CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

040702

The AOAC Research Institute hereby certifies the method known as:

BAX® System Real-Time PCR Assay for *Campylobacter jejuni*, *coli*, and *lari*

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 *Campylobacter jejuni*, *coli*, and *lari*
Formerly DuPont™ BAX® System Real-Time PCR Assay for *Campylobacter jejuni*, *coli*, and *lari*

CATALOG NUMBERS
BAX® System Assay KIT2018 (D12683449)

INDEPENDENT LABORATORY
Cherney Microbiological Services
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APPLICABILITY OF METHOD
Target organism – *Campylobacter jejuni, C. coli, and C. lari.*

Matrixes – Ready-to-eat turkey product (25 g), chicken carcass rinses (30 mL)

Performance claims – Sensitivity equivalent to the reference ISO culture-based method and specificity ≥ 99%.

REFERENCE METHOD

International Organization for Standardization (ISO) (2006) ISO FDIS 10272-1: Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method (4)

ORIGINAL CERTIFICATION DATE
April 16, 2007

CERTIFICATION RENEWAL RECORD
Renewed through December 2025.

METHOD MODIFICATION RECORD

1. **July 2013 Level 2**
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**
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. **Editorial updates to inserts, manuals, and labels for Hygiena.**
4. **Editorial updates to inserts and corporate address update.**
5. **Editorial changes.**
6. **Editorial changes.**
7. **Editorial changes.**
8. **Editorial changes.**

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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 [2]. 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 [3]. 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 target DNA and associated primers. This Internal Positive Control (INPC) must be shown to amplify in the absence of a 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 multi-dye 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 [3]. The BAX system Q7 instrument uses multiple filters to measure signal at the end of each cycle and report results for each target in less than 90 minutes.

DISCUSSION OF THE VALIDATION STUDY (1)

Results from the method comparison studies demonstrate BAX system performance that is statistically indistinguishable from the ISO FSIS 10272-1 (2006) reference method for detection of Campylobacter in sliced vacuum packaged turkey and chicken rinses. Several discordant results were found in the chicken rinses. It is likely that these results were due to sampling error (failure to have any target cells in the sub-sample of rinse inoculated in either the test or reference enrichment). All BAX positive samples were found to culture confirm, with the exception of the BAX enrichment from the external laboratory study, even if their paired reference enrichment sample was negative for the presence of *Campylobacter*. The one sample which did not confirm was positive at both BAX time points, had high levels of presumptive *Campylobacter* byy direct plating, and the paire ISO enrichment from the sample was demonstrated to contain *Campylobacter*.
The inclusivity/exclusivity study showed 100% agreement with expected results for the test panel
Lot-to-lot and stability studies showed consistent performance. The ruggedness study demonstrated that the BAX system was not sensitive to changes in factors most likely to adversely impact assay performance including lysis and protease inactivation temperatures, lysis sample volume, and PCR sample volume. Initial ruggedness testing revealed that incubation only slightly above the originally suggested incubation temperature of 42±2°C gave inconsistent results at the high incubation temperature abuse condition of 45°C. In order to reduce the risk of similar issues as users run the assay, and to highlight the sensitivity of this portion of the assay, the suggested incubation range was tightened to 42±1°C. Incubations at 44°C gave consistently positive results. The BAX System User Guide was edited to reflect this change in temperature tolerance.

Table 5. Real-Time BAX *Campylobacter* PCR Assay Inclusivity (1)

Strain #	Genus / Species	Source	BAX Result	Strain #	Genus / Species	Source	BAX Result
TD4604	*C. coli*	Avian	POS	TD6529	*C. coli*	Avian	POS
TD4631	*C. coli*	Avian	POS	TD6531	*C. coli*	Avian	POS
TD4923	*C. coli*	Avian	POS	TD6539	*C. coli*	Avian	POS
TD4928	*C. jejuni*	Avian	POS	TD6540	*C. coli*	Avian	POS
TD4937	*C. jejuni*	Avian	POS	TD6551	*C. jejuni*	Avian	POS
TD4960	*C. jejuni*	Avian	POS	TD6553	*C. jejuni*	Avian	POS
TD6295	*C. jejuni*	Avian	POS	TD6555	*C. jejuni*	Avian	POS
TD6296	*C. jejuni*	Avian	POS	TD6557	*C. jejuni*	Avian	POS
TD6297	*C. jejuni*	Avian	POS	TD6560	*C. jejuni*	Avian	POS
TD6300	*C. jejuni*	Avian	POS	TD6561	*C. jejuni*	Avian	POS
TD6301	*C. jejuni*	Avian	POS	TD6562	*C. lari*	Avian	POS
TD6308	*C. coli*	Avian	POS	TD6563	*C. lari*	Avian	POS
TD6311	*C. coli*	Avian	POS	TD6564	*C. lari*	Avian	POS
TD6312	*C. coli*	Avian	POS	TD6566	*C. lari*	Avian	POS
TD6321	*C. coli*	Avian	POS	TD6567	*C. lari*	Avian	POS
TD6423	*C. lari*	Avian	POS	TD6568	*C. lari*	Avian	POS
TD6424	*C. lari*	Avian	POS	TD6569	*C. lari*	Avian	POS
TD6425	*C. lari*	Avian	POS	TD6570	*C. lari*	Avian	POS
TD6481	*C. lari*	Clinical	POS	TD6571	*C. lari*	Avian	POS
TD6483	*C. lari*	Clinical	POS	TD6577	*C. lari*	Avian	POS
TD6484	*C. lari*	Clinical	POS	TD6622	*C. lari*	Avian	POS
TD6485	*C. lari*	Clinical	POS	TD7012	*C. jejuni*	Avian	POS
TD6486	*C. lari*	Clinical	POS	TD7018	*C. jejuni*	Avian	POS
TD6525	*C. coli*	Avian	POS	TD7019	*C. jejuni*	Avian	POS
TD6526	*C. coli*	Avian	POS	TD7023	*C. coli*	Avian	POS
TD6527	*C. coli*	Avian	POS	TD7026	*C. jejuni*	Avian	POS

All from DuPont Qualicon Culture Collection

Table 6. Real-Time BAX *Campylobacter* PCR Assay exclusivity (1)

	Genus / Species	Source	BAX Result	ID #	Genus / Species	Source	BAX Result
DD 2901	*Bacillus cereus*	Cream cake	NEG	TD 6537	*Campylobacter fetus venerealis*	Unknown	NEG
ATCC 25408	*Citrobacter diversus*	Human clinical	NEG	ATCC BAA-1059	*Campylobacter upsaliensis*	human	NEG
ATCC 33379	*Edwardsiella hoshinae*	Avian	NEG	ATCC 33562	*Campylobacter sputorum*	Bovine	NEG
DD 10549	*Enterococcus cecorum*	Avian	NEG	ATCC 51210	*Campylobacter helveticus*	Feline	NEG
ATCC 35038	*Enterococcus gallinarum*	Avain	NEG	ATCC 43264	*Campylobacter mucosalis*	Porcine	NEG
DD 10674	*Enterococcus saccharolyticus*	Straw bedding - Avain	NEG	DD 6832	*Shigella sonnei*	Unknown	NEG
DD 1722	*Escherichia coli O127:HNM*	PSU E. coli Reference Laboratory	NEG	ATCC 43952	*Staphylococcus arlettae*	Avian	NEG
ATCC 33821	*Escherichia vulnaris*	Human clinical	NEG	ATCC 35539	*Staphylococcus gallinarum*	Avain	NEG
DD 6523	*Klebsiella oxytoca*	Ground beef	NEG	ATCC 9610	*Yersinia enterocolitica*	Human clinical	NEG
ATCC 33403	*Kurthia zopfii*	Avain	NEG	DD 2992	*Salmonella ser.* Lille		NEG
ATCC 19111	*Listeria monocytogenes*	Avain	NEG	DD 1261	*Salmonella ser.* Newport	Avain	NEG
DD 3064	*Morganella morganii*	Environmental swab	NEG	ATCC 49616	*Acrobacter butzleri*	Human clinical	NEG
DD 6121	*Proteus mirabilis*	Avain	NEG	TD 6513	*Arcobacter butzleri*	Unknown	NEG
ATCC 27853	*Pseudomonas aeruginosa*	Human clinical	NEG	TD 7030	*Arcobacter cryaerophilus*	Unknown	NEG
ATCC 43972	*Salmonella* enterica salame	Unknown	NEG	TD 7011	*Campylobacter fetus fetus*	Unknown	NEG
DD 1550	*Salmonella ser.* Abaetetuba	Unknown	NEG	TD 7013	*Campylobacter fetus fetus*	Unknown	NEG
DD 3017	*Salmonella ser.* Dublin	Unknown	NEG	ATCC 13076	*Salmonella ser.* Enteritidis	Unknown	NEG
TD 6536	*Campylobacter fetus venerealis*	Unknown	NEG	DD 626	*Lactobacillus viridescens*	Cured meat	NEG
DD 659	*Lactobacillus lactis*	Unknown	NEG	DD 687	*Lactobacillus carnis*	*Vacuum pack lamb*	NEG

Table 1a. Internal Study of Vacuum Packaged Sliced Turkey (25 g) Samples Tested with BAX System Compared with the ISO 10272-1:2006(E) Reference Method (1)

Enrichment Time	Method	Total	CFU / 25g inoculated	MPN / 25g[1]	BAX Assay Positive	Culture Confirmed[2]	Sensitivity %	False Neg %	False Pos %	Specificity %	Chi Square Test vs Reference
24 hr	BAX	20	7.6	0.4	9	10	90	10	0	100	0.1
		5	0		0	0		0	0		
48 hr	BAX	20	7.6	0.4	10	10	100	0	0	100	0.4
		5	0		0	0		0	0		
	ISO	20	7.6	0.4		8	100	0	0	100	
		5	0			0		0	0		
24 hr	BAX	20	76	52.5	20	20	100	0	0	100	0
		5	0		0	0		0	0		
48 hr	BAX	20	76	52.5	20	20	100	0	0	100	0
		5	0		0	0		0	0		
	ISO	20	76	52.5		20	0	0	0	100	
		5	0			0		0	0		

[1] A 3-tube MPN (with 100, 10, and 1, and 0.1 g test portions) was conducted using the reference method beginning 2 days post inoculation and run concurrently with the study enrichments

[2] BAX ® enrichments were confirmed using the ISO reference method plating media and subsequent ISO isolate confirmatory tests. Culture confirmed results are from 48 hr of liquid enrichment.

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

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

Sensitivity is calculated as 100% – false negative rate = 100%

Specificity is calculated as 100% – false positive rate = 100%

Table 2a. Internal Study of Naturally Contaminated Chicken Carcass Rinses Tested with BAX System Compared with the ISO 10272-1:2006(E) Reference Method (1)

Method	Total	Presump.Pos /Confirmed	Sensitivity %	False Neg %	False Pos %	Specificity %	Chi Square Test Method vs Culture
24 hr BAX vs culture from BAX enrichment	20	16/17	94	6	0	100	0
48 hr BAX vs culture from BAX enrichment	20	17/17	100	0	0	100	0.3
ISO	20	16	100	0	0	100	

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 *Campylobacter jejuni/coli/lari*	Chicken rinses	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	-0.18, 0.18
	Processed turkey	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, 1.00	17	1	0.82, 1.0	0	-0.18, 0.18
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.18, 0.18
		High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18

REFERENCES CITED

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4. International Organization for Standardization (ISO) (2006) ISO FDIS 10272-1: Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method.
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