# Design and Evaluation of a Real-Time PCR Method for Detecting O157:H7 and non-O157 STEC strains from Beef Samples

**Sharon A. Matheny[1], Aisha Abdul-Walkeel[2], Robert S. Tebbs[1], Craig Cummings[1], Jason K. Wall[1], Quoc H. Hoang[1], Rixun Fang[1], Lily Wong[1], Lavorka Degoricija[1], Pius Brzoska[1], Manohar Furtado[1], Catherine O'Connell[1] and Pina Fratamico[2]**

**[1]Life Technologies Corporation, Carlsbad, CA, USA [2]USDA-ARS-ERRC, Wyndmoor, PA,USA**

## INTRODUCTION

*E. coli* O157:H7 was first recognized as a human pathogen in 1982 and until recently was the only *E. coli* strain mandated for testing by the USDA. In late 2011, the USDA announced it will declare 6 additional Shiga-toxin producing *E. coli* serotypes as adulterants, namely O26, O45, O103, O111, O121, and O145. These 6 non-O157 STECs will be classified as adulterants if they also contain virulence genes for *eae* and *stx1* and/or *stx2*.

## PURPOSE

To design a real-time PCR assay for detecting STEC adulterants including O157:H7 and the 6 non-O157 STEC bacteria using multiplex designs to reduce the number of tests to one screening assay and one confirmation assay.

## MATERIALS AND METHODS

A minimum of six TaqMan® real-time PCR assays were designed against each of the 6 non-O157 STEC O serotypes using Applied Biosystems assay design software. Additional assays were also designed against virulence factors *stx1*, *stx2*, and *eae*. Each assay was tested against an inclusion/exclusion panel of 241 *E. coli* strains consisting of 167 of the 180 known *E. coli* O-types to determine assay specificity and sensitivity. High throughput screening by real-time PCR was performed on the 7900 real-time PCR system and multiplex optimization was performed on the 7500 Fast real-time PCR system using standard cycling conditions (95 ºC for 10 min, followed by 40 cycles at 95 ºC for 15 seconds and 60 ºC for 60 seconds).

## RESULTS

Multiple assays for each of the 6 non-O157 STECs detected all inclusion strains within the targeted serotype, and no exclusion strains from other serotypes. The *stx* assays detected all variants of *stx1* and *stx2* tested, including *stx2f* and *stx2g*. The assays were combined into two separate multiplex assays and optimized using statistical methods based on Design of Experiments. The assays will be evaluated for detecting STEC in ground beef and beef trim.

## SIGNIFICANCE

Regulations are moving toward increased testing for food borne pathogens. Multiplex real-time PCR can combine up to four PCR tests into a single reaction by using four different fluorescent dyes, reducing time and costs.

**Figure 1. RapidFinder™ STEC Product Workflow**

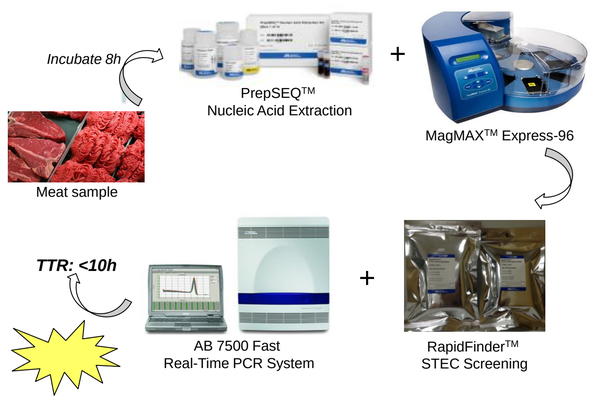



**Table 1. Internal and External Evaluation of RapidFinder™ STEC Assays: Inclusion / Exclusion Panels**

| | Organism | Serotype/species | # strains | % detected | %FalsePos | %FalseNeg |
|---|---|---|---|---|---|---|
| **Life Tech** | | | | | | |
| *Inclusion* | E. coli | O157:H7 | 8 | 100 | 0 | 0 |
| | E. coli | O26 | 13 | 100 | 0 | 0 |
| | E. coli | O45 | 11 | 100 | 0 | 0 |
| | E. coli | O103 | 13 | 100 | 0 | 0 |
| | E. coli | O111 | 13 | 100 | 0 | 0 |
| | E. coli | O121 | 10 | 100 | 0 | 0 |
| | E. coli | O145 | 7 | 100 | 0 | 0 |
| *Exclusion* | E. coli | non Big 6, non-o157:H7 | 65 | 0 | 0 | 0 |
| | Salmonella | typhi, typhimurium, enteriditis | 8 | 0 | 0 | 0 |
| | Shigella | dysenteriae | 3 | 0 | 0 | 0 |
| | Listeria | monocytogenes | 1 | 0 | 0 | 0 |
| | Legionella | pneumoniae | 1 | 0 | 0 | 0 |
| | Vibrio | cholerae | 1 | 0 | 0 | 0 |
| **USDA** | | | | | | |
| *Inclusion* | E. coli | O157:H7 | 5 | 100 | 0 | 0 |
| | E. coli | O26 | 9 | 100 | 0 | 0 |
| | E. coli | O45 | 16 | 100 | 0 | 0 |
| | E. coli | O103 | 7 | 100 | 0 | 0 |
| | E. coli | O111 | 7 | 100 | 0 | 0 |
| | E. coli | O121 | 7 | 100 | 0 | 0 |
| | E. coli | O145 | 6 | 100 | 0 | 0 |
| *Exclusion* | E. coli | non Big 6, non-o157H7 | 180 | 0 | 0 | 0 |
| | Salmonella | typhimurium, enteriditis, many | 12 | 0 | 0 | 0 |
| | Shigella | dysenteriae, flexneri, sonnei | 3 | 0 | 0 | 0 |
| | Listeria | monocytogenes, welshmeri | 2 | 0 | 0 | 0 |
| | Bacillus | cereus, brevis, subtilis, spp | 4 | 0 | 0 | 0 |
| | Pseudomonas | fluorescens, aeruginosa | 2 | 0 | 0 | 0 |
| | Citrobacter | freundi | 1 | 0 | 0 | 0 |
| | Serratia | enterriditis | 1 | 0 | 0 | 0 |

**Figure 2. RapidFinder™ STEC Product Configuration**

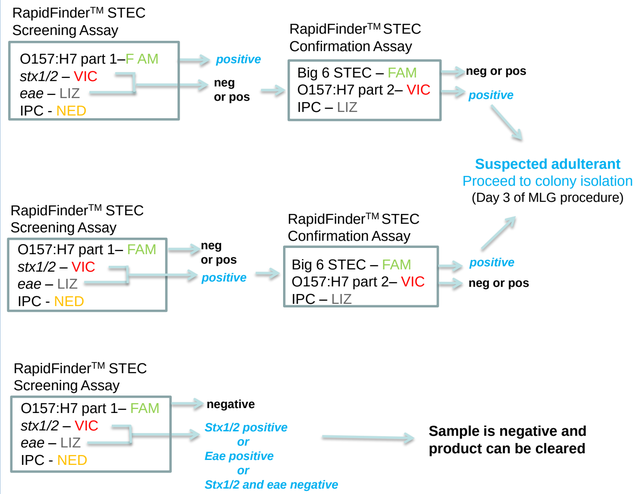
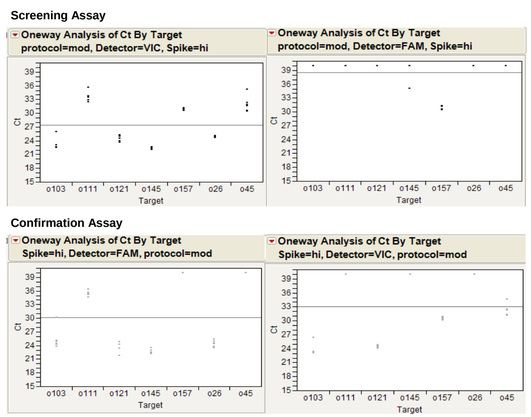



**Figure 2. Performance of RapidFinder™ STEC Screening and Confirmation Assays in Ground Beef cultures**




375g ground beef was inoculated at 10cfu/L and incubated in 1L tryptic soy broth for 8h 42ºC. DNA was extracted with PrepSEQ™ Nucleic Acid Extraction Kit by automated protocol on MagMax™ Express 96. 30ul of sample prep was used to hydrate lyophilized RapidFinder™ STEC Screening and Confirmation Assays. qPCR was performed on AB 7500 Fast Real-Time PCR System for 40 cycles with default settings.

**Table 2. USDA Evaluation of RapidFinder™ STEC Screening and Confirmation Assays (Short Study of lyophilized product)**

| | RapidFinder™ STEC Screening Assay | | | | | RapidFinder™ STEC Confirmation Assay | | |
|---|---|---|---|---|---|---|---|---|
| **Detector** | FAM | VIC | LIZ | NED | | FAM | VIC | LIZ |
| **Target** | O157:H7 (P1) | *stx1/2* | *eae* | IPC | | Big 6 O-types | O157:H7 (P2) | IPC |
| **Serotype** | | | | | | | | |
| *Inclusion* | | | | | | | | |
| O157:H7 | 22.54 | 21.82 | 22.70 | NR | | neg | 26.13 | NR |
| O157:H7 | 22.84 | 20.94 | 22.88 | NR | | neg | 23.24 | NR |
| O157 (not H7) | neg | neg | neg | NR | | neg | neg | NR |
| O26:H11 | neg | 19.11 | 19.18 | NR | | 18.85 | neg | NR |
| O45:H12 | neg | neg | 37.65^ | NR | | 20.53 | neg | NR |
| O103:H26 | neg | 20.87 | 21.23 | NR | | 21.80 | 19.98* | NR |
| O103:H2 | neg | 21.57 | 21.79 | NR | | 23.06 | 21.85* | NR |
| O111:NM | neg | 20.97 | 21.97 | NR | | 21.41 | neg | NR |
| O121:NM | neg | 22.04 | 22.31 | NR | | 22.69 | 30.28* | NR |
| *Exclusion* | | | | | | | | |
| O2 | neg | 21.37 | neg | NR | | neg | neg | NR |
| O128ac:H2 | neg | 21.09 | 20.95 | NR | | neg | neg | NR |
| O128 | neg | neg | 22.67 | NR | | neg | neg | NR |
| O138 | neg | 24.51 | neg | NR | | neg | neg | NR |
| *NTC* | | | | | | | | |
| water | neg | neg | neg | 33.01 | | neg | neg | 30.31 |
| water | neg | neg | neg | 33.08 | | neg | neg | 30.25 |

NR = Not relevant. Our Internal Positive Control (IPC) is designed to not compete with target-specific amplification; IPC amplification only serves as a positive control for the PCR reaction.
^Our cutoff for positive amplification is Ct </= 37
*Our O157:H7 (P2) assay also picks up some Big 6 serotypes, but *not* any non-Big 6

## CONCLUSIONS

➢The RapidFinder™ STEC Screening and Confirmation real-time PCR assays are part of a complete food testing solution designed to make food pathogen detection as rapid and easy as possible.

➢ Ease-of-use with a streamlined, lyophilized format.

➢ Minimal hands-on time with automated MagMAX Express-96 protocol.

➢ Total time to results is <10h (including 8h incubation) for raw ground beef or beef trim

➢ Results of validation studies demonstrated 100% detection of inclusion strains and 0% detection of exclusion strains.

➢ Internal positive control significantly reduces the risk of false-negatives.

## REFERENCES

1. Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli (STEC)* from Meat Products (MLG 5B.02). United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science. Effective: 6/4/12