CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

031002

The AOAC Research Institute hereby certifies the method known as:

BAX® System Real-Time 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

Issue Date	December 10, 2024
Expiration Date	December 31, 2025

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METHOD NAME
BAX® System Real-Time PCR Assay for *E. coli* O157:H7

CATALOG NUMBERS
BAX® Assay KIT2000, MED2003 (MP Media)

INDEPENDENT LABORATORY
Food Safety Net Laboratory Services USA

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

Matrixes – Ground beef (65 g), beef trim (375 g), spinach (25 g), and lettuce (25 g)

Performance claims – Equivalent or better performance than the appropriate reference method

REFERENCE METHOD
***Microbiology Laboratory Guidebook* (January 28, 2008) MLG 5.04, USDA Food Safety and Inspection Service, Office of Public Health and Science (2)**

ORIGINAL CERTIFICATION DATE
March 09, 2010

CERTIFICATION RENEWAL RECORD
Renewed through December 2025.

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. July 2013	1. Addition of Thermal Block for automated sample lysis.
2. March 2017 Level 1	2. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company.
3. January 2018 Level 1	3. Editorial update inserts, manuals, labels to Hygiena.
4. May 2019 Level 1	4. Editorial updates to inserts and corporate address update.
5. December 2019 Level 1	5. Editorial changes.
6. December 2021 Level 1	6. Editorial changes.
7. December 2023 Level 1	7. Editorial changes.
8. December 2024 Level 1	8. Editorial changes.

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NONE

PRINCIPLE OF THE METHOD (1)
PCR Amplification - The BAX® System Real-Time E. coli O157:H7 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, 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 product. One PCR primer for each target (two E. coli O157:H7 specific targets and an internal control) contains a fluorescent dye (three different dyes, one for each target) as a constituent of the primer as well as a quencher (the uni-molecular combination of a primer, fluorescent dye and quencher constitute a Scorpion™ Probe). When incorporated into a PCR product, the dye and quencher are spatially separated, which causes an increase in emission signal. The BAX System measures the magnitude and characteristics 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.

DISCUSSION OF THE VALIDATION STUDY (1)
Multiple replicates of the beef trim protocol were performed since this is the most heavily tested matrix for *E. coli* O157:H7 in the U.S. In addition, many corporate customers, as well as governments outside the U.S. are requesting data from samples comprising >50 positive samples at a spike level in the 1-2 cfu per analytical portion range. The use of data from multiple strains for validation on beef trim has also been requested. Across all matrixes the BAX® System demonstrated equivalent or statistically better performance compared to the corresponding appropriate reference method. Since this study was completed, the FDA BAM method for the detection of EHEC has been substantively altered. Additional studies are planned to address the performance of the BAX method relative to the revised reference method and if necessary, changes will be made using the PTM method modification process. Additionally, the USDA-FSIS is considering new validation guidelines for 375g trim testing, but since these guidelines are not available yet, and since the protocol for these studies was approved in April, these differences will be addressed through the AOAC-RI method modification process when the new USDA requirements are available.

Table 10. Test Assay Inclusivity (1)

Strain	Source	Strain	Assay Result	Strain	Source	Strain	Assay Result
12836	PSU ReferenceLab	*E. coli O157 :H7*	POS	12848	PSU Reference Lab	*E. coli O157 :H7*	POS
12830	PSU ReferenceLab	*E. coli O157 :H7*	POS	12859	PSU Reference Lab	*E. coli O157 :H7*	POS
12832	PSU Reference Lab	*E. coli O157 :H7*	POS	12860	PSU Reference Lab	*E. coli O157 :H7*	POS
12833	PSU Reference Lab	*E. coli O157 :H7*	POS	12861	PSU Reference Lab	*E. coli O157 :H7*	POS
12844	PSU Reference Lab	*E. coli O157 :H7*	POS	12862	PSU Reference Lab	*E. coli O157 :H7*	POS
12845	PSU Reference Lab	*E. coli O157 :H7*	POS	12863	PSU Reference Lab	*E. coli O157 :H7*	POS
12846	PSU Reference Lab	*E. coli O157 :H7*	POS	12874	PSU Reference Lab	*E. coli O157 :H7*	POS
12835	PSU Reference Lab	*E. coli O157 :H7*	POS	12875	PSU Reference Lab	*E. coli O157 :H7*	POS
12834	PSU Reference Lab	*E. coli O157 :H7*	POS	12876	PSU Reference Lab	*E. coli O157 :H7*	POS
12839	PSU Reference Lab	*E. coli O157 :H7*	POS	12857	PSU Reference Lab	*E. coli O157 :H7*	POS
12840	PSU Reference Lab	*E. coli O157 :H7*	POS	12858	PSU Reference Lab	*E. coli O157 :H7*	POS
12841	PSU Reference Lab	*E. coli O157 :H7*	POS	12869	PSU Reference Lab	*E. coli O157 :H7*	POS
12842	PSU Reference Lab	*E. coli O157 :H7*	POS	12870	PSU Reference Lab	*E. coli O157 :H7*	POS
12843	PSU Reference Lab	*E. coli O157 :H7*	POS	12871	PSU Reference Lab	*E. coli O157 :H7*	POS
12854	PSU Reference Lab	*E. coli O157 :H7*	POS	12873	PSU Reference Lab	*E. coli O157 :H7*	POS
12855	PSU Reference Lab	*E. coli O157 :H7*	POS	12884	PSU Reference Lab	*E. coli O157 :H7*	POS
12856	PSU Reference Lab	*E. coli O157 :H7*	POS	12885	PSU Reference Lab	*E. coli O157 :H7*	POS
12837	PSU Reference Lab	*E. coli O157 :H7*	POS	12887	PSU Reference Lab	*E. coli O157 :H7*	POS
12849	PSU Reference Lab	*E. coli O157 :H7*	POS	12867	PSU Reference Lab	*E. coli O157 :H7*	POS
12850	PSU Reference Lab	*E. coli O157 :H7*	POS	12868	PSU Reference Lab	*E. coli O157 :H7*	POS
12851	PSU Reference Lab	*E. coli O157 :H7*	POS	12879	PSU Reference Lab	*E. coli O157 :H7*	POS
12852	PSU Reference Lab	*E. coli O157 :H7*	POS	12880	PSU Reference Lab	*E. coli O157 :H7*	POS
12853	PSU Reference Lab	*E. coli O157 :H7*	POS	12881	PSU Reference Lab	*E. coli O157 :H7*	POS
12864	PSU Reference Lab	*E. coli O157 :H7*	POS	12882	PSU Reference Lab	*E. coli O157 :H7*	POS
12865	PSU Reference Lab	*E. coli O157 :H7*	POS	12883	PSU Reference Lab	*E. coli O157 :H7*	POS
12847	PSU Reference Lab	*E. coli O157 :H7*	POS	12810	PSU Reference Lab	*E. coli O157 :H7*	POS
12813	PSU Reference Lab	*E. coli O157 :H7*	POS	12816	PSU Reference Lab	*E. coli O157 :H7*	POS
2485	Unknown	*E. coli O157:HNM*	POS	8301	Unknown	*E. coli O157 :HNM*	POS
5893	Unknown	*E. coli O157 :HNM*	POS	8302	Unknown	*E. coli O157 :HNM*	POS
5894	Unknown	*E. coli O157 :HNM*	POS	TD8136	Bovine	*E. coli O157 :H7 Cluster A*	POS
MA06	Peter Feng, FDA	*E. coli O157:H7 rough*	POS				

Table 11. Test Assay Exclusivity (1)

Strain #	Source	Strain	Result	Strain #	Source	Strain	Result
DD1081	Unknown	*Shigella boydii*	NEG	DD2434	Unknown	E. coli O1:H7	NEG
DD11348	Unknown	*Enterobacter sakazakii*	NEG	DD2443	Unknown	*E. coli O157 :H19*	NEG
DD1152	Pate'	*Listeria monocytogenes*	NEG	DD2491	Unknown	*E. coli O2:H7*	NEG
DD1261	Duck	*Salmonella newport*	NEG	DD2520	Unknown	*E. coli* O113:H7	NEG
DD13249	raw shrimp	*Vibrio parahemolyticus*	NEG	DD2614	Human feces	*Edwardsiella tarda*	NEG
DD1716	Unknown	*E. coli O158:H23*	NEG	DD2901	Cream cake	*Bacillus cereus*	NEG
DD1718	Unknown	*E. coli O128:H2*	NEG	DD2992	Unknown	*Salmonella enterica* serovar Lille	NEG
DD1719	Unknown	*E. coli O28:HNM*	NEG	DD3017	Unknown	*Salmonella dublin*	NEG
DD1720	Unknown	*E. coli O26:HNM*	NEG	DD3019	Unknnown	*Salmonella dublin*	NEG
DD1721	Unknown	*E. coli O114:H32*	NEG	DD3064	Environmental swab	*Morganella morganii*	NEG
DD1722	Unknown	*E. coli O127: HNM*	NEG	DD3981	urine	*Enterococcus faecalis*	NEG
DD1723	Unknown	*E. coli* O119:H27	NEG	DD3982	Blood culture	*Pseudomonas aeruginosa*	NEG
DD1724	Unknown	*E. coli* O18:H14	NEG	DD3998	Bovine mastitis	*Streptococcus equi*	NEG
DD1725	Unknown	*E. coli O125:H19*	NEG	DD4160	Howler monkey	*Staphylococcus aureus*	NEG
DD1777	Unknown	*Salmonella enterica*	NEG	DD5588	Ground beef	*Hafnia alvei*	NEG
DD1810	Unknown	*E. coliO28:H16*	NEG	DD577	Human	*Pseudomonas stutzeri*	NEG
DD1811	Unknown	*E. coli O127:H40*	NEG	DD5883	Unknown	*E. coli O55 :H10*	NEG
DD1812	Unknown	*E. coli O127:H10*	NEG	DD610	ham	*Staphylococcus aureus*	NEG
DD1814	Unknown	*E. coli O6:H-*	NEG	DD6121	Gull, cloacal swab	*Proteus mirabilis*	NEG
DD1817	Unknown	*E. coli O29:H-*	NEG	DD649	sheep	*Listeria ivanovii*	NEG
DD1818	Unknown	*E. coli O136:H8*	NEG	DD6523	Ground beef	*Klebsiella oxytoca*	NEG
DD1819	Unknown	*E. coli O18:HNM*	NEG	DD655	Calf Intestine	*E. coli O101:K-:K99*	NEG
DD1820	Unknown	*E. coli O86:H8*	NEG	DD661	pre-filter tanks	*Pseudomonas fluorescens*	NEG
DD1821	Unknown	*E. coli O55:H-*	NEG	DD6719	Sesame seeds	*Escherichia hermanii*	NEG
DD1822	Unknown	*E. coli O28:H8,4,3*	NEG	DD6832	Unknown	*Shigella sonnei*	NEG
DD1824	Unknown	*E. coli O125:HNM*	NEG	DD687	vacuum packed lamb	*Lactobacillus carnis*	NEG

DD1825	Unknown	*E. coli O25:H8*	NEG	DD7005	Unknown	*Salmonella enterica* serovar *Dublin*	NEG
DD1827	Unknown	*E. coli O20:HNM*	NEG	DD7344	Human	*Lactobacillus acidophilus*	NEG
DD1831	Unknown	*E. coliO26:H11*	NEG	DD846	Cockroach	*Escherichia blattae*	NEG
DD1833	Unknown	*E. coliO55:H9*	NEG	DD847	Human feces	*Escherichia ferguson*	NEG
DD1834	Unknown	*E. coliO29:H51*	NEG	DD849	Unknown	*Escherichia intermedia*	NEG
DD1835	Unknown	*E. coli O127:H-*	NEG	DD850	Human wound	*Escherichia vulnaris*	NEG
DD1908	Unknown	*E. coli* O25:H7	NEG	DD922	cured ham	*Listeria innocua*	NEG
DD2166	Unknown	*Salmonella abaetetuba*	NEG	TD2631	Unknown	*Vibrio fluvialis*	NEG
DD2274	Unknown	*Salmonella* enterica serovar Anatum	NEG	TD3122	Unknown	*Vibrio vulnificus*	NEG
DD2341	Unknown	*Salmonella* enterica serovar Mbandaka	NEG	TD3136	Unknown	*Vibrio cholera*	NEG

Table 2. Results of 65g Ground Beef Internal Study (1)

Method	MPN Per 65g[a]	Spike Level[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
9 hr BAX	0.59	0.70	BAX MP	20	5/5	100	0	0/5	100	0	0
24 hr BAX	0.59	0.70	BAX MP	20	5/5	100	0	0/5	100	0	0
Reference (22 hr)	0.59	0.70	mTSB + n	20	5/4	100	0	0/5	80	20	

[a] Most probable number of colony forming units per test portion. ***MPN was performed on the day of testing using the reference method.*** *MPN values were calculated using the tables found in the FDA-BAM. Spike level was determined by performing a standard plate count on the incident cultures which were diluted for spiking each matrix on the day of introduction to the master sample.*

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

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

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

[e] False positive rate: 100 minus specificity rate

[f] Mantel Haenszel Chi-square for unpaired samples..

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

Table 3. Results of 65g Ground Beef Independent Laboratory Study (1)

Method	MPN Per 65g[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
9 hr BAX	1.0	BAX MP	20	11/12	92	8	0/5	100	0	0.94
24 hr BAX	1.0	BAX MP	20	12/12	100	0	0/5	100	0	0.42
Reference (22 hr)	1.0	mTSB + n	20	14/14	100	0	0/5	100	0	

[a] Most probable number of colony forming units per test portion. ***MPN was performed on the day of testing using the reference method.*** *MPN values were calculated using the tables found in the FDA-BAM. .*

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

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

[d] Specificity rate: 100 times the number of assay-negative results divided by total number of true negative results, including unspiked samples. Specificity is determined by the USDA presumptive result compared with the actual culture confirmation.

[e] False positive rate: 100 minus specificity rate

[f] Mantel Haenszel Chi-square for unpaired samples.

Table 4. Results of 375g Beef Trim Replicate 1 Strain DD12850 (1)											
Method	MPN Per 25g[a]	Spike Level[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
10 hr BAX	0.16	1.0	BAX MP	20	10/10	100	0	0/5	100	0	5.4
24 hr BAX	0.16	1.0	BAX MP	20	10/10	100	0	0/5	100	0	5.4
Reference (22 hr)	0.16	1.0	mTSB + n	20	3/3	100	0	0/5	80	20	

[a] Most probable number of colony forming units per test portion. ***MPN was performed on the day of testing using the reference method.*** *MPN values were calculated using the tables found in the FDA-BAM. Spike level was determined by performing a standard plate count on the incident cultures which were diluted for spiking each matrix on the day of introduction to the master sample.*

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

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

[d] Specificity rate: 100 times the number of assay-negative results divided by total number of true negative results, including unspiked samples. Specificity is determined by the USDA presumptive result compared with the actual culture confirmation.

[e] False positive rate: 100 minus specificity rate

[f] Mantel Haenszel Chi-square for unpaired samples..

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

Table 5. Results of 375g Beef Trim Replicate 2 Strain DD642 (1)											
Method	MPN Per 25g[a]	Spike Level[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
10 hr BAX	0.28	0.65	BAX MP	20	11/11	100	0	0/5	100	0	5.1
24 hr BAX	0.28	0.65	BAX MP	20	11/11	100	0	0/5	100	0	5.1
Reference (22 hr)	0.28	0.65	mTSB + n	20	5/4	100	0	0/5	80	20	

[a] Most probable number of colony forming units per test portion. ***MPN was performed on the day of testing using the reference method.*** *MPN values were calculated using the tables found in the FDA-BAM. Spike level was determined by performing a standard plate count on the incident cultures which were diluted for spiking each matrix on the day of introduction to the master sample.*

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

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

[d] Specificity rate: 100 times the number of assay-negative results divided by total number of true negative results, including unspiked samples. Specificity is determined by the USDA presumptive result compared with the actual culture confirmation.

[e] False positive rate: 100 minus specificity rate

[f] Mantel Haenszel Chi-square for unpaired samples.

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

Table 6. Results of 375g Beef Trim Replicate 3 Strain DD1979 (1)											
Method	MPN Per 25g[a]	Spike Level[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
10 hr BAX	2.1	2.0	BAX MP	20	18/18	100	0	0/5	100	0	0.22
24 hr BAX	2.1	2.0	BAX MP	20	18/18	100	0	0/5	100	0	0.22
Reference (22 hr)	2.1	2.0	mTSB + n	20	17/17	100	0	0/5	100	100	

[a] Most probable number of colony forming units per test portion. MPN was performed on the day of testing using the reference method. MPN values were calculated using the tables found in the FDA-BAM. Spike level was determined by performing a standard plate count on the incident cultures which were diluted for spiking each matrix on the day of introduction to the master sample.

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

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

[d] Specificity rate: 100 times the number of assay-negative results divided by total number of true negative results, including unspiked samples. Specificity is determined by the USDA presumptive result compared with the actual culture confirmation.

[e] False positive rate: 100 minus specificity rate

[f] Mantel Haenszel Chi-square for unpaired samples.

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

Table 7. Results of 375g Beef Trim Replicate 4 Strain DD12835 (1)											
Method	MPN Per 25g[a]	Spike Level[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
10 hr BAX	1.8	1.9	BAX MP	20	17/17	100	0	0/5	100	0	0
24 hr BAX	1.8	1.9	BAX MP	20	17/17	100	0	0/5	100	0	0
Reference (22 hr)	1.8	1.9	mTSB + n	20	17/17	100	0	0/5	100	100	

[a] Most probable number of colony forming units per test portion. ***MPN was performed on the day of testing using the reference method.*** *MPN values were calculated using the tables found in the FDA-BAM. Spike level was determined by performing a standard plate count on the incident cultures which were diluted for spiking each matrix on the day of introduction to the master sample.*

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

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

[d] Specificity rate: 100 times the number of assay-negative results divided by total number of true negative results, including unspiked samples. Specificity is determined by the USDA presumptive result compared with the actual culture confirmation.

[e] False positive rate: 100 minus specificity rate

[f] Mantel Haenszel Chi-square for unpaired samples.

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

Table 8. Results of 25g Lettuce Strain DD12835 (1)											
Method	MPN Per 25g[a]	Spike Level[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
8 hr BAX	1.1	1.0	BAX MP	20	15/16	94	6	0/5	100	0	6.3
10 hr BAX	1.1	1.0	BAX MP	20	15/16	94	6	0/5	100	0	6.3
24 hr BAX	1.1	1.0	BAX MP	20	16/16	100	0	0/5	100	0	8.1
Reference (24 hr)	1.1	1.0	EEB	20	7	NA	NA	0/5	NA	NA	

[a] Most probable number of colony forming units per test portion. ***MPN was performed on the day of testing using the reference method.*** *MPN values were calculated using the tables found in the FDA-BAM. Spike level was determined by performing a standard plate count on the incident cultures which were diluted for spiking each matrix on the day of introduction to the master sample.*

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

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

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

[e] False positive rate: 100 minus specificity rate

[f] Mantel Haenszel Chi-square for unpaired samples.

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

Table 9. Results of 25g Lettuce Strain DD1450 (1)											
Method	MPN Per 25g[a]	Spike Level[a]	Enrichment Method (Media)	Total spiked	Presump.Pos /Confirmed	Sensitivity[b] %	False Neg [c] %	Presump. Pos /Unspiked	Specificity[d] %	False Pos[e] %	Chi-square[f]
8 hr BAX	0.23	1.0	BAX MP	20	12/13	92	8	0/5	100	0	3.6
10 hr BAX	0.23	1.0	BAX MP	20	13/13	100	0	0/5	100	0	4.8
24 hr BAX	0.23	1.0	BAX MP	20	13/13	100	0	0/5	100	0	4.8
Reference (24 hr)	0.23	1.0	EEB	20	6	NA	NA	0/5	NA	NA	

[a] Most probable number of colony forming units per test portion. ***MPN was performed on the day of testing using the reference method.*** *MPN values were calculated using the tables found in the FDA-BAM. Spike level was determined by performing a standard plate count on the incident cultures which were diluted for spiking each matrix on the day of introduction to the master sample.*

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

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

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

[e] False positive rate: 100 minus specificity rate

[f] 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 used for paired samples, Mantel Haenszel for unpaired samples.

**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]		
Real-time *E. coli* O157:H7	Ground beef	Low	17	17	1	0.82, 1.0	16	0.94	0.73, 0.99	0.059	-0.13, 0.27
		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
	Beef trim	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
		High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
	Spinach	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
		High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18

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