



CERTIFICATION

AOAC[®] Performance TestedSM

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 in the AOAC[®] Performance Tested MethodsSM 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 TestedSM 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 06, 2021 – December 31, 2022). 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 06, 2021

Date

METHOD AUTHORS ORIGINAL VALIDATION: Frank Burns, Lois Fleck, Bridget Andalaro, Eugene Davis, Jeff Rohrbeck, George Tice, and Morgan Wallace MODIFICATION JULY 2013: Steve Hoelzer, F. Morgan Wallace, Lois Fleck, Deana DiCosimo, Jacqueline Harris, Bridget Andalaro, Andrew Farnum, Eugene Davis, and Jeff Rohrbeck	SUBMITTING COMPANY DuPont ESL Building 400 Route 141 & Henry Clay Road Wilmington, DE 19880-0400	CURRENT SPONSOR Hygiena 2 Boulden Circle New Castle, DE 19720 USA
METHOD NAME BAX® System Real-Time PCR Assay for <i>E. coli</i> O157:H7	CATALOG NUMBERS BAX® Assay KIT2000, MED2003 (MP Media)	
INDEPENDENT LABORATORY Food Safety Net Laboratory Services USA	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Michael Brodsky ² , Wayne Ziemer ³ ¹ US FDA, FCSAN, College Park, MD, USA ² Brodsky Consulting, Thornhill, Ontario, CANADA ³ Consultant, Loganville, GA, USA	
APPLICABILITY OF METHOD Target organism – <i>E. coli</i> 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 <i>Microbiology Laboratory Guidebook</i> (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 annually through December 2022.	
METHOD MODIFICATION RECORD 1. July 2013 2. March 2017 Level 1 3. January 2018 Level 1 4. May 2019 Level 1 5. December 2019 Level 1 6. December 2021 Level 1	SUMMARY OF MODIFICATION 1. Addition of Thermal Block for automated sample lysis. 2. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company. 3. Editorial update inserts, manuals, labels to Hygiena. 4. Editorial updates to inserts and corporate address update. 5. Editorial/clerical changes to insert. 6. Editorial changes to insert.	
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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.

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

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

DD1825	Unknown	<i>E. coli</i> O25:H8	NEG	DD7005	Unknown	<i>Salmonella enterica</i> serovar Dublin	NEG
DD1827	Unknown	<i>E. coli</i> O20:HNM	NEG	DD7344	Human	<i>Lactobacillus acidophilus</i>	NEG
DD1831	Unknown	<i>E. coli</i> O26:H11	NEG	DD846	Cockroach	<i>Escherichia blattae</i>	NEG
DD1833	Unknown	<i>E. coli</i> O55:H9	NEG	DD847	Human feces	<i>Escherichia ferguson</i>	NEG
DD1834	Unknown	<i>E. coli</i> O29:H51	NEG	DD849	Unknown	<i>Escherichia intermedia</i>	NEG
DD1835	Unknown	<i>E. coli</i> O127:H-	NEG	DD850	Human wound	<i>Escherichia vulnaris</i>	NEG
DD1908	Unknown	<i>E. coli</i> O25:H7	NEG	DD922	cured ham	<i>Listeria innocua</i>	NEG
DD2166	Unknown	<i>Salmonella abaeetuba</i>	NEG	TD2631	Unknown	<i>Vibrio fluvialis</i>	NEG
DD2274	Unknown	<i>Salmonella enterica</i> serovar Anatum	NEG	TD3122	Unknown	<i>Vibrio vulnificus</i>	NEG
DD2341	Unknown	<i>Salmonella enterica</i> serovar Mbandaka	NEG	TD3136	Unknown	<i>Vibrio cholera</i>	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 *Campylobacter 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 <i>E. coli</i> 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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