# CERTIFICATION

## AOAC Research Institute

## *Performance Tested Methods*[SM]

Certificate No.

**091301**

**The AOAC Research Institute hereby certifies the method known as:**

## BAX® System Real-Time PCR Assay Suite for STEC

manufactured by

**Hygiena**
**2 Boulden Circle**
**New Castle, DE 19720**
**USA**

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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Expiration Date December 31, 2024

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**METHOD NAME**
BAX® System Real-Time PCR Assay Suite for STEC
Formerly DuPont™ BAX® System Real-Time PCR Assay Suite for STEC

**CATALOG NUMBERS**
BAX® Screening Kit KIT2021 (D14642964), BAX® System Panel 1 KIT2008 (D14642970), BAX® System Panel 2 KIT2009 (D14642987)

**INDEPENDENT LABORATORY**
Aegis Food Testing Laboratories, Inc.
224 N. Derby Lane
North Sioux City, SD 57049
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**INDEPENDENT LABORATORY MODIFICATION JANUARY 2022**
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**APPLICABILITY OF METHOD**
**Target organism**
**STEC Screening Assay – *stx* and *eae* virulence genes**
**STEC Panel 1 assay – *E. coli* O26, O111, O121**
**STEC Panel 2 assay – *E. coli* O45, O103, O145**

**Matrixes – raw beef trim (375g), raw ground beef (325 g, 375g), raw ground beef plus soy (325g).**
**MODIFICATION DECEMBER 2017 – raw ground beef (25 g) and all-purpose flour (25 g).**
**MODIFICATION JANUARY 2022 – (AOAC SMPR 2020.12; 10 g) dried cannabis flower [>0.3% delta 9-tetrahydrocannabinol (THC)] and dried hemp flower (≤0.3% THC).**
**MODIFICATION APRIL 2023 – Sampling cloths swabbed from 375 g beef trim portions.**

***Performance claims* – Sensitivity equal to or better than the corresponding reference method.**
**MODIFICATION JANUARY 2022: BAX System Real-Time PCR Assay for STEC Suite test kit is effective in screening for STEC species (O26, O111, O121, O45, O103, O145) in dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC) at a 10 g test portion size and met the requirements of *Standard Method Performance Requirements* (SMPRs®) for Detection of Shiga toxin-producing *Escherichia coli* in Cannabis and Cannabis Products (AOAC SMPR 2020.012; 8).**
**MODIFICATION APRIL 2023: The study data were unable to find a statistically detectable difference in results from zero between the BAX System Real-Time PCR Assay for STEC Suite and the United States Department of Agriculture Food Safety and Inspection Service MLG 5C.03 Detection, Isolation, and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products, Carcass, and Environmental Sponges (8) from sampling cloths swabbed from 375 g beef trim test portions in 8–24 h using MP media or modified Tryptic Soy Broth with casamino acids (mTSB +caa).**

**REFERENCE METHODS**

**Least Cost Formulations, Ltd., MPN Calculator-Version 1.6 (http://www.lcfltd.com/customer/LCFMPNCalculator.exe) (2)**

**USDA FSIS (2011) Microbiology Laboratory Guidebook (3)**

**US FDA (2017) *FDA Bacteriological Analytical Manual,* Chapter 4A, Diarrheagenic *Escherichia coli* (6)**

**USDA FSIS (2014) *Microbiology Laboratory Guidebook,* Chapter 5B.05, Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products and Carcass and Environmental Sponges (7)**

***Standard Method Performance Requirements* (SMPRs®) for Detection of Shiga toxin-producing *Escherichia coli* in Cannabis and Cannabis Products (AOAC SMPR 2020.012) (8)**

**United States Department of Agriculture Food Safety and Inspection Service MLG 5C.03 Detection, Isolation, and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STEC) from Meat Products, Carcass, and Environmental Sponges (10)**

**BAX® System Real-Time PCR Assay Suite for STEC, AOAC *Performance Tested Methods*℠ Certification Number 091301**

| ORIGINAL CERTIFICATION DATE | CERTIFICATION RENEWAL RECORD |
|---|---|
| September 24, 2013 | Renewed annually through December 2024. |

| METHOD MODIFICATION RECORD | SUMMARY OF MODIFICATION |
|---|---|
| 1. March 2017 Level 1 | 1. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company. |
| 2. December 2017 Level 1 | 2. Inserts, manuals, and labels updated to Hygiena. |
| 3. December 2017 Level 2 | 3. Matrix extension to add raw ground beef (25 g) and all-purpose flour (25 g). |
| 4. January 2018 Level 1 | 4. Editorial changes to insert and labels to update Hygiena. |
| 5. May 2019 Level 1 | 5. Editorial insert updates and corporate address. |
| 6. December 2019 Level 1 | 6. Editorial/clerical changes. |
| 7. December 2021 Level 1 | 7. Editorial changes. |
| 8. January 2022 Level 2 | 8. Matrix extension to include dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC). |
| 9. April 2023 Level 2 | 9. Matrix extension to include sampling cloths for 375 g beef trim portions. |
| 10. January 2024 Level 1 | 10. Editorial/clerical changes. |

| Under this AOAC *Performance Tested Methods*℠ License Number, 091301 this method is distributed by: | Under this AOAC *Performance Tested Methods*℠ License Number, 091301 this method is distributed as: |
|---|---|
| NONE | NONE |

**PRINCIPLE OF THE METHOD (1)**

*PCR Amplification* - The BAX® System uses the Polymerase Chain Reaction (PCR) to amplify specific fragments of bacterial DNA, which are stable and unaffected by growth environment. The fragments are genetic sequences that are unique to each of the *E. coli* serotypes and the associated *stx* and *eae* virulence factors, providing a highly reliable indicator that the target organisms are present in the sample. The BAX System simplifies the PCR process by combining the requisite primers, polymerase and nucleotides into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed for the detection phase, thus significantly reducing the potential for contamination with one or more molecules of amplified PCR product.

*Fluorescent Real-Time Detection* - This automated BAX System method uses fluorescent detection to analyze PCR products. One PCR primer for each associated target (*stx* and *eae* in the STEC Screening assay and three of the O-types of interest in each of the STEC Panel assays) and one for the internal DNA control target contains a fluorescent dye (a different one for each target) as a constituent of the primer as well as a quencher (the uni-molecular combination of a primer and fluorescent dye with an associated quencher either on the same molecule or on a separate oligonucleotide constitute a Scorpion™ Probe). When incorporated into a PCR product, the dye and quencher are spatially separated at the temperature at which detection occurs, which causes an increase in emission signal. The BAX® System instrument measures the magnitude and characteristics of fluorescent signal change from cycle to cycle of the PCR process. An analysis by the BAX® System software algorithm then evaluates that data to determine a positive or negative result which is displayed as described below.

The BAX System real-time STEC suite is a two-stage screening method. After appropriate sample enrichment, the Screening assay is used to determine the presence or absence of the Shiga toxin genes (*stx* encoding genes) and intimin (*eae* encoding genes) and clear negative samples quickly. If the Screening assay returns positive results for both virulence factors, then the two multiplex panel assays are run to detect and differentiate the top six STEC serogroups of public health concern which are regulated by the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) as adulterants in beef.

**DISCUSSION OF THE VALIDATION STUDY (1)**

For the internal and independent laboratory validation studies, POD analysis for all food indicated that the test method performed in a fashion not statistically different than the reference method, with the exception of one beef trim replicate (spiked with an O26 strain). The one replicate that produced a statistically distinguishable difference in method performance indicated that the BAX System method had a greater recovery of the target pathogen than the reference method. The results of the inclusivity and exclusivity studies demonstrate 100% inclusivity and exclusivity for the BAX® System real-time STEC suite for detecting both the stx and eae virulence genes and identifying the six targeted serogroups.

**Table 3. Method comparison results POD (1)**

| Matrix | Strain | MPN[a]/test portion | N[c] | Test Method | | | Reference Method | | | dPOD$_c$[g] | 95% CI[h] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | x[d] | POD$_c$[e] | 95% CI | x | POD$_R$[f] | 95% CI | | |
| Beef Trim / MP Media (11 and 24 hours) | O26:H11 | 7.9 (3.0, 21) | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | (-0.43, 0.43) |
| | | 0.51 (0.24, 0.90) | 20 | 17 | 0.85 | (0.64, 0.95) | 7 | 0.35 | (0.18, 0.57) | 0.50 | (0.20, 0.30) |
| | | Negative Control | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Beef Trim / MP Media (11 and 24 hours) | O145:H- | 10 (3.9, 26) | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | (-0.43, 0.43) |
| | | 0.42 (0.19, 0.73) | 20 | 7 | 0.35 | (0.18, 0.57) | 7 | 0.35 | (0.18, 0.57) | 0 | (-0.28, 0.28) |
| | | Negative Control | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Ground beef / mTSB + 2 mg/L novobiocin (11 and 24 hours) | O103:H11 | 18 (7.3, 54) | 5 | 5 | 1.0 | (0.57, 1.0) | 5 | 1.0 | (0.57, 1.0) | 0 | -(0.43, 0.43) |
| | | 0.78 (0.41, 1.3) | 20 | 15 | 0.75 | (0.53, 0.89) | 8 | 0.40 | (0.22, 0.061) | 0.35 | (-0.05, 0.58) |
| | | Negative Control | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.43, 0.43) |
| Ground beef with soy /mTSB+caa+n (12 and 24 hours) | O26:H11 | 0.87 (0.54, 1.4) | 30 | 19 | 0.63 | (0.46, 0.78) | 19 | 0.63 | (0.46, 0.78) | 0 | (-0.25, 0.25) |
| | | Negative Control | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Ground beef with soy /mTSB+caa+n (12 and 24 hours) | O111:H- | 0.91 (0.57, 1.4) | 30 | 18 | 0.60 | (0.42, 0.75) | 18 | 0.60 | (0.42, 0.75) | 0 | (-0.23, 0.23) |
| | | Negative Control | 5 | 0 | 0 | (0, 0.43) | 0 | 0 | (0, 0.43) | 0 | (-0.45, 0.45) |
| Beef Trim / MP Media 11 hrs[i] | O145:H28 | 3.01 | 5 | 4 | 0.80 | (0.38, 1.00) | 5 | 1.00 | (0.57, 1.00) | -0.2 | (-0.62, 0.28) |
| | | 0.56 | 20 | 6 | 0.30 | (0.15, 0.52) | 9 | 0.45 | (0.26, 0.66) | -0.15 | (-0.41, 0.14) |
| | | Negative Control | 5 | 0 | 0.00 | (0.00, 0.44) | 0 | 0.00 | (0.00, 0.44) | 0.00 | (-0.44, 0.44) |
| Beef Trim / TSB Media 11 hrs[i] | O145:H28 | 3.01 | 5 | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.44, 0.44) |
| | | 0.56 | 20 | 7 | 0.35 | (0.18, 0.57) | 9 | 0.45 | (0.26, 0.66) | -0.1 | (-0.37, 0.19) |
| | | Negative Control | 5 | 0 | 0.00 | (0.00, 0.44) | 0 | 0.00 | (0.00, 0.44) | 0.00 | (-0.44, 0.44) |
| Beef Trim / TSB Media 24 hrs[i] | O145:H28 | 3.01 | 5 | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.44, 0.44) |
| | | 0.56 | 20 | 7 | 0.35 | (0.18, 0.57) | 9 | 0.45 | (0.26, 0.66) | -0.1 | (-0.37, 0.19) |
| | | Negative Control | 5 | 0 | 0.00 | (0.00, 0.44) | 0 | 0.00 | (0.00, 0.44) | 0.00 | (-0.44, 0.44) |
| Beef Trim / mTSB + caa +n 24 hrs[i] | O145:H28 | 3.01 | 5 | 5 | 1.00 | (0.57, 1.00) | 5 | 1.00 | (0.57, 1.00) | 0.00 | (-0.43, 0.43) |
| | | 0.56 | 20 | 9 | 0.45 | (0.26, 0.66) | 9 | 0.45 | (0.26, 0.66) | 0.00 | (-0.25, 0.25) |
| | | Negative Control | 5 | 0 | 0.00 | (0.00, 0.44) | 0 | 0.00 | (0.00, 0.44) | 0.00 | (-0.44, 0.44) |

**Table 4. Inclusivity Results for STEC Screening (*stx* and eae containing strains) (1)**

| Strain ID | Other ID | *E. coli* serotype | Source | BAX result *eae* | BAX result *stx* | *stx*$_1$ gene | *stx*$_2$ gene | *eae* gene |
|---|---|---|---|---|---|---|---|---|
| DEC 101 | - | O145:H16 | MSU>USDA | Pos | Pos | Present | Absent | Present |
| DD13417 | CDC 85-337 | O4:HNM | US CDC>USDA | Pos | Pos | Present | Present | Present |
| DD13418 | CDC 95-3209 | O14:HNM | US CDC>USDA | Pos | Pos | Absent | Present | Present |
| DD1450 | - | O157:H7 | Human Clinical | Pos | Pos | Absent | Present | Present |
| DD13430 | CDC 86-3153 | O125:HNM | US CDC>USDA | Pos | Pos | Present | Absent | Present |
| DD13435 | CDC 88-3001 | O165:H25 | US CDC>USDA | Pos | Pos | Absent | Present | Present |
| DD13439 | PHAC 03-2682 | O5:HNM | PHAC>USDA | Pos | Pos | Present | Absent | Present |
| DD13440 | PHAC 05-0376 | O55:H7 | PHAC>USDA | Pos | Pos | Present | Absent | Present |
| DD13448 | SJ87 | O63:HNM | US CDC>USDA | Pos | Pos | Present | Absent | Present |
| TD8136 | | O157:H7 | Cattle | Pos | Pos | Present | Present | Present |
| DD13459 | FDA BB2 | O55:H7 | US FDA>USDA | Pos | Pos | Present | Absent | Present |
| DD13461 | FDA DD4 | O177:H25 | US FDA>USDA | Pos | Pos | Absent | Present | Present |
| DD13462 | FDA EE5 | O111:H8 | US FDA>USDA | Pos | Pos | Present | Present | Present |
| DD13464 | GG7 | O103:H2 | US FDA>USDA | Pos | Pos | Present | Absent | Present |
| DD13465 | HH8 | O26:H11 | US FDA>USDA | Pos | Pos | Present | Absent | Present |
| DD13368 | SJ18 | O121:H19 | US CDC>USDA | Pos | Pos | Present | Present | Present |
| MA6 | - | O157:H7 (rough) | FDA | Pos | Pos | Present | Present | Present |
| 05-6545 | - | O45:H2 | US CDC>USDA | Pos | Pos | Present | Absent | Present |
| BCL 17 | - | O5:N | MSU>USDA | Pos | Pos | Present | Absent | Present |
| DD640 | ATCC 43889 | O157:H7 | ATCC | Pos | Pos | Absent | Present | Present |
| DD641 | ATCC 43890 | O157:H7 | ATCC | Pos | Pos | Present | Absent | Present |
| DD642 | ATCC 43895 | O157:H7 | ATCC | Pos | Pos | Present | Present | Present |
| DD914 | ATCC 43894 | O157:H7 | ATCC | Pos | Pos | Present | Present | Present |
| DD916 | ATCC 35150 | O157:H7 | ATCC | Pos | Pos | Present | Present | Present |
| DD8297 | C984 | O157:H7 | Korea | Pos | Pos | Present | Unknown | Present |
| DD8298 | 86-24 | O157:H7 | Human Clinical | Pos | Pos | Absent | Present | Present |

HC = Health Canada , US CDC = United States Centers for Disease Control and Prevention
PHAC = Public Health Agency of Canada, USDA = United States Department of Agriculture,
US FDA = United States Food and Drug Administration

**Table 5. Inclusivity Results for STEC Panel 1 (*E. coli* O26, O111, O121) (1)**

| Strain ID | *E. coli* serotype | Source | BAX result | Strain ID | *E. coli* serotype | Source | BAX result |
|---|---|---|---|---|---|---|---|
| DD1720 | O26 | Unknown | POS O26 | DD1858 | O111 | Unknown | POS O111 |
| DD1807 | O26 | USDA | POS O26 | DD1927 | O111 | Unknown | POS O111 |
| DD1831 | O26 | USDA | POS O26 | R70 | O111 | Human Clinical | POS O111 |
| DD1913 | O26 | USDA | POS O26 | R71 | O111 | Human Clinical | POS O111 |
| DD5902 | O26 | Unknown | POS O26 | R72 | O111 | Human Clinical | POS O111 |
| DD5903 | O26 | Unknown | POS O26 | DD13362 | O121 | USDA | POS O121 |
| DD5904 | O26 | Unknown | POS O26 | DD13363 | O121 | USDA | POS O121 |
| DD5905 | O26 | Unknown | POS O26 | DD13364 | O121 | USDA | POS O121 |
| DD9704 | O26 | Unknown | POS O26 | DD13365 | O121 | USDA | POS O121 |
| DD9705 | O26 | Unknown | POS O26 | DD13366 | O121 | USDA | POS O121 |
| DD9706 | O26 | Unknown | POS O26 | DD13367 | O121 | USDA | POS O121 |
| DD9707 | O26 | Unknown | POS O26 | DD13368 | O121 | USDA | POS O121 |
| R144 | O26 | USDA MDP | POS O26 | DD13370 | O121 | USDA | POS O121 |
| R58 | O26 | USDA MDP | POS O26 | DD2440 | O121 | Unknown | POS O121 |
| R59 | O26 | USDA MDP | POS O26 | DD2460 | O121 | Unknown | POS O121 |
| R60 | O26 | USDA MDP | POS O26 | R184 | O121 | USDA | POS O121 |
| DD133400 | O111 | Unknown | POS O111 | R185 | O121 | USDA | POS O121 |
| DD133401 | O111 | Unknown | POS O111 | R186 | O121 | USDA | POS O121 |
| DD133402 | O111 | Unknown | POS O111 | R187 | O121 | USDA | POS O121 |
| DD133403 | O111 | Unknown | POS O111 | R188 | O121 | USDA | POS O121 |
| DD1729 | O111 | Unknown | POS O111 | R75 | O121 | USDA | POS O121 |
| DD1808 | O111 | Unknown | POS O111 | R76 | O121 | USDA | POS O121 |
| DD1809 | O111 | Unknown | POS O111 | R84 | O121 | USDA | POS O121 |

**Table 6. Inclusivity Results for STEC Panel 2 (*E. coli* O45, O103, O145) (1)**

| Strain ID | *E. coli* serotype | Source | BAX result | Strain ID | *E. coli* serotype | Source | BAX result |
|---|---|---|---|---|---|---|---|
| DD13349 | O45 | USDA | POS O45 | DD13388 | O103 | University of Washington | POS O103 |
| DD13350 | O45 | USDA | POS O45 | DD13389 | O103 | University of Washington | POS O103 |
| DD13351 | O45 | USDA | POS O45 | DD2521 | O103 | Unknown | POS O103 |
| DD13352 | O45 | USDA | POS O45 | DD2530 | O103 | Unknown | POS O103 |
| DD13353 | O45 | USDA | POS O45 | R163 | O103 | USDA | POS O103 |
| DD13354 | O45 | USDA | POS O45 | R164 | O103 | USDA | POS O103 |
| DD13355 | O45 | USDA | POS O45 | R165 | O103 | USDA | POS O103 |
| DD13358 | O45 | USDA | POS O45 | R166 | O103 | USDA | POS O103 |
| DD13390 | O145 | USDA | POS O45 | R167 | O103 | USDA | POS O103 |
| DD13361 | O45 | USDA | POS O45 | R168 | O103 | USDA | POS O103 |
| DD2450 | O45 | Unknown | POS O45 | R66 | O103 | Human Clinical | POS O103 |
| R62 | O45 | Human Clinical | POS O45 | R67 | O103 | Human Clinical | POS O103 |
| R63 | O45 | Human Clinical | POS O45 | R68 | O103 | Human Clinical | POS O103 |
| R64 | O45 | Human Clinical | POS O45 | DD13391 | O145 | USDA | POS O145 |
| DD13373 | O103 | USDA | POS O103 | DD13392 | O145 | USDA | POS O145 |
| DD13374 | O103 | USDA | POS O103 | DD13393 | O145 | USDA | POS O145 |
| DD13375 | O103 | USDA | POS O103 | DD13394 | O145 | USDA | POS O145 |
| DD13376 | O103 | USDA | POS O103 | DD13395 | O145 | USDA | POS O145 |
| DD13377 | O103 | USDA | POS O103 | DD13397 | O145 | USDA | POS O145 |
| DD13378 | O103 | USDA | POS O103 | DD13398 | O145 | USDA | POS O145 |
| DD13379 | O103 | USDA | POS O103 | DD2439 | O145 | Unknown | POS O145 |
| DD13380 | O103 | USDA | POS O103 | DD2483 | O145 | Unknown | POS O145 |
| DD13381 | O103 | USDA | POS O103 | DD2526 | O145 | Unknown | POS O145 |
| DD13382 | O103 | USDA | POS O103 | R198 | O145 | USDA | POS O145 |
| DD13383 | O103 | USDA | POS O103 | R77 | O145 | Human Clinical | POS O145 |
| DD13384 | O103 | USDA | POS O103 | R78 | O145 | Human Clinical | POS O145 |
| DD13386 | O103 | USDA | POS O103 | R79 | O145 | Human Clinical | POS O145 |
| DD13387 | O103 | USDA | POS O103 | R80 | O145 | Human Clinical | POS O145 |

**Table 7. Exclusivity Results for Non-*E. coli* Strains (1)**

| Strain name | Strain ID | STEC Screening result | STEC Panel 1 result | STEC Panel 2 result |
|---|---|---|---|---|
| *Citrobacter freundii* | DD2558 | NEG | NEG | NEG |
| *Citrobacter freundii* | DD383 | NEG | NEG | NEG |
| *Enterobacter amnigenus* | DD13186 | NEG | NEG | NEG |
| *Enterobacter amnigenus* | DD13187 | NEG | NEG | NEG |
| *Enterobacter asburiae* | DD13161 | NEG | NEG | NEG |
| *Enterobacter cloacae* | DD13135 | NEG | NEG | NEG |
| *Enterobacter hormaechei* | DD13162 | NEG | NEG | NEG |
| *Enterobacter sakazakii* | DD13094 | NEG | NEG | NEG |
| *Enterobacter sakazakii* | DD13099 | NEG | NEG | NEG |
| *Enterobacter sakazakii* | DD13134 | NEG | NEG | NEG |
| *Enterobacter turicensis* | DD13163 | NEG | NEG | NEG |
| *Escherichia hermanii* | DD13151 | NEG | NEG | NEG |
| *Hafnia alvei* | DD5588 | NEG | NEG | NEG |
| *Klebsiella oxytoca* | DD658 | NEG | NEG | NEG |
| *Klebsiella ozaenae* | DD657 | NEG | NEG | NEG |
| *Klebsiella pneumoniae* | DD373 | NEG | NEG | NEG |
| *Morganella morganii* | DD13142 | NEG | NEG | NEG |
| *Morganella morganii* | DD3064 | NEG | NEG | NEG |
| *Listeria monocytogenes* | DD1309 | NEG | NEG | NEG |
| *Bacillus subtilis* | DD1939 | NEG | NEG | NEG |
| *Enterococcus faecalis* | DD2425 | NEG | NEG | NEG |
| *Carnobacterium divergens* | DD2539 | NEG | NEG | NEG |
| *Citrobacter diversus* | DD2561 | NEG | NEG | NEG |
| *Pantoea agglomerans* | DD2599 | NEG | NEG | NEG |
| *Vibrio vulnificus* | DD2633 | NEG | NEG | NEG |
| *Cronobacter sakazaki* | DD2847 | NEG | NEG | NEG |
| *Lactococcus lactis* | DD3590 | NEG | NEG | NEG |
| *Stapylococus epidermis* | DD3624 | NEG | NEG | NEG |
| *Streptococcus equi* | DD3998 | NEG | NEG | NEG |
| *Leuconostoc mesenteroides* | DD4001 | NEG | NEG | NEG |
| *Carnobacterium gallinarum* | DD4063 | NEG | NEG | NEG |
| *Pediococcus damnosus* | DD4303 | NEG | NEG | NEG |
| *Shigella sonnei* | DD6832 | NEG | NEG | NEG |
| *Yersinia entercolitica* | DD7120 | NEG | NEG | NEG |
| *Edwardsiella tarda* | DD13139 | NEG | NEG | NEG |
| *Kluyvera georgiana* | DD13261 | NEG | NEG | NEG |
| *Yersinia enterocolitica* | DD7120 | NEG | NEG | NEG |
| *Burkholderia cepacia* | DD11946 | NEG | NEG | NEG |
| *Xanthomonas maltophilia* | DD6263 | NEG | NEG | NEG |
| *Providencia alcalofaciens* | DD960 | NEG | NEG | NEG |
| *Shigella boydii* | DD1081 | NEG | NEG | NEG |
| *Shigell flexneri* | DD1083 | NEG | NEG | NEG |
| *Brocothrix thermosphacta* | DD666 | NEG | NEG | NEG |
| *Hafnia alvei* | DD2389 | NEG | NEG | NEG |

**Table 8. Exclusivity Results for Non-Target *E. coli* Strains (1)**

| *E. coli* serogroup | Strain ID | stx/eae presence | STEC Screening result | STEC Panel 1 result | STEC Panel 2 result |
|---|---|---|---|---|---|
| O1 | DD2434 | No/No | Neg | Neg | Neg |
| O104 | DD13427 | No/No | Neg | Neg | Neg |
| O113 | DD13437 | No/No | Neg | Neg | Neg |
| O113 | DD13450 | No/No | Neg | Neg | Neg |
| O113 | DD13451 | No/No | Neg | Neg | Neg |
| O113 | DD13452 | No/No | Neg | Neg | Neg |
| O113 | DD13463 | No/No | Neg | Neg | Neg |
| O113 | DD2445 | No/No | Neg | Neg | Neg |
| O114 | DD1721 | No/No | Neg | Neg | Neg |
| O115 | DD1770 | No/No | Neg | Neg | Neg |
| O117 | DD13428 | No/No | Neg | Neg | Neg |
| O117 | DD2441 | No/No | Neg | Neg | Neg |
| O118 | DD2438 | No/No | Neg | Neg | Neg |
| O119 | DD13429 | No/No | Neg | Neg | Neg |
| O125 | DD1836 | No/No | Neg | Neg | Neg |
| O126 | DD13431 | No/No | Neg | Neg | Neg |
| O126 | DD1861 | No/No | Neg | Neg | Neg |
| O127 | DD1835 | No/No | Neg | Neg | Neg |
| O128 | DD13432 | No/No | Neg | Neg | Neg |
| O128 | DD13445 | No/No | Neg | Neg | Neg |
| O128 | DD13446 | No/No | Neg | Neg | Neg |
| O128 | DD13460 | No/No | Neg | Neg | Neg |
| O128 | DD1718 | No/No | Neg | Neg | Neg |
| O137 | DD13433 | No/No | Neg | Neg | Neg |
| O139 | DD1769 | No/No | Neg | Neg | Neg |
| O143 | DD1732 | No/No | Neg | Neg | Neg |
| O146 | DD13434 | No/No | Neg | Neg | Neg |
| O152 | DD1889 | No/No | Neg | Neg | Neg |

**DISCUSSION OF THE MODIFICATION STUDY APPROVED DECEMBER 2017 (4)**

The results of the BAX STEC Suite were compared to the results of the reference culture methods using probability of detection (POD) and difference in probability of detection (dPOD), according to the AOAC Micro Guidelines Appendix J. For flour samples enriched using the BAX System method, the real-time PCR assays (STEC Screening and Panel 1) detected *stx*, *eae* and the O121 serogroup for 8/20 low spiked samples and 5/5 high spiked samples at 24 h enrichment. All BAX System results were confirmed by culture (Table 1). The corresponding unpaired samples enriched using the FDA BAM reference method resulted in 7/20 culture positives for the low spike samples and 5/5 culture positives for the high spike samples. All uninoculated controls were negative. Inoculation levels were determined to be 0.43 CFU/test portion for the low level, and 4.3 CFU/portion for the high level, as determined using the BAM Ch. 4A method with the Least Cost Formulations (LCF) MPN calculator (2). At each inoculation level, the BAX STEC Suite method and the reference method demonstrated no significant statistical difference as indicated by POD analysis (the 95% confidence interval of the dPOD included 0 in all cases) as indicated in Table 2.

For raw ground beef samples enriched using the BAX System method, the real-time PCR assays detected *stx*, *eae* and the O126 serogroup for 11/20 low spiked samples and 5/5 high spiked samples at both 10 and 24 h enrichment times. All BAX® System results were confirmed by culture. The corresponding unpaired samples enriched using the USDA-FSIS culture reference method resulted in 8/20 culture positives for the low spike samples and 5/5 culture positives for the high spike samples. All uninoculated controls were negative. In addition, the BAX STEC suite was run on the reference method enriched test portions (mTSB), with results of 8/20 for the low spiked samples and 5/5 for the high spiked samples at both 10 and 24 h, matching the reference method results (see Tables 1 and 2). Inoculation levels were determined to be 0.51 CFU/test portion for the low level, and 3.36 CFU/portion for the high level, as determined using the USDA-FSIS MLG 5B.05 method with the LCF MPN calculator. At each inoculation level, the BAX STEC Suite method and the reference method, regardless of enrichment, demonstrated no significant statistical difference as indicated by POD analysis.

The results of these statistical analyses demonstrate no significant difference in the presumptive results compared to the confirmed results for flour and ground beef using either enrichment (BPW or mTSB) (Tables 1 and 2). While the flour and ground beef matrixes (enriched in BPW) produced more positives with the BAX System method than with the reference methods (Table 2), the differences were not statistically significant.

**Table 1. BAX System STEC Presumptive vs. Confirmed Results for 25 g of All-Purpose Flour and Ground Beef (4)**

| Sample Type | Strain | MPN[a]/test portion | N[b] | BAX Presumptive | | | BAX Confirmed | | | dPOD$_{CP}$[f] | 95% CI[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | X[c] | POD$_{CP}$[d] | 95% CI | X | POD$_{CC}$[e] | 95% CI | | |
| Flour (25 g) | STEC O121 DD13363 | 0 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (-0.45, 0.45) |
| | | 0.43 | 20 | 8 | 0.4 | (0.22, 0.61) | 8 | 0.4 | (0.22, 0.61) | 0 | (-0.28, 0.28) |
| | | 4.3 | 5 | 5 | 1 | (0.57, 1.00) | 5 | 1 | (0.57, 1.00) | 0 | (-0.43, 0.43) |
| Ground Beef (25 g) in BPW[h] | STEC O26 MSU TW00971 | 0 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (-0.43, 0.43) |
| | | 0.51 | 20 | 11 | 0.55 | (0.34, 0.74) | 11 | 0.55 | (0.34, 0.74) | 0 | (-0.28, 0.28) |
| | | 3.36 | 5 | 5 | 1 | (0.57, 1.00) | 5 | 1 | (0.57, 1.00) | 0 | (-0.43, 0.43) |
| Ground Beef (25 g) in mTSB[h] | STEC O26 MSU TW00971 | 0 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (-0.43, 0.43) |
| | | 0.51 | 20 | 8 | 0.4 | (0.22, 0.61) | 8 | 0.4 | (0.22, 0.61) | 0 | (-0.28, 0.28) |
| | | 3.36 | 5 | 5 | 1 | (0.57, 1.00) | 5 | 1 | (0.57, 1.00) | 0 | (-0.43, 0.43) |

[a]MPN/test portion = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator.
[b]N = Number of test portions.
[c]X = Number of positive test portions.
[d]POD$_{CP}$ = BAX® method presumptive positive results divided by the total number of test portions.
[e]POD$_{CC}$ = BAX® method confirmed positive results divided by the total number of test portions.
[f]dPOD$_{CP}$ = Difference between the BAX® method presumptive result and BAX® method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Portions were tested by the BAX STEC Suite System at 10 and 24 h with no difference in results.

**Table 2. BAX System method vs. the Reference method for the Detection of non-O157 STEC in 25g Flour and Ground Beef**

| Sample Type | Strain | MPN[a]/test portion | N[b] | BAX Method | | | Reference Method | | | dPOD$_{C}$[f] | 95% CI[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | X[c] | POD$_{C}$[d] | 95% CI | X | POD$_{R}$[e] | 95% CI | | |
| Flour (25 g) | STEC O121 DD13363 | 0 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (-0.45, 0.45) |
| | | 0.43 | 20 | 8 | 0.4 | (0.22, 0.61) | 7 | 0.35 | (0.18, 0.57) | 0.05 | (-0.23, 0.32) |
| | | 4.3 | 5 | 5 | 1 | (0.57, 1.00) | 5 | 1 | (0.57, 1.00) | 0 | (-0.43, 0.43) |
| Fresh Raw Ground Beef (25 g) BPW[h] | STEC O26 MSU TW00971 | 0 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (-0.43, 0.43) |
| | | 0.51 | 20 | 11 | 0.55 | (0.34, 0.74) | 8 | 0.4 | (0.22, 0.61) | 0.15 | (-0.15, 0.41) |
| | | 3.36 | 5 | 5 | 1 | (0.57, 1.00) | 5 | 1 | (0.57, 1.00) | 0 | (-0.43, 0.43) |
| Fresh Raw Ground Beef (25 g) mTSB[h] | STEC O26 MSU TW00971 | 0 | 5 | 0 | 0 | (0.00, 0.43) | 0 | 0 | (0.00, 0.43) | 0 | (-0.43, 0.43) |
| | | 0.51 | 20 | 8 | 0.4 | (0.22, 0.61) | 8 | 0.4 | (0.22, 0.61) | 0 | (-0.28, 0.28) |
| | | 3.36 | 5 | 5 | 1 | (0.57, 1.00) | 5 | 1 | (0.57, 1.00) | 0 | (-0.43, 0.43) |

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator.
[b]N/A = Not applicable.
[c]N = Number of test potions.
[d]X = Number of positive test portions.
[e]POD$_{C}$ = Confirmed candidate method positive outcomes divided by the total number of trials.
[f]POD$_{R}$ = Confirmed reference method positive outcomes divided by the total number of trials.
[g]dPOD$_{C}$ = Difference between the candidate method and reference method POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Portions were tested by the BAX STEC Suite System at 10 and 24 h with no difference in results.

**DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2022 (7)**

The BAX System Real-time PCR Assays successfully detected the target STEC species in dried cannabis flower and dried hemp flower at a 10 g sample size. Difference in POD analysis for the presumptive versus confirmed positives showed no statistically significant differences, with all ranges of the 95% confidence intervals containing the zero point. There was one unconfirmed BAX presumptive positive in the STEC screen PCR assay in dried hemp flower that could not be confirmed for any of the Top 7 STEC. It's possible that there was a non-Top 7 STEC in the material, but further investigation was not conducted by the independent laboratory. According to independent laboratory feedback, processing samples for these assays was very user friendly with a standard heat dependent lysis step and transfer into pre-aliquoted lyophilized pellets in PCR wells. Short run times on the instrument helped improve throughput for processing samples.

The BAX Real-time PCR Assays for detecting STEC species allow users to obtain presumptive positive results after 24 h of incubation and 1–2 h of processing and assay run time for STEC analysis. Presumptive results are easily visualized, denoted by a plus or minus sign within the software.

**Table 1. Matrix study: BAX Real-time PCR Assays for STEC Suite and *E. coli* O157:H7 Exact (used together for Top 7 STEC results) presumptive vs. confirmed results in dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC) (7)**

| | MPN[a] / Test | | | Presumptive | | | Confirmed | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Matrix and Inoculum | Portion | N[b] | x[c] | $POD_{cp}$[d] | 95% CI | x | $POD_{cc}$[e] | 95% CI | $dPOD_{cp}$[f] | 95% CI[g] |
| Dried cannabis | NA[i] | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.47, 0.47) |
| flower 10 g (*E. coli* | 0.88 (0.40, 2.02) | 20 | 10 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0.00 | (-0.13, 0.13) |
| O157:H7 ATCC[h] | 2.96 (1.54, 9.78) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |
| 43895) | | | | | | | | | | |
| Dried hemp Flower | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.47, 0.47) |
| 10 g (*E. coli* O157:H7 | 1.48 (0.77, 3.74) | 20 | 14 | 0.70 | 0.48, 0.86 | 14 | 0.70 | 0.48, 0.86 | 0.00 | (-0.13, 0.13) |
| ATCC 43890) | 6.77 (3.95, 16.2) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]$POD_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]$POD_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]American Type Culture Collection, Manassas, VA.
[i]Not applicable.

**Table 3. Matrix study: BAX Real-time PCR Assays for STEC Suite presumptive vs. confirmed results in dried hemp flower (≤0.3% THC) (7)**

| Matrix and | MPN[a] / Test | | | Presumptive | | | Confirmed | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Inoculum | Portion | N[b] | x[c] | $POD_{cp}$[d] | 95% CI | x | $POD_{cc}$[e] | 95% CI | $dPOD_{cp}$[f] | 95% CI[g] |
| Dried hemp Flower | NA[i] | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.47, 0.47) |
| 10 g (*E. coli* O26 | 1.15 (0.61, 2.45) | 20 | 11 | 0.55 | 0.34, 0.74 | 10 | 0.50 | 0.30, 0.70 | 0.05 | (-0.11, 0.21) |
| CDC[h] 03-3014) | 2.96 (1.54, 9.78) | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]$POD_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]$POD_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Centers for Disease Control and Prevention, Atlanta, GA.
[i]Not applicable.

**DISCUSSION OF THE MODIFICATION STUDY APPROVED APRIL 2023 (10)**

The BAX System Real-time PCR Assays for STEC Suite successfully detected the target STEC species in beef trim sampling cloths at a 375 g test portion size. Although the initial study MP media data set showed 9 presumptive positives at 8 h but 10 presumptive positives at 10 and 24 h, subsequent repeat studies demonstrated the method's ability to detect all presumptive positives at 8, 10 and 24 h. All presumptive positives in these second sets of data also confirmed positive. The study data were unable to find a statistical difference between the BAX STEC method's presumptive and confirmed results, nor between the BAX STEC and the MLG 5C.03 reference method results with 95% confidence.

**Table 1. Matrix study: BAX Real-time PCR Assay for STEC Suite presumptive vs. confirmed results in beef trim (375 g) sampling cloths (10)**

| Matrix and Inoculum | cfu[a]/ Test Portion | N[b] | x[c] | Presumptive $POD_{cp}$[d] | Presumptive 95% CI | x | Confirmed $POD_{cc}$[e] | Confirmed 95% CI | $dPOD_{cp}$[f] | 95% CI[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 8 h, MP media | NA[i] | 5 | 0 | 0.00 | 0.00, 0.00 | 0 | 0.00 | 0.00, 0.00 | 0.00 | (-0.47, 0.47) |
| | 0.64 | 20 | 9 | 0.45 | 0.26, 0.66 | 9 | 0.45 | 0.26, 0.66 | 0.00 | (-0.13, 0.13) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 10 h, MP media | NA | 5 | 0 | 0.00 | 0.00, 0.00 | 0 | 0.00 | 0.00, 0.00 | 0.00 | (-0.47, 0.47) |
| | 0.64 | 20 | 9 | 0.45 | 0.26, 0.66 | 9 | 0.45 | 0.26, 0.66 | 0.00 | (-0.13, 0.13) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 24 h, MP media | NA | 5 | 0 | 0.00 | 0.00, 0.00 | 0 | 0.00 | 0.00, 0.00 | 0.00 | (-0.47, 0.47) |
| | 0.64 | 20 | 9 | 0.45 | 0.26, 0.66 | 9 | 0.45 | 0.26, 0.66 | 0.00 | (-0.13, 0.13) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |

[a]cfu/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]$POD_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]$POD_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Hygiena Culture Collection, New Castle, DE.
[i]Not applicable.

**Table 2. BAX Real-time PCR Assay for STEC Suite method vs. reference method results in beef trim (375 g) sampling cloths (10)**

| Matrix and Inoculum | cfu[a]/ Test Portion | N[b] | x[c] | BAX Method $POD_c$[d] | BAX Method 95% CI | x | Reference Method $POD_g$[e] | Reference Method 95% CI | $dPOD_c$[f] | 95% CI[g] |
|---|---|---|---|---|---|---|---|---|---|---|
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 8 h, MP media[i] | NA[j] | 5 | 0 | 0.00 | 0.00, 0.00 | 0 | 0.00 | 0.00, 0.00 | 0.00 | (-0.43, 0.43) |
| | 0.64 | 20 | 9 | 0.45 | 0.26, 0.66 | 13 | 0.65 | 0.43, 0.82 | -0.20 | (-0.46, 0.10) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.43, 0.43) |
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 10 h, MP media | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.43, 0.43) |
| | 0.64 | 20 | 9 | 0.45 | 0.26, 0.66 | 13 | 0.65 | 0.43, 0.82 | -0.20 | (-0.46, 0.10) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.43, 0.43) |
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 24 h, MP media | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.43, 0.43) |
| | 0.64 | 20 | 9 | 0.45 | 0.26, 0.66 | 13 | 0.65 | 0.43, 0.82 | -0.20 | (-0.46, 0.10) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.43, 0.43) |
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 8 h, mTSB+caa[k] | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.47, 0.47) |
| | 0.64 | 20 | 13 | 0.65 | 0.43, 0.82 | 13 | 0.65 | 0.43, 0.82 | 0.00 | (-0.13, 0.13) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |
| Beef trim Sampling cloth (*E. coli* O26:H11 DD 9703[h]) 24 h, mTSB+caa | NA | 5 | 0 | 0.00 | 0.00, 0.43 | 0 | 0.00 | 0.00, 0.43 | 0.00 | (-0.47, 0.47) |
| | 0.64 | 20 | 13 | 0.65 | 0.43, 0.82 | 13 | 0.65 | 0.43, 0.82 | 0.00 | (-0.13, 0.13) |
| | 4.76 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | (-0.47, 0.47) |

[a]cfu/portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.
[b]N = Number of test potions.
[c]X = Number of positive test portions.
[d]$POD_C$ = Confirmed candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f]$POD_R$ = Confirmed reference method positive outcomes divided by the total number of trials.
[e]$dPOD_C$ = Difference between the candidate method and reference method POD values.
[f]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Hygiena Culture Collection, New Castle, DE.
[i]Results calculated using unpaired POD statistical analysis.
[i]Not applicable.
[k]Results calculated using paired POD statistical analysis.

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