CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

050501

The AOAC Research Institute hereby certifies that the performance of the test kit known as:

DuPont™ BAX® System PCR Assay for *E. coli* O157:H7 MP and the BAX® System MP Media

manufactured by

DuPont Nutrition & Health
Experimental Station 400
200 Powder Mill Road
Wilmington, DE 19803
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 (January 1, 2016 – December 31, 2016). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

November 30, 2015
Date

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KIT NAME(S)
DuPont™ BAX® System PCR Assay for *E. coli* O157:H7 MP and the BAX® System MP Media

CATALOG NUMBERS
D12404903 Test Kit
D12404925 Media

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APPLICABILITY OF METHOD
Target organism – *E. coli* O157:H7
Matrices – Raw ground beef (25 g), beef trim (65 g), spinach (25 g), & lettuce (25 g)
Performance claims - Sensitivity and Specificity ≥ 99% with equivalent performance between BAX® system MP and MP Express protocols.

REFERENCE METHOD
***Microbiology Laboratory Guidebook* (October 25, 2002) MLG 5.03, USDA Food Safety and Inspection Service, Office of Public Health and Science (Accessed March 4, 2005), http://www.fsis.usda.gov/ophs/microlab/mlg5.03.pdf (9)**

PRINCIPLE OF THE METHOD
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 result which is displayed as described below (section 11.2.1).

DISCUSSION OF THE VALIDATION STUDY
For both ground beef and beef trim, the USDA-FSIS culture method using single25g or 65g samples demonstrated false negative rate of 20-100%, possibly due to background flora naturally found in these food matrices. 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.

DISCUSSION OF MODIFICAITON APPROVED 2009
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.

DISCUSSION OF JULY 2013 MODIFICATION (11)

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.

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

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		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	PSU Reference Lab	*E. coli* O29:H51	Neg
1725	PSU Reference Lab	*E.coli* O125:H19	Neg	1835	PSU Reference Lab	*E. coli* O127:H-	Neg
1726	PSU Reference Lab	*E.coli* O126:H2	Neg	1836	PSU Reference Lab	*E. coli* O125:H-	Neg
1727	PSU Reference Lab	*E.coli* O44:H18	Neg	1839	PSU Reference Lab	*E. coli* O12:H-	Neg
1728	PSU Reference Lab	*E.coli* O55:HNM	Neg	1841	PSU Reference Lab	*E. coli* O124	Neg
1730	PSU Reference Lab	*E.coli* O86:H25	Neg	1842	PSU Reference Lab	*E. coli* O78:HNM	Neg
1731	PSU Reference Lab	*E.coli* O167:H5	Neg	1844	PSU Reference Lab	*E. coli* O119:HSM	Neg
1732	PSU Reference Lab	*E.coli* O143:HNM	Neg	1847	PSU Reference Lab	*E. coli* O128:H2,3,6	Neg
1733	PSU Reference Lab	*E.coli* O142:H6	Neg	1848	PSU Reference Lab	*E. coli* O126:H27	Neg
1734	PSU Reference Lab	*E.coli* O124:H30	Neg	1849	PSU Reference Lab	*E. coli* O27:HNT	Neg
1756	PSU Reference Lab	*E.coli* O25:H12	Neg	1852	PSU Reference Lab	*E. coli* O152:H2,3,7	Neg
1757	PSU Reference Lab	*E.coli* O152:HNM	Neg	1853	PSU Reference Lab	*E. coli* O124:H8	Neg
1758	Unknown	*E. coli* O63:HNM	Neg	1854	PSU Reference Lab	*E. coli* O44:HNT	Neg
1759	PSU Reference Lab	*E.coli* O15:H4	Neg	1855	PSU Reference Lab	*E. coli* O119 :H-	Neg
1760	PSU Reference Lab	*E.coli* O6:H1	Neg	1856	PSU Reference Lab	*E. coli* O144 :H25	Neg
1761	PSU Reference Lab	*E.coli* O27:HNM	Neg	1857	PSU Reference Lab	*E. coli* O144 :H42	Neg
1762	PSU Reference Lab	*E.coli* O164:HNM	Neg	1860	PSU Reference Lab	*E. coli* O126 :H27,6	Neg
1764	PSU Reference Lab	*E.coli* O8:H4	Neg	1861	PSU Reference Lab	*E. coli* O126:H-	Neg
1766	PSU Reference Lab	*E.coli* O80:H26	Neg	1865	PSU Reference Lab	*E. coli* O144:HNM	Neg
1767	PSU Reference Lab	*E.coli* O85:H1	Neg	1866	PSU Reference Lab	*E. coli* O18:H-	Neg
1768	PSU Reference Lab	*E.coli* O153:H7	Neg	1871	PSU Reference Lab	*E. coli* O119:H26	Neg
1769	PSU Reference Lab	*E.coli* O139:H1	Neg	1872	PSU Reference Lab	*E. coli* O126:H10	Neg
1770	PSU Reference Lab	*E.coli* O115:H18	Neg	1873	PSU Reference Lab	*E. coli* O27:H12	Neg
1771	PSU Reference Lab	*E.coli* O148:H28	Neg	1875	PSU Reference Lab	*E. coli* O15:H6	Neg
1772	PSU Reference Lab	*E.coli* O159:H20	Neg	1876	PSU Reference Lab	*E. coli* O124:H8,6,2	Neg
1796	PSU Reference Lab	*E.coli* O86: HNM	Neg	1877	PSU Reference Lab		Neg
1798	PSU Reference Lab	*E.coli* O28:HSM	Neg	1878	PSU Reference Lab	*E. coli* O152:H6,8	Neg
1799	PSU Reference Lab	*E.coli* O142:H-	Neg	1882	PSU Reference Lab	*E. coli* O114:H10	Neg
1800	PSU Reference Lab	*E.coli* O128:HNM	Neg	1883	PSU Reference Lab	*E. coli* O125:HNM	Neg
1801	PSU Reference Lab	*E.coli* O142:HNM	Neg	1884	PSU Reference Lab	*E. coli* O158:H7	Neg
1802	PSU Reference Lab	*E.coli* O6:HNM	Neg	1889	PSU Reference Lab	*E. coli* O152:H10	Neg
1803	PSU Reference Lab	*E.coli* O25:H-	Neg	1893	PSU Reference Lab	*E. coli* O114:H8,10	Neg
1804	PSU Reference Lab	*E.coli* O124:H-	Neg	1894	PSU Reference Lab	*E. coli* O114:H-	Neg
1807	PSU Reference Lab	*E.coli* O26:H-	Neg	2477	PSU Reference Lab	*E. coli* O55:H7	Pos
1550	Unknown	*Salmonella abaetetuba*	Neg	706	Unknown	*Salmonella enteritidis*	Neg
2166	Unknown	*Salmonella abaetetuba*	Neg	846		*Escherichia blattae*	Neg
2341	Unknown	*Salmonella mbandaka*	Neg	847	ATCC/Human feces	*Escherichia ferguson*	Neg
2992	Unknown	*Salmonella Lille*	Neg	849	ATCC/soil	*Escherichia intermedia*	Neg
1261	Duck	*Salmonella newport*	Neg	850	ATCC/Human wound	*Escherichia vulnaris*	Neg
1777	Unknown	*Salmonella enterica*	Neg	2901	Cream cake	*Bacillus cereus*	Neg
2274	Unknown	*Salmonella anatum*	Neg	3017	Unknown	*Salmonella dublin*	Neg
2614	Human feces	*Edwardsiella tarda*	Neg	3019	Unknnown	*Salmonella dublin*	Neg
3982	Blood culture	*Pseudomonas aeruginosa*	Neg	3064	Environmental swab	*Morganella morganii*	Neg
3998	Bovine mastitis	*Streptococcus equi*	Neg	6121	Gull, cloacal swab	*Proteus mirabilis*	Neg
4160	Howler monkey	*Staphylococcus aureus*	Neg	6523	Ground beef	*Klebsiella oxytoca*	Neg
5588	Ground beef	*Hafnia alvei*	Neg	6719	Sesame seeds	*Escherichia hermanii*	Neg
7005	Unknown	*Salmonella dublin*	Neg	6832	Unknown	*Shigella sonnei*	Neg
7344	Human	*Lactobacillus acidophilus*	Neg	11348	Unknown	*Enterobacter sakazakii*	Neg

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)

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)

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)

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.

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

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

Modification Data Approved 2009 (11)

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)

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 spinach (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)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed[b]	Sensitivity[c] %	False Neg [d] %	Presump. Pos /Unspiked	Specifi-city[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.*

Modification Data Approved July 2013 (12)

Table 3. BAX® System Results – DuPont™ Thermal Block vs. Analog Heating/Cooling Blocks

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

ORIGINAL CERTIFICATION DATE
June 01, 2005

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2016

METHOD MODIFICATION RECORD
1. **2009**
2. **July 2013**

SUMMARY OF MODIFICATION
1. **Matrix extension to include Spinach and lettuce**
2. **Addition of Thermal Block for automated sample lysis**

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