CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

050902

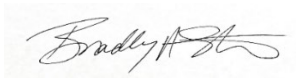
The AOAC Research Institute hereby certifies the method known as:

BAX® System Real-Time PCR Assay for *Vibrio cholerae, parahaemolyticus,* and *vulnificus*

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.



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Signature for AOAC Research Institute

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New Castle, DE 19720
USA

METHOD NAME
BAX® System Real-Time PCR Assay for *Vibrio cholerae, parahaemolyticus,* and *vulnificus*
Formerly DuPont™ BAX® System Real-Time PCR Assay for *Vibrio cholerae, parahaemolyticus,* and *vulnificus*

CATALOG NUMBER
BAX® Assay KIT2010 (D12863877)

INDEPENDENT LABORATORY
Texas State Department of Health Services
Consumer Microbiology Team
Austin, TX 78756 USA

APPLICABILITY OF METHOD
Target organism – *Vibrio cholerae, parahaemolyticus, and vulnificus*.

Matrixes – (25 g) – Shrimp, oysters, tuna, and scallops

Performance claims – Sensitivity and specificity equivalent to the official FDA-BAM culture-based method.

REFERENCE METHOD
US FDA, Bacteriological Analytical Manual (4)

ORIGINAL CERTIFICATION DATE
May 10, 2009

CERTIFICATION RENEWAL RECORD
Renewed through December 2025.

METHOD MODIFICATION RECORD

1. **July 2013 Level 2**
2. **March 2017 Level 1**
3. **December 2017 Level 1**
4. **May 2019 Level 1**
5. **December 2019 Level 1**
6. **December 2021 Level 1**
7. **December 2023 Level 1**
8. **December 2024 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. **Inserts, manuals, and labels updated to Hygiena.**
4. **Editorial updates to inserts and corporate address.**
5. **Editorial changes.**
6. **Editorial changes.**
7. **Editorial changes.**
8. **Editorial changes.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)

The BAX® system uses the Polymerase Chain Reaction (PCR) to amplify specific DNA fragments, which are stable and unaffected by growth conditions [3]. Each fragment is a genetic sequence that is unique to the targeted organism, thus providing a highly reliable indicator that the organism is present. The BAX system simplifies the PCR process by combining the requisite PCR reagents into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After hydrating these tablets with prepared samples, the tubes remain sealed to reduce the potential for contamination.

In a typical PCR application, sample DNA is combined with DNA polymerase, nucleotides and primers that are specific for a given nucleotide sequence. The mixture then undergoes a series of timed heating and cooling cycles. Heating denatures the DNA, separating it into single strands. As the mixture cools, the primers recognize and anneal (bind) to the targeted DNA sequence. DNA polymerase then uses nucleotides to extend the primers, thus creating two copies of the targeted fragment (amplification). Repeating cycles of denaturing, annealing and extending produces an exponential increase in the number of target DNA fragments, creating millions of copies in a very short time. If the target sequence is not present, no detectable amplification takes place [2]. Inhibitors to PCR are present in some food matrixes. In particular, phenolic compounds found in some spices and other plant-based materials such as high purity cocoa can cause the PCR reaction to shut down. Because of this, each BAX reagent tablet is formulated with a low level control DNA molecule and associated primers. This Internal Positive Control (INPC) must be shown to amplify in the absence of specific pathogen target amplification product for the BAX instrument to report a negative result. In the absence of any target or INPC associated product, the instrument reports an indeterminate result.

The BAX system PCR tablets used in real-time assays also contain multiple dye-labeled probes. Intact probes are short oligonucleotides with quencher dye at one end that absorbs the signal from fluorescent reporter dye at the opposite end. During PCR cooling cycles, probes bind to a specific area within the targeted fragment. During extension, DNA polymerase encounters the probe in its path and breaks the probe apart. This releases the reporter dye, resulting in increased fluorescent signal [5]. In multiplex reactions such as in this test kit, each species specific probe is labeled with a different fluorescent reporter dye, allowing independent detection of the presence or absence of each target. The BAX system Q7 instrument uses multiple filters to measure specific signal resulting from the presence of each target at the end of each cycle and report results for the presence or absence of *Vibrio cholerae*, *vulnificus*, or *parahaemolyticus* in less than 90 minutes.

DISCUSSION OF THE VALIDATION STUDY (1)

In initial development studies, some enriched samples were found to test positive by the BAX PCR assay but negative by the reference culture method. Often, this is the case when non-target competitive flora, either non-*Vibrio*, or non-target *Vibrio* species are present in an enrichment with cell densities at a much higher level than the target organism. In such cases, an additional plating media, CHROMagar *Vibrio*, has been found to be useful. For each sample tested for most studies (with the exception of the oyster studies performed at Dauphin Island), a CHROMagar *Vibrio* plate was also struck from each enriched sample to reflect this fact. In one study (the naturally contaminated frozen raw shrimp work) two samples were found to be pcr positive/culture negative. For these samples that tested pcr positive, but from which no confirmed colonies of a positive species were found from the FDA-BAM media, more colonies than required by the FDA BAM procedure were picked from the TCBS, mCPC and CHROMagar *Vibrio* plates into cluster tubes containing 500 µl APW (up to 24 per sample per media where available). Individual isolates were allowed to grow in the cluster tubes overnight at room temperature and tested by BAX assay. Presumptive positive cluster tubes were struck onto TCBS or T_1N_3 agar and confirmed using the FDA-BAM methods. Both of these samples were then found to be positive using this enhanced protocol, yielding at least one confirmed *V. cholerae* isolate. Qualicon has also demonstrated the presence of atypical *V. parahaemolyticus* strains (confirmed by DNA sequence-based characterization) that do not present with typical characteristics on *Vibrio* selective and differential agars. All enrichments which tested positive by PCR, with the exception of two MPN tubes from the oyster study, were also positive for typical confirmed colonies on one or more of the three agars above. In the oyster studies, only three typical colonies per MPN tube were selected as per the FDA-BAM protocols, and a greater number of colonies selected per tube would have made the experiment unmanageable. This highlights a potential issue with the reference method in that typical colony morphology on plates is a critical step in the reference method and the complex microbial ecology of an oyster can potentially lead to less than optimal results when non-target isolates with a typical phenotype on *Vibrio* selective agars are present in significant numbers relative to the levels of target *Vibrio*. In other non-AOAC studies conducted at Qualicon some instances of PCR positive enrichments have yielded phenotypically atypical isolates that test positive by PCR. These isolates have been characterized by sequence-based identification (microSeq®, Applied Biosystems, Foster City, CA) as target *Vibrio* species and are being shared with the community of *Vibrio* experts for further characterization (data not shown). The above described work supports continued work on the natural phenotypic and genetic variation of pathogenic species of *Vibrio* occurring in foods.

Table 1. BAX vs. Reference Results for Presence/Absence Testing (1)

Sample type	MPN or Spike Level	Samples	BAX pos	BAX Confirmed	Reference pos	Sensitivity[1]	Specificity[2]	Chi Square[3]
Tuna	0.5 MPN/25g (*V. choleraee*)	20	3	3	3	100%	100%	-
	1.9 MPN/25g (*V. choleraee*)	20	13	13	13	100%	100%	-
	3.75 MPN/25g (*V. choleraee*)	20	19	19	19	100%	100%	-
	0 cfu/25g	5	0	0	0		100%	
Tuna (Independent Laboratory)	6 MPN/25g (*V. choleraee*)	20	9	9	9	100%	100%	-
	0 cfu/25g	5	0	0	0		100%	
Frozen raw shrimp	Naturally contaminated (*V. choleraee*)	20	5	5	5	100%	100%	-

[1] *Sensitivity - Total number of confirmed positive test portions by the method divided by total number of confirmed positive test portions by both the alternative and reference methods.*

[2] Specificity - Total number of analyzed negative test portions by the method divided by total number of confirmed negative test portions by both the alternative and reference methods.

[3] McNemar Chi-Square test statistic used for calculating significance of results

Table 2. BAX System Results for Samples with Presence/Absence and MPN Testing (1)

Sample type	Presence/Absence in 25g sample			MPN (3 tube, 3 dilution – 1g, 0.1g, 0.01g)				
	Inoculation level	BAX positive / confirmed	Reference positive / confirmed	Sample	BAX positive (1g, 0.1g, 0.01g)	Reference positive (1g, 0.1g, 0.01g)	BAX MPN[1]	Reference MPN[1]
Cooked shrimp (*V. parahaemolyticus*)	1.8 cfu/g	5/5	5/5	1	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				2	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				3	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				4	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				5	1, 0, 0	1, 0, 0	0.36/g	0.36/g
Cooked shrimp (*V. parahaemolyticus*)	18 cfu/g	5/5	5/5	1	2, 0, 0	2, 0, 0	0.92/g	0.92/g
				2	2, 2, 0	2, 2, 0	2.1/g	2.1/g
				3	2, 0, 0	2, 0, 0	0.92/g	0.92/g
				4	3, 0, 0	3, 0, 0	2.3/g	2.3/g
				5	2, 1, 0	2, 1, 0	1.5/g	1.5/g
Scallops (*V. vulnificus*)	1.4×10^4 cfu/g	5/5	5/5	1	1, 0, 0	1, 0, 0	0.36/g	0.36/g
				2	0, 0, 0	0, 0, 0	<0.3/g	<0.3/g
				3	2, 0, 0	2, 0, 0	0.92/g	0.92/g
				4	0, 0, 0	0, 0, 0	<0.3/g	<0.3/g
				5	0, 0, 0	0, 0, 0	<0.3/g	<0.3/g

[1] MPN values determined using the FDA-BAM MPN tables.

Table 3. BAX System Results for Oysters with MPN Testing *V. parahaemolyticus* (3 tube, 8 dilution) (1)

Sample Set	BAX positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	Reference positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	BAX MPN[1]	Reference MPN[1]
3°C	3, 3, 3, 1, 0, 0, 0, 0	3, 3, 3, 1, 0, 0, 0, 0	42 MPN/g	42 MPN/g
25°C	3, 3, 3, 3, 3, 3, 3, 2	3, 3, 3, 3, 3, 3, 3, 2	1.1 X 10^6 MPN/g	1.1 X 10^6 MPN/g
35°C	3, 3, 3, 3, 3, 3, 3, 3	3, 3, 2, 3, 3, 3, 3, 3	>1.1 X 10^6 MPN/g	>1.1 X 10^6 MPN/g *

[1] MPN values determined using the FDA-BAM MPN tables.

*An MPN of 3,3,3 for the Reference MPN was used for the 10^{-4}, 10^{-5} and 10^{-6} replicates. This MPN calculation assumes that the one 10^{-1} g MPN tube from which no confirmed *V. parahaemolyticus* strain was recovered was a failure to pick a true typical isolate present in the background of non-*V. parahaemolyticus* which exhibited typical morphology for the target. Since all three replicates for the MPN tubes up to 5 orders of magnitude more dilute than the 10-1 tube were culture confirmed, it is unlikely that the culture result from this one discordant tube was correct..

Table 4. BAX System Results for Oysters with MPN Testing *V. vulnificus* (3 tube, 8 dilution) (1)

Sample Set	BAX positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	Reference positive (10g, 1g, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6})	BAX MPN[1]	Reference MPN[1]
3°C	3, 3, 1, 0, 0, 0, 0, 0	3, 3, 1, 0, 0, 0, 0, 0	4.6 MPN/g	4.6 MPN/g
25°C	3, 3, 3, 3, 3, 1, 0, 0	3, 3, 3, 3, 3, 1, 0, 0	4,200 MPN/g	4,200 MPN/g
35°C	3, 3, 3, 3, 3, 2, 0, 1	3, 2, 3, 3, 3, 2, 0, 1	14,000 MPN/g	14,000 MPN/g *

[1] MPN values determined using the FDA-BAM MPN tables

* An MPN of 2,0,1 for the Reference MPN was used for the 10^{-4}, 10^{-5} and 10^{-6} replicates. This MPN calculation assumes that the one 1 g MPN tube from which no confirmed *V. vulnificus* strain was recovered was a failure to pick a true typical isolate present in the background of non-*V. vulnificus* which exhibited typical morphology for the target. Since all three replicates for the MPN tubes up to 3 orders of magnitude more dilute than the 10-1 tube were culture confirmed, it is unlikely that the culture result from this one discordant tube was correct.

Table 5. BAX vs. Reference Results Aggregate (1)

Sample type	Target Level by MPN or cfu per 25 gram	Samples or Number of MPN Tubes	BAX pos	Reference pos	Sensitivity %[1]	Specificity %[2]	False Pos %[3]	False Neg %[4]	Chi Square[5]
Tuna	0.5 MPN/25g	20	3	3	100	100	0	0	-
	1.9 MPN /25g	20	13	13	100	100	0	0	-
	3.75 MPN /25g	20	19	19	100	100	0	0	-
	0 cfu/25g	5	0	0		100	0	0	-
Tuna (Independent Laboratory Study)	MPN/25g	20	9	9	100	100	0	0	-
	0 cfu/25g	5	0	0		100	0	0	-
Frozen raw shrimp	Naturally contaminated	20	5	5	100	100	0	0	-
Cooked shrimp (MPN)	1.8 cfu/g	45	5	5	100	100	0	0	-
Cooked shrimp (25g)	1.8 cfu/g	5	5	5	100		0	0	-
Cooked shrimp (MPN)	18 cfu/g	45	14	14	100	100	0	0	-
Cooked shrimp (25g)	18 cfu/g	5	5	5	100		0	0	-
Frozen Scallops (MPN)	1.4 x 10^4 cfu/g	45	3	3	100	100	0	0	-
Frozen Scallops (25g)	1.4 x 10^4 cfu/g	5	5	5	100		0	0	-
Oysters 3°C	Naturally contaminated – *V. parahaemolyticus*	24	10	10	100	100	0	0	-
Oysters 25°C Abuse		24	23	23	100	100	0	0	-
Oysters 35°C Abuse		24	24	23	100	96	4	0	0
Oysters 3°C	Naturally contaminated – *V. vulnificus*	24	7	7	100	100	0	0	-
Oysters 25°C Abuse		24	16	16	100	100	0	0	-
Oysters 35°C Abuse		24	18	17	100	94	6	0	0

[1] *Sensitivity - Total number of confirmed positive test portions by the method divided by total number of confirmed positive test portions by both the alternative and reference methods.*

[2] Specificity - Total number of analyzed negative test portions by the method divided by total number of confirmed negative test portions by both the alternative and reference methods.

[3] False negative rate is calculated as BAX (-) Ref (+) BAX enrichment samples / Tot Ref (+) samples

[4] False positive rate is calculated as BAX (+) Ref (-) / Tot Ref (-) samples

[5] McNemar Chi-Square test statistic used for calculating significance of results

Table 6. Inclusivity Results for *Vibrio choleraee/parahaemolyticus/vulnificus* (1)

Strain ID	Other strain designation	Source	Location of testing	Species (serotype)	Result *V. cholerae*	Result *V. parahaemolyticus*	Result *V. vulnificus*
VcJVY212		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
VcJVB52		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
Vc5439/62		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
Vc569B		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
VcS171		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
VcNAG12		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
VcATCC25874		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
Vc8		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
VcB1307 Dacca		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
VcA5		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
VcI10		Unknown	UF	*V. choleraee*	Pos	Neg	Neg

Vc646 Ogawa01		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
Vc395 Classical Ogawa01		Unknown	UF	*V. choleraee*	Pos	Neg	Neg
TD3192		Unknown	Qualicon	*V. choleraee*	Pos	Neg	Neg
TD7000	ATCC 9459	Unknown	Qualicon	*V. choleraee*	Pos	Neg	Neg
DD9892		Unknown	Qualicon	*V. choleraee*	Pos	Neg	Neg
DD13084	ATCC 14035	Unknown	Qualicon	*V. choleraee*	Pos	Neg	Neg
TD3161		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3162		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3163		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3164		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3165		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3167		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3170		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3171		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3173		Unknown	Qualicon	*V. choleraee (non-O1, O139)*	Pos	Neg	Neg
TD3180		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3183		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3185		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3186		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3187		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3858		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3859		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3860		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3861		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3862		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3863		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3864		Unknown	Qualicon	*V. choleraee O1*	Pos	Neg	Neg
TD3203		Unknown	Qualicon	*V. choleraee O139*	Pos	Neg	Neg
TD3211		Unknown	Qualicon	*V. choleraee O139*	Pos	Neg	Neg
TD3213		Unknown	Qualicon	*V. choleraee O139*	Pos	Neg	Neg
TD3214		Unknown	Qualicon	*V. choleraee O139*	Pos	Neg	Neg
VpTx2103		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
VpTx3547		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
VpDAL1094		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
Vp17802		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
Vp43996		Unknown	UF	*V. parahaemolyticus*	Neg	Pos	Neg
DD2633	ATCC 17802	Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3129		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3130		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3131		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3132		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3133		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3134		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3135		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3153		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3154		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3155		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3156		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3157		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3159		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
TD3160		Unknown	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
Vv FLA141		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
Vv FLA126		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA134		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
Vv Fla 129		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA127		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA135		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA115		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA149		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvB3-313/98		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA121		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA137		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvB3-302/99		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA119		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA116		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA102		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvB2-2		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos
VvFLA108		Unknown	UF	*V. vulnificus*	Neg	Neg	Pos

TD3121		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3148		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3149		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3204		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3207		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3208		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3210		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3212		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3217		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD3219		Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
TD4527	ATCC 27562	Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13082	ATCC BAA-86	Unknown	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13231		Shrimp	Qualicon	*V. cholerae*	Pos	Neg	Neg
DD13232		Shrimp	Qualicon	*V. cholerae*	Pos	Neg	Neg
DD13208		Shrimp	Qualicon	*V. cholerae*	Pos	Neg	Neg
DD13209		Shrimp	Qualicon	*V. cholerae*	Pos	Neg	Neg
DD13212		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13216		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13217		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13218		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13211		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13222		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13223		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13224		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13225		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13226		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13228		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13229		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13230		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13233		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13234		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13235		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13236		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13204		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13207		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13200		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13202		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13201		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13203		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13211		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13214		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13215		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13210		Shrimp	Qualicon	*V. parahaemolyticus*	Neg	Pos	Neg
DD13205		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13206		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13227		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos
DD13213		Shrimp	Qualicon	*V. vulnificus*	Neg	Neg	Pos

Table 7. Inclusivity Results for *Vibrio choleraee/parahaemolyticus/vulnificus* (1)

Strain ID	Other strain designation	Source	Species	Result *V. cholerae*	Result *V. parahaemolyticus*	Result *V. vulnificus*
DD2558		Unknown	*Citrobacter freundii*	Neg	Neg	Neg
DD383		Unknown	*Citrobacter freundii*	Neg	Neg	Neg
DD2560		Unknown	*Citrobacter kosei*	Neg	Neg	Neg
DD2561		Unknown	*Citrobacter kosei*	Neg	Neg	Neg
DD12835		Unknown	E. coli O157:H7	Neg	Neg	Neg
DD1450		Unknown	E. coli O157:H7	Neg	Neg	Neg
DD1979		Unknown	E. coli O157:H7	Neg	Neg	Neg
TD8136		Unknown	E. coli O157:H7	Neg	Neg	Neg
DD2554		Unknown	*Enterococcus faecalis*	Neg	Neg	Neg
DD6523		Unknown	*Klebsiella oxytoca*	Neg	Neg	Neg
DD2546		Unknown	*Klebsiella pneumoniae*	Neg	Neg	Neg
DD1144		Unknown	*Listeria monocytogenes*	Neg	Neg	Neg
DD1283		Unknown	*Listeria monocytogenes*	Neg	Neg	Neg
DD1309		Unknown	*Listeria monocytogenes*	Neg	Neg	Neg
DD3572	ATCC 9459	Unknown	*Listeria innocua*	Neg	Neg	Neg
DD3376		Unknown	*Listeria ivanovii*	Neg	Neg	Neg
DD2874	ATCC 14035	Unknown	*Listeria seeligeri*	Neg	Neg	Neg
DD3354		Unknown	*Listeria welshimeri*	Neg	Neg	Neg
DD3411		Unknown	*Listeria welshimeri*	Neg	Neg	Neg
DD2357		Unknown	*Proteus mirabilis*	Neg	Neg	Neg
DD374		Unknown	*Proteus mirabilis*	Neg	Neg	Neg
DD13148		Unknown	*Pseudomonas aeruginosa*	Neg	Neg	Neg
DD3982		Unknown	*Pseudomonas aeruginosa*	Neg	Neg	Neg
DD3019		Unknown	*Salmonella ser.* Dublin	Neg	Neg	Neg
DD706		Unknown	*Salmonella ser.* Enteritidis	Neg	Neg	Neg
DD1261		Unknown	*Salmonella ser.* Newport	Neg	Neg	Neg
DD13060		Unknown	*Salmonella ser.* Senftenburg	Neg	Neg	Neg
DD586		Unknown	*Salmonella ser.* Typhimurium	Neg	Neg	Neg
DD1083		Unknown	*Shigella flexneri*	Neg	Neg	Neg
DD699		Unknown	*Shigella soneii*	Neg	Neg	Neg
DD10156		Unknown	*Staphylococcus aureus*	Neg	Neg	Neg
DD7426		Unknown	*Staphylococcus aureus*	Neg	Neg	Neg
DD9775		Unknown	*Staphylococcus aureus*	Neg	Neg	Neg
DD11233		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3146		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3195		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3200		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD3658		Unknown	*Vibrio alginolyticus*	Neg	Neg	Neg
TD4501		Unknown	*Vibrio anguillarum*	Neg	Neg	Neg
TD4498		Unknown	*Vibrio carchariae*	Neg	Neg	Neg
TD3194		Unknown	*Vibrio damsela*	Neg	Neg	Neg
TD4524		Unknown	*Vibrio damsela*	Neg	Neg	Neg
DD2631		Unknown	*Vibrio fluvialis*	Neg	Neg	Neg
TD4526		Unknown	*Vibrio fluvialis*	Neg	Neg	Neg
TD4497		Unknown	*Vibrio harveyi*	Neg	Neg	Neg
DD11232		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
DD13083		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD3137	ATCC 17802	Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD3147		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD3216		Unknown	*Vibrio mimicus*	Neg	Neg	Neg
TD4500		Unknown	*Vibrio natriegens*	Neg	Neg	Neg
TD4528		Unknown	*Vibrio pelagia*	Neg	Neg	Neg
TD4523		Unknown	*Vibrio tubiashii*	Neg	Neg	Neg
DD2399		Unknown	*Yersinia aldovae*	Neg	Neg	Neg
DD592		Unknown	*Yersinia enterocolitica*	Neg	Neg	Neg

DISCUSSION OF MODIFICATION APPROVED JULY 2013 (5)

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 (5)

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 *Vibrio choleraee/ parahaemolyticus/ vulnificus*	Shrimp	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
	Scallops	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
	Ahi tuna	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	0.18, 0.18
		Low	17	3	0.18	0.062, 0.41	4	0.24	0.10, 0.47	0.059	-.032, 0.21
		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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