

CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

100201

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

BAX[®] System PCR Assay for *Salmonella*
BAX[®] System PCR Assay for *Salmonella* 2
BAX[®] System X5 PCR Assay for *Salmonella*

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 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

METHOD AUTHORS

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MODIFICATION NOVEMBER 2008 – F. Morgan Wallace, Siqun Wang, George Tice, Bridget Andaloro, Jack Janes, Viviana Fino, and Eugene Davis
MODIFICATION APRIL 2009 – Morgan Wallace
MODIFICATION JUNE 2009 – Linda X. Peng, George Tice, Morgan Wallace, Bridget Andaloro, Dawn Fallon, Lois Fleck, and Dan Delduco
MODIFICATION OCTOBER 2010 – Frank R. Burns, Andrew Famum, George Tice, Bridget Andaloro, Eugene Davis, Jeff Rohrbeck, and Morgan Wallace
MODIFICATION NOVEMBER 2015 – Jeff Rohrbeck, Alain Minelli, Eugene Davis, Gongbo Wang, Lois Fleck, Dawn Fallon, Steve Hoeizer, and Morgan Wallace

SUBMITTING COMPANY

Qualicon, Inc.
3531 Silverside Road
Bedford Building
Wilmington, DE 19810

CURRENT SPONSOR

Hygiena
2 Boulden Circle
New Castle, DE 19720
USA

KIT NAME(S)

DuPont™ BAX® System PCR Assay for *Salmonella*
DuPont™ BAX® System PCR Assay for *Salmonella* 2
DuPont™ BAX® System X5 PCR Assay for *Salmonella*
BAX® System PCR Assay for *Salmonella*
BAX® System PCR Assay for *Salmonella* 2
BAX® System X5 PCR Assay for *Salmonella*

CATALOG NUMBERS

BAX® System Salmonella 1 Kit KIT2012 (D11000133), BAX® System Salmonella 2 Kit KIT2011 (D14368501), BAX® System X5 Assay KIT2025 (D15407187), MP Media MED2003 (D12404925)

INDEPENDENT LABORATORY

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2015 Modification: Yi Chen, USDA CFSAN, College Park, Maryland, USA

APPLICABILITY OF METHOD

Target organism – *Salmonella*

Matrices – Original Validation - (25 g) black pepper, custard, 2% milk, chilled ready meal, chipped ham, chocolate, cooked chicken, raw ground chicken, hot dogs, nonfat dry milk, orange juice, peanut butter, alfalfa sprouts, cooked fish, prawns, macaroni, pizza dough, frozen peas, dry pet food, soy protein flour, ground beef, spinach, lettuce, liquid egg, cream cheese, peanut butter (ERV 2009)
(65 g) beef trim
(4 x 4 inch sponge) concrete, stainless steel, ceramic tile, plastic, epoxy material affixed to a support matrix

Performance claims – Method performs equivalent to reference methods.

REFERENCE METHODS

US Department of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science. Microbiology Laboratory Guidebook [Internet]. Washington: The Dept; c1998 [rev 2001 January 10; cited 2002 August 27]. Chapter 4, Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg Products [about 14 screens]. Available from <http://www.fsis.usda.gov/OPHS/microlab/mlgchp4.pdf>. (6)
US Food & Drug Administration, Center for Food Safety & Applied Nutrition. Bacteriological Analytical Manual Online [Internet]. Washington: The Admin; c1998 [rev 2001 October; cited 2002 August 27]. Chapter 5, *Salmonella* [about 12 screens]. Available from <http://vm.cfsan.fda.gov/~ebam/bam-5.htm> (7)

ORIGINAL CERTIFICATION DATE	CERTIFICATION RENEWAL RECORD
November 2002	Renewed annually through December 2020
METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
<ol style="list-style-type: none"> 2006 November 2008 April 2009 June 2009 October 2010 September 2013 November 2015 March 2017 Level 1 January 2018 Level Renewal Modification May 2019 Level 1 December 2019 Level 1 Renewal Modification 	<ol style="list-style-type: none"> Addition of Q7 BAX® Instrument Additional of Environmental sponges using BAX® and BAX® Q7 Matrix Extension to include Peanut Butter Screening of beef and produce using BAX® System PCR Assay for <i>E. coli</i> O157:H7 MP enrichment media and protocol. Addition of hot start functionality Inclusion of DuPont™ Thermal Block for automated sample lysis Certification of BAX® X5 instrument Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiene company Editorial updates to Inserts, manuals, and labels to Hygiene Editorial updates to inserts and corporate address Editorial/clerical changes.
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PRINCIPLE OF THE METHOD (1)

The BAX® System uses the Polymerase Chain Reaction (PCR) to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. The fragment is a genetic sequence that is unique to *Salmonella*, thus providing a highly reliable indicator that the organism is present. The automated BAX® System then uses fluorescent detection [2] to analyze PCR product for positive or negative results. (The non-automated BAX® system, which uses gel-detection methods, has already received AOAC-RI Performance Tested certification [3].)

PCR offers the potential for rapid and definitive detection of *Salmonella*, and has been demonstrated to be effective for other organisms [5,6]. In order to realize this potential, however, some of the drawbacks of PCR must be overcome. The first of these is the complexity of the PCR procedure itself. The second is the potential for false positives due to contamination with one or more molecules of amplified PCR product in the laboratory. This most often happens during the many pipetting steps associated with traditional PCR. The BAX® System with automated detection alleviates these drawbacks by combining the reagents needed for the PCR process into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed for the detection phase, thus significantly reducing the potential for contamination.

The BAX® System combines primers, polymerase and nucleotides needed for PCR into a single tablet. The specificity of a PCR assay is determined by the DNA sequences of the primers employed. The basis for primer design for the BAX® System assay is a collection of 1572 strains of *Salmonella*, which have been analyzed by ribotyping [5]. This large, well-characterized culture collection has facilitated the design of primers that can detect all strains of *Salmonella*, while remaining highly specific for *Salmonella* and no other genus.

The product of the PCR reaction is automatically analyzed by fluorescent detection. Each PCR tablet contains a fluorescent dye, which binds with double-stranded DNA and emits a signal in response to excitation light. During the automated detection phase, the temperature of the samples is slowly increased to denature the DNA, which releases the dye causing a drop in emission signal. The temperature of denaturation and the magnitude of fluorescent signal change are measured, and the BAX® system software analyzes those data to determine a positive or negative result.

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

The method comparison studies, both internal and external, support equivalence of the BAX® system method to the reference method for detection of *Salmonella* in the 19 foods analyzed from the same original pre-enrichment. All chi-square values were below the required level of 3.84 indicating no statistically significant difference at $P \leq 0.05$.

The details of the performance indicator calculations are shown below:

Accuracy is the fraction of confirmed positive samples that are correctly identified by the detection method. In this study, confirmation is the reference method.

Method positives / confirmed positives = $527/530 = 99.4\%$

False negative rate is calculated as BAX (-), Ref (+) samples / Total Ref (+) samples = $7/530 = 1.3\%$

False positive rate is calculated as BAX (+), Ref (-) samples / Total Ref (-) samples = $4/245 = 1.6\%$

Sensitivity is calculated as $1 - \text{false negative rate} = 100 - 1.3 = 98.7\%$.

Specificity is calculated as $1 - \text{false positive rate} = 100 - 1.6 = 98.3\%$.

Alfalfa sprouts were analyzed using a Z-test for Differences in Means of Two Populations [13] with a 5% significance level. The results indicate no significant difference in recovery rate.

The independent lab method comparison was done for ground beef and liquid egg. Again there was no significant difference by chi-square analysis indicating equivalent detection sensitivity and specificity.

The inclusivity/exclusivity study showed 100% agreement with expected results for the 194 *Salmonella* strains and the 35 non-*Salmonella* strains.

The ruggedness study demonstrated that the BAX® system was not sensitive to changes in lysis temperature, lysis sample volume, nor PCR sample volume. Each of the variations tested was beyond the tolerances indicated for the assay, i.e., +/- 1°C for the heaters and +/-10% for the volumes.

Table 3 . Method performances (1)

Sample			Samples Positive				Chi sq ^b	Sensitivity rate ^c		Indicence of false negatives among total positive samples, % ^d		Specificity rate ^e
			Total	BAX system		Ref. ^a		BAX®	Ref.	BAX	Ref.	BAX
				Pres. ^a	Conf. ^a							
Alfalfa sprouts	Low	1.08	20	15	15	4	0	100	26.7	0	73.3	100
	High	5.25	20	18	18	20	0.5	90	100	10	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Ground Beef	Low	1.08	20	18	18	19	0	94.7	100	5.3	0	100
	High	11.5	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Cooked chicken	Low	1.08	20	13	13	13	0	100	100	0	0	100
	High	2.3	20	17	17	17	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Custard	Low	6	20	14	14	15	0	93.3	100	6.7	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Chilled ready meal	Low	6	20	18	18	18	0	100	100	0	0	100
	High	23.75	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Chocolate	Low	0.575	20	7	7	8	0	87.5	100	12.5	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Liquid eggs	Low	1.075	20	18	18	18	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Cooked fish	Low	2.3	20	13	13	13	0	100	100	0	0	100
	High	27.5	20	17	17	17	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Frankfurters	Low	2.3	20	10	10	10	0	100	100	0	0	100
	High	0.375	20	18	18	18	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Chipped ham	Low	2.3	20	12	12	12	0	100	100	0	0	100
	High	2.3	20	17	17	17	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	0	–	–	–	–	–

Table 3. Method performances (cont'd.) (1)												
			Samples Positive				Chi sq ^b	Sensitivity rate ^c		Indicence of false negatives among total positive samples, % ^d		Specificity rate ^e
			Total	BAX system		Ref. ^a		BAX®	Ref.	BAX	Ref.	BAX
Sample	Level	MPN/g		Pres. ^a	Conf. ^a		BAX®					
Elbow macaroni	Low	1.08	20	11	11	12	0	91.7	100	8.3	0	100
	High	11.5	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	0	–	–	–	–	–
Liquid milk	Low	1.08	20	9	9	9	0	100	100	0	0	100
	High	10.75	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Nonfat dry milk	Low	1.08	20	15	15	16	0	93.8	100	6.2	0	100
	High	2.33	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Orange juice	Low	6	20	18	18	19	0	94.7	100	5.3	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Peanut butter	Low	1.88	20	17	17	17	0	100	100	0	0	100
	High	11.5	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Frozen peas	Low	0.53	20	5	5	5	0	100	100	0	0	100
	High	0.98	20	12	12	13	0	92.3	100	7.7	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Black pepper	Low	6	20	19	19	19	0	100	100	0	0	100
	High	10.75	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	0	–	–	–	–	–
Dry pet food	Low	0.575	20	13	13	13	0	100	100	0	0	100
	High	2.325	20	20	20	20	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Pizza dough	Low	0.95	20	13	13	11	0	100	84.6	0	15.4	100
	High	23.25	20	20	20	18	0	100	90.0	0	10.0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–
Seafood - prawns	Low	0.375	20	5	5	5	0	100	100	0	0	100
	High	1.075	20	15	15	15	0	100	100	0	0	100
	Control	< 0.003	0	0	0	0	–	–	–	–	–	–

^aPres. = presumptive, Conf. = confirmed

^bChi Sq = Chi square as defined by McNemar – (|a-b|-1)²/(a+b) where a = samples positive by BAX and negative by the reference method, and b = samples negative by BAX and positive by the reference method. A chi square value greater than 3.84 indicates significance at P<0.05.

^cSensitivity rate is defined as the total number of positive test samples among the confirmed positive samples for a method divided by the total number of confirmed positive samples by either method.

^dIncidence of false negatives = 100 – sensitivity rate.

^eSpecificity rate is defined as the number of negative samples by the BAX method divided by the number of confirmed negative samples by both BAX and reference method.

Table 4. BAX® System Inclusivity (1)									
Strain number	Salmonella serovar	O-Group	Source	BAX result	Strain number	Salmonella serovar	O-Group	Source	BAX result
919	<i>S. paratyphi A</i>	A	unknown	+	1331	<i>S. berta</i>	D1	sausages	+
1548	<i>S. abony</i>	B	unknown	+	1641	<i>S. durban</i>	D1	feces	+
1335	<i>S. agona</i>	B	chicken	+	1236	<i>S. enteritidis</i>	D1	duck	+
1360	<i>S. agona</i>	B	chicken	+	741	<i>S. gallinarum</i>	D1	unknown	+
1531	<i>S. altendorf</i>	B	unknown	+	1691	<i>S. inverness</i>	D1	feces	+
1553	<i>S. ball</i>	B	unknown	+	1701	<i>S. miami</i>	D1	unknown	+
1338	<i>S. brandenburg</i>	B	milk	+	966	<i>S. napoli</i>	D1	unknown	+
1435	<i>S. brandenburg</i>	B	unknown	+	1476	<i>S. napoli</i>	D1	unknown	+
964	<i>S. bredeney</i>	B	raw chicken	+	1482	<i>S. pullorum</i>	D1	chicken liver	+
1356	<i>S. bredeney</i>	B	pork	+	1507	<i>S. pullorum</i>	D1	unknown	+
1238	<i>S. heidelberg</i>	B	chicken	+	4029	<i>S. dublin</i>	D1	unknown	+
1244	<i>S. heidelberg</i>	B	unknown	+	3792	<i>S. enteritidis</i>	D1	chicken	+
1655	<i>S. reading</i>	B	unknown	+	3794	<i>S. enteritidis</i>	D1	chicken	+
1330	<i>S. saintpaul</i>	B	bean sprouts	+	4013	<i>S. enteritidis</i>	D1	chicken	+
1372	<i>S. saintpaul</i>	B	milk powder	+	4020	<i>S. enteritidis</i>	D1	chicken	+
1478	<i>S. saintpaul</i>	B	unknown	+	4022	<i>S. enteritidis</i>	D1	mayonaise	+
1237	<i>S. typhimurium</i>	B	raw egg	+	2294	<i>S. pullorum</i>	D1	unknown	+
1467	<i>S. typhimurium</i>	B	unknown	+	1558	<i>S. canastel</i>	D1	feed	+
4084	<i>S. africana</i>	B	unknown	+	1552	<i>S. alabama</i>	D2	unknown	+
3920	<i>S. agona</i>	B	environmental	+	1530	<i>S. amager</i>	E1	unknown	+
4033	<i>S. brandenburg</i>	B	unknown	+	1649	<i>S. lexington</i>	E1	unknown	+
2316	<i>S. bredeney</i>	B	coconut	+	1652	<i>S. london</i>	E1	unknown	+
3461	<i>S. heidelberg</i>	B	egg yolk	+	1705	<i>S. muenster</i>	E1	unknown	+
3868	<i>S. heidelberg</i>	B	poultry feed	+	3851	<i>S. anatum</i>	E1	chicken	+
3871	<i>S. heidelberg</i>	B	chicken	+	3907	<i>S. anatum</i>	E1	poultry feed	+
4038	<i>S. heidelberg</i>	B	chicken	+	3919	<i>S. anatum</i>	E1	chicken	+
3852	<i>S. indiana</i>	B	poultry feed	+	3922	<i>S. anatum</i>	E1	environmental	+
3898	<i>S. neumuenster</i>	B	poultry feed	+	4025	<i>S. anatum</i>	E1	chicken	+
2645	<i>S. reading</i>	B	turkey intestine	+	2189	<i>S. give</i>	E1	unknown	+
2637	<i>S. swarzensgrund</i>	B	chicken	+	2353	<i>S. kristianstad</i>	E1	unknown	+
2641	<i>S. swarzensgrund</i>	B	chicken	+	3883	<i>S. lexington</i>	E1	poultry feed	+
2647	<i>S. swarzensgrund</i>	B	llama	+	2676	<i>S. orion</i>	E1	chicken feed	+
3537	<i>S. typhimurium</i>	B	unknown	+	4012	<i>S. orion</i>	E1	chicken	+
3854	<i>S. typhimurium</i>	B	poultry feed	+	1491	<i>S. weltevreden</i>	E1	prawns	+
3858	<i>S. typhimurium</i>	B	environmental	+	1248	<i>S. anatum</i>	E2	unknown	+
4017	<i>S. typhimurium</i>	B	unknown	+	1332	<i>S. anatum</i>	E2	shrimp	+
1526	<i>S. austin</i>	C1	unknown	+	1365	<i>S. anatum</i>	E2	paprika	+
1510	<i>S. bareilly</i>	C1	unknown	+	1366	<i>S. anatum</i>	E2	chicken	+
2181	<i>S. bareilly</i>	C1	unknown	+	1085	<i>S. binza</i>	E2	dried spice	+
1329	<i>S. branderup</i>	C1	dried egg	+	1707	<i>S. newbrunswick</i>	E2	unknown	+

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1628	<i>S. colorado</i>	C1	unknown	+	3903	<i>S. binza</i>	E2	poultry feed	+
1235	<i>S. infantis</i>	C1	avian meal	+	2349	<i>S. drypool</i>	E2	unknown	+
1255	<i>S. montevideo</i>	C1	egg	+	4610	<i>S. newbrunswick</i>	E2	cereal	+
1260	<i>S. montevideo</i>	C1	animal feed	+	6177	<i>S. arkansas</i>	E3	chicken giblets	+
1267	<i>S. montevideo</i>	C1	chicken	+	2639	<i>S. thomasville</i>	E3	turkey intestine	+
1274	<i>S. montevideo</i>	C1	animal feed	+	3924	<i>S. thomasville</i>	E3	poultry feed	+
1492	<i>S. montevideo</i>	C1	unknown	+	740	<i>S. senftenberg</i>	E4	unknown	+
1710	<i>S. oranienburg</i>	C1	unknown	+	3882	<i>S. broughton</i>	E4	poultry feed	+
1336	<i>S. thompson</i>	C1	chicken	+	1624	<i>S. chandans</i>	F	unknown	+
3458	<i>S. braenderup</i>	C1	egg albumin	+	1712	<i>S. pretoria</i>	F	pig	+
3003	<i>S. choleraesuis</i>	C1	unknown	+	2220	<i>S. senftenberg</i>	F	unknown	+
3984	<i>S. choleraesuis</i>	C1	gallbladder	+	2340	<i>S. senftenberg</i>	F	coconut	+
3985	<i>S. choleraesuis</i>	C1	unknown	+	1562	<i>S. montgomery</i>	F	unknown	+
3986	<i>S. choleraesuis</i>	C1	unknown	+	1489	<i>S. poona</i>	G1	clinical isolate	+
3987	<i>S. choleraesuis</i>	C1	unknown	+	1632	<i>S. cubana</i>	G2	chicken	+
3869	<i>S. infantis</i>	C1	poultry feed	+	1251	<i>S. kedougou</i>	G2	turkey	+
3902	<i>S. infantis</i>	C1	poultry feed	+	1703	<i>S. mississippi</i>	G2	feces	+
4028	<i>S. infantis</i>	C1	liquid egg	+	2245	<i>S. havana</i>	G2	pancake	+
2263	<i>S. lille</i>	C1	pancake	+	2271	<i>S. havana</i>	G2	pet food	+
3155	<i>S. lille</i>	C1	environmental	+	3876	<i>S. havana</i>	G2	poultry feed	+
4036	<i>S. livingstone</i>	C1	chicken	+	4039	<i>S. kedougou</i>	G2	chicken	+
3870	<i>S. mbandaka</i>	C1	poultry feed	+	1509	<i>S. bovismorbificans</i>	H	unknown	+
3878	<i>S. mbandaka</i>	C1	poultry feed	+	1621	<i>S. carrau</i>	H	unknown	+
6206	<i>S. mbandaka</i>	C1	chicken giblets	+	1686	<i>S. fayed</i>	H	unknown	+
3760	<i>S. montevideo</i>	C1	unknown	+	1620	<i>S. carmel</i>	J	unknown	+
4027	<i>S. montevideo</i>	C1	chicken	+	1622	<i>S. cerro</i>	K	unknown	+
3892	<i>S. ohio</i>	C1	poultry feed	+	3459	<i>S. cerro</i>	K	whole egg	+
3863	<i>S. othmarschen</i>	C1	environmental	+	3460	<i>S. cerro</i>	K	whole egg	+
3874	<i>S. othmarschen</i>	C1	poultry feed	+	3926	<i>S. cerro</i>	K	poultry feed	+
3536	<i>S. tennessee</i>	C1	unknown	+	1557	<i>S. chicago</i>	M	unknown	+
6687	<i>S. tennessee</i>	C1	sesame seeds	+	6696	<i>S. cotham</i>	M	unknown	+
3866	<i>S. thompson</i>	C1	environmental	+	1469	<i>S. ealing</i>	O	dried milk	+
738	<i>S. virchow</i>	C1	unknown	+	1543	<i>S. adelaide</i>	O	unknown	+
1257	<i>S. virchow</i>	C1	turkey	+	3884	<i>S. adelaide</i>	O	poultry feed	+
1551	<i>S. aequatoria</i>	C2	unknown	+	3885	<i>S. adelaide</i>	O	poultry feed	+
1521	<i>S. amersfoort</i>	C2	unknown	+	1684	<i>S. emmastad</i>	P	unknown	+
1343	<i>S. blockley</i>	C2	environment	+	1429	<i>S. anfo</i>	Q	box meat	+
1344	<i>S. blockley</i>	C2	chicken	+	1623	<i>S. champaign</i>	Q	chicken liver	+
1424	<i>S. manchester</i>	C2	yeast	+	2313	<i>S. wandsworth</i>	Q	unknown	+
1653	<i>S. manhatan</i>	C2	unknown	+	1695	<i>S. johannesburg</i>	R	unknown	+
1261	<i>S. newport</i>	C2	duck	+	1608	<i>S. seminole</i>	R	snake feces	+
1262	<i>S. newport</i>	C2	cotton seed	+	2346	<i>S. vietnam</i>	S	unknown	+
1481	<i>S. newport</i>	C2	raw burger	+	1523	<i>S. berkeley</i>	U	diseased turkey	+
3916	<i>S. hadar</i>	C2	chicken	+	1680	<i>S. dugbe</i>	W	unknown	+
3917	<i>S. hadar</i>	C2	chicken	+	1569	<i>S.sp.</i>	ND	unknown	+
3918	<i>S. hadar</i>	C2	chicken	+	1568	<i>S. arizona</i>	ND	unknown	+
2673	<i>S. manhattan</i>	C2	avian	+	1525	<i>S. betioky</i>	ND	unknown	+

4014	<i>S. newport</i>	C2	chicken	+	1554	<i>S. branalia</i>	ND	unknown	+
6174	<i>S. newport</i>	C2	chicken giblets	+	1535	<i>S. brookfield</i>	ND	frog	+
1231	<i>S. hadar</i>	C3	turkey	+	1775	<i>S. sp.</i>	ND	unknown	+
3157	<i>S. corvalis</i>	C3	environmental	+	1616	<i>S. houten</i>	ND	bird feces	+
3915	<i>S. haardt</i>	C3	chicken	+	1566	<i>S. sp.</i>	ND	rattlesnake skin	+
3921	<i>S. haardt</i>	C3	environmental	+	2376	<i>S. sculcoates</i>	ND	unknown	+
2195	<i>S. kentucky</i>	C3	unknown	+	2379	<i>S. sp.</i>	ND	unknown	+
6250	<i>S. santiago</i>	C3	dried onion	+	2380	<i>S. sp.</i>	ND	unknown	+
6253	<i>S. santiago</i>	C3	dried onion	+	3804	<i>S. sp.</i>	ND	unknown	+
2229	<i>S. theilalle</i>	C4	unknown	+	3805	<i>S. sp.</i>	ND	unknown	+

Table 5. BAX® System exclusivity (1)

Strain DD#	Strain ID	Source	BAX result	Strain DD#	Strain ID	Source	BAX result
715	<i>Bacillus cereus</i>	unknown	-	6247	<i>Klebsiella spp.</i>	avian	-
6712	<i>Citrobacter freundii</i>	vegetables	-	659	<i>Lactococcus lactis</i>	unknown	-
8990	<i>Citrobacter freundii</i>	soil	-	6121	<i>Proteus mirabilis</i>	avian	-
2560	<i>Citrobacter freundii</i>	throat	-	2357	<i>Proteus mirabilis</i>	poultry	-
3097	<i>Citrobacter freundii</i>	Cake mix	-	6921	<i>Proteus mirabilis</i>	chicken	-
1074	<i>Enterobacter cloacae</i>	skim milk powder	-	960	<i>Providencia alcalifaciens</i>	prawns	-
3108	<i>Enterobacter cloacae</i>	cereal	-	295	<i>Pseudomonas aeruginosa</i>	unknown	-
3112	<i>Enterobacter cloacae</i>	unknown	-	9022	<i>Pseudomonas aeruginosa</i>	soil	-
1458	<i>Escherichia coli</i>	human bloody	-	569	<i>Pseudomonas fluorescens</i>	pre-filter tank	-
2443	<i>Escherichia coli</i>	unknown	-	7083	<i>Serratia marcesens</i>	unknown	-
2513	<i>Escherichia coli</i>	unknown	-	700	<i>Shigella sonnei</i>	unknown	-
3785	<i>Escherichia coli</i>	unknown	-	6723	<i>Shigella sonnei</i>	sandwich	-
3786	<i>Escherichia coli</i>	meninges - baby	-	6832	<i>Shigella sonnei</i>	unknown	-
3789	<i>Escherichia coli</i>	unknown	-	1396	<i>Staphylococcus aureus</i>	margarine	-
3790	<i>Escherichia coli</i>	unknown	-	765	<i>Staphylococcus warneri</i>	chicken	-
6719	<i>Escherichia hermannii</i>	sesame seeds	-	3990	<i>Stomatococcus mucilaginosus</i>	pharynx	-
6588	<i>Hafnia alvei</i>	raw vegetables	-	7120	<i>Yersinia enterocolitica</i>	unknown	-
6708	<i>Hafnia alvei</i>	vegetables	-				

DISCUSSION OF MODIFICATION APPROVED 2006 (8)

The data in these studies, within their statistical uncertainty, support the product claims and demonstrate that the BAX® system *Salmonella* pcr test kit when used with the Q7 instrument is comparable to standard reference methods for detecting *Salmonella* in a variety of foods. All chi-square values were below the required level of 3.84 indicating no statistically significant difference at $P \leq 0.05$. In addition the assay demonstrated excellent inclusivity and exclusivity with all *Salmonella* isolates screened testing positive and all non-*Salmonella* isolates testing negative.

Table 1. Inclusivity test panel for BAX® <i>Salmonella</i> test kit using the Q7 Instrument (8)				
DD#	Other Designation (Non-Qualicon Origin)	Serotype	Source (where available)	Result
586		<i>Salmonella</i> Typhimurium	Chicken hearts and livers	POS
706	ATCC 13076	<i>Salmonella</i> Enteritidis	Human clinical	POS
707	ATCC 6962	<i>Salmonella</i> Newport	Human clinical fatal	POS
725	ATCC 13314	<i>Salmonella</i> Arizonae	NCTC	POS
917	ATCC 13312	<i>Salmonella</i> Choleraesuis	NCTC	POS
1084		<i>Salmonella</i> Typhimurium	Unknown	POS
1261		<i>Salmonella</i> Newport	Duck	POS
1329		<i>Salmonella</i> Branderup	Dried egg	POS
1330		<i>Salmonella</i> Saintpaul	Bean sprouts	POS
1331		<i>Salmonella</i> Berta	Sausages	POS
1332		<i>Salmonella</i> Anatum	Shrimp	POS
1335		<i>Salmonella</i> Agona	Chicken	POS
1336		<i>Salmonella</i> Thompson	Chicken	POS
1338		<i>Salmonella</i> Brandenburg	Milk	POS
1343		<i>Salmonella</i> Blockley	Environment	POS
1344		<i>Salmonella</i> Blockley	Chicken	POS
1356		<i>Salmonella</i> Bredeney	Pork	POS
1360		<i>Salmonella</i> Agona	Chicken	POS
1365		<i>Salmonella</i> Anatum	Paprika	POS
1366		<i>Salmonella</i> Anatum	Chicken	POS
1372		<i>Salmonella</i> Saintpaul	Milk powder	POS
1424		<i>Salmonella</i> Manchester	Yeast	POS

1429		<i>Salmonella</i> Anfo	Box meat	POS
1435		<i>Salmonella</i> Brandenburg	Unknown	POS
1483	CRA 728 ¹	<i>Salmonella</i> Hadar	Unknown	POS
1492	CRA 72 ¹	<i>Salmonella</i> Montevideo	Unknown	POS
1662		<i>Salmonella</i> Tranoroa	Unknown	POS
1711		<i>Salmonella</i> Pomona	Turkey intestine	POS
1773	ATCC 43975	<i>Salmonella</i> Brookfield	Unknown	POS
1777	ATCC 43972	<i>Salmonella</i> Salamae	ATCC	POS
2181		<i>Salmonella</i> Bareilly	Unknown	POS
2274		<i>Salmonella</i> Anatum	ATCC	POS
2317		<i>Salmonella</i> Othmarschen	Unknown	POS
2341		<i>Salmonella</i> Barry	Unknown	POS
2380		<i>Salmonella</i> Sya	Unknown	POS
2628	ATCC 9263	<i>Salmonella</i> Kentucky	ATCC	POS
2791		<i>Salmonella</i> Binza	Chicken	POS
2824		<i>Salmonella</i> Cerro	Chicken	POS
2992		<i>Salmonella</i> Lille	Unknown	POS
3017		<i>Salmonella</i> Dublin	Unknown	POS
3019		<i>Salmonella</i> Dublin	Unknown	POS
3096		<i>Salmonella</i> Tennessee	Unknown	POS
3984	ATCC 8759	<i>Salmonella</i> Choleraesuis	Gall bladder	POS
3988	ATCC 13428	<i>Salmonella</i> Choleraesuis	ATCC	POS
4022		<i>Salmonella</i> Enteritidis	Mayonnaise	POS
4102		<i>Salmonella</i> Species	Nuts	POS
5533		<i>Salmonella</i> Infantis	Thyme	POS
7005		<i>Salmonella</i> Dublin	CMCC	POS
8034		<i>Salmonella</i> Species	Raw chicken	POS
8047		<i>Salmonella</i> Cubana	Oats	POS

¹ Campden & Chorleywood Food Research Association Culture Collection (CRA)

Table 2. Exclusivity test panel for BAX® *Salmonella* test kit using the Q7 Instrument (8)

DD#	Other Designation (Non-Qualicon Origin)	Serotype	Source (where available)	Result
295		<i>Pseudomonas aeruginosa</i>	Unknown	NEG
569	ATCC 13525	<i>Pseudomonas fluorescens</i>	Pre-filter tank	NEG
715	ATCC 14579	<i>Bacillus cereus</i>	Unknown	NEG
765		<i>Staphylococcus warneri</i>	Chicken	NEG
846	ATCC 29907	<i>Escherichia blattae</i>	Insect	NEG
960		<i>Providencia alcalifaciens</i>	Prawns	NEG
1074		<i>Enterobacter cloacae</i>	Skim milk powder	NEG
1396		<i>Staphylococcus aureus</i>	Margarine	NEG
2357		<i>Proteus mirabilis</i>	Poultry	NEG
2443		<i>E.coli O157 :H19</i>	PSU Reference Lab	NEG
2560	ATCC 25408	<i>Citrobacter diversus</i>	Throat Swab	NEG
3097		<i>Citrobacter freundii</i>	Cake mix	NEG
3790		<i>Escherichia coli</i>	Unknown	NEG
3990	ATCC 25296	<i>Stomatococcus mucilaginosus</i>	Pharynx	NEG
6121		<i>Proteus mirabilis</i>	Avian	NEG
6588		<i>Hafnia alvei</i>	Raw vegetables	NEG
6719		<i>Escherichia hermanii</i>	Sesame seeds	NEG
6832		<i>Shigella sonnei</i>	Unknown	NEG
7083		<i>Serratia marcesens</i>	Unknown	NEG
7120		<i>Yersinia enterocolitica</i>	Unknown	NEG

Table 3. Results of method comparison on 3 food matrices (8)					
Frankfurters					
		BAX pos	BAX neg	Total	
MPN = 3.8 / 25g	MLG pos	18	0	18	Chi-square = 0.00 Sensitivity = 100%
APC = TFTC	MLG neg	0	2	2	
Est. APC ¹ = 0	Total	18	2	20	
Negative Controls					
	MLG pos	0	0	0	Specificity = 100%
	MLG neg	0	5	5	False positive = 0%
	Total	0	5	5	False negative = 0%
Raw Ground Chicken					
		BAX pos	BAX neg	Total	
MPN = 1.1 / 25g	MLG pos	10	0	10	Chi-square = 0.00 Sensitivity = 100%
APC ² = 2.5 X 10 ⁶ cfu/g	MLG neg	0	10	10	
APC ³ = 1.8 X 10 ⁷ cfu/g	Total	10	10	20	
Negative Controls					
	MLG pos	0	0	0	Specificity = 100%
	MLG neg	0	5	5	False positive = 0%
	Total	0	5	5	False negative = 0%
Orange Juice					
		BAX pos	BAX neg	Total	
MPN = 0.38 / 25 ml	BAM pos	11	0	11	Chi-square = 0.00 Sensitivity = 100%
APC = TFTC	BAM neg	0	9	9	
Est. APC = 15 cfu/ml	Total	11	9	20	
Negative Controls					
	MLG pos	0	0	0	Specificity = 100%
	MLG neg	0	5	5	False positive = 0%
	Total	0	5	5	False negative = 0%

¹ Est. APC – Results reported are from aerobic plate count plates that have too few colonies to accurately count from the most concentrated dilution (the 1:10 initial dilution in Preenrichment broth).

² At prescreening for *Salmonella*.

³ At start of experiment.

DISCUSSION OF JUNE 2008 MODIFICATION (9)

For laboratory contaminated samples, all LB enrichments that would ultimately be confirmed as containing *Salmonella* through the reference method protocol were positive by the BAX® test method (tables 3a and 3c). No statistically significant differences were obtained when comparing the BAX® test kit and the FDA-BAM reference method for any of the studies performed except for the naturally contaminated LB protocol with the standard *Salmonella* re-growth procedure. This protocol performed significantly differently (with a chi-square value of > 3.84 indicative of significance at the 95% confidence level) than the reference method. A modified re-growth protocol in which 1 ml of the LB enrichment is added to 9 ml of BHI and the re-growth time is extended from 3 to 5 hours was found to improve the method performance to give statistical, though not absolute, equivalence. In order to achieve absolute, as opposed to statistical, equivalence BPW enrichment with the standard re-growth protocol was used to support *Salmonella* growth to BAX® detectable levels in these high background microflora sample types (Tables 3b and 3d). Studies using BPW and LB for the detection of *Salmonella* from artificially contaminated “clean” surfaces, however, indicated better performance with LB (data not shown but available upon request). Qualicon thus recommends that in testing from the less microbiologically complex finished product side of manufacturing facilities (surfaces similar to the artificially contaminated samples), LB enrichment should be used for best results, but when testing for the presence of *Salmonella* from concrete surfaces with heavy levels of competing microbial flora from the raw ingredients side of a manufacturing facility, BPW should be used. Environmental sponges collected from surface types other than concrete and containing high levels of non-*Salmonella* competing microbial flora would likely behave in a similar fashion; however validation studies should be performed by end-users of this test kit on their particular surface type(s) other than concrete to determine whether the conclusions of this study are applicable to their matrices.

Table 3a Method performance for the detection of <i>Salmonella</i> species from environmental surfaces by the BAX® System. Artificially inoculated surfaces. BAX® testing was performed from the same LB enrichments used for subsequent culture confirmation as per the FDA-BAM. (9)										
Surface	Level (cfu applied per analytical unit)	Total Samples (each treatment)	BAX® Presumptive (# positive)	BAX® Confirmed (# positive)	Reference Method (# positive)	Sensitivity % ¹	Specificity % ²	False Negative % ³	False Positive % ⁴	χ ² Value ⁵
Ceramic Tile	114	20	15	15	15	100	100	0	0	-
	0	5	0	0	0		100	0	0	
Plastic	162	20	13	13	13	100	100	0	0	-
	0	5	0	0	0		100	0	0	
Stainless Steel	50	20	13	13	13	100	100	0	0	-
	0	5	0	0	0		100	0	0	
Stainless Steel (Independent Laboratory)	2.7	20	8	7	7	100	92.3	0	7.7	0.0
	0	5	0	0	0		100	0	0	
Epoxy Coated Flooring	836	20	10	10	10	100	100	0	0	-
	0	5	0	0	0		100	0	0	

¹ Sensitivity is calculated as 100% – false negative rate = 100%

² Specificity is calculated as 100% – false positive rate = 100%

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 calculated for paired enrichments

Table 3b Method performance for the detection of <i>Salmonella</i> species from environmental surfaces by the BAX® System. Naturally contaminated surfaces. (9)										
Surface	Re-growth Scheme	Total Samples (each treatment)	BAX® Presumptive (# positive)	BAX® Confirmed (# positive)	Reference Method (# positive)	Sensitivity %	Specificity %	False Negative %	False Positive %	χ ² Value ^{1,2}
Concrete (LB Enrichment)	10 ul in 500 ul 3 hr	20	6	4	10	40	80	60	20	1.1
Concrete (LB Enrichment)	1 ml in 9 ml 5 hr	20	9	8	10	80	90	20	10	0.0
Concrete (BPW Enrichment)	10 ul in 500 ul 3 hr	20	10	10	10	100	100	0	0	0.0

¹ McNemar Chi-Square test statistic calculated for paired enrichments (used for calculating data from single enrichments using LB)

² Mantel-Haenszel Chi-Square test statistic (used for calculating significance of results for adjacently collected sponges enriched in BPW for the test method)

Table 3c Method performance for the detection of <i>Salmonella</i> species from environmental surfaces by the BAX Q7® System. Artificially inoculated surfaces. BAX® testing was performed from the same LB enrichments used for subsequent culture confirmation as per the FDA-BAM. (9)										
Surface	Level (cfu applied per analytical unit)	Total Samples (each treatment)	BAX® Presumptive (# positive)	BAX® Confirmed (# positive)	Reference Method (# positive)	Sensitivity %	Specificity %	False Negative %	False Positive %	χ ² Value
Ceramic Tile	114	20	15	15	15	100	100	0	0	-
	0	5	0	0	0		100	0	0	
Plastic	162	20	13	13	13	100	100	0	0	-
	0	5	0	0	0		100	0	0	
Stainless Steel	50	20	13	13	13	100	100	0	0	-
	0	5	0	0	0		100	0	0	
Stainless Steel (Independent Laboratory)	2.7	20	7	7	7	100	100	0	0	-
	0	5	0	0	0		100	0	0	
Epoxy Coated Flooring	836	20	10	10	10	100	100	0	0	-
	0	5	0	0	0		100	0	0	

Table 3d Method performance for the detection of <i>Salmonella</i> species from environmental surfaces by the BAX Q7® System. Naturally contaminated surfaces. (9)										
Surface	Re-growth Scheme	Total Samples (each treatment)	BAX® Presumptive (# positive)	BAX® Confirmed (# positive)	Reference Method (# positive)	Sensitivity %	Specificity %	False Negative %	False Positive %	χ ² Value
Concrete (LB Enrichment)	10 ul in 500 ul 3 hr	20	7	5	10	40	80	60	20	0.6
Concrete (LB Enrichment)	1 ml in 9 ml 5 hr	20	9	8	10	80	90	20	10	0.0
Concrete (BPW Enrichment)	10 ul in 500 ul 3 hr	20	10	10	10	100	100	0	0	0.0

DISCUSSION OF APRIL 2009 MODIFICATION (10)

In this three laboratory collaborative study, results obtained were consistent across the participating sites. Each site also demonstrated identical results between platforms (BAX® and BAX® Q7 instruments). In the 135 samples assayed, one false negative result was found at one of the participating laboratories. It is not known whether this result is due to a candidate method related issue (*Salmonella* cell density reached a high enough level to make the transfer into the BAM secondary enrichments, but did not reach a level high enough to trigger a positive result by PCR or a failure to transfer from the correct primary enrichment to the BHI re-growth tube), or due to a contamination event in the reference method (though this seems unlikely since the discordant sample demonstrated positive results from both the TT and RV secondary enrichment broths). Since the theoretical limit of detection of the reference method from the primary LB enrichment is on the order of 1 cfu/ml (since 1 ml is transferred to the TT secondary enrichment broth tube) and the theoretical limit of detection of the BAX® method from the primary LB enrichment is on the order of 10³ cfu/ml, it may be possible that the one discordant sample fell into this category. Another possibility is that the sample preparation may have been responsible. Since the sample matrix of peanut butter is very hydrophobic, if there is a less than complete homogenization of the sample in the enrichment broth, then the *Salmonella* cell(s) may not be exposed to the media for some period of the enrichment. Sample preparation is thus a critical step in the detection of *Salmonella* from this matrix (much more so than for matrices that easily go into solution, or are only susceptible to surface contamination) by this candidate method, and presumably by any other rapid method. Overall, though, the results of this study demonstrate that the BAX® System method is comparable to the reference culture method for the detection of *Salmonella* from peanut butter even when the viable cell count approaches 1 cfu per analytical portion.

Modification Validation Data Approved April 2009 (10)

Table 1. Summary Data Table – Qualicon, BAX® Instrument

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	20	20	20	20	1.00	1.00	1.00	1.00	-
Low	1.08	20	2	2	2	1.00	1.00	1.00	1.00	-
Control	-	5	0	0	0	-	1.00	-	1.00	-

Table 2. Summary Data Table – Q Laboratories, BAX® Instrument

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	20	20	20	20	1.00	1.00	1.00	1.00	-
Low	1.08	20	5	4	5	0.80	1.00	1.00	1.00	0
Control	-	5	0	0	0	-	1.00	-	1.00	-

Table 3. Summary Data Table – retch laboratories, BAX® Instrument

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	20	18	18	18	1.00	1.00	1.00	1.00	-
Low	1.08	20	4	4	4	1.00	1.00	1.00	1.00	-
Control	-	5	0	0	0	-	1.00	-	1.00	-

Table 4. Summary Data Table – Aggregate Data, BAX® Instrument (10)

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	60	58	58	58	1.00	1.00	1.00	1.00	-
Low	1.08	60	11	10	11	0.91	1.00	1.00	1.00	0
Control	-	15	0	0	0	-	1.00	-	1.00	-

Table 5. Summary Data Table – Qualicon, BAX® Q7 Instrument (10)

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	20	20	20	20	1.00	1.00	1.00	1.00	-
Low	1.08	20	2	2	2	1.00	1.00	1.00	1.00	-
Control	-	5	0	0	0	-	1.00	-	1.00	-

Table 6. Summary Data Table – Q Laboratories, BAX® Q7 Instrument (10)

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	20	20	20	20	1.00	1.00	1.00	1.00	-
Low	1.08	20	5	4	5	0.80	1.00	1.00	1.00	0
Control	-	5	0	0	0	-	1.00	-	1.00	-

Table 7. Summary Data Table – retch laboratories, BAX® Q7 Instrument (10)

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	20	18	18	18	1.00	1.00	1.00	1.00	-
Low	1.08	20	4	4	4	1.00	1.00	1.00	1.00	-
Control	-	5	0	0	0	-	1.00	-	1.00	-

Table 8. Summary Data Table – Aggregate Data, BAX® Q7 Instrument (10)

Level	MPN per 25 g	Replicates per method	RM Pos.	CM Pres. Pos.	CM Conf. Pos.	CM Sens.	CM Spec.	RM Sens.	RM Spec.	χ ²
High	11.5	60	58	58	58	1.00	1.00	1.00	1.00	-
Low	1.08	60	11	10	11	0.91	1.00	1.00	1.00	0
Control	-	15	0	0	0	-	1.00	-	1.00	-

DISCUSSION OF JUNE 2009 MODIFICATION (11)

The results in these studies, within their statistical uncertainty, support the product claims that the BAX® System is an effective method and comparable to standard reference methods for detecting *Salmonella* in beef and produce enriched in the BAX® System *E. coli* O157:H7 MP media and for detecting *Salmonella* in soy protein flour when enriched in lactose broth as primary enrichment followed by 3-hour re-growth in BHI broth.

Table 2. Internal study of spiked and unspiked spinach (25 g) samples tested with BAX® system method and FDA- BAM reference culture methods. (0.016 MPN^a/g) (11)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed ^b	Sensitivity ^c %	False Neg ^d %	Presump. Pos /Unspiked	Specificity ^e %	False Pos ^f %	Chi-square ^g
8 hr	BAX® Classic	20	7/7	87.5	12.5	0/5	100	0	0.1
	BAX® Q7	20	8/8	100	0	0/5	100	0	0
10 hr	BAX® Classic	20	8/8	100	0	0/5	100	0	0
	BAX® Q7	20	8/8	100	0	0/5	100	0	0
24 hr	BAX® Classic	20	8/8	100	0	0/5	100	0	0
	BAX® Q7	20	8/8	100	0	0/5	100	0	0
FDA-BAM		20	8	--	--	0/5	--	--	--

^a Most probable number of colony forming units per test portion.

^b Presump Pos: Positive by BAX® System assay for BAX® enrichments. Confirmed: At least one confirmed *E. coli* O157:H7 isolate was obtained by culture.

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

^d False negative rate: 100 minus sensitivity rate.

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

^f False positive rate: 100 minus specificity rate

^g Chi-square: Mantel-Haenszel chi square. *Chi-square value > 3.84 indicates significance at P < 0.05.

Table 3. Internal study of spiked and unspiked iceberg lettuce (25 g) samples tested with BAX® system method and FDA- BAM reference culture methods. (0.092 MPN^a/g) (11)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed ^b	Sensitivity ^c %	False Neg ^d %	Presump. Pos /Unspiked	Specificity ^e %	False Pos ^f %	Chi-square ^g
7.5 hr	BAX® Classic	20	6/6	75	25	0/5	100	0	1.63
	BAX® Q7	20	6/6	75	25	0/5	100	0	1.63
10 hr	BAX® Classic	20	8/8	100	0	0/5	100	0	0.39
	BAX® Q7	20	8/8	100	0	0/5	100	0	0.39
24 hr	BAX® Classic	20	8/8	100	0	0/5	100	0	0.39
	BAX® Q7	20	8/8	100	0	0/5	100	0	0.39
FDA-BAM		20	10	--	--	0/5	--	--	--

^a Most probable number of colony forming units per test portion.

^b Presump Pos: Positive by BAX® System assay for BAX® enrichments. Confirmed: At least one confirmed *E. coli* O157:H7 isolate was obtained by culture.

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

^d False negative rate: 100 minus sensitivity rate.

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

^f False positive rate: 100 minus specificity rate

^g Chi-square: Mantel-Haenszel chi square. *Chi-square value > 3.84 indicates significance at P < 0.05.

Table 4. Internal study of spiked and un-spiked ground beef (25 g) samples tested with BAX® system method and FDA-BAM reference culture methods. (0.02 MPN ^a /g) (11)									
Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed ^b	Sensitivity ^c %	False Neg ^d %	Presump. Pos /Unspiked	Specifi-city ^e %	False Pos ^f %	Chi-square ^g
8 hr	BAX® Classic	20	6/6	75	25	0/5	100	0	0.94
	BAX® Q7	20	5/5	62.5	37.5	0/5	100	0	1.71
10 hr	BAX® Classic	20	7/7	87.5	12.5	0/5	100	0	0.41
	BAX® Q7	20	7/7	87.5	12.5	0/5	100	0	0.41
12 hr	BAX® Classic	20	8/8	100	0	0/5	100	0	0.1
	BAX® Q7	20	7/7	87.5	12.5	0/5	100	0	0.41
24 hr	BAX® Classic	20	8/8	100	0	0/5	100	0	0.1
	BAX® Q7	20	8/8	100	0	0/5	100	0	0.1
FDA-BAM		20	9	--	--	0/5	--	--	--

^a Most probable number of colony forming units per test portion.

^b Presump Pos: Positive by BAX® System assay for BAX® enrichments. Confirmed: At least one confirmed *E. coli* O157:H7 isolate was obtained by culture.

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

^d False negative rate: 100 minus sensitivity rate.

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

^f False positive rate: 100 minus specificity rate

^g Chi-square: Mantel-Haenszel chi square. *Chi-square value > 3.84 indicates significance at P < 0.05.

Table 5. Internal study of spiked and un-spiked beef trim (65 g) samples tested with BAX® system method and FDA- BAM reference culture methods. (0.0092 MPN ^a /g) (11)									
Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed ^b	Sensitivity ^c %	False Neg ^d %	Presump. Pos /Unspiked	Specifi-city ^e %	False Pos ^f %	Chi-square ^g
8 hr	BAX® Classic	20	14/14	100	0	0/5	100	0	0.11
	BAX® Q7	20	13/13	92.9	7.1	0/5	100	0	0
10 hr	BAX® Classic	20	14/14	100	7.1	0/5	100	0	0.11
	BAX® Q7	20	13/13	92.9	14.3	0/5	100	0	0.1
12 hr	BAX® Classic	20	14/14	100	0	0/5	100	0	0.11
	BAX® Q7	20	14/14	100	0	0/5	100	0	0.11
24 hr	BAX® Classic	20	14/14	100	0	0/5	100	0	0.11
	BAX® Q7	20	14/14	100	7.1	0/5	100	0	0
FDA-BAM		20	13	--	--	0/5	--	--	--

^a Most probable number of colony forming units per test portion.

^b Presump Pos: Positive by BAX® System assay for BAX® enrichments. Confirmed: At least one confirmed *E. coli* O157:H7 isolate was obtained by culture.

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

^d False negative rate: 100 minus sensitivity rate.

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

^f False positive rate: 100 minus specificity rate

^g Chi-square: Mantel-Haenszel chi square. *Chi-square value > 3.84 indicates significance at P < 0.05.

Table 6. Internal study of spiked and un-spiked soy protein flour (25 g) samples tested with BAX® system method and FDA-BAM reference culture methods. (0.02 MPN/g) (11)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed ^b	Sensitivity ^c %	False Neg ^d %	Presump. Pos /Unspiked	Specifi -city ^e %	False Pos ^f %	Chi-square ^g
20 hr	BAX® Classic	20	8/8	100	0	0/5	100	0	0
FDA-BAM		20	8	--	--	0/5	--	--	--

^a Most probable number of colony forming units per test portion.

^b Presump Pos: Positive by BAX® System assay for BAX® enrichments. Confirmed: At least one confirmed *E. coli* O157:H7 isolate was obtained by culture.

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

^d False negative rate: 100 minus sensitivity rate.

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

^f False positive rate: 100 minus specificity rate

^g Chi-square: Mantel-Haenszel chi square. *Chi-square value > 3.84 indicates significance at P < 0.05.

Table 7. Independent laboratory study of spiked and un-spiked ground beef (25 g) samples tested with BAX® system method and FDA-BAM reference culture methods. (0.024 MPN^a/g) (11)

Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed ^b	Sensitivity ^c %	False Neg ^d %	Presump. Pos /Unspiked	Specifi -city ^e %	False Pos ^f %	Chi-square ^g
8 hr	BAX® Classic	20	13/13	100	0	0/5	100	0	0.11
	BAX® Q7	20	12/12	92.3	7.7	0/5	100	0	0.43
10 hr	BAX® Classic	20	13/13	100	0	0/5	100	0	0.11
	BAX® Q7	20	13/13	100	0	0/5	100	0	0.11
12 hr	BAX® Classic	20	13/13	100	0	0/5	100	0	0.11
	BAX® Q7	20	13/13	100	0	0/5	100	0	0.11
24 hr	BAX® Classic	20	13/13	100	0	0/5	100	0	0.11
	BAX® Q7	20	13/13	100	0	0/5	100	0	0.11
FDA-BAM		20	14	--	--	0/5	--	--	--

^a Most probable number of colony forming units per test portion.

^b Presump Pos: Positive by BAX® System assay for BAX® enrichments. Confirmed: At least one confirmed *E. coli* O157:H7 isolate was obtained by culture.

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

^d False negative rate: 100 minus sensitivity rate.

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

^f False positive rate: 100 minus specificity rate

^g Chi-square: Mantel-Haenszel chi square. *Chi-square value > 3.84 indicates significance at P < 0.05.

Table 12. Internal study of spiked and unspiked Romaine lettuce (25 g) samples tested with BAX® system method and FDA-BAM reference culture methods. (0.0083 MPN ^a /g) (11)									
Enrichment Time	Method	Total spiked	Presump.Pos /Confirmed ^b	Sensitivity ^c %	False Neg d %	Presump. Pos /Unspiked	Specificity ^e %	False Pos ^f %	Chi-square ^g
8 hr	BAX® Classic	20	15/14	93.3	6.7	0/5	80	20	12.1
	BAX® Q7	20	15/14	93.3	6.7	0/5	80	20	12.1
22 hr	BAX® Classic	20	16/15	100	0	0/5	80	20	14.2
	BAX® Q7	20	16/15	100	0	0/5	80	20	14.2
FDA-BAM		20	3	--	--	0/5	--	--	--

Table 8. Salmonella BAX® system Inclusionity

Strain No.	Serovar	O-Group	Source	Result	Strain No.	Serovar	O-Group	Source	Result
1550	S. Abaetetuba	F	unknown	Positive	3915	S. Haardt	C3	Broiler breeders	Positive
1548	S. Abony	B	unknown	Positive	1689	S. Hartford	ND	unknown	Positive
3885	S. Adelaide	O	poultry feed	Positive	13067	S. Havana	G2	Solae	Positive
1551	S. Aequatorla	C2	Unknown	Positive	12907	S. Heidelberg	B	Tyson 03/2006	Positive
4084	S. Africana	B	Unknown	Positive	3852	S. Indiana	B	poultry feed	Positive
1552	S. Alabama	D2	unknown	Positive	5533	S. infantis	C1	Thyme	Positive
1531	S. Altendorf	B	unknown	Positive	1693	S. Javiana	ND	unknown	Positive
1530	S. Amager	E1	unknown	Positive	2628	S. Kentucky	C3	ATCC9263	Positive
1521	S. Amersfoort	C2	unknown	Positive	2353	S. Kristianstad	E1	unknown	Positive
1332	S. Anatum	E2	shrimp	Positive	13068	S. Lexington	E1	Solae	Positive
725	S. Arizonae	C	ATCC13314	Positive	2253	S. Lille	C1	pancake	Positive
1526	S. Austin	C1	unknown	Positive	4036	S. Livingstone	C1	chicken	Positive
1553	S. Ball	B	unknown	Positive	1653	S. Manhattan	C2	unknown	Positive
1554	S. Banaila	ND	unknown	Positive	2309	S. Maregrosso	ND	unknown	Positive
1510	S. Bareilly	C1	unknown	Positive	13069	S. Mbandaka	C1	Solae	Positive
2341	S. Barry	ND	unknown	Positive	1703	S. Mississippi	G2	feces	Positive
3185	S. Bellevue	ND	Cocoa bean environment	Positive	1492	S. Montevideo	C1	unknown	Positive
1523	S. Berkeley	U	diseased turkey	Positive	3156	S. Muenchen	C2	Cocoa bean environment	Positive
1525	S. Betokoy	ND	unknown	Positive	2203	S. Muenster	E1	unknown	Positive
1085	S. Binza	E2	dried spice	Positive	966	S. Napoli	D1	unknown	Positive
1343	S. Blockley	C2	environment	Positive	3698	S. Neumuenster	B	poultry feed	Positive
2343	S. Bockenheim	ND	unknown	Positive	1707	S. Newbrunswick	E2	unknown	Positive
1329	S. Braenderup	C1	dried egg	Positive	707	S. Newport	C2	ATCC6962	Positive
1338	S. Brandenburg	B	milk	Positive	2735	S. Ohio	C1	Protein supplement for feed	Positive
1356	S. Bredeney	B	pork	Positive	2676	S. Orton	E1	chicken feed	Positive
1535	S. Brookfield	ND	frog	Positive	3863	S. Othmarschen	C1	environmental	Positive
3882	S. Broughton	E4	poultry feed	Positive	918	S. Paratyphi A	A	ATCC9150	Positive
1558	S. Canastel	D1	feed	Positive	1711	S. Pomona	M	Turkey intestine in 1941	Positive
1620	S. Carmel	J	unknown	Positive	1712	S. Pretoria	F	plg	Positive
1621	S. Carrau	H	unknown	Positive	1482	S. Pullorum	D1	chicken liver	Positive
2629	S. Cerro	K	ATCC10723	Positive	1655	S. Reading	B	unknown	Positive
1615	S. Chameleon	ND	Lizard liver	Positive	1372	S. Saintpaul	B	milk powder	Positive
1623	S. Champaign	Q	chicken liver	Positive	6250	S. Santiago	C3	dried onion	Positive
1625	S. Chester	ND	unknown	Positive	1608	S. Seminole	R	snake feces	Positive
1557	S. Chicago	M	unknown	Positive	13168	S. Senftenberg	F	environmental sponge	Positive
13167	S. Chingola	ND	environmental sponge	Positive	1566	S. species	ND	rattlesnake skin	Positive
917	S. Choleraesuis	C1	ATCC13312	Positive	6177	S. species	ND	Chicken gblets	Positive
1628	S. Colorado	C1	unknown	Positive	6735	S. species	ND	Sesame seeds	Positive
3157	S. Corvallis	C3	environmental	Positive	1590	S. species	ND	Snake regurgitate	Positive
2870	S. Corvallis	ND	Cocoa bean environment	Positive	739	S. Stanley	ND	unknown	Positive
1632	S. Cubana	G2	chicken	Positive	2637	S. Swarzengrund	B	chicken	Positive
1675	S. Daressalaam	ND	#N/A	Positive	3536	S. Tennessee	C1	unknown	Positive
13169	S. Desau	ND	environmental sponge	Positive	2639	S. Thomasville	E3	turkey intestine	Positive
2349	S. Drypool	E2	unknown	Positive	1336	S. Thompson	C1	chicken	Positive
3017	S. Dublin	D1	unknown	Positive	1613	S. Tuindorp	ND	Zoo animal liver	Positive
1680	S. Dugbe	W	unknown	Positive	584	S. Typhi	D	ATCC6539	Positive
1641	S. Durban	D1	feces	Positive	586	S. Typhimurium	B	ATCC14028, Animal tissue	Positive
1469	S. Ealing	O	dried milk	Positive	13081	S. Virchow	C1	Basil	Positive
1684	S. Emmastad	P	unknown	Positive	1614	S. Volksdorf	ND	Iguana bladder	Positive
1773	S. Enterica	ND	unknown	Positive	2313	S. Wandsworth	Q	unknown	Positive
706	S. Enteritidis	D1	ATCC13076	Positive	1609	S. Wassenaar	ND	Iguana swab	Positive
1686	S. Fayed	H	unknown	Positive	1560	S. Westpark	ND	Tortoise intestine	Positive

DISCUSSION OF OCTOBER 2010 MODIFICATION (12)

Tables 1a - 1d (Modification results October 2010 below) show that the BAX® system using the *Salmonella* 2 test kit performed in a statistically indistinguishable manner to the reference methods for detecting *Salmonella* in frankfurters, raw ground beef, cream cheese and orange juice analyzed from the same pre-enrichment. Additionally, results from samples tested from enrichments conducted using the BAX® MP test media were statistically equivalent to the samples enriched using the reference method. All chi-square values were below the level of 3.84, indicating no statistically significant difference at P < 0.05. As shown in Tables 3 and 4, inclusivity and exclusivity of the modified test kit testing 352 inclusivity and 46 exclusivity strains gave correct results.

Within the statistical constraints of this study, the BAX® system *Salmonella* 2 test kit supports the performance claims of the BAX® system PCR assay for the detection of *Salmonella* that were validated in previous AOAC studies (≥ 98% sensitivity/specificity) when compared to the appropriate reference method.

Table 1a. Results of Hot Dogs Strain *Salmonella enterica* Serotype Agona DD1333 (12)

Method	MPN Per 25g ^a	Enrichment Method (Media)	Total sp	Presump.Pos /Confirmed	Sensitivity ^b	False Neg ^c %	Presump. Pos /Unspiked	Specificity ^d %	False Pos ^e %	Chi-square
22 hr BAX	0.67	BAX MP	20	6/6	100	0	0/5	100	0	0
22 hr BAX	0.67	BPW	20	6/6	100	0	0/5	100	0	-
Reference cult	0.67	BPW	20	6	-	-	-	-	-	-

Table 1b. Results of Ground Beef Strain *Salmonella enterica* Serotype Heidelberg Agona DD13017 (12)

Method	MPN Per 25g ^a	Enrichment Method (Media)	Total sp	Presump.Pos /Confirmed	Sensitivity ^b	False Neg ^c %	Presump. Pos /Unspiked	Specificity ^d %	False Pos ^e %	Chi-square
22 hr BAX	0.79	BAX MP	20	12/12	100	0	0/5	100	0	0
22 hr BAX	0.79	BPW	20	12/12	100	0	0/5	100	0	-
Reference cult	0.79	BPW	20	12	-	-	-	-	-	-

Table 1c. Results of Orange Juice Strain *Salmonella enterica* Serotype Worthington DD403 (12)

Method	MPN Per 25g ^a	Enrichment Method (Media)	Total sp	Presump.Pos /Confirmed	Sensitivity ^b	False Neg ^c %	Presump. Pos /Unspiked	Specificity ^d %	False Pos ^e %	Chi-square
22 hr BAX	0.80	BAX MP	20	7/7	100	0	0/5	100	0	2.1
22 hr BAX	0.80	UPB	20	3/3	100	0	0/5	100	0	0
Reference cult	0.80	UPB	20	3	-	-	-	-	-	-

Table 1d. Results of Cream Cheese Strain *Salmonella enterica* Serotype Typhimurium DD586 (12)

Method	MPN Per 25g ^a	Enrichment Method (Media)	Total sp	Presump.Pos /Confirmed	Sensitivity ^b	False Neg ^c %	Presump. Pos /Unspiked	Specificity ^d %	False Pos ^e %	Chi-square ^{f, 2}
22 hr BAX	0.67	BAX MP	20	7/7	100	0	0/5	100	0	0.46
22 hr BAX	0.67	BPW	20	5/5	100	0	0/5	100	0	0
Reference cult	0.67	BPW	20	5	-	-	-	-	-	-

^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. McNemar's Chi-square for paired samples.

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

Table 2. Inclusivity of the BAX® *Salmonella* 2 Test Kit (12)

DuPont ID Number	Genus and Serotype	Isolate Source	Serogroup	BAX® <i>Salmonella</i> 2 Result
1550	<i>Salmonella</i> Abaetetuba		F	POS
2166	<i>Salmonella</i> Abaetetuba		F	POS
1547	<i>Salmonella</i> Aberdeen		F	POS
1548	<i>Salmonella</i> Abony		B	POS
1543	<i>Salmonella</i> Adelaide		O	POS
1551	<i>Salmonella</i> Aequatoria		C1	POS
4084	<i>Salmonella</i> Africana		B	POS

3218	<i>Salmonella</i> Agama	Cocoa bean environment	B	POS
1335	<i>Salmonella</i> Agona	Chicken	B	POS
1352	<i>Salmonella</i> Agona	Cotton seeds	B	POS
1552	<i>Salmonella</i> Alabama		D1	POS
1556	<i>Salmonella</i> Alachua	Soil, abbatoir	O	POS
2966	<i>Salmonella</i> Albany		C3	POS
1531	<i>Salmonella</i> Altendorf		B	POS
1530	<i>Salmonella</i> Amager		E1	POS
3432	<i>Salmonella</i> Amager		E1	POS
1521	<i>Salmonella</i> Amersfoort		C1	POS
7072	<i>Salmonella</i> Amsterdam		E1	POS
1332	<i>Salmonella</i> Anatum	Shrimp	E1	POS
1334	<i>Salmonella</i> Anatum	Egg	E1	POS
2274	<i>Salmonella</i> Anatum		E1	POS
725	<i>Salmonella</i> Arizonae			POS
726	<i>Salmonella</i> Arizonae			POS
2980	<i>Salmonella</i> Arkansas		B	POS
2981	<i>Salmonella</i> Arkansas		B	POS
1527	<i>Salmonella</i> Atlanta		G	POS
1526	<i>Salmonella</i> Austin		C1	POS
1553	<i>Salmonella</i> Ball		B	POS
1554	<i>Salmonella</i> Banalia		C2	POS
1510	<i>Salmonella</i> Bareilly		C1	POS
2172	<i>Salmonella</i> Bareilly		C1	POS
2341	<i>Salmonella</i> Barry		O54	POS
3185	<i>Salmonella</i> Bellevue	Cocoa bean environment	C3	POS
1523	<i>Salmonella</i> Berkeley	Diseased turkey	U	POS
1606	<i>Salmonella</i> Bern	Opossum	R	POS
1331	<i>Salmonella</i> Berta	Sausages	D1	POS
2795	<i>Salmonella</i> Berta	Chicken intestine	D1	POS
1525	<i>Salmonella</i> Betioky		O59	POS
1085	<i>Salmonella</i> Binza	Dried spice	E2	POS
2786	<i>Salmonella</i> Binza	Chicken	E2	POS
1343	<i>Salmonella</i> Blockley	Environment	C2	POS
2343	<i>Salmonella</i> Bockenheim			POS
1509	<i>Salmonella</i> Bovismorbificans		C2	POS
1329	<i>Salmonella</i> Braenderup	Dried egg	C1	POS
1337	<i>Salmonella</i> Braenderup	Chicken	C1	POS
1555	<i>Salmonella</i> Brancaster		B	POS
1338	<i>Salmonella</i> Brandenburg	Milk	B	POS
964	<i>Salmonella</i> Bredeney	Fresh chicken	B	POS
1356	<i>Salmonella</i> Bredeney	Pork	B	POS
1535	<i>Salmonella</i> Brookfield	Frog	O66	POS
3882	<i>Salmonella</i> Broughton	Poultry feed	E4	POS

1668	<i>Salmonella</i> California		B	POS
2178	<i>Salmonella</i> California		B	POS
1558	<i>Salmonella</i> Canastel	Feed	D1	POS
1620	<i>Salmonella</i> Carmel		O17	POS
1621	<i>Salmonella</i> Carrau		H	POS
2629	<i>Salmonella</i> Cerro		K	POS
2813	<i>Salmonella</i> Cerro	Chicken chilled water tank	K	POS
1615	<i>Salmonella</i> Chameleon	Lizard liver	O16	POS
1623	<i>Salmonella</i> Champaign	Liver of hen	Q	POS
2180	<i>Salmonella</i> Champaign		Q	POS
1624	<i>Salmonella</i> Chandans		F	POS
3153	<i>Salmonella</i> Chandans	Cocoa bean environment	F	POS
1625	<i>Salmonella</i> Chester		B	POS
1557	<i>Salmonella</i> Chicago		M	POS
917	<i>Salmonella</i> Choleraesuis		UNK	POS
3984	<i>Salmonella</i> Choleraesuis paratyphi B	Gallbladder	B	POS
3988	<i>Salmonella</i> Choleraesuis paratyphi C		C1	POS
1665	<i>Salmonella</i> Colombo		P	POS
1628	<i>Salmonella</i> Colorado		C1	POS
2870	<i>Salmonella</i> Corvallis	Cocoa bean environment	C3	POS
3157	<i>Salmonella</i> Corvallis	Cocoa bean environment	C3	POS
3217	<i>Salmonella</i> Cotham	Cocoa bean environment	O28	POS
6966	<i>Salmonella</i> Cotham		O28	POS
1632	<i>Salmonella</i> Cubana	Chicks	G2	POS
1675	<i>Salmonella</i> Daressalaam			POS
1635	<i>Salmonella</i> Daytona		C1	POS
1638	<i>Salmonella</i> Derby		B	POS
2186	<i>Salmonella</i> Drypool		O15	POS
2349	<i>Salmonella</i> Drypool		O15	POS
3015	<i>Salmonella</i> Dublin		D1	POS
3017	<i>Salmonella</i> Dublin		D1	POS
3019	<i>Salmonella</i> Dublin		D1	POS
7005	<i>Salmonella</i> Dublin		D1	POS
1680	<i>Salmonella</i> Dugbe		W	POS
1641	<i>Salmonella</i> Durban	Faeces	D1	POS
2869	<i>Salmonella</i> Durham	Cocoa bean environment	G2	POS
3187	<i>Salmonella</i> Durham	Cocoa bean environment	G2	POS
1469	<i>Salmonella</i> Ealing	Dried baby milk	O	POS
1644	<i>Salmonella</i> Ealing	Dried baby milk (1985-1986)	O	POS
1684	<i>Salmonella</i> Emmastad		P	POS
1773	<i>Salmonella</i> enterica unk serotype			POS
1775	<i>Salmonella</i> enterica Typhimurium		B	POS
1777	<i>Salmonella</i> enterica Dar-es-salaam			POS

BAX® System PCR Assay for *Salmonella* AOAC® Certification Number 100201

13035	<i>Salmonella enterica</i> Choleraesuis			POS
13036	<i>Salmonella enterica</i> Typhimurium		B	POS
706	<i>Salmonella</i> Enteritidis		D1	POS
737	<i>Salmonella</i> Enteritidis		D1	POS
4022	<i>Salmonella</i> Enteritidis	Mayonnaise	D1	POS
1686	<i>Salmonella</i> Fayed		C2	POS
1687	<i>Salmonella</i> Ferlac	Ceylonese dessicated coconut	H	POS
5908	<i>Salmonella</i> Ferlac		H	POS
741	<i>Salmonella</i> Gallinarum		D1	POS
2350	<i>Salmonella</i> Gallinarum		D1	POS
2189	<i>Salmonella</i> Give		E1	POS
3915	<i>Salmonella</i> Haardt	Broiler breeders	C3	POS
12967	<i>Salmonella</i> Haardt	Poultry	C3	POS
12968	<i>Salmonella</i> Haardt	Poultry	C3	POS
12969	<i>Salmonella</i> Haardt	Poultry	C3	POS
12985	<i>Salmonella</i> Haardt	Poultry	C3	POS
3917	<i>Salmonella</i> Hadar	Broilers	C2	POS
3918	<i>Salmonella</i> Hadar	Broilers	C2	POS
1689	<i>Salmonella</i> Hartford		C1	POS
2290	<i>Salmonella</i> Hartford	Cheesecake, Dover	C1	POS
2245	<i>Salmonella</i> Havana	Pancake	G2	POS
13067	<i>Salmonella</i> Havana	Soy Plant Environmental	G2	POS
6667	<i>Salmonella</i> Heidelberg		B	POS
12907	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12908	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12909	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12910	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12911	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12913	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12919	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12920	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12922	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12928	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12929	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12931	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12932	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12933	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12935	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12936	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12937	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12945	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12947	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12952	<i>Salmonella</i> Heidelberg	Poultry	B	POS

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12953	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12954	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12958	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12959	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12995	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12996	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12998	<i>Salmonella</i> Heidelberg	Poultry	B	POS
12999	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13000	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13001	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13004	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13014	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13017	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13018	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13019	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13020	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13021	<i>Salmonella</i> Heidelberg	Poultry	B	POS
13022	<i>Salmonella</i> Heidelberg	Poultry	B	POS
1616	<i>Salmonella</i> Houten	Imported bird feces	O43	POS
3699	<i>Salmonella</i> Hvitvingfoss	Herbs or spices	I	POS
1480	<i>Salmonella</i> Indiana	Turkey	B	POS
3852	<i>Salmonella</i> Indiana	Poultry feed	B	POS
7011	<i>Salmonella</i> Indiana		B	POS
5533	<i>Salmonella</i> infantis	Thyme	C1	POS
7111	<i>Salmonella</i> Infantis		C1	POS
1693	<i>Salmonella</i> Javiana		D1	POS
1695	<i>Salmonella</i> Johannesburg		R	POS
3043	<i>Salmonella</i> Jjohannesburg		R	POS
1251	<i>Salmonella</i> Kedougou	Turkey	G2	POS
2628	<i>Salmonella</i> Kentucky		C3	POS
12912	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12914	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12915	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12916	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12917	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12918	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12921	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12924	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12925	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12926	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12927	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12941	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12943	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12946	<i>Salmonella</i> Kentucky	Poultry	C3	POS

12948	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12949	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12950	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12951	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12955	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12956	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12957	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12981	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12989	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12990	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12993	<i>Salmonella</i> Kentucky	Poultry	C3	POS
12997	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13002	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13003	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13006	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13007	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13008	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13009	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13010	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13012	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13013	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13015	<i>Salmonella</i> Kentucky	Poultry	C3	POS
13016	<i>Salmonella</i> Kentucky	Poultry	C3	POS
2196	<i>Salmonella</i> Kiambu		B	POS
2312	<i>Salmonella</i> Kottbus		C2	POS
3038	<i>Salmonella</i> Krefeld		E4	POS
2353	<i>Salmonella</i> Kristianstad		E1	POS
7061	<i>Salmonella</i> Kubacha		B	POS
7062	<i>Salmonella</i> Kubacha		B	POS
2199	<i>Salmonella</i> Lexington		E1	POS
13068	<i>Salmonella</i> Lexington	Soy Plant Environmental	E1	POS
2263	<i>Salmonella</i> Lille	Pancake	C1	POS
2868	<i>Salmonella</i> Lille	Cocoa bean environment	C1	POS
2992	<i>Salmonella</i> Lille		C1	POS
1650	<i>Salmonella</i> Livingstone	Faeces	C1	POS
4036	<i>Salmonella</i> Livingstone	Chicken	C1	POS
1652	<i>Salmonella</i> London		E1	POS
1698	<i>Salmonella</i> Madelia	Liver of hen	H	POS
2201	<i>Salmonella</i> Madelia		H	POS
1424	<i>Salmonella</i> Manchester	Autolysed yeast	C2	POS
1653	<i>Salmonella</i> Manhattan		C2	POS
2673	<i>Salmonella</i> Manhattan	Avian	C2	POS
6729	<i>Salmonella</i> Manila	Sesame seeds	E2	POS
2309	<i>Salmonella</i> Maregrasso			POS

2755	<i>Salmonella</i> Mbandaka	Swine tissue composite	C1	POS
13069	<i>Salmonella</i> Mbandaka	Soy Plant Environmental	C1	POS
1701	<i>Salmonella</i> Miami		D1	POS
2204	<i>Salmonella</i> Minnesota		L	POS
1703	<i>Salmonella</i> Mississippi	Faeces in 1942	G2	POS
2205	<i>Salmonella</i> Mississippi		G2	POS
1255	<i>Salmonella</i> Montevideo	Egg	C1	POS
1492	<i>Salmonella</i> Montevideo		C1	POS
13071	<i>Salmonella</i> Montevideo	Soy Plant Environmental	C1	POS
1704	<i>Salmonella</i> Muenchen		C2	POS
3156	<i>Salmonella</i> Muenchen	Cocoa bean environment	C2	POS
2748	<i>Salmonella</i> Muenster	Chicken	E1	POS
966	<i>Salmonella</i> Napoli		D1	POS
1476	<i>Salmonella</i> napoli		D1	POS
3898	<i>Salmonella</i> Neumuenster	Poultry feed	B	POS
1707	<i>Salmonella</i> Newbrunswick		E1	POS
2283	<i>Salmonella</i> Newbrunswick	Malted barley flour	E1	POS
707	<i>Salmonella</i> Newport	Fatal case of food poisoning	C2	POS
1261	<i>Salmonella</i> Newport	Duck	C2	POS
13079	<i>Salmonella</i> Newport	Basil	C2	POS
2735	<i>Salmonella</i> Ohio	Protein supplement for feed	C1	POS
1710	<i>Salmonella</i> Oranienburg		C1	POS
3863	<i>Salmonella</i> Othmarschen	Poultry hatchery	C1	POS
1248	<i>Salmonella</i> Panama	Pork sausages	D1	POS
918	<i>Salmonella</i> Paratyphi A		A	POS
919	<i>Salmonella</i> Paratyphi A		A	POS
1711	<i>Salmonella</i> Pomona	Turkey intestine in 1941	M	POS
2215	<i>Salmonella</i> Poona		G1	POS
1712	<i>Salmonella</i> Pretoria	Pig	F	POS
1482	<i>Salmonella</i> Pullorum	Chicks livers	D1	POS
1507	<i>Salmonella</i> Pullorum	Chicks livers	D1	POS
1655	<i>Salmonella</i> Reading		B	POS
4558	<i>Salmonella</i> Redlands		I	POS
2289	<i>Salmonella</i> Rubislaw	Barley malt berries	F	POS
1372	<i>Salmonella</i> Saintpaul	Milk powder	B	POS
13080	<i>Salmonella</i> Saintpaul	Basil	B	POS
1657	<i>Salmonella</i> Sandiego		B	POS
2218	<i>Salmonella</i> Sandiego		B	POS
2935	<i>Salmonella</i> Sandiego		B	POS
6250	<i>Salmonella</i> Santiago	Dried onion	C3	POS
6586	<i>Salmonella</i> Santiago	Bourgignon powder	C3	POS
2352	<i>Salmonella</i> Saphra		I	POS

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1658	<i>Salmonella</i> Schwarzengrund		B	POS
2637	<i>Salmonella</i> Schwarzengrund	Chicken	B	POS
2641	<i>Salmonella</i> Schwarzengrund	Chicken	B	POS
3184	<i>Salmonella</i> Sculcoates	Cocoa bean environment	I	POS
1610	<i>Salmonella</i> Seminole	Lizard coelomic fluid	O40	POS
12960	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12961	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12962	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12963	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12964	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12965	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12966	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12970	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12971	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12972	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12973	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12975	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12978	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12980	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12982	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12983	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12984	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12986	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12987	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
12988	<i>Salmonella</i> Senftenberg	Poultry	E4	POS
13056	<i>Salmonella</i> Senftenberg	Soy Plant Environmental	E4	POS
13057	<i>Salmonella</i> Senftenberg	Soy Plant Environmental	E4	POS
13058	<i>Salmonella</i> Senftenberg	Soy Plant Environmental	E4	POS
13059	<i>Salmonella</i> Senftenberg	Soy Plant Environmental	E4	POS
13060	<i>Salmonella</i> Senftenberg	Soy Plant Environmental	E4	POS
1659	<i>Salmonella</i> Shangani		E1	POS
2373	<i>Salmonella</i> species			POS
2376	<i>Salmonella</i> species			POS
2380	<i>Salmonella</i> species			POS
4102	<i>Salmonella</i> species	Nuts		POS
6177	<i>Salmonella</i> species	Chicken giblets		POS
6696	<i>Salmonella</i> species	Chicken giblets		POS
6735	<i>Salmonella</i> species	Sesame seeds		POS
8034	<i>Salmonella</i> species	Raw chicken		POS
12241	<i>Salmonella</i> species	Gum arabic		POS
13037	<i>Salmonella</i> Species	Isolate from NYS Department of Ag and Markets		POS
13070	<i>Salmonella</i> Species	Soy Plant Environmental		POS

13075	<i>Salmonella</i> Species	celery seed		POS
739	<i>Salmonella</i> Stanley		B	POS
1333	<i>Salmonella</i> Stanley	Chicken	B	POS
3194	<i>Salmonella</i> Stanleyville	Cocoa bean environment	B	POS
1660	<i>Salmonella</i> Sundsvall		H	POS
2867	<i>Salmonella</i> Sya	Cocoa bean environment	X	POS
3186	<i>Salmonella</i> Sya	Cocoa bean environment	X	POS
1661	<i>Salmonella</i> Tennessee		C1	POS
3536	<i>Salmonella</i> Tennessee		C1	POS
13061	<i>Salmonella</i> Tennessee	Soy Plant Environmental	C1	POS
13062	<i>Salmonella</i> Tennessee	Soy Plant Environmental	C1	POS
13063	<i>Salmonella</i> Tennessee	Soy Plant Environmental	C1	POS
13064	<i>Salmonella</i> Tennessee	Soy Plant Environmental	C1	POS
13065	<i>Salmonella</i> Tennessee	Soy Plant Environmental	C1	POS
13066	<i>Salmonella</i> Tennessee	Soy Plant Environmental	C1	POS
2229	<i>Salmonella</i> Theilalle			POS
2639	<i>Salmonella</i> Thomasville	Turkey intestine	E3	POS
3924	<i>Salmonella</i> Thomasville	Poultry feed	E3	POS
1336	<i>Salmonella</i> Thompson	Chicken	C1	POS
1339	<i>Salmonella</i> Thompson	Egg	C1	POS
12904	<i>Salmonella</i> Tranorora	700148	O55	POS
1613	<i>Salmonella</i> Tuindorp	Zoo animal liver	O43	POS
584	<i>Salmonella</i> Typhi		D1	POS
585	<i>Salmonella</i> Typhi		D1	POS
586	<i>Salmonella</i> Typhimurium	Animal tissue	B	POS
1084	<i>Salmonella</i> Typhimurium		B	POS
1467	<i>Salmonella</i> Typhimurium		B	POS
13005	<i>Salmonella</i> Typhimurium	Poultry	B	POS
13011	<i>Salmonella</i> Typhimurium	Poultry	B	POS
2238	<i>Salmonella</i> Urbana		N	POS
2239	<i>Salmonella</i> Uzaramo		H	POS
2346	<i>Salmonella</i> Vietnam		S	POS
738	<i>Salmonella</i> Virchow		C1	POS
13081	<i>Salmonella</i> Virchow	Basil	C1	POS
1614	<i>Salmonella</i> Volksdorf	Iguana bladder	O43	POS
2313	<i>Salmonella</i> Wandsworth		Q	POS
1609	<i>Salmonella</i> Wassenaar	Iguana swab	O50	POS
1714	<i>Salmonella</i> Wassenaar	Human	O50	POS
4035	<i>Salmonella</i> Waycross		S	POS
1491	<i>Salmonella</i> Weltevreden	Prawns	E1	POS
1560	<i>Salmonella</i> Westpark	Tortoise intestine	O3,10	POS
4043	<i>Salmonella</i> Worthington		G2	POS
1429	<i>Salmonella</i> species	African box meat (1967)		POS

Table 3. Exclusivity of the BAX® *Salmonella* 2 Test Kit (12)

DuPont Strain ID Number	ATCC Strain Number	Genus and Species	Isolate Source	BAX® <i>Salmonella</i> 2 Result
373	13883	<i>Klebsiella pneumoniae</i>		NEG
374	29906	<i>Proteus mirabilis</i>		NEG
383	8090	<i>Citrobacter freundii</i>		NEG
640	43889	<i>Escherichia coli</i> O157:H7	HUS Case Stool	NEG
641	43890	<i>Escherichia coli</i> O157:H7	Human Feces	NEG
657	11296	<i>Klebsiella ozaenae</i>		NEG
658	13182	<i>Klebsiella oxytoca</i>	Pharyngeal Tonsil	NEG
2389	13337	<i>Hafnia alvei</i>		NEG
2417		<i>Serratia liquefaciens</i>	Raw Mince	NEG
2558	43864	<i>Citrobacter freundii</i>		NEG
3064		<i>Morganella morganii</i>	Environmental Swab	NEG
3982	27853	<i>Pseudomonas aeruginosa</i>	Blood Culture	NEG
5588		<i>Hafnia alvei</i>	Ground Beef	NEG
6121		<i>Proteus mirabilis</i>	Herring Gull Cloacae	NEG
13142		<i>Morganella morganii</i>		NEG
13147		<i>Providencia rettgeri</i>		NEG
13148		<i>Pseudomonas aeruginosa</i>		NEG
13186		<i>Enterobacter amnigenus</i>		NEG
13187		<i>Enterobacter amnigenus</i>		NEG
ES9		<i>Enterobacter sakazakii</i>		NEG
ES14		<i>Enterobacter sakazakii</i>		NEG
ES53		<i>Enterobacter sakazakii</i>		NEG
ES1		<i>Enterobacter sakazakii</i>		NEG
ES20		<i>Enterobacter sakazakii</i>		NEG
ES34		<i>Enterobacter sakazakii</i>		NEG
ES35		<i>Enterobacter sakazakii</i>		NEG
ES38		<i>Enterobacter sakazakii</i>		NEG
700		<i>Shigella sonnei</i>		NEG
1083		<i>Shigella flexneri</i>		NEG
702		<i>Shigella sonnei</i>		NEG
846	29907	<i>Escherichia blattae</i>	Hindgut of Cockroach	NEG
847	35469	<i>Escherichia fergusonii</i>	Human Feces	NEG
848	33650	<i>Escherichia hermannii</i>	Human Toe	NEG
849	21073	<i>Escherichia intermedia</i>		NEG

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850	33821	<i>Escherichia vulneris</i>	Human Wound	NEG
854	35539	<i>Staphylococcus gallinarum</i>	Chicken Nares	NEG
862	4698	<i>Micrococcus luteus</i>		NEG
863	12600	<i>Staphylococcus aureus</i>	Human Clinical	NEG
864	14990	<i>Staphylococcus epidermidis</i>	Nose	NEG
3354		<i>Listeria welshimeri</i>		NEG
1309		<i>Listeria monocytogenes</i>	Soft Cheese	NEG
1154		<i>Listeria innocua</i>	Pate	NEG
QC201	13048	<i>Enterobacter aerogenes</i>	Sputum	NEG
QC203	51113	<i>Citrobacter brakii</i>	Snake	NEG
QC204	700814	<i>Bacillus pumilus</i>		NEG
QC102	51740	<i>Staphylococcus aureus</i>	Margarine	NEG

DISCUSSION OF NOVEMBER 2015 MODIFICATION (13)

The method comparison study demonstrated that the alternative method as performed using the X5 BAX® instrument is equivalent to the USDA-FSIS reference method for ground beef. Data demonstrated positive results across a wide titer of target in both pure culture and in enriched food matrix demonstrating that the X5 instrument performs in a similar fashion to the currently validated instruments when using the *Salmonella* 2 assay. Additional studies showed broad inclusivity and the ability to discriminate against non-target species. Robustness studies indicated that several of the factors tested could have a negative impact on assay performance. These factors will be explicitly called out in the instrument user guide and/or end user training materials.

Table 1. Method results POD Food Matrices – Candidate Method Compared to Culture Results from Candidate Enrichments (13)											
Matrix	Strain	MPN ^a /test portion	N	Candidate Method Presumptive			Candidate Method Confirmed			dPOD _{CP} ^e	95% CI ^f
				X ^b	POD _{CP} ^c	95% CI	x	POD _{CC} ^d	95% CI		
Ground Beef BAX® System X5 BPW	S. Typhimurium DD586	5.0 (2.9, 8.8)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.5 (0.28, 0.88)	20	6	0.30	(0.14, 0.52)	6	0.30	(0.14, 0.52)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

Table 2. Method results POD Food Matrices – Candidate Method Compared to Culture Results from Candidate Enrichments											
Matrix	Strain	MPN ^a /test portion	N	Candidate Method Presumptive			Candidate Method Confirmed			dPOD _{CP} ^e	95% CI ^f
				X ^b	POD _{CP} ^c	95% CI	x	POD _{CC} ^d	95% CI		
Ground Beef BAX® System X5 mTSB	S. Typhimurium DD586	5.0 (2.9, 8.8)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.5 (0.28, 0.88)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

Table 3. Method results POD Food Matrices – Candidate Method Compared to Reference Culture Method											
Matrix	Strain	MPN ^a /test portion	N	Test Method			Reference Method			dPOD _C ^e	95% CI ^f
				X ^b	POD _C ^c	95% CI	x	POD _R ^d	95% CI		
Ground Beef Reference method mTSB v Candidate method BPW	S. Typhimurium DD586	5.0 (2.9, 8.8)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.43, 0.43)
		0.5 (0.28, 0.88)	20	6	0.30	(0.11, 0.46)	5	0.25	(0.14, 0.52)	0.050	(-0.22, 0.31)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.43, 0.43)
Ground Beef Reference method mTSB v Candidate method mTSB	S. Typhimurium DD586	5.0 (2.9, 8.8)	5	5	1.0	(0.57, 1.0)	5	1.0	(0.57, 1.0)	0	(-0.45, 0.45)
		0.5 (0.28, 0.88)	20	5	0.25	(0.11, 0.47)	5	0.25	(0.11, 0.47)	0	(-0.14, 0.14)
		Negative Control	5	0	0	(0, 0.43)	0	0	(0, 0.43)	0	(-0.45, 0.45)

^aMPN = Most Probable Number based on the POD of reference method test portions using the AOAC MPN calculator (with 95% confidence interval).

^bX = Number of positive test portions.

^cPOD_C = Confirmed candidate method positive outcomes divided by the total number of trials.

^dPOD_R = Confirmed reference method positive outcomes divided by the total number of trials.

^edPOD_C = Difference between the candidate method and reference method POD values.

^f95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 4. Inclusivity Results for the BAX® System X5 PCR Assay for <i>Salmonella</i> (13)							
DuPont Strain ID	<i>Salmonella</i> Serotype and Serogroup	Strain Source	Result	DuPont Strain ID	<i>Salmonella</i> Serotype and Serogroup	Strain Source	Result
584	Typhi	Unknown	Pos	1705	Muenster	Unknown	Pos
585	Typhi	Unknown	Pos	1712	Pretoria	Pig	Pos
586	Typhimurium	Animal Tissue	Pos	2189	Give	Unknown	Pos
707	Newport	Fatal Case of Food Poisoning	Pos	2263	Lille	Pancake	Pos
739	Stanley	Unknown	Pos	2283	Newbrunswick	Malted Barley Flour	Pos
741	Gallinarum	Unknown	Pos	2289	Rubislaw	Barley Malt Berries	Pos
919	Paratyphi	Unknown	Pos	2290	Hartford	Cheesecake	Pos
966	Napoli	Unknown	U	2313	Wandsworth	Unknown	Pos
1085	Binza	Dried Spice	Pos	2343	Bockenheim	Unknown	Pos
1248	Panama	Pork Sausages	Pos	2349	Drypool	Unknown	Pos
1251	Kedougou	Turkey	Pos	2353	Kristianstad	Unknown	Pos
1329	Braenderup	Dried Egg	Pos	2376	Sculcoates	Unknown	Pos
1332	Anatum	Shrimp	Pos	2637	Schwarzengrund	Chicken	Pos
1336	Thompson	Chicken	Pos	2639	Thomasville	Turkey Intestine	Pos
1352	Agona	Cotton Seeds	Pos	2735	Ohio	Protein Supplement for Feed	Pos
1356	Bredeney	Pork	Pos	2813	Cerro	Chicken Chilled Water Tank	Pos
1429	Anfo	African Box Meat (1967)	Pos	2867	Sya	Cocoa Bean Environment	Pos
1435	Brandenburg	Unknown	Pos	2870	Corvallis	Cocoa Bean Environment	Pos
1469	Ealing	Dried Baby Milk	Pos	3019	Dublin	Unknown	Pos
1482	Pullorum	Chicks Livers	Pos	3156	Muenchen	Cocoa Bean Environment	Pos
1509	Bovismorbificans	Unknown	Pos	3217	Cotham	Cocoa Bean Environment	Pos
1510	Abaetetuba	Unknown	Pos	3218	Agama	Cocoa Bean Environment	Pos
1521	Bareilly	Unknown	Pos	3699	Hvittingfoss	Herbs or Spices	Pos
1523	Berkeley	Diseased Turkey	Pos	3852	Indiana	Poultry Feed	Pos
1525	Betioky	Unknown	Pos	3863	Oranienburg	Poultry Hatchery	Pos
1526	Austin	Unknown	Pos	3882	Broughton	Poultry Feed	Pos
1530	Altendorf	Unknown	Pos	3898	Thompson	Poultry Feed	Pos
1535	Brookfield	Frog	Pos	3915	Haardt	Broiler Breeders	Pos
1543	Adelaide	Unknown	Pos	3984	Java	Gallbladder	Pos
1547	Aberdeen	Unknown	Pos	4022	Enteritidis	Mayonnaise	Pos
1548	Abony	Unknown	Pos	4036	Livingstone	Chicken	Pos
1552	Alabama	Unknown	Pos	4102	Saintpaul	Nuts	Pos
1553	Ball	Unknown	Pos	5533	Infantis	Thyme	Pos
1557	Chicago	Unknown	Pos	6177	Arkansas	Chicken Giblets	Pos
1568	Arizonae	Turkey Egg	Pos	6250	Santiago	Dried Onion	Pos

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1585	Arizonae	Turtle Soil	Pos	6735	Albany	Sesame Seeds	Pos
1590	<i>Salmonella</i> 3b	Snake Regurgitate	Pos	7111	Infantis	Unknown	Pos
1597	<i>Salmonella</i> 3b	Gila Monster Feces	Pos	12907	Heidelberg	Poultry	Pos
1608	Seminole	Snake Feces	Pos	12914	Kentucky	Poultry	Pos
1609	Wassenaar	Iguana Swab	Pos	12960	Senftenberg	Poultry	Pos
1614	Kralendyk	Iguana Bladder	Pos	12968	Blockley	Unknown	Pos
1616	Houten IV	Imported Bird Feces	Pos	13005	Typhimurium	Poultry	Pos
1620	Carmel	Unknown	Pos	13035	Choleraesuis	ATCC 10708	Pos
1621	Carrau	Unknown	Pos	13056	Senftenberg	Soy Manufacturing	Pos
1623	Champaign	Liver of Hen	Pos	13064	Tennessee	Soy Manufacturing	Pos
1638	Derby	Unknown	Pos	13066	Tennessee	Soy Manufacturing	Pos
1652	London	Unknown	Pos	13067	Havana	Soy Manufacturing	Pos
1653	Yovokome	Unknown	Pos	13068	Lexington	Soy Manufacturing	Pos
1657	Reading	Unknown	Pos	13069	Mbandaka	Soy Manufacturing	Pos
1668	California	Unknown	Pos	13071	Montevideo	Soy Manufacturing	Pos
1680	Dugbe	Unknown	Pos	13075	Cubana	Celery Seed	Pos
1684	Emmastad	Unknown	Pos	13079	Newport	Basil	Pos
1686	Fayed	Unknown	Pos	13081	Virchow	Basil	Pos
1687	Ferlac	Ceylonese Dessicated Coconut	Pos	13344	Berta	Unknown	Pos
1698	Madelia	Liver of Hen	Pos	13630	Hadar	Unknown	Pos
1703	Mississippi	Faeces in 1942	Pos	SAFE73	<i>Salmonella</i> 14,[5],12:i:-	Unknown	Pos

Table 5. Exclusivity Results for the BAX® System X5 PCR Assay for *Salmonella* (13)

DuPont Strain ID	Genus and Species	Result	DuPont Strain ID	Genus and Species	Result
373	<i>Klebsiella pneumoniae</i>	Neg	2554	<i>Enterococcus faecalis</i>	Neg
375	<i>Enterobacter cloacae</i>	Neg	2558	<i>Citrobacter freundii</i>	Neg
383	<i>Citrobacter freundii</i>	Neg	2559	<i>Citrobacter amalonaticus</i>	Neg
569	<i>Pseudomonas fluorescens</i>	Neg	2560	<i>Citrobacter koseri</i>	Neg
572	<i>Aeromonas hydrophila</i>	Neg	2584	<i>Enterobacter hormaechei</i>	Neg
576	<i>Pseudomonas mendocina</i>	Neg	2586	<i>Klebsiella planticola</i>	Neg
577	<i>Pseudomonas stutzeri</i>	Neg	2604	<i>Enterobacter amnigenus</i>	Neg
592	<i>Yersinia enterocolitica</i>	Neg	2631	<i>Vibrio fluvialis</i>	Neg
610	<i>Staphylococcus aureus</i>	Neg	2632	<i>Vibrio vulnificus</i>	Neg
657	<i>Klebsiella ozaenae</i>	Neg	3097	<i>Citrobacter freundii</i>	Neg
659	<i>Lactococcus lactis</i>	Neg	3785	<i>Escherichia coli</i>	Neg
700	<i>Shigella sonnei</i>	Neg	3982	<i>Pseudomonas aeruginosa</i>	Neg
715	<i>Bacillus cereus</i>	Neg	5588	<i>Hafnia alvei</i>	Neg
1081	<i>Shigella boydii</i>	Neg	6121	<i>Prot. Mirabilis</i>	Neg
1082	<i>Shigella dysenteriae</i>	Neg	6523	<i>Klebsiella oxytoca</i>	Neg
1450	<i>Escherichia coli</i>	Neg	6719	<i>Escherichia hermanni</i>	Neg
1458	<i>Escherichia coli</i>	Neg	6832	<i>Shigella sonnei</i>	Neg
2357	<i>Proteus mirabilis</i>	Neg	7083	<i>Serratia marcesens</i>	Neg
2389	<i>Hafnia alvei</i>	Neg	8877	<i>Xanthomonas maltophilia</i>	Neg
2399	<i>Yersinia aldovae</i>	Neg	10006	<i>Enterobacter sakazakii</i>	Neg
2435	<i>Escherichia coli</i>	Neg	11232	<i>Vibrio mimicus</i>	Neg
2443	<i>Escherichia coli</i>	Neg	12720	<i>Enterobacter sakazakii</i>	Neg
2514	<i>Escherichia coli</i>	Neg	12760	<i>Enterobacter cloacae</i>	Neg
2552	<i>Enterococcus faecium</i>	Neg	13041	<i>Escherichia coli</i>	Neg

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