

# Effects of Media Type on Shiga Toxigenic *E. coli* Growth Patterns

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## Introduction

*Escherichia coli* O157:H7 was declared to be an adulterant in raw ground beef in 1994 by the United States Department of Agriculture Food Safety and Inspection Service following a large and deadly foodborne disease outbreak in the Pacific Northwest involving undercooked hamburgers sold at Jack-in-the-Box restaurants. Due to their recognition as significant human foodborne pathogens, six additional strains (serotypes) of Shiga toxin-producing *E. coli* (STEC) were also deemed to be adulterants in raw beef products in 2012.

The beef processing industry has worked diligently since the mid-1990s to control the presence of *E. coli* O157:H7 in finished raw products through the implementation of aggressive microbial testing programs and the incorporation of antimicrobial intervention technologies validated to substantially reduce the presence of this pathogenic organism. This effort has occurred within the framework of Hazard Analysis and Critical Control Points (HACCP) programs. With the addition of six additional STEC strains that also must be controlled through these programs, laboratory-testing methods must be developed and implemented to afford the industry a means to accurately document their control programs. Shiga toxin-producing *E. coli* cultivation, identification, and quantification methods are currently lacking.

Establishing behavior patterns for these STECs will allow the beef processing industry to better develop methods for controlling or eliminating them in the food supply. To accomplish this, the prevalence of these organisms must first be established through sampling, but research into which media type is best for enriching samples to recover and identify all STEC organisms has been limited. To determine which media type was best suited for recovery of STECs, we inoculated multiple enrichment media types with the target strains and observed their growth patterns.

## Experimental Procedures

Strains of the newly designated STEC adulterants (O26, O45, O103, O111, and O145), O157:H7, and O104:H4 (isolate from a German sprout outbreak) were evaluated. Eight types of liquid enrichment media were evaluated to compare STEC growth profiles over incubation time: buffered peptone water, universal pre-enrichment broth, tryptic soy broth, tryptic soy broth with 8mg/L novobiocin (mTSB M), *Escherichia coli* broth, as well as three levels of novobiocin added at 4, 8, and 20 mg/L (mEC L, mEC M, and mEC H, respectively). Each of these media types was individually inoculated with a separate strain of STEC and placed in an incubator at 99°F. At 4, 8, 12, 18, and 24-hour points, aliquots of each media/strain combination were removed and plated on tryptic soy agar. These plates were then placed in a separate incubator for 24 hours at 99°F. The plates were removed from the incubator and enumerated.

## Results and Discussion

The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 held in each enrichment media at 99°F for up to 24 hours and then plated on tryptic soy agar for enumeration are shown in Figures 1–8. Of the media types used, buffered peptone water, universal pre-enrichment broth, and tryptic soy broth are classified as general, non-selective enrichment media and allow growth of most organisms present in a sample. *E. coli* broth is selective for *E. coli* serotypes, and novobiocin antibiotic is commonly added to enrichments to detect *E. coli* O157:H7 to further suppress growth of non-*E. coli* competing microflora. One of the standard *E. coli* O157:H7 enrichments currently used in the beef industry is *E. coli* broth with high levels of novobiocin, but our results indicated that growth of most of the other non-O157:H7 STEC strains was severely inhibited when subjected to any level of novobiocin in *E. coli* broth. Because the use of *E. coli* broth containing no novobiocin showed growth levels for all STEC serotypes comparable to non-selective media types, it can be surmised that the use of unmodified *E. coli* broth may be preferable when enriching beef samples to determine possible presence of STEC.

## Implications

The documented ability of *E. coli* broth containing no novobiocin to recover all target STECs will help the beef processing industry by allowing for the use of a single enrichment that will promote growth of target STEC organisms while simultaneously suppressing natural background flora, thereby reducing testing costs. Common selective enrichment broths utilized in beef testing for *E. coli* O157:H7 containing novobiocin may lead to false negatives for the other STEC strains that cannot tolerate the presence of this antibiotic.

## Acknowledgements

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## MEAT AND FOOD SAFETY

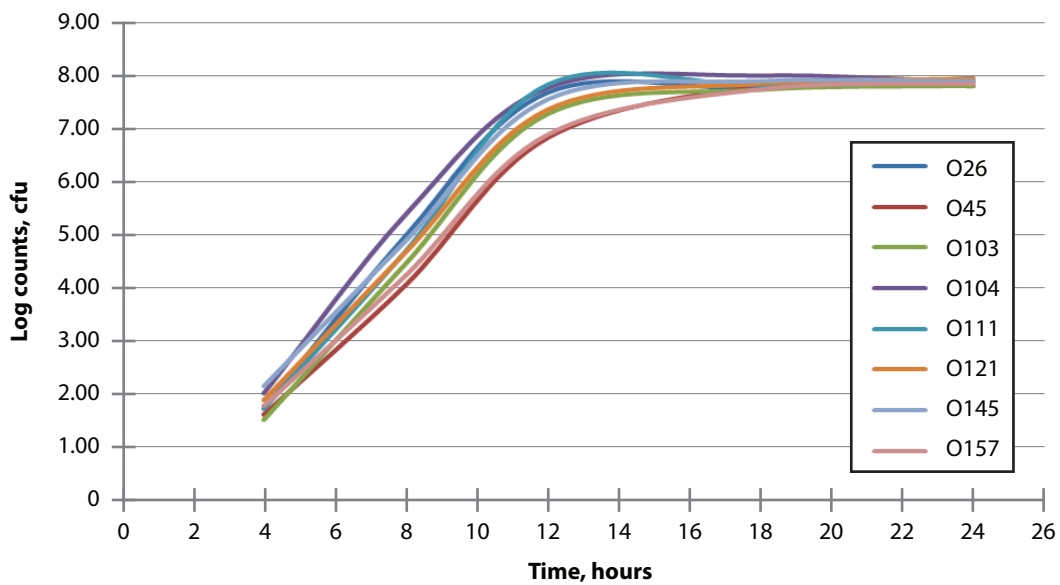


Figure 1. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using buffered peptone water at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.

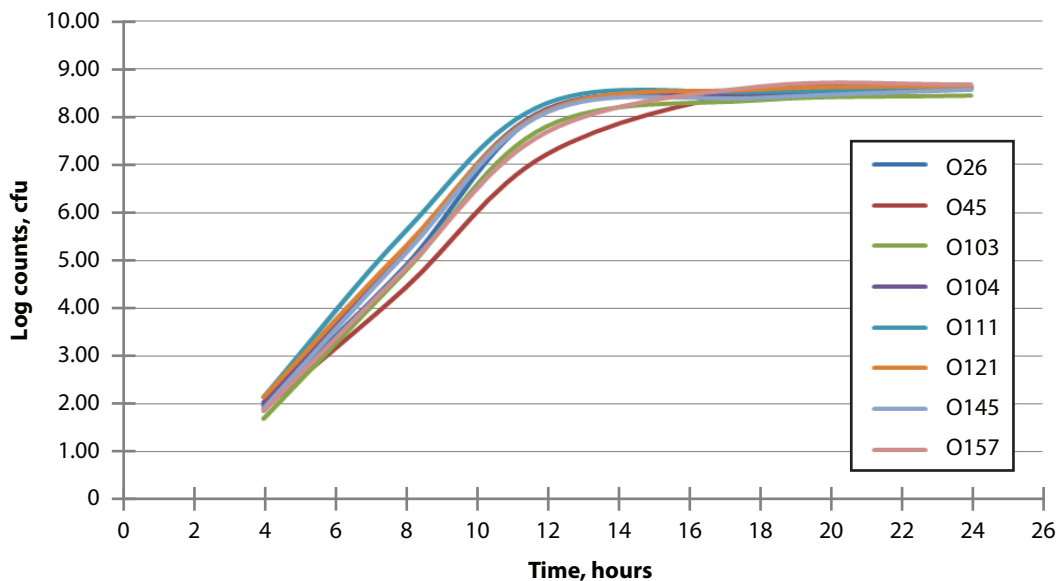


Figure 2. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using universal pre-enrichment broth at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.

MEAT AND FOOD SAFETY

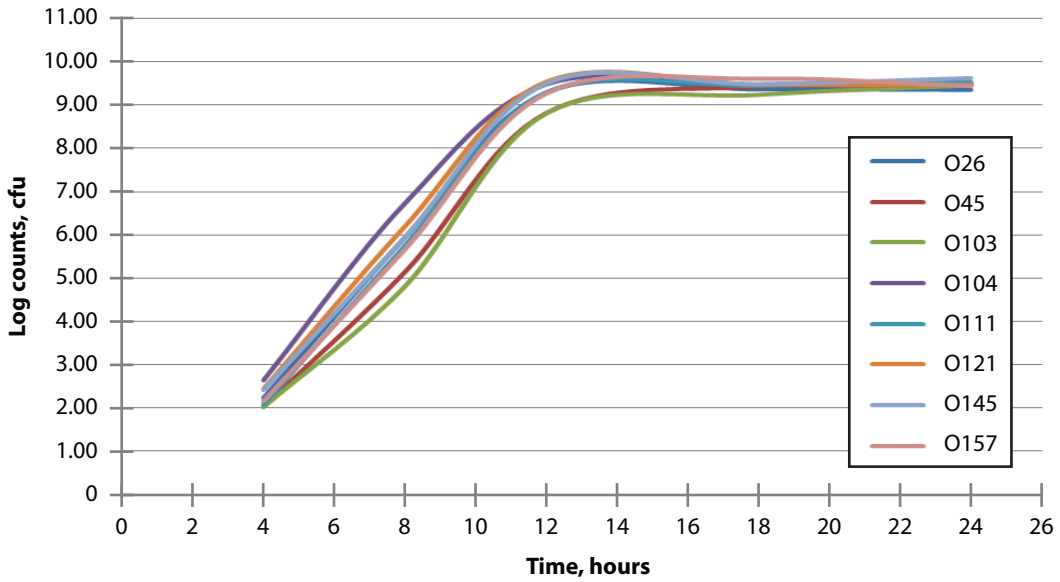


Figure 3. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using tryptic soy broth at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.

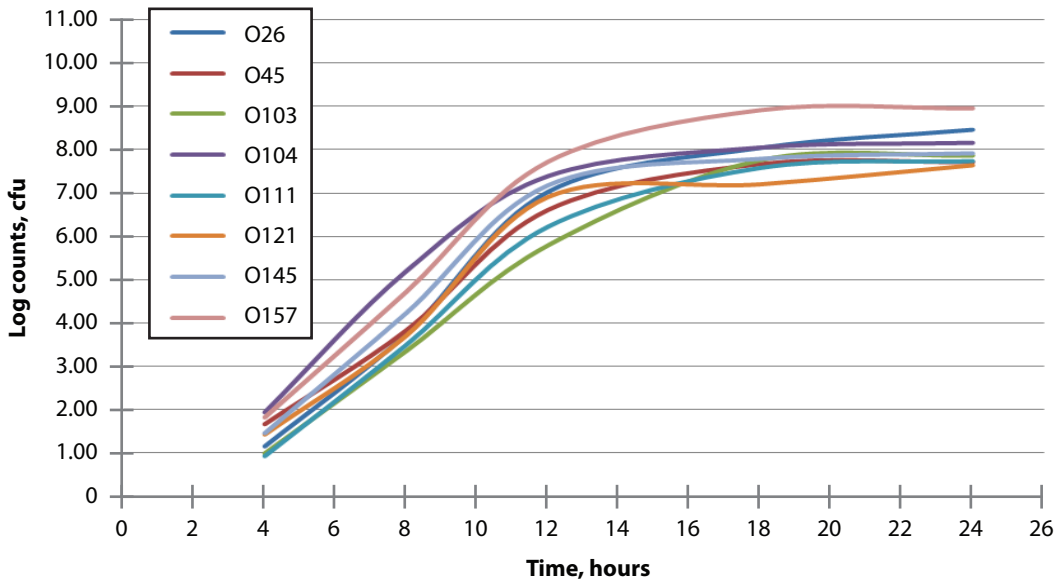


Figure 4. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using modified tryptic soy broth with 8 mg/L Novobiocin at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.

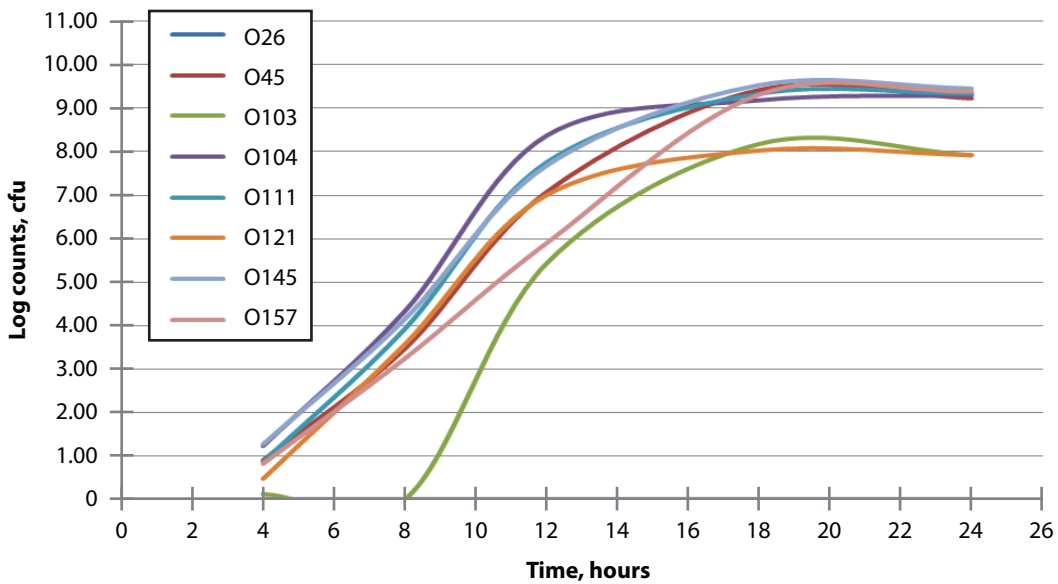


Figure 5. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using *E. coli* broth at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.

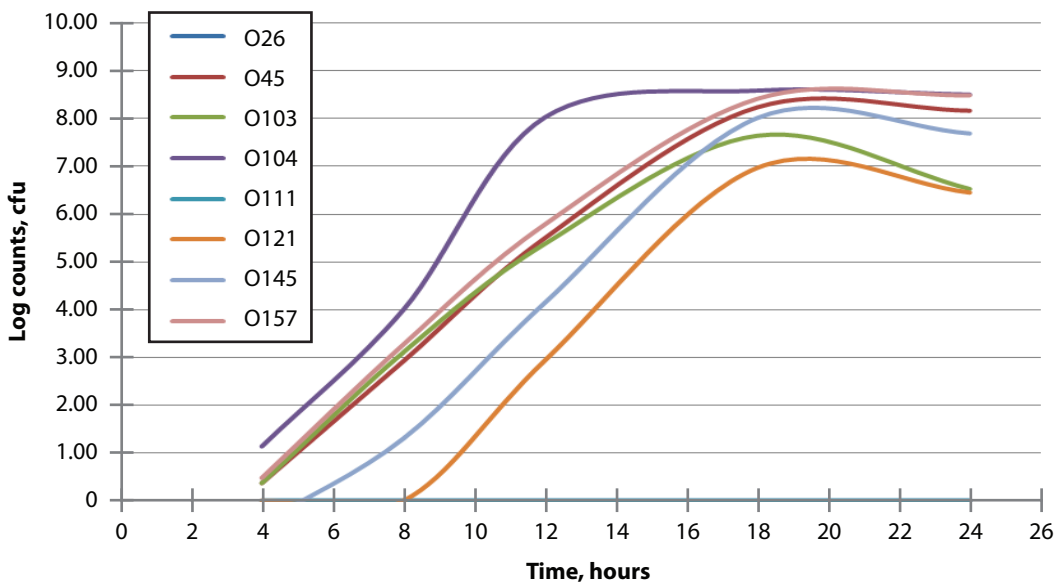


Figure 6. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using modified *E. coli* broth with 4mg/L Novobiocin at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.

MEAT AND FOOD SAFETY

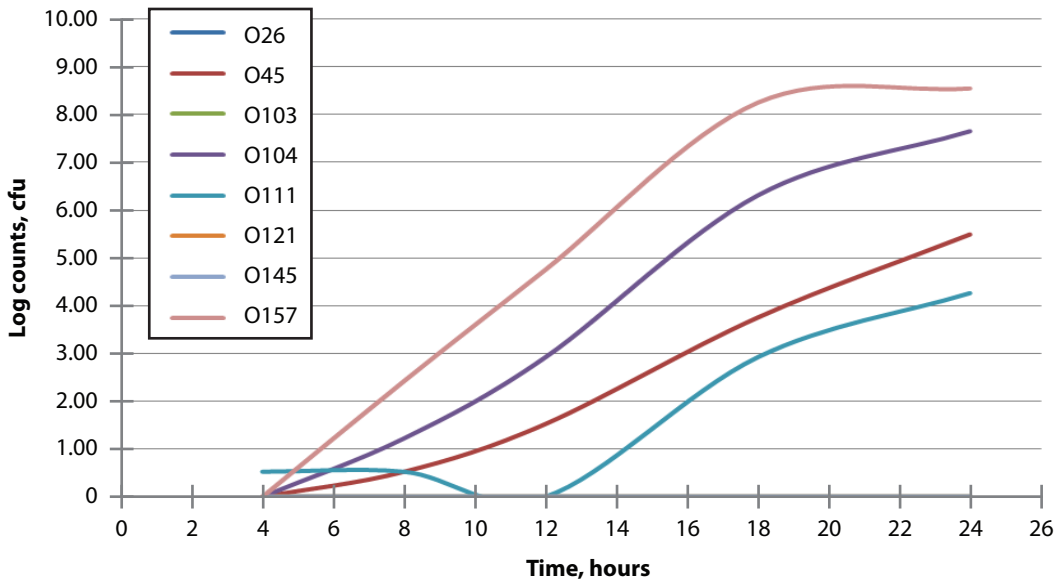


Figure 7. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using modified *E. coli* broth with 8mg/L Novobiocin at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.

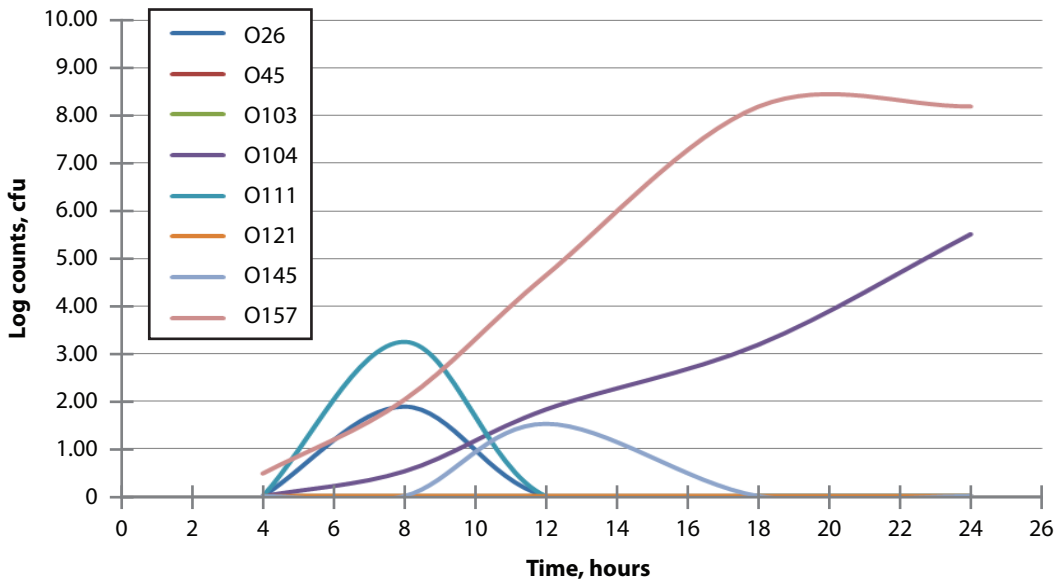


Figure 8. The average log count (cfu) of *E. coli* O26, O45, O103, O111, O145, O157:H7, and O104:T4 enriched using modified *E. coli* broth with 20mg/L Novobiocin buffered peptone water at 99°F for up to 24 hours then plated on tryptic soy agar for enumeration.