CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

050501

The AOAC Research Institute hereby certifies the method known as:

BAX® System PCR Assay for *E. coli* O157:H7 MP
BAX® System X5 PCR Assay for *E. coli* O157:H7

manufactured by

Hygiena
2 Boulden Circle
New Castle, DE 19720 USA

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. 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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METHOD NAMES
BAX® System PCR Assay for *E. coli* O157:H7 MP
BAX® System X5 PCR Assay for *E. coli* O157:H7
Formerly DuPont™ BAX® System PCR Assay for *E. coli* O157:H7 MP and the BAX® System MP Media

CATALOG NUMBERS
BAX® System Assay KIT2004 (D12404903), Media MED2003 (D12404925), BAX® System X5 Assay KIT2022 (D15407214)

INDEPENDENT LABORATORY
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APPLICABILITY OF METHOD
Target organism – *E. coli* O157:H7.

Matrixes – Raw ground beef (25 g, 65 g), beef trim (65 g, 325 g, 375 g), spinach (25 g), lettuce (25 g), red leaf lettuce (200 g, 375 g)

Performance claims – Method performed equivalent to the appropriate reference culture method depending on matrix type.

REFERENCE METHODS
***Microbiology Laboratory Guidebook* (October 25, 2002) MLG 5.03, USDA Food Safety and Inspection Service, Office of Public Health and Science (2)**
FDA Bacteriological Analytical Manual Online (February 2011) Chapter 4a, Diarrheagenic *Escherichia coli* (6)
USDA FSIS Microbiology Laboratory Guidebook (January 2015) MLG 5.09, Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges, USDA Food Safety and Inspection Service, Office of Public Health and Science (7)

ORIGINAL CERTIFICATION DATE
June 01, 2005

CERTIFICATION RENEWAL RECORD
Renewed through December 2025.

METHOD MODIFICATION RECORD

1. **June 2009 Level 2**
2. **July 2013 Level 2**
3. **May 2016 Level 2**
4. **March 2017 Level 1**
5. **January 2018 Level 1**
6. **May 2019 Level 1**
7. **December 2021 Level 1**
8. **December 2023 Level 1**
9. **December 2024 Level 1**

SUMMARY OF MODIFICATION

1. **Matrix extension to include Spinach and lettuce.**
2. **Addition of Thermal Block for automated sample lysis.**
3. **Addition of X5 Instrument .**
4. **Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company.**
5. **Editorial changes to update insert, labels, etc.**
6. **Editorial updates to insert and corporate address.**
7. **Editorial changes.**
8. **Editorial changes.**
9. **Editorial changes.**

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NONE

PRINCIPLE OF THE METHOD (1)

PCR Amplification - The BAX® system E. coli O157:H7 MP assay 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 the *E. coli* O157:H7 serotype, thus providing a highly reliable indicator that the organism is present. 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 detection - The automated BAX system uses fluorescent detection to analyze PCR product. Each PCR tablet contains a fluorescent dye, which binds with double-stranded DNA and emits a signal in response to excitation light. During the detection phase, the temperature of the samples is slowly increased to denature the DNA. This releases the dye and causes a drop in emission signal. The BAX system measures the denaturation temperature and the magnitude of fluorescent signal change. An analysis by the BAX System software algorithm then evaluates that data to determine a positive or negative.

DISCUSSION OF THE VALIDATION STUDY (1)

For both ground beef and beef trim, the USDA-FSIS culture method using single 25 g or 65 g samples demonstrated false negative rate of 20-100%, possibly due to background flora naturally found in these food matrixes. In comparison, the BAX system demonstrated 100% specificity. Sensitivity for 65-g samples of both food types was 100% at 24 hours. The 25 g ground beef samples demonstrated 94% sensitivity at 22 hours.

Chi-square analysis indicates that the BAX system performed significantly better than the USDA-FSIS culture method at 7, 8, 22 and 24 hours in ground beef. The difference between methods in beef trim was not statistically significant.

Table 4 shows a comparison of the paired samples processed with two BAX system protocols, MP and MP Express. Results indicate consistent performance between the protocols.

Table 1. Internal Study of Twenty Spiked and Five Unspiked Ground Beef (25 g) Samples Tested with BAX System MP and MP Express Methods and Twenty Spiked and Five Unspiked Ground Beef (25 g) Samples tested with USDA Methods (0.71 MPN[a]/25g: direct plate 2.2 cfu/25g) (1)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed[b]	Sensitivity[c] %	False Neg [d] %	Presump. Pos /Unspiked	Specificity[e] %	False Pos[f] %	Chi-square[g]
7 hr	BAX MP	20	9/16	56	44	0/5	100	0	5.1*
	BAX MP-Express	20	8/16	50	50	0/5	100	0	4.2*
8 hr	BAX MP	20	13/16	81	19	0/5	100	0	9.1*
	BAX MP-Express	20	13/16	81	19	0/5	100	0	9.1*
22 hr	BAX MP	20	15/16	94	6	0/5	100	0	11.1*
	BAX MP-Express	20	15/16	94	6	0/5	100	0	11.1*
24 hr	USDA - FSIS	20	2/10	20	80	0/5	100	0	--

[a] Most probable number of colony forming units per test portion.

[b] Presump Pos: Positive either by BAX System assay for BAX enrichments or by lateral flow device for FSIS enrichments. Confirmed: At least one confirmed E. coli O157:H7 isolate was obtained by culture.

[c] Sensitivity rate: 100 times the number of presumptive positive results divided by total true positive results confirmed from enrichment of spiked samples.

[d] False negative rate: 100 minus sensitivity rate.

[e] Specificity rate: 100 times the number of assay-negative results divided by total number of true negative results, including unspiked samples.

[f] False positive rate: 100 minus specificity rate

[g] Chi-square: McNemar formula $(|a-b|-1)^2/(a+b)$, where a = results that were positive by BAX and negative by reference method, and b= results that were negative by BAX and positive by reference method.

Table 2. Internal Study of Twenty Spiked and Five Unspiked Ground Beef (65 g) Samples Tested with BAX System MP and MP Express Methods and Twenty Spiked and Five Unspiked Ground Beef (65 g) Samples Tested with USDA Methods (0.23 MPN/65g: direct plate 2.1cfu/65g) (1)

Enrichment Time	Method	Total Spiked	Presump.Pos /Confirmed	Sensitivity %	False Neg %	Presump. Pos /Unspiked	Specificity %	False Pos %	Chi-Square
8 hr	BAX MP	20	14/14	100	0	0/5	100	0	12*
	BAX MP-Express	20	14/14	100	0	0/5	100	0	12*
24 hr	BAX MP	20	14/14	100	0	0/5	100	0	12*
	BAX MP-Express	20	14/14	100	0	0/5	100	0	12*
	USDA - FSIS	20	0/1	0	100	0/5	100	0	--

See Table 1 for descriptions of methods of analysis used to calculate Sensitivity%, False Negative %, Specificity%, False Positive % and Chi-Square. Chi-square value > 3.84 indicates significance at P < 0.05.

Table 3. Internal Study of Twenty Spiked and Five Unspiked Beef Trim (65 g) Samples Tested with BAX System MP and MP Express Methods and Twenty Spiked and Five Unspiked Beef Trim (65 g) Samples tested with USDA Methods (2.8 MPN/65g: direct plate 1.7 cfu/65g) (1)

Enrichment Time	Method	Total Spiked	Presump.Pos /Confirmed	Sensitivity %	False Neg %	Presump. Pos /Unspiked	Specificity %	False Pos %	Chi-Square
8 hr	BAX MP	20	19/20	95	5	0/5	100	0	2.3
	BAX MP-Express	20	19/20	95	5	0/5	100	0	2.3
24 hr	BAX MP	20	20/20	100	0	0/5	100	0	3.2
	BAX MP-Express	20	20/20	100	0	0/5	100	0	3.2
	USDA - FSIS	20	15/20	75	25	0/5	100	0	--

See Table 1 for descriptions of methods of analysis used to calculate Sensitivity%, False Negative %, Specificity%, False Positive % and Chi-Square. Chi-square value > 3.84 indicates significance at P < 0.05.

Table 4. Comparison of MP vs. MP Express Protocols - Internal + External Study Data (1)

n = 250	MP Positive	MP Negative	Total
MP Express Positive	145	0	145
MP Express Negative	1	104	105
Total	146	104	250

Table 8. BAX system exclusivity (*E. coli* O157:H7 MP assay and BHI) (1)

Strain DD #	Source	Strain	BAX MP	Strain DD #	Source	Strain	BAX MP
2434	PSU Reference Lab	E.coli O1:H7	Neg	1810	PSU Reference Lab	*E. coli* O28:H16	Neg
2520	PSU Reference Lab	*E.coli* O113:H7	Neg	1811	PSU Reference Lab	*E. coli* O127:H40	Neg
2491	PSU Reference Lab	*E.coli* O2:H7	Neg	1812	PSU Reference Lab	*E. coli* O127:H10	Neg
1908	PSU Reference Lab	E.coli *O25:H7*	Neg	1814	PSU Reference Lab	*E. coli* O6:H-	Neg
2443	PSU Reference Lab	*E.coli* O157 :H19	Neg	1817	PSU Reference Lab	*E. coli* O29:H-	Neg
5883	Unknown	*E.coli* O55 :H10	Neg	1818	PSU Reference Lab	*E. coli* O136:H8	Neg
655	ATCC/Calf Intestine	*E.coli* O101:K-:K99	Neg	1819	PSU Reference Lab	*E. coli* O18:HNM	Neg
656	ATCC/Calf Intestine	*E.coli* O101:K30:K99	Neg	1820	PSU Reference Lab	*E. coli* O86:H8	Neg
1716	PSU Reference Lab	*E.coli* O158:H23	Neg	1821	PSU Reference Lab	*E. coli* O55:H-	Neg
1718	PSU Reference Lab	*E.coli* O128:H2	Neg	1822	PSU Reference Lab	*E. coli* O28:H8,4,3	Neg
1719	PSU Reference Lab	*E.coli* O28:HNM	Neg	1824	PSU Reference Lab	*E. coli* O125:HNM	Neg
1720	PSU Reference Lab	*E.coli* O26:HNM	Neg	1825	PSU Reference Lab	*E. coli* O25:H8	Neg
1721	PSU Reference Lab	*E.coli* O114:H32	Neg	1827	PSU Reference Lab	*E. coli* O20:HNM	Neg
1722	PSU Reference Lab	*E.coli* O127: HNM	Neg	1831	PSU Reference Lab	*E. coli* O26:H11	Neg
1723	PSU Reference Lab	*E.coli* O119:H27	Neg	1833	PSU Reference Lab	*E. coli* O55:H9	Neg

1724	PSU Reference Lab	*E.coli* O18:H14	Neg	1834
1725	PSU Reference Lab	*E.coli* O125:H19	Neg	1835
1726	PSU Reference Lab	*E.coli* O126:H2	Neg	1836
1727	PSU Reference Lab	*E.coli* O44:H18	Neg	1839
1728	PSU Reference Lab	*E.coli* O55:HNM	Neg	1841
1730	PSU Reference Lab	*E.coli* O86:H25	Neg	1842
1731	PSU Reference Lab	*E.coli* O167:H5	Neg	1844
1732	PSU Reference Lab	*E.coli* O143:HNM	Neg	1847
1733	PSU Reference Lab	*E.coli* O142:H6	Neg	1848
1734	PSU Reference Lab	*E.coli* O124:H30	Neg	1849
1756	PSU Reference Lab	*E.coli* O25:H12	Neg	1852
1757	PSU Reference Lab	*E.coli* O152:HNM	Neg	1853
1758	Unknown	*E. coli* O63:HNM	Neg	1854
1759	PSU Reference Lab	*E.coli* O15:H4	Neg	1855
1760	PSU Reference Lab	*E.coli* O6:H1	Neg	1856
1761	PSU Reference Lab	*E.coli* O27:HNM	Neg	1857
1762	PSU Reference Lab	*E.coli* O164:HNM	Neg	1860
1764	PSU Reference Lab	*E.coli* O8:H4	Neg	1861
1766	PSU Reference Lab	*E.coli* O80:H26	Neg	1865
1767	PSU Reference Lab	*E.coli* O85:H1	Neg	1866
1768	PSU Reference Lab	*E.coli* O153:H7	Neg	1871
1769	PSU Reference Lab	*E.coli* O139:H1	Neg	1872
1770	PSU Reference Lab	*E.coli* O115:H18	Neg	1873
1771	PSU Reference Lab	*E.coli* O148:H28	Neg	1875
1772	PSU Reference Lab	*E.coli* O159:H20	Neg	1876
1796	PSU Reference Lab	*E.coli* O86: HNM	Neg	1877
1798	PSU Reference Lab	*E.coli* O28:HSM	Neg	1878
1799	PSU Reference Lab	*E.coli* O142:H-	Neg	1882
1800	PSU Reference Lab	*E.coli* O128:HNM	Neg	1883
1801	PSU Reference Lab	*E.coli* O142:HNM	Neg	1884
1802	PSU Reference Lab	*E.coli* O6:HNM	Neg	1889
1803	PSU Reference Lab	*E.coli* O25:H-	Neg	1893
1804	PSU Reference Lab	*E.coli* O124:H-	Neg	1894
1807	PSU Reference Lab Unknown	*E.coli* O26:H-	Neg	2477
1550	Unknown	*Salmonella abaetetuba*	Neg	706
2166	Unknown	*Salmonella abaetetuba*	Neg	846
2341	Unknown	*Salmonella mbandaka*	Neg	847
2992	Unknown	*Salmonella Lille*	Neg	849
1261	Duck	*Salmonella newport*	Neg	850
1777	Unknown	*Salmonella enterica*	Neg	2901
2274	Unknown	*Salmonella anatum*	Neg	3017
2614	Human feces	*Edwardsiella tarda*	Neg	3019
3982	Blood culture	*Pseudomonas aeruginosa*	Neg	3064
3998	Bovine mastitis	*Streptococcus equi*	Neg	6121
4160	Howler monkey	*Staphylococcus aureus*	Neg	6523
5588	Ground beef	*Hafnia alvei*	Neg	6719
7005	Unknown	*Salmonella dublin*	Neg	6832
7344	Human	*Lactobacillus acidophilus*	Neg	11348

DISCUSSION OF MODIFICATION APPROVED 2009 (3)

For spinach and iceberg lettuce, both the BAX System Classic and Q7 instruments demonstrated 100% sensitivity and 100% specificity from 8 to 24 hours. Chi-square analysis indicates that the BAX System performed significantly better than the FDA-BAM culture method at 8, 10 and 24 hours in spinach, and equivalent to the reference method at 8 and 22 hours in iceberg lettuce.

Table 1. Internal study of spiked and unspiked spinach (25 g) samples tested with BAX system method and FDA-BAM reference culture methods. (0.23 MPN[a]/25g: direct plate 1 cfu/25g) (3)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed[b]	Sensitivity[c] %	False Neg [d] %	Presump. Pos /Unspiked	Specificity[e] %	False Pos[f] %	Chi-square[g]
8 hr	BAX classic	20	13/13	100	0	0/5	100	0	4.8*
	BAX Q7	20	13/13	100	0	0/5	100	0	4.8*
10 hr	BAX classic	20	13/13	100	0	0/5	100	0	4.8*
	BAX Q7	20	13/13	100	0	0/5	100	0	4.8*
24 hr	BAX classic	20	13/13	100	0	0/5	100	0	4.8*
	BAX Q7	20	13/13	100	0	0/5	100	0	4.8*
FDA-BAM		20	6	--	--	0/5	--	--	--

[a] Most probable number of colony forming units per test portion.

[b] Presump Pos: Positive by BAX System assay for BAX enrichments. Confirmed: At least one confirmed E. coli O157:H7 isolate was obtained by culture.

[c] Sensitivity rate: 100 times the number of presumptive positive results divided by total true positive results confirmed from enrichment of spiked samples.

[d] False negative rate: 100 minus sensitivity rate.

[e] Specificity rate: 100 times the number of assay-negative results divided by total number of true negative results, including unspiked samples.

[f] False positive rate: 100 minus specificity rate

*[g] Chi-square: Mantel-Haenszel chi square. *Chi-square value > 3.84 indicates significance at P < 0.05.*

Table 2. Internal Study of spiked and unspiked iceberg lettuce (25 g) samples tested with BAX system method and FDA-BAM reference culture methods. (0.2 MPN[a]/25g: direct plate 0.2 cfu/25g) (3)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed[b]	Sensitivity[c] %	False Neg [d] %	Presump. Pos /Unspiked	Specificity[e] %	False Pos[f] %	Chi-square[g]
8 hr	BAX classic	20	7/7	100	0	0/5	100	0	0.1
	BAX Q7	20	7/7	100	0	0/5	100	0	0.1
22 hr	BAX classic	20	7/7	100	0	0/5	100	0	0.1
	BAX Q7	20	7/7	100	0	0/5	100	0	0.1
FDA-BAM		20	8	--	--	0/5	--	--	--

See Table 1 for descriptions of methods of analysis used to calculate Sensitivity%, False Negative %, Specificity%, False Positive % and Chi-Square.

** Chi-square value > 3.84 indicates significance at P < 0.05.*

DISCUSSION OF MODIFICATION APPROVED JULY 2013 (4)

The results of the method comparison between the digital DuPont™ Thermal Block and the analog heating/cooling blocks are provided in Table 3 below. For all sample types and BAX System assays evaluated, the results for samples processed with the DuPont Thermal Block and the original heating/cooling blocks demonstrated no significant statistical difference as indicated by POD analysis (the 95% confidence interval of the dPOD included 0 in all cases). For additional figures illustrating the target responses of the individual BAX System assays, see Appendix B.

All 544 samples inoculated with high levels of the target organism returned positive results with the BAX System using both sample preparation methods, and all 544 samples tested as unspiked negative controls returned negative results with the BAX System using both sample preparation methods with the exception of the non-inoculated poultry rinse samples that gave positive results *for Campylobacer jejuni*, while giving negative results for the target *C. coli* that was spiked into the test samples. For samples inoculated with low levels of target organism, the two preparation methods returned identical results for 530 of the 544 samples tested. The results for the 14 samples that returned different results between the two methods are summarized in Table 3. Because the low-spike samples were tested at levels near the limit of detection for the BAX System assays, some discrepancy between the two methods is expected based on factors such as the distribution of the target organism within the sample.

Analysis of target response in cases where a fractional response was not generated, while demonstrating significant differences from a statistical standpoint in some cases, were not indicative of any difference that would likely be significant in a practical sense. All average differences were less than 10% for melt curve based target peak height, or target peak area to target plus internal control peak areas (for the Yeast and Mold assay) and all average C_t differences were less than 1 for all real time assay.

Because the difference in results between the two methods demonstrated no significant statistical difference as indicated by the POD analysis, these differences are found to be acceptable in this study for demonstrating equivalency between the two methods.

Table 3. BAX System Results – DuPont Thermal Block vs. Analog Heating/Cooling Blocks (4)

BAX System Assay	Sample Type	Spike Level	Test Portions	Heating/Cooling Blocks			DuPont Thermal Block			$dPOD_{TB}$[d]	95% CI[e]
				X[a]	POD_{2B}[b]	95% CI[e]	X[a]	POD_{TB}[c]	95% CI[e]		
***E. coli* O157:H7 MP**	Ground beef	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	0.18, 0.18
		Low	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	0.18, 0.18
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.19, 0.19
	Beef trim	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	0.18, 0.18
		Low	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	0.18, 0.18
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.19, 0.19

Table 3. BAX System Results – DuPont Thermal Block vs. Analog Heating/Cooling Blocks (con't)

BAX System Assay	Sample Type	Spike Level	Test Portions	Heating/Cooling Blocks			DuPont Thermal Block			$dPOD_{TB}$[d]	95% CI[e]
				X[a]	POD_{2B}[b]	95% CI[e]	X[a]	POD_{TB}[c]	95% CI[e]		
***E. coli* O157:H7 MP (con't)**	Spinach	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Low	17	14	0.82	0.29, 0.94	14	0.8235	0.29, 0.94	0	-0.26, 0.26
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.19, 0.19
		High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18

DISCUSSION OF MODIFICATION APPROVED MAY 2016 (5)

Studies conducted on the two categories of matrixes previously validated (meat and leafy greens) demonstrated equivalent performance of the BAX MP test kit to culture when run using the BAX X5 instrument. Since the reference methods for both of these matrix types had changed considerably since the previous validation studies, both in enrichment conditions and in sample size, the latest USDA and FDA culture methods were compared to the BAX System method.

Of 45 target strains tested, all gave positive results with the BAX System method using the X5 instrument. Of the 46 strains used for exclusivity testing, none were positive.

For diluted *E. coli* O157:H7 in pure culture and in spinach or ground beef matrix, results were as expected based on previous work.

Table 1. Method Results POD Food Matrixes – BAX System X5 Presumptive Results Compared to Confirmed Results (5)

Matrix/Enrichment	Strain	MPN[a]/test portion	N[b]	BAX System X5 Presumptive			BAX System X5 Confirmed			POD_{CP}[f]	95% CI[g]
				x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI		
Beef Trim (375 g) BAX System X5 MP Media 10 h incubation	*E. coli* O157:H7 DD1450	2.0 (1.1, 3.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.32 (0.16, 0.62)	20	5	0.25	(0.11, 0.47)	6	0.30	(0.15, 0.52)	-0.05	(-0.31, 0.22)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Beef Trim (375 g) BAX System X5 MP Media 24 h incubation	*E. coli* O157:H7 DD1450	2.0 (1.1, 3.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.32 (0.16, 0.62)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Beef Trim (325 g) BAX System X5 mTSB Media 20 h incubation	*E. coli* O157:H7 DD1450	2.0 (1.1, 3.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.32 (0.16, 0.62)	20	4	0.20	(0.08, 0.42)	4	0.20	(0.08, 0.42)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Red Leaf Lettuce (375 g) BAX System X5 MP Media 10 + 3 h	*E. coli* O157:H7 DD1450	18 (11, 30)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.8 (1.1, 3.0)	20	16	0.80	(0.53, 0.89)	15	0.30	(0.14, 0.52)	0	(-0.21, 0.30)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Red Leaf Lettuce (375 g) BAX System X5 MP Media 22 + 3 h	*E. coli* O157:H7 DD1450	18 (11, 30)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.8 (1.1, 3.0)	20	15	0.75	(0.53, 0.89)	15	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Red Leaf Lettuce (200 g) BAX System X5 BPWp Media, Batch 1	*E. coli* O157:H7 DD1450	18 (11, 30)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.8 (1.1, 3.0)	20	18	0.90	(0.70, 0.97)	18	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Red Leaf Lettuce (200 g) BAX System X5 BPWp Media, Batch 2	*E. coli* O157:H7 DD1450	7 (3.6, 134)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.7 (0.38, 1.3)	20	10	0.5	(0.3, 0.7)	10	0.5	(0.3, 0.7)	0	(-0.28, 0.28)
		Negative Control	5	4	0.8	(0.38, 0.96)	4	0.8	(0.38, 0.96)	0	(-0.45, 0.45)
Red Leaf Lettuce (200 g) BAX System X5 BPWp Media, Batch 3	*E. coli* O157:H7 DD1450	24 (13, 40)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		2.4 (1.3, 4)	20	18	0.90	(0.70, 0.97)	18	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

[a]Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator (7), 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.

Table 2. Method Results POD Food Matrixes – Candidate Method Compared to Reference Culture Method (USDA-FSIS MLG 5.09 for beef trim, and FDA BAM 4A for red leaf lettuce) (5)

Matrix/Enrichment	Strain	MPN[a]/test portion	N[b]	BAX System X5 Confirmed			Reference Method			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Beef Trim (375 g) BAX System X5 MP Media 10 h incubation	*E. coli* O157:H7 DD1450	2.0 (1.1, 3.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.32 (0.16, 0.62)	20	5	0.25	(0.11, 0.47)	4	0.20	(0.08, 0.42)	0.05	(-0.21, 0.30)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Beef Trim (375 g) BAX System X5 MP Media 24 h incubation	*E. coli* O157:H7 DD1450	2.0 (1.1, 3.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.32 (0.16, 0.62)	20	6	0.3	(0.15, 0.52)	4	0.20	(0.08, 0.42)	0.10	(-0.17, 0.35)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Beef Trim (325 g) BAX System X5 mTSB Media 20 h incubation	*E. coli* O157:H7 DD1450	2.0 (1.1, 3.9)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.32 (0.16, 0.62)	20	4	0.2	(0.08, 0.42)	4	0.20	(0.08, 0.42)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Red Leaf Lettuce (375 g) BAX System X5 MP Media 10 & 22 h	*E. coli* O157:H7 DD1450	18 (11, 30)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		1.8 (1.1, 3.0)	20	15	0.75	(0.53, 0.89)	18	0.90	(0.70, 0.97)	-0.15	(-0.38, 0.09)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Red Leaf Lettuce (200 g) BAX System X5 mBPWp Media, Batch 1	*E. coli* O157:H7 DD1450	18 (11, 30)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		1.8 (1.1, 3.0)	20	18	0.9	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)
Red Leaf Lettuce (200 g) BAX System X5 mBPWp Media, Batch 2	*E. coli* O157:H7 DD1450	7 (3.6, 13)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.7 (0.38, 1.3)	20	10	0.5	(0.3, 0.7)	10	0.5	(0.3, 0.7)	0	(-0.28, 0.28)
		Negative Control	5	4	0.8	(0.38, 0.96)	4	0.8	(0.38, 0.96)	0	(-0.45, 0.45)
Red Leaf Lettuce (200 g) BAX System X5 mBPWp Media, Batch 3	*E. coli* O157:H7 DD1450	24 (13, 40)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		2.4 (1.3, 4)	20	18	0.9	(0.70, 0.97)	18	0.90	(0.70, 0.97)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator [6], with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD$_C$ = Confirmed candidate method positive outcomes divided by the total number of trials.
[e]POD$_R$ = Confirmed reference method positive outcomes divided by the total number of trials.
[f]dPOD$_C$ = Difference between the candidate method and reference method 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.

Table 3. Inclusivity Results for the BAX System X5 PCR Assay for *E. coli* O157:H7 (5)

DuPont Strain ID	*E. coli* Strain	Result	DuPont Strain ID	*E. coli* Serotype	Result
1979	*E. coli* O157:H7	Pos	12813	*E. coli* O157:H7	Pos
5893	*E. coli* O157:HNM	Pos	12814	*E. coli* O157:H7	Pos
5894	*E. coli* O157:HNM	Pos	12815	*E. coli* O157:H7	Pos
8301	*E. coli* O157:HNM	Pos	12816	*E. coli* O157:H7	Pos
8302	*E. coli* O157:HNM	Pos	12817	*E. coli* O157:H7	Pos
12787	*E. coli* O157:H7	Pos	12818	*E. coli* O157:H7	Pos
12789	*E. coli* O157:H7	Pos	12820	*E. coli* O157:H7	Pos
12790	*E. coli* O157:H7	Pos	12821	*E. coli* O157:H7	Pos
12791	*E. coli* O157:H7	Pos	12822	*E. coli* O157:H7	Pos
12792	*E. coli* O157:H7	Pos	12823	*E. coli* O157:H7	Pos
12796	*E. coli* O157:H7	Pos	12824	*E. coli* O157:H7	Pos
12797	*E. coli* O157:H7	Pos	12825	*E. coli* O157:H7	Pos
12798	*E. coli* O157:H7	Pos	12826	*E. coli* O157:H7	Pos
12799	*E. coli* O157:H7	Pos	12827	*E. coli* O157:H7	Pos
12802	*E. coli* O157:H7	Pos	12828	*E. coli* O157:H7	Pos
12803	*E. coli* O157:H7	Pos	12829	*E. coli* O157:H7	Pos
12805	*E. coli* O157:H7	Pos	12830	*E. coli* O157:H7	Pos
12806	*E. coli* O157:H7	Pos	12832	*E. coli* O157:H7	Pos
12807	*E. coli* O157:H7	Pos	12833	*E. coli* O157:H7	Pos
12810	*E. coli* O157:H7	Pos	12834	*E. coli* O157:H7	Pos
12811	*E. coli* O157:H7	Pos	12835	*E. coli* O157:H7	Pos
12812	*E. coli* O157:H7	Pos	12836	*E. coli* O157:H7	Pos
12837	*E. coli* O157:H7	Pos			

Table 4. Exclusivity Results for the BAX System X5 PCR Assay for *E. coli* O157:H7

DuPont Strain ID	Strain	Result	DuPont Strain ID	Strain	Result
373	*Klebsiella pneumoniae*	Neg	2558	*Citrobacter freundii*	Neg
375	*Enterobacter cloacae*	Neg	2559	*Citrobacter amalonaticus*	Neg
383	*Citrobacter freundii*	Neg	2560	*Citrobacter koseri*	Neg
569	*Pseudomonas fluorescens*	Neg	2584	*Enterobacter hormaechei*	Neg
572	*Aeromonas hydrophila*	Neg	2586	*Klebsiella planticola*	Neg
576	*Pseudomonas mendocina*	Neg	2604	*Enterobacter amnigenus*	Neg
577	*Pseudomonas stutzeri*	Neg	2631	*Vibrio fluvialis*	Neg
592	*Yersinia enterocolitica*	Neg	2632	*Vibrio vulnificus*	Neg
610	*Staphylococcus aureus*	Neg	3097	*Citrobacter freundii*	Neg
657	*Klebsiella ozaenae*	Neg	3785	*Escherichia coli*	Neg
659	*Lactococcus lactis*	Neg	3982	*Pseudomonas aeruginosa*	Neg
700	*Shigella sonnei*	Neg	5588	*Hafnia alvei*	Neg
715	*Bacillus cereus*	Neg	6121	*Proteus mirabilis*	Neg
1081	*Shigella boydii*	Neg	6523	*Klebsiella oxytoca*	Neg
1082	*Shigella dysenteriae*	Neg	6719	*Escherichia hermanni*	Neg
2357	*Proteus mirabilis*	Neg	6832	*Shigella sonnei*	Neg
2389	*Hafnia alvei*	Neg	7083	*Serratia marcesens*	Neg
2399	*Yersinia aldovae*	Neg	8877	*Xanthomonas maltophilia*	Neg
2435	*Escherichia coli*	Neg	10006	*Enterobacter sakazakii*	Neg
2443	*Escherichia coli*	Neg	11232	*Vibrio mimicus*	Neg
2514	*Escherichia coli*	Neg	12720	*Enterobacter sakazakii*	Neg
2552	*Enterococcus faecium*	Neg	12760	*Enterobacter cloacae*	Neg
2554	*Enterococcus faecalis*	Neg	13041	*Escherichia coli*	Neg

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