CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

120701

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

BAX® System Real-Time PCR Assay for *Staphylococcus aureus*

manufactured by

Hygiena
2 Boulden Circle
New Castle, DE 19720
USA

This method has been evaluated in the AOAC® *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 05, 2019 – December 31, 2020). 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 05, 2019
Date

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KIT NAME(S)
DuPont™ BAX® System Real-Time PCR Assay for *Staphylococcus aureus*
March 01, 2017, BAX® System Real-Time PCR Assay for *Staphylococcus aureus*

CATALOG NUMBERS
BAX® System Assay KIT2020 (D12762689)

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APPLICABILITY OF METHOD
Target organism – *Staphylococcus aureus*

Matrices - ground beef, soy protein isolate, soy-based powedered infant formula, milk-based powdered infant formula

Performance claims - Sensitivity equivalent to the reference ISO or FDA-BAM culture-based methods and specificity ≥ 99%.

REFERENCE METHOD
International Organization for Standardization (ISO) (2004) ISO 6888-3: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 3: Detection and MPN technique for low numbers (4)

ORIGINAL CERTIFICATION DATE
December 03, 2007

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2020

METHOD MODIFICATION RECORD
1. **July 2013**
2. **March 2017 Level 1**
3. **December Level 1 Renewal Modification**
4. **May 2019 Level 1**
5. **December 2019 Level 1 Renewal Modification**

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 insert updates and corporate address**
5. **Editorial/clerical changes.**

Under this AOAC® *Performance Tested*℠ License Number, 120701 this method is distributed by:
NONE

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NONE

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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 [2].

The BAX® system PCR tablets used in real-time assays including this test kit also contain dye-linked 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

Results from the method comparison studies demonstrate BAX® system performance that is statistically indistinguishable from the ISO 6888-1: 2003 (E), ISO 6888-3: 2003 (E), and FDA-BAM reference methods for detection of S. aureus in ground beef, powdered infant formula, and soy protein isolate for comparably enriched samples. In addition, for infant formula, the enrichment of non-diluted samples was found to allow for the analysis of a larger weight of sample without any apparent negative effect on S. aureus growth or PCR detection. This method was significantly more sensitive, despite the use of the same amount of enrichment media and should thus be considered as an alternate enrichment method where applicable. All BAX ® positive samples were found to culture confirm, with the exception of one BAX ® enrichment from the independent laboratory study, which was characterized by the independent laboratory as a contamination event and upon re-test gave the expected result which was in concordance with the culture confirmation. The inclusivity/exclusivity study showed 100% agreement with expected results for the test panel. For infant formula enrichments, adding product directly to the enrichment media (10 g vs 10 ml of a 1:10 dilution of the product) was found to give a significant improvement in the detection indicating no growth or pcr issues with this modification.

Lot-to-lot and stability (stability study data used from the Campylobacter PTM evaluation as reagents used were chemically analogous in every meaningful way) 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 as well as the enrichment condition of increased and decreased temperature outside the method defined.

Table 6. Composite Table of Samples Tested with the BAX ® System Method for the Detection of *Staphylococcus aureus* Compared with the ISO 6888-1:2003(E), ISO 6888-3:2003(E), and FDA-BAM Reference Methods (1)

Matrix	Methoc	Total	MPN / 1g	Amount Assayed	BAX ® Assay Positive	Culture Confirmed	Sensitivity %	False Neg %	False Pos %	Specificity %	Chi Square Test vs Reference
Ground Beef (Naturally Contaminated)	BAX	20	0.092	1 ml (0.1 g)	18/20	18/20	100	0	0	100	0.0
	ISO	20	0.092	1 ml (0.1 g)	-	18/20	-	-	-	-	
	BAX	20	2.4	10 ml (1.0 g)	12/20	12/20	100	0	0	100	0
	BAX	5	0	10 ml (1.0 g)	0/5	0/5			0		
Soy-Based Powdered Infant Formula	BAX	20	2.4	10 g	20/20	20/20	100	0	0	100	9.75
	BAX	5	0	10 g	0/5	0/5			0		
	ISO	20	2.4	10 ml (1.0 g)	-	12/20					
	ISO	5	0	10 ml (1.0 g)	-	0/5					
	BAX	20	0.43	10 ml (1.0 g)	10/20	10/20	100	0	0	100	0
	BAX	5	0	10 ml (1.0 g)	0/5	0/5			0		
Milk-Based Powdered Infant Formula	BAX	20	0.43	10 g	20/20	20/20	100	0	0	100	13
	BAX	5	0	10 g	0/5	0/5			0		
	ISO	20	0.43	10 ml (1.0 g)	-	10/20					
	ISO	5	0	10 ml (1.0 g)	-	0/5					
	BAX	20	2.4	1 ml (0.1 g)	16/20	16/20	100	0	0	100	0
Soy Protein Isolate	BAX	5	0	1 ml (0.1 g)	0/5	0/5			0		
	BAM	20	2.4	1 ml (0.1 g)	-	16/20					
	BAM	5	0	1 ml (0.1 g)	-	0/5					
	BAX	20	0.93	10 ml (1.0 g)	19/20	19/20	100	0	0	100	1.08
	BAX	5	0	10 ml (1.0 g)	0/5	0/5			0		
Milk-Based Powdered Infant Formula (Independent Study)	BAX	20	0.93	10 g	20/20	20/20	100	0	0	100	1.54
	BAX	5	0	10 g	0/5	0/5			0		
	ISO	20	0.93	10 ml (1.0 g)		17/20					
	ISO	5	0	10 ml (1.0 g)		0/5					

Table 7. *S. aureus* Inclusivity Panel (1)

Strain #	Isolate	Source	Result	Strain #	Isolate	Source	Result
DD10156	*S. aureus aureus*	ATCC 12600 Clinical	POS	TD1467	*S. aureus*	Processed Food	POS
DD1096	*S. aureus*	Unknown	POS	TD1468	*S. aureus*	Processed Food	POS
DD1098	*S. aureus*	Unknown	POS	TD1469	*S. aureus*	Processed Food	POS
DD1379	*S. aureus_ Coagulase negative*	Unknown	POS	TD7445	*S. aureus*	ATCC 33591 Clinical	POS
DD1385	*S. aureus_ Coagulase negative*	Unknown	POS	TD7446	*S. aureus*	ATCC 43300 Clinical	POS
DD4160	*S. aureus*	Howler monkey	POS	TD1472	*S. aureus*	Clinical	POS
DD613	*S. aureus*	Chicken	POS	TD1527	*S. aureus*	Clinical	POS
DD912	*S. aureus*	Unknown	POS	TD1528	*S. aureus*	Clinical	POS
DD9766	*S. aureus*	Agricultural	POS	TD1529	*S. aureus*	Clinical	POS
DD9769	*S. aureus*	Ovine mastitis	POS	TD1530	*S. aureus*	Clinical	POS
DD9771	*S. aureus*	Clinical	POS	TD7426	*S. aureus*	ATCC 13709	POS
DD9772	*S. aureus*	Clinical	POS	TD1471	*S. aureus*	Processed Food	POS
DD9774	*S. aureus*	Clinical	POS	DD9780	*S. aureus*	Clinical	
DD9775	*S. aureus*	Clinical	POS	TD7447	*S. aureus*	ATCC 29213	POS
DD9776	*S. aureus*	Clinical	POS	TD7448	*S. aureus*	ATCC 10390	POS
DD9778	*S. aureus*	Clinical	POS	TD1470	*S. aureus*	Processed Food	POS
TD7449	*S. aureus*	ATCC 14154 Clinical	POS	DD9784	*S. aureus*	Clinical	POS
TD7450	*S. aureus*	ATCC 19636 Clinical	POS	DD9781	*S. aureus*	Clinical	POS
TD7451	*S. aureus*	ATCC 25923 Clinical	POS	TD7454	*S. aureus*	ATCC 29247 CDC	POS
DD9803	*S. aureus*	Agricultural	POS	TD7550	*S. aureus*	Unknown	POS
DD9867	*S. aureus*	Mixed vegetables	POS	TD 13043*	*S. aureus*	Ground Beef	POS
TD 13044*	*S. aureus*	Ground Beef	POS	TD 13045*	*S. aureus*	Ground Beef	POS
TD 13046*	*S. aureus*	Ground Beef	POS	TD 13047*	*S. aureus*	Ground Beef	POS
TD 13048*	*S. aureus*	Ground Beef	POS	TD 13049*	*S. aureus*	Ground Beef	POS
TD 13050*	*S. aureus*	Ground Beef	POS	TD 13051*	*S. aureus*	Ground Beef	POS
TD 13052*	*S. aureus*	Ground Beef	POS	TD 13053*	*S. aureus*	Ground Beef	POS
TD 13054*	*S. aureus*	Ground Beef	POS				

Ground Beef isolates indicated are those obtained during methods development for this study. All isolates included are independent in origin and have novel Riboprint ™ patterns by EcoRI molecular subtyping using the DuPont Qualicon Riboprinter ™.
All confirmed *S. aureus* isolates obtained during methods development demonstrated a positive BAX ® test kit result.

Table 8. *S. aureus* Exclusivity Panel (1)

Strain #	Isolate	Source	Result	Strain #	Isolate	Source	Result
DD871	*Staphylococcus arlettae*	Poultry	NEG	DD854	*Staphylococcus gallinarum*	Poultry	NEG
DD7397	*Staphylococcus auricularis*	Unknown	NEG	DD668	*Staphylococcus haemolyticus*	Human	NEG
DD872	*Staphylococcus auricularis*	Human	NEG	DD865	*Staphylococcus hominis*	Human	NEG
DD873	*Staphylococcus capitis*	Human	NEG	DD887	*Staphylococcus hyicus*	Swine	NEG
DD4213	*Staphylococcus caprae*	Human	NEG	DD856	*Staphylococcus intermedius*	Avian	NEG
DD4214	*Staphylococcus caprae*	Human	NEG	DD8423	*Staphylococcus saprophyticus*	Urine	NEG
DD874	*Staphylococcus caprae*	Goat milk	NEG	DD861	*Staphylococcus schleiferi*	Clinical	NEG
DD1092	*Staphylococcus carnosus*	Fermented sausage	NEG	DD1113	*Staphylococcus sciuri*	Human	NEG
DD1093	*Staphylococcus carnosus*	Fermented sausage	NEG	DD4277	*Staphylococcus scuiri*	Squirrel	NEG
DD1094	*Staphylococcus carnosus*	Dry sausage	NEG	DD8357	*Staphylococcus simulans*	Human	NEG
DD1095	*Staphylococcus carnosus*	Fermented sausage	NEG	DD5366	*Staphylococcus warneri*	Human	NEG
DD851	*Staphylococcus chromogenes*	Swine	NEG	DD756	*Staphylococcus xylosus*	Unknown	NEG
DD8455	*Staphylococcus cohnii*	Human	NEG	DD913	*Staphylococcus xylosus*	Human	NEG
DD2704	*Staphylococcus delphini*	Unknown	NEG	DD4510	*Macrococcus caseolyticus*	Unknown	NEG
DD864	*Staphylococcus epidermidis*	Nose	NEG	DD4508	*Macrococcus caseolyticus*	Unknown	NEG
DD2636	*Staphylococcus felis*	Cat	NEG	TD 5946	*Staphylococcus piscifermentans*	Fermented shrimp	NEG
DD659	*Lactobacillus lactis*	Unknown	NEG	DD 687	*Lactobacillus carnis*	Vacuum pack lamb	NEG
DD626	*Lactobacillus viridescens*	Cured meat	NEG	DD558	*Pseudomonas diminuta*	Unknown	NEG
TD1517	*Stenotrophomonas maltophilia*	Environmental	NEG	DD563	*Pseudomonas putida*	Unknown	NEG
TD1518	*Stenotrophomonas maltophilia*	Environmental	NEG	DD576	*Pseudomonas mendocino*	Unknown	NEG
TD2936	*Shewanella putrefaciens*	Unknown	NEG	DD577	*Pseudomonas stutz*	Unknown	NEG
TD2965	*Shewanella putrefaciens*	Unknown	NEG	DD3982	*Pseudomonas aeruginosa*	Unknown	NEG

DISCUSSION OF JULY 2013 MODIFICATION (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 *Staphylococcus aureus*	Ground beef	High	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Low	17	17	1	0.82, 1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.18, 0.18
	Soy infant formula	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
	Powdered infant formula	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	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

REFERENCES CITED

1. Wallace, Morgan, Dambaugh, Tim, Tice, George, Andaloro, Bridget, Fallon, Dawn, Davis, Eugene, and Wang, Siqun., Evaluation of the DuPont™ BAX® System Real-Time PCR Assay for *Staphylococcus aureus*, AOAC® *Performance Tested℠* certification number 120701.
2. Innis, MA , and Gelfand, DH (1989) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, Burlington, MA.
3. Livak, K.J., Flood, S.J.A., Marmaro, J., and Mullah, K.B., inventors Perkin-Elmer Corporation (Foster City, CA), assignee. (1999) Hybridization assay using self-quenching fluorescence probe. United States patent 5,876,930.
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