CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

091301

The AOAC Research Institute hereby certifies the test kit 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® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 05, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 05, 2019
Date

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KIT NAME(S)
DuPont™ BAX® System Real-Time PCR Assay Suite for STEC March 01, 2017, 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
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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

Matrices - raw beef trim (375g), raw ground beef (325 g, 375g), raw ground beef plus soy (325g)
December 2017: raw ground beef (25 g) and all-purpose flour (25 g)

Performance claims - Sensitivity equal to or better than the corresponding reference method.

REFERENCE METHODS
Least Cost Formulations, Ltd. , MPN Calculator-Version 1.6 (http://www.lcfltd.com/customer/LCFMPNCalculator.exe) (2)
U.S. Department of Agriculture, Food Safety and Inspection Service (2011) Microbiology Laboratory Guidebook (http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp) (3)
U.S. Food and Drug Administration (2017) *FDA Bacteriological Analytical Manual,* Chapter 4A, Diarrheagenic *Escherichia coli,* https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm (6)
U.S. Department of Agriculture, Food Safety and Inspection Service (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, https://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/MLG-5B.pdf?MOD=AJPERES (7)

ORIGINAL CERTIFICATION DATE
September 24, 2013

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2019

METHOD MODIFICATION RECORD
1. **March 2017 Level 1**
2. **December 2017 Level 1 Renewal Modification**
3. **December 2017 Level 2**
4. **January 2018 Level 1 Renewal Modification**
5. **May 2019 Level 1**
6. **December 2019 Level 1 Renewal Modification**

SUMMARY OF MODIFICATION
1. **Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company**
2. **Inserts, manuals, and labels updated to Hygiena**
3. **Matrix extension to add raw ground beef (25 g) and all-purpose flour (25 g)**
4. **Editorial changes to insert and labels to update Hygiena.**
5. **Editorial insert updates and corporate address**
6. **Editorial/clerical changes.**

Under this AOAC® *Performance Tested*℠ License Number, 091301 this method is distributed by:
NONE

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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.

In the lot-to-lot study, consistent performance was demonstrated across multiple production lots of each BAX® System real-time assay for STEC.

The stability study indicated a predicted stability of 5.2 years for the BAX® System real-time PCR assays for STEC, which supports the shelf life of 3 years assigned to the kits by the manufacturer.

Failure mode analysis performed in the robustness studies had to be conducted three times, with the last replicate conducted at multiple dilutions to attain some conditions that yielded fractional results. As the PCR sensitivity for the O type assays is near single target detection and the analytes are diluted from many log orders over the sensitivity of the test this is not unexpected. The final results demonstrated a high degree of flexibility in the test method when subjected to most deviations from the manufacturer's specifications. When the inactivation temperature was increased to 99°C, however, the performance of the test method was negatively affected, likely due to the fact that the cluster tube caps detached during heating at this temperature and a significant amount of volume was lost during the inactivation step. As a result, it is strongly recommended that heating block temperatures be checked using thermometers in the heating block before each use of the inactivation process for these assays to avoid the detrimental effects of over-heating samples. Training conducted by DuPont personnel will emphasize this parameter as a point of interest for end users.

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 (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 matrices (enriched in BPW) produced more positives with the BAX® System method than with the reference methods (Table 2), the differences were not statistically significant.

MODIFICATION DATA APPROVED DECEMBER 2017 (4)

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

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.

REFERENCES CITED

1. Varkey, Stephen, DeMarco, Daniel, Thompson, Leslie, Jensen Mark, Andaloro, Bridget, Fallon, Dawn, Rohrbeck Jeff, Hoelzer, Steve, Tadler, Monica, Kraynak, Julie, Davis, Eugene, Tice, George, and Wallace, Morgan., Evaluation of the DuPont™ BAX® System Real-Time PCR Assay Suite for STEC, AOAC® *Performance Tested*℠ certification number 091301.
2. Least Cost Formulations, Ltd. , MPN Calculator-Version 1.6 (http://www.lcfltd.com/customer/LCFMPNCalculator.exe)
3. U.S. Department of Agriculture, Food Safety and Inspection Service (2011) Microbiology Laboratory Guidebook (http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp)
4. Corrigan, N., Weller, J., Wallace, M., Post, L., Bastin, B.., and Bird, P., Evaluation of BAX® System Real-Time PCR Assay Suite for STEC – Level 2 Matrix Extension for 25 g Raw ground beef and All-purpose Flour, AOAC® *Performance Tested*℠ certification number 091301. Approved December 2017.
5. U.S. Food and Drug Administration (2017) *FDA Bacteriological Analytical Manual,* Chapter 4A, Diarrheagenic *Escherichia coli,* https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm
6. U.S. Department of Agriculture, Food Safety and Inspection Service (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, https://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/MLG-5B.pdf?MOD=AJPERES