$	7.7 ± 0.1 $_{A}$	7.4 ± 0.1 $_{AB}$	7.5 ± 0.1 $_{BC}$	7.4 ± 0.1 $_{CD}$	7.1 ± 0.1 $_{CD}$	6.3 ± 0.1 $_{EF}$
BAA-2217	7.6 ± 0.1 $_{DEF}$	7.5 ± 0.1 $_{AB}$	7.6 ± 0.1 $_{A}$	7.5 ± 0.1 $_{BC}$	7.4 ± 0.1 $_{BCD}$	7.0 ± 0.1 $_{CD}$	6.4 ± 0.1 $_{CDEF}$
BAA-2215	7.6 ± 0.1 $_{EF}$	7.3 ± 0.1 $_{CD}$	7.0 ± 0.1 $_{CDE}$	7.0 ± 0.1 $_{D}$	7.0 ± 0.1 $_{EF}$	7.0 ± 0.1 $_{CD}$	6.7 ± 0.1 $_{BCD}$
BAA-2196	7.2 ± 0.1 $_{G}$	7.0 ± 0.1 $_{EF}$	6.8 ± 0.1 $_{EFG}$	6.7 ± 0.1 $_{EF}$	6.8 ± 0.1 $_{GH}$	6.8 ± 0.1 $_{DEF}$	6.8 ± 0.1 $_{BC}$
BAA-2193	7.7 ± 0.1 $_{BCDE}$	7.5 ± 0.1 $_{ABC}$	7.5 ± 0.1 $_{AB}$	7.7 ± 0.1 $_{AB}$	7.7 ± 0.1 $_{A}$	7.6 ± 0.1 $_{A}$	7.5 ± 0.1 $_{A}$
BAA-2192	7.7 ± 0.1 $_{ABCD}$	7.5 ± 0.1 $_{AB}$	7.3 ± 0.1 $_{BC}$	7.5 ± 0.1 $_{C}$	7.4 ± 0.1 $_{CD}$	7.3 ± 0.1 $_{ABC}$	7.0 ± 0.1 $_{B}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] rif = rif-resistant organism
[e] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

Table 17. Mean counts (log CFU/ml) for *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline stored at low temperatures

	Mean reduction[a] ± SEM[b]						
	Time (days)						
	0	7	14	21	28	60	90
Organism	Stationary/−20°C						
E. coli surrogates[d]	7.8 ± 0.0 $_{A}$[c]	6.6 ± 0.1 $_{A}$	5.9 ± 0.1 $_{AB}$	5.6 ± 0.1 $_{AB}$	5.3 ± 0.1 $_{B}$	5.3 ± 0.1 $_{A}$	4.7 ± 0.2 $_{A}$
rif-resistant[e] *E. coli* surrogates	7.8 ± 0.0 $_{A}$	6.6 ± 0.1 $_{A}$	6.2 ± 0.1 $_{A}$	5.8 ± 0.1 $_{A}$	5.6 ± 0.1 $_{A}$	5.2 ± 0.1 $_{A}$	4.8 ± 0.2 $_{A}$
Non-O157:H7 STECs[f]	7.8 ± 0.0 $_{A}$	6.4 ± 0.1 $_{A}$	5.8 ± 0.1 $_{B}$	5.5 ± 0.1 $_{B}$	5.2 ± 0.1 $_{B}$	4.9 ± 0.1 $_{A}$	4.6 ± 0.1 $_{A}$
	Stationary/4°C						
E. coli surrogates	7.9 ± 0.0 $_{A}$	7.7 ± 0.1 $_{A}$	7.5 ± 0.1 $_{A}$	7.5 ± 0.1 $_{A}$	7.5 ± 0.1 $_{A}$	7.2 ± 0.1 $_{A}$	6.8 ± 0.1 $_{A}$
rif-resistant *E. coli* surrogates	7.8 ± 0.0 $_{A}$	7.6 ± 0.1 $_{A}$	7.4 ± 0.1 $_{AB}$	7.3 ± 0.1 $_{B}$	7.2 ± 0.1 $_{B}$	6.9 ± 0.1 $_{B}$	6.8 ± 0.1 $_{A}$
Non-O157:H7 STECs	7.8 ± 0.0 $_{A}$	7.6 ± 0.1 $_{A}$	7.3 ± 0.1 $_{B}$	7.3 ± 0.1 $_{AB}$	7.3 ± 0.1 $_{AB}$	6.9 ± 0.1 $_{B}$	6.7 ± 0.1 $_{A}$
	Acid-adapted/−20°C						
E. coli surrogates	7.8 ± 0.1 $_{A}$	5.8 ± 0.1 $_{A}$	5.4 ± 0.1 $_{A}$	5.1 ± 0.1 $_{A}$	4.4 ± 0.1 $_{B}$	4.5 ± 0.1 $_{A}$	3.9 ± 0.2 $_{A}$
rif-resistant *E. coli* surrogates	7.6 ± 0.1 $_{B}$	6.0 ± 0.1 $_{A}$	5.4 ± 0.1 $_{A}$	5.1 ± 0.1 $_{A}$	4.9 ± 0.1 $_{A}$	4.7 ± 0.1 $_{A}$	3.8 ± 0.2 $_{A}$
Non-O157:H7 STECs	7.7 ± 0.1 $_{AB}$	6.0 ± 0.1 $_{A}$	5.5 ± 0.1 $_{A}$	5.2 ± 0.1 $_{A}$	4.6 ± 0.1 $_{AB}$	4.6 ± 0.1 $_{A}$	4.0 ± 0.2 $_{A}$
	Acid-adapted/4°C						
E. coli surrogates	7.7 ± 0.0 $_{A}$	7.4 ± 0.1 $_{A}$	7.0 ± 0.1 $_{AB}$	7.1 ± 0.1 $_{AB}$	7.0 ± 0.1 $_{B}$	6.9 ± 0.1 $_{AB}$	6.7 ± 0.1 $_{A}$
rif-resistant *E. coli* surrogates	7.6 ± 0.0 $_{B}$	7.1 ± 0.1 $_{B}$	6.9 ± 0.1 $_{B}$	6.9 ± 0.1 $_{B}$	6.8 ± 0.1 $_{B}$	6.7 ± 0.1 $_{B}$	6.4 ± 0.1 $_{A}$
Non-O157:H7 STECs	7.6 ± 0.0 $_{AB}$	7.4 ± 0.1 $_{A}$	7.3 ± 0.1 $_{A}$	7.3 ± 0.1 $_{A}$	7.3 ± 0.1 $_{A}$	7.1 ± 0.1 $_{A}$	6.8 ± 0.1 $_{A}$

[a] Mean values were obtained from three independent replicates
[b] SEM = Standard error of the least squares means
[c] Means in the same column with the same letter (ABCDEFG) are not different ($P > 0.05$)
[d] *E. coli* surrogates include BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431 strains
[e] Rif-resistant *E. coli* surrogates include rif-resistant strains BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BAA-1431
[f] BAA-2219 = O121:H19, BAA-2217 = O111, BAA-2215 = O103:H11, BAA-2196 = O26:H11, BAA-2193 = O45:H2, BAA-2192 = O145:NM

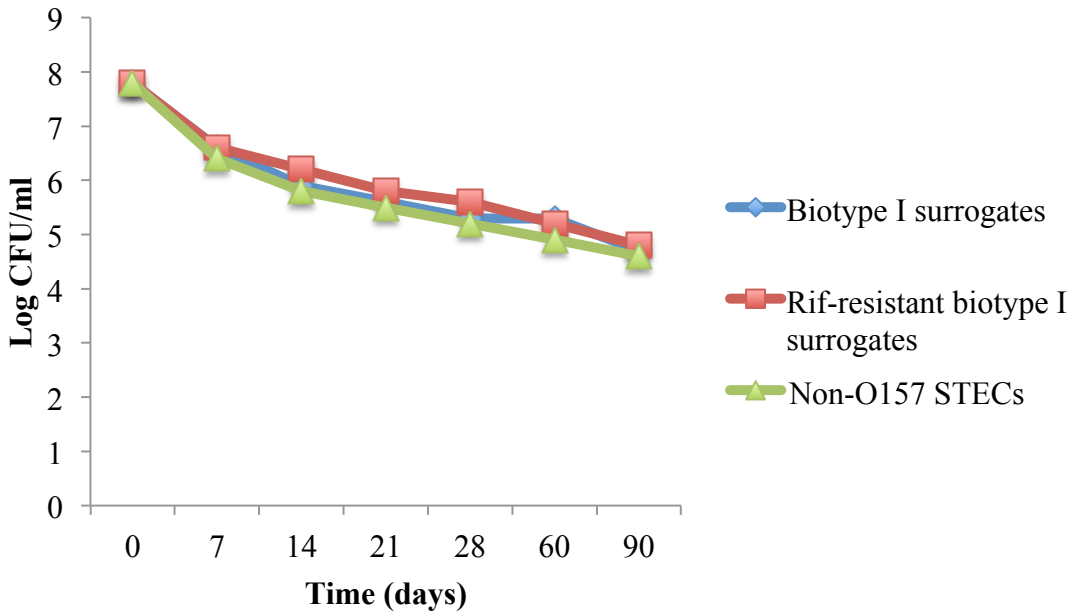

Figure 15. Mean counts for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline stored at −20°C

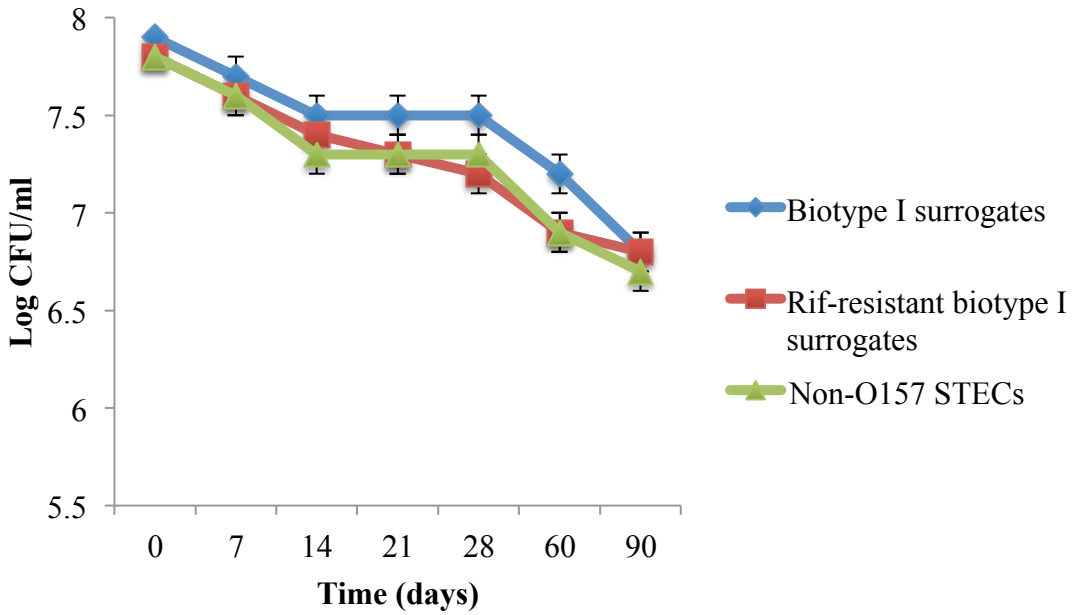

Figure 16. Mean counts for stationary phase *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline stored at 4°C

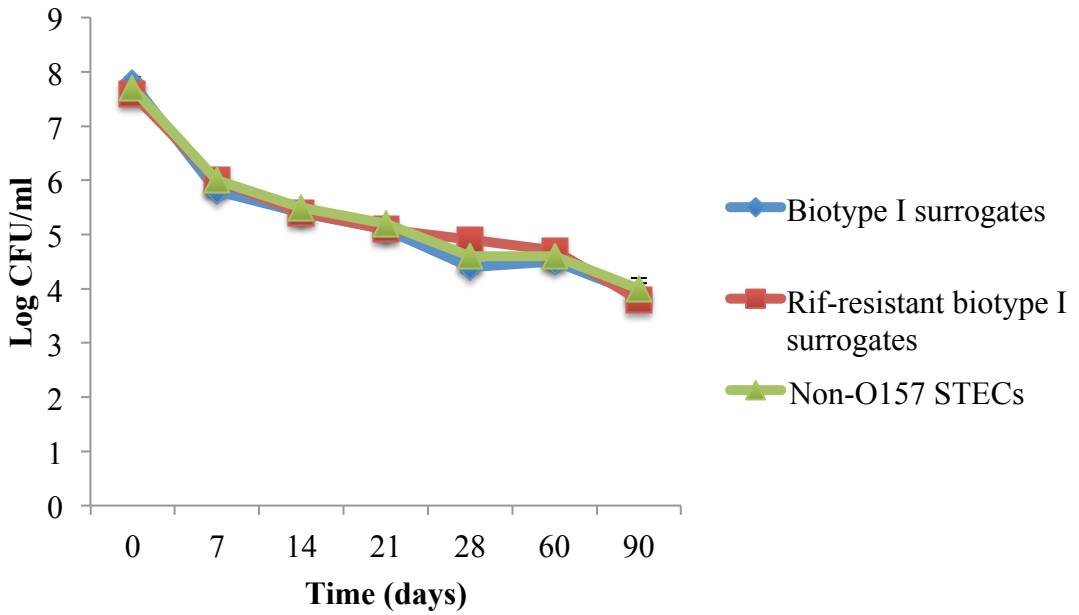

Figure 17. Mean counts for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline stored at −20°C

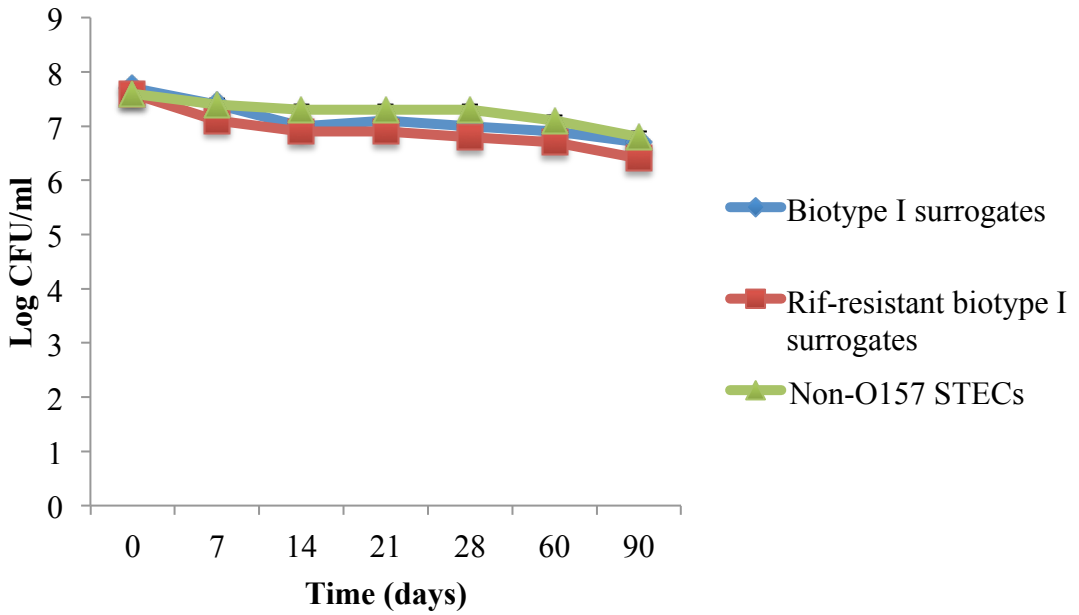

Figure 18. Mean counts for acid-adapted *E. coli* biotype I surrogates, rif-resistant *E. coli* biotype I surrogates, and non-O157 STECs strains after exposure to phosphate buffered saline stored at 4°C

5. CONCLUSIONS

These data suggest that *E. coli* biotype I surrogates may be used to predict growth, acid, and thermal resistance, and storage conditions of non-O157 STECs within a small amount of error. Findings for growth, acid, and thermal resistance, and storage conditions support previous research stating that surrogates may best be used as cocktail rather than as single strains *(8, 19, 29)*. The beef industry may use these data to conduct in-plant validation studies to determine the efficacy of antimicrobial interventions on both *E. coli* O157:H7 and non-O157 STECs, and these data may also assist the beef industry in fulfilling regulatory expectations.

USDA-FSIS requires scientific validation on interventions, and has published draft guidelines for in-plant validations. Rif-resistant *E. coli* biotype I surrogates are not recommended for use by FSIS for in-plant validation. Antibiotic resistant organisms are useful in laboratory settings to eliminate potential background microflora, and allow for the researcher to prove that the organisms that were used for the inoculation procedure are the same organisms that are recovered. The biotype I surrogates, if taken into a plant for use in a validation study, may be used to inoculate the product at a high rate. For example, the inoculum used may be close to 10^8 log CFU/ml. The benefit in this application, although not necessarily representative of what may be coming into a plant on a daily basis, is to be able to show reductions, and help in maneuvering different processing steps. The use of the higher inoculation procedure and attachment may allow a facility to evaluate their interventions and determine if the use of their antimicrobial

spray is appropriate, or does it need to be altered. A higher inoculation attachment also allows the facility to show a greater reduction or kill or microorganisms, which is useful in validating a system.

The biotype I surrogates (parents strains) are approved for use in a facility by FSIS to validate a system; however, FSIS recommends that the use of bacterial surrogate strains that have been "marked" using an antibiotic resistant genes be avoided *(35)*. The surrogates are nonpathogenic, and have been shown by the data in this study to be appropriate for use to represent the non-O157 STECs. The non-O157 STECs can cause illness, and it is important for processing facilities to validate their systems for these organisms. These data may help support decisions related to food safety programs in beef slaughter and further processing establishments. Continued data collection will provide information critical for use of the surrogates in validation studies.

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APPENDIX

BAA-2217™ CERTIFICATE OF ANALYSIS

61161460

ATCC® Number:	Escherichia coli Strain 10C-3114
Lot Number:	Bacterial cells suspended in an appropriate cryoprotectant
Organism:	07/31/2017
Product Format:	2°C to 8°C for freeze-dried cultures; - 80°C or colder for frozen cultures
Expiration Date:	
Storage Conditions:	Note: Do not store frozen vials in freezers with a defrost cycle, as this will expose the vials to increased temperatures.

Test	Specification	Result
Gram stain and cell morphology	Gram stain and cell morphology are consistent with the organism being tested.	Gram negative, motile, rods in singles and pairs.
Colony description	Colony description is consistent with the organism being tested.	Circular, entire, low convex, translucent.
Purity	Sample material is inoculated onto non-selective media. Cultures are examined macroscopically and microscopically after incubation. Cultures show no evidence of aberrant growth.	No evidence of aberrant growth.
Viability	Sample material is checked for titer. Results are ≥ 10^6 cfu/vial, ccu/vial, or bacteria/vial.	3.5 x 10^9 cfu/vial
Phenotypic testing	Sample material is evaluated with a defined battery of phenotypic tests including evaluation by bioMérieux VITEK® 2 Compact. Results are consistent with the organism being tested.	Catalase – positive Oxidase – negative Pigment Production – negative MacConkey agar growth - positive 99% identification to Escherichia coli using bioMérieux VITEK® 2 Compact
Genotypic testing	Sample material is evaluated by 16S ribosomal gene sequencing and by PCR profile for select virulence genes. Results are consistent with the organism being tested.	Identification is consistent with the organism being tested Positive for stx2 gene; Negative for eae and stx1 genes

CERTIFICATE OF ANALYSIS

ATCC® Number: BAA-2217™

Lot Number: 61161460

Revised on 11/07/2013: Updated serogroup information from O111 to O146 as per the results of additional testing. Added results information for virulence genes.

Manager of Material Release; Quality Assurance

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