CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

081201

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

BAX® System Real-Time 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 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 04, 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 04, 2019
Date

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METHOD AUTHORS

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MODIFICATION AUGUST 2015: Sergiy Olishevskyy, Melissa Buzinhani, Cathy St-Laurent, Benoit Crevier, Renaud Tremblay, and F. Morgan Wallace

SUBMITTING COMPANY

DuPont Nutrition & Health
Experimental Station 400
200 Powder Mill Road
Wilmington, DE 19803
USA

CURRENT SPONSOR

Hygiena
2 Boulden Circle
New Castle, DE 19720
USA

KIT NAME(S)

DuPont™ BAX® System Real-Time PCR Assay for *Salmonella* March 01, 2017, BAX® System Real-Time PCR Assay for *Salmonella*

CATALOG NUMBERS

BAX® System Assay KIT2006 (D14306040), MP Media MED2003 (D12404925)

INDEPENDENT LABORATORY

Original Validation:
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, OH 45214
USA

August 2015 Modification:
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AOAC EXPERTS AND PEER REVIEWERS

Original Validation: Yi Chen[1,5], Maria Cristina Fernandez[2], Wayne Ziemer[3]
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[5] July 2013 Modification

APPLICABILITY OF METHOD

Target organism – *Salmonella*

Matrices - USDA/FSIS MLG 4.04 Raw ground beef (25g, 375g), Chicken carcass rinse (30mL)
FDA BAM Ch. 5 – Bagged lettuce (25g), cream cheese (25g), dry pet food (375g), stainless steel
Health Canada Compendium of Methods MFHPB 20 – Raw ground beef (25g, 375g), dry pet food (375g), stainless steel
August 2015 Matrix Extension (USDA BAM Ch 5): dry pet food, milk chocolate, chocolate liquor, cocoa powder, shell egg, stainless steel, plastic

Performance claims - Sensitivity and specificity equivalent to the appropriate reference culture methods.

REFERENCE METHODS

Andrews, W. H. and Hammack T.S. Bacteriological Analytical Manual Online. Revised 11/2011. US Food & Drug Administration, Center for Food Safety & Applied Nutrition. Chapter 5, *Salmonella*. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm (2)

Reid, A. MFHPB-20, Isolation and Identification of *Salmonella* from Food and Environmental Samples. 2009 In: Health Canada Compendium, Vol. 3, Laboratory Procedures for the Microbiological Examination of Foods. Health Canada, Health Products and Food Branch. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhpb20-01-eng.php (3)

Dey, B.P. and Lattuada, C.P. eds. 2011. Microbiology Laboratory Guidebook. 3rd ed Revised 1/20/2011. US Department of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science. http://www.fsis.usda.gov/PDF/MLG_4_05.pdf (4)

ORIGINAL CERTIFICATION DATE

August 07, 2012

CERTIFICATION RENEWAL RECORD

Renewed Annually through December 2020

METHOD MODIFICATION RECORD

1. **July 2013**
2. **August 2015 Level 2**
3. **March 2017 Level 1**
4. **January 2018 Level 1 Renewal Modification**
5. **May 2019 Level 1**
6. **December 2019 Level 1 Renewal Modification**

SUMMARY OF MODIFICATION

1. **Addition of Thermal Block for automated sample lysis**
2. **Matrix Extension in collaboration with FoodChek PTM 041303**
3. **Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company**
4. **Inserts, labels, manuals updated to Hygiena**
5. **Editorial updates to insert and corporate address**
6. **Editorial/clerical changes.**

Under this AOAC® *Performance Tested*SM License Number, 081201 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)

PCR amplification - 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 the genus Salmonella, thus providing a highly reliable indicator that the organism is present. The BAX® System simplifies the PCR process by combining the requisite primers, polymerase and nucleotides into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed thus significantly reducing the potential for contamination with one or more molecules of amplified PCR product in future tests.

Fluorescent real time detection - This automated BAX® System method uses fluorescent detection to analyze PCR product. One PCR primer for each target (one *Salmonella*-specific target and an internal control) contains a fluorescent dye (two different dyes, one for each target) as a constituent of the primer as well as a quencher (the uni-molecular combination of a primer, fluorescent dye and quencher constitute a Scorpion™ Probe).

When not incorporated into a PCR product, the Scorpion™ Probe has a hair-pin loop structure which keeps the dye and quencher in close proximity. When incorporated into a PCR product, the dye and quencher are spatially separated due to an internal hybridization, which causes an increase in emission signal. The BAX® System measures the magnitude and characteristics of fluorescent signal change. An analysis by the BAX® System software algorithm then evaluates that data to determine a positive or negative result which is displayed as described below.

DISCUSSION OF THE VALIDATION STUDY (1)

The data in these studies, within their statistical uncertainty, support the product claims of the BAX® System Real-Time Assay for Salmonella from ground beef, cream cheese, bagged lettuce, dry pet food, chicken carcass rinses, and stainless steel. Additional studies showed broad inclusivity and the ability to discriminate against non-target species, a high degree of robustness when subjected to deviations from the manufacturer's specifications and consistent performance across different lots of the test kit.

Table 3. Data Summary – Test method vs Reference Method (1)												
Matrix and Enrichment Media	Strain	MPN[a]/ Test Portion	N[b]	BAX® Method			Reference Method			dPOD$_c$[f]	95% CI[g]	X^2 [h]
				x[c]	POD$_c$[d]	95% CI	x[c]	POD$_R$[e]	95% CI			
ground Beef BPW (25g)	*S.* Heidelberg DD13017	0.37	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	4	0.20	0.081-0.42	4	0.20	0.081-0.42	0	-0.25-0.25	-
ground Beef mTSB (375g test 25g reference)	*S.* Heidelberg DD13017	0.37	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	5	0.25	0.11-0.47	4	0.20	0.08-0.42	0.05	-0.21-0.30	0.140
Chicken Rinse	Naturally Occurring	NA	20	11	0.55	0.34-0.74	11	0.55	0.34-0.74	0	-0.28-0.28	-
CREAM CHEESE LB	*S.* Typhimurium DD586	0.63	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	5	0.25	0.028-0.30	5	0.25	0.11-0.47	-0.15	-0.38-0.022	-
CREAM CHEESE MP	*S.* Typhimurium DD586	0.63	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	2	0.1	0.028-0.30	5	0.25	0.11-0.47	-0.15	-0.38-0.022	1.52
LETTUCE MP	*S.* Newport DD1261	0.85	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	10	0.5	0.30-0.70	10	0.5	0.30-0.70	0	-0.28-0.28	0
LETTUCE LB	*S.* Newport DD1261	0.85	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	10	0.5	0.30-0.70	10	0.5	0.30-0.70	0	-0.28-0.28	0
DRY PET FOOD BPW	*S.* Tennessee DD13062	0.30	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	5	0.25	0.11-0.47	5	0.25	0.11-0.47	0	-0.26-0.26	-
DRY PET FOOD LB	*S.* Tennessee DD13062	0.30	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	0
			20	5	0.25	0.11-0.47	5	0.25	0.11-0.47	0	-0.26-0.26	0
STAINLESS STEEL SURFACES BPW[i]	*S.* Senftenberg DD12960 *C. brakii* DD13477	NA	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	13	0.65	0.43-0.82	13	0.65	0.43-0.82	0	-0.28-0.28	-

STAINLESS STEEL SURFACES LB[I]	***S.* Senftenberg DD12960 *C. brakii* DD13477**	NA	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	13	0.65	0.43-0.82	13	0.65	0.43-0.82	0	-0.28-0.28	-
CREAM CHEESE[J]	***S.* Typhimurium ATCC 14028**	0.76	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	14	0.70	0.48-0.85	10	0.50	0.30-0.70	0.2	-0.097-0.45	1.63
STAINLESS STEEL SURFACES BPW [I,J]	***S.* Senftenberg ATCC 43845 *C. braaki*i ATCC 43162**	NA	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	7	0.35	0.18-0.57	7	0.35	0.18-0.57	0	-0.28-0.28	-
STAINLESS STEEL SURFACES LB [I,J]	***S.* Senftenberg ATCC 43845 *C. braaki*i ATCC 43162**	NA	5	0	0	0-0.43	0	0	0-0.43	0	-0.43-0.43	-
			20	5	0.25	0.11-0.47	5	0.25	0.11-0.47	0	-0.26-0.26	-

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the AOAC MPN calculator

[b]N = Number of test potions

[c]x = Number of positive test portions

[d]POD_C = Confirmed candidate method positive outcomes divided by the total number of trials

[e]POD_R = Confirmed reference method positive outcomes divided by the total number of trials

[f]$dPOD_C$ = Difference between the candidate method and reference method POD values

[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

[h] Chi Square is McNemar's Chi Square for paired samples (those tested from the same enrichment broth as the primary enrichment broth for the reference method) and Mantel-Haenszel for unpaired samples (those tested from an alternative enrichment broth for the test method)

[I]BPW Health Canada MFHPB-20 results are considered as reference method results for this matrix tested by the candidate method from BPW for statistical analysis, while LB FDA-BAM results are considered as reference method results for this matrix tested by the candidate method from LB for statistical analysis

[J]Independent Laboratory Study

Table 7. Inclusivity of the BAX ® Real Time *Salmonella* Test Kit (1)				
DuPont ID Number	**Genus, Serotype and Subgroup**	**Isolate Source**	**Serogroup**	**BAX ® RT *Salmonella* Result**
1550	*Salmonella* Abaetetuba I		F	POS
2166	*Salmonella* Abaetetuba I		F	POS
1547	*Salmonella* Aberdeen I		F	POS
1548	*Salmonella* Abony I		B	POS
1543	*Salmonella* Adelaide I		O	POS
1551	*Salmonella* Aequatoria I		C1	POS
4084	*Salmonella* Africana I		B	POS
3218	*Salmonella* Agama I	Cocoa bean environment	B	POS
1335	*Salmonella* Agona I	Chicken	B	POS
1352	*Salmonella* Agona I	Cotton seeds	B	POS
1552	*Salmonella* Alabama I		D1	POS
1556	*Salmonella* Alachua I	Soil, abbatoir	O	POS
2966	*Salmonella* Albany I		C3	POS
1531	*Salmonella* Altendorf I		B	POS
1530	*Salmonella* Amager I		E1	POS
3432	*Salmonella* Amager I		E1	POS
1521	*Salmonella* Amersfoort I		C1	POS
7072	*Salmonella* Amsterdam I		E1	POS
1332	*Salmonella* Anatum I	Shrimp	E1	POS
1334	*Salmonella* Anatum I	Egg	E1	POS
2274	*Salmonella* Anatum I		E1	POS
725	*Salmonella* Arizonae IIIa	ATCC13314	51:z4,z23	POS
726	*Salmonella* Arizonae IIIa	ATCC12324	40:z4,z23	POS
2980	*Salmonella* Arkansas I		B	POS
2981	*Salmonella* Arkansas I		B	POS
1527	*Salmonella* Atlanta I		G	POS
1526	*Salmonella* Austin I		C1	POS
1553	*Salmonella* Ball I		B	POS
1554	*Salmonella* Banalia I		C2	POS
1510	*Salmonella* Bareilly I		C1	POS
2172	*Salmonella* Bareilly I		C1	POS
2341	*Salmonella* Barry I		O54	POS
3185	*Salmonella* Bellevue I	Cocoa bean environment	C3	POS
1523	*Salmonella* Berkeley I	Diseased turkey	U	POS
1331	*Salmonella* Berta I	Sausages	D1	POS
2795	*Salmonella* Berta I	Chicken intestine	D1	POS
1525	*Salmonella* Betioky II		59:k:(z)	POS
1085	*Salmonella* Binza I	Dried spice	E2	POS
2786	*Salmonella* Binza I	Chicken	E2	POS
1343	*Salmonella* Blockley I	Environment	C2	POS
2343	*Salmonella* Bockenheim IV		1,53:z36,z38:-	POS

1509	*Salmonella* Bovismorbificans I		C2	POS
1329	*Salmonella* Braenderup I	Dried egg	C1	POS
1337	*Salmonella* Braenderup I	Chicken	C1	POS
1555	*Salmonella* Brancaster I		B	POS
1338	*Salmonella* Brandenburg I	Milk	B	POS
964	*Salmonella* Bredeney I	Fresh chicken	B	POS
1356	*Salmonella* Bredeney I	Pork	B	POS
1535	*Salmonella* bongori serotype Brookfield	Frog	66:z41:-	POS
3882	*Salmonella* Broughton I	Poultry feed	E4	POS
1668	*Salmonella* California I		B	POS
2178	*Salmonella* California I		B	POS
1558	*Salmonella* Canastel II	Feed	D1	POS
1620	*Salmonella* Carmel I		O17	POS
1621	*Salmonella* Carrau I		H	POS
2629	*Salmonella* Cerro I		K	POS
2813	*Salmonella* Cerro I	Chicken chilled water tank	K	POS
1615	*Salmonella* Chameleon IV	Lizard liver	16:z4,z32:-	POS
1623	*Salmonella* Champaign I	Liver of hen	Q	POS
2180	*Salmonella* Champaign I		Q	POS
1624	*Salmonella* Chandans I		F	POS
3153	*Salmonella* Chandans I	Cocoa bean environment	F	POS
1625	*Salmonella* Chester I		B	POS
1557	*Salmonella* Chicago I		M	POS
917	*Salmonella* Choleraesuis I		UNK	POS
3984	*Salmonella* Choleraesuis paratyphi B I	Gallbladder	B	POS
3988	*Salmonella* Choleraesuis paratyphi C I		C1	POS
1665	*Salmonella* Colombo I		P	POS
1628	*Salmonella* Colorado I		C1	POS
2870	*Salmonella* Corvallis I	Cocoa bean environment	C3	POS
3157	*Salmonella* Corvallis I	Cocoa bean environment	C3	POS
3217	*Salmonella* Cotham I	Cocoa bean environment	O28	POS
6966	*Salmonella* Cotham I		O28	POS
1632	*Salmonella* Cubana I	Chicks	G2	POS
1675	*Salmonella enterica* subspecies *salamae* serovar Daressalaam II		1,9,12:l,w:e,n,x	POS
1635	*Salmonella* Daytona I		C1	POS
1638	*Salmonella* Derby I		B	POS
2186	*Salmonella* Drypool I		O15	POS
2349	*Salmonella* Drypool I		O15	POS
3015	*Salmonella* Dublin I		D1	POS
3017	*Salmonella* Dublin I		D1	POS
3019	*Salmonella* Dublin I		D1	POS
7005	*Salmonella* Dublin I		D1	POS
1680	*Salmonella* Dugbe I		W	POS

1641	*Salmonella* Durban I	Faeces	D1	POS
2869	*Salmonella* Durham I	Cocoa bean environment	G2	POS
3187	*Salmonella* Durham I	Cocoa bean environment	G2	POS
1469	*Salmonella* Ealing I	Dried baby milk	O	POS
1644	*Salmonella* Ealing I	Dried baby milk (1985-1986)	O	POS
1684	*Salmonella* Emmastad I		P	POS
1775	*Salmonella* Typhimurium I		B	POS
1777	*Salmonella enterica* subspecies *salamae* serovar Dar-es-salaam II		1,9,12:l,w:e,n,x	POS
13035	*Salmonella* Choleraesuis I	ATCC 10708	C1	POS
13036	*Salmonella* Typhimurium I		B	POS
706	*Salmonella* Enteritidis I		D1	POS
737	*Salmonella* Enteritidis I		D1	POS
4022	*Salmonella* Enteritidis I	Mayonnaise	D1	POS
1686	*Salmonella* Fayed I		C2	POS
1687	*Salmonella* Ferlac VI	Ceylonese dessicated coconut	1,6,14,25:a:e,n,x	POS
5908	*Salmonella* Ferlac VI		1,6,14,25:a:e,n,x	POS
741	*Salmonella* Gallinarum I		D1	POS
2350	*Salmonella* Gallinarum I		D1	POS
2189	*Salmonella* Give I		E1	POS
3915	*Salmonella* Haardt I	Broiler breeders	C3	POS
12967	*Salmonella* Haardt I	Poultry	C3	POS
12968	*Salmonella* Haardt I	Poultry	C3	POS
12969	*Salmonella* Haardt I	Poultry	C3	POS
12985	*Salmonella* Haardt I	Poultry	C3	POS
3917	*Salmonella* Hadar I	Broilers	C2	POS
3918	*Salmonella* Hadar I	Broilers	C2	POS
1689	*Salmonella* Hartford I		C1	POS
2290	*Salmonella* Hartford I	Cheesecake, Dover	C1	POS
2245	*Salmonella* Havana I	Pancake	G2	POS
13067	*Salmonella* Havana I	Soy Plant Environmental	G2	POS
6667	*Salmonella* Heidelberg I		B	POS
12907	*Salmonella* Heidelberg I	Poultry	B	POS
12908	*Salmonella* Heidelberg I	Poultry	B	POS
12909	*Salmonella* Heidelberg I	Poultry	B	POS
12910	*Salmonella* Heidelberg I	Poultry	B	POS
12911	*Salmonella* Heidelberg I	Poultry	B	POS
12913	*Salmonella* Heidelberg I	Poultry	B	POS
12919	*Salmonella* Heidelberg I	Poultry	B	POS
12920	*Salmonella* Heidelberg I	Poultry	B	POS
12922	*Salmonella* Heidelberg I	Poultry	B	POS
12928	*Salmonella* Heidelberg I	Poultry	B	POS
12929	*Salmonella* Heidelberg I	Poultry	B	POS

12931	*Salmonella* Heidelberg I	Poultry	B	POS
12932	*Salmonella* Heidelberg I	Poultry	B	POS
12933	*Salmonella* Heidelberg I	Poultry	B	POS
12935	*Salmonella* Heidelberg I	Poultry	B	POS
12936	*Salmonella* Heidelberg I	Poultry	B	POS
12945	*Salmonella* Heidelberg I	Poultry	B	POS
12947	*Salmonella* Heidelberg I	Poultry	B	POS
12952	*Salmonella* Heidelberg I	Poultry	B	POS
1616	*Salmonella* Houten IV	Imported bird feces	43:z4,z23:-	POS
3699	*Salmonella* Hvittingfoss	Herbs or spices	I	POS
1480	*Salmonella* Indiana I	Turkey	B	POS
3852	*Salmonella* Indiana I	Poultry feed	B	POS
7011	*Salmonella* Indiana I		B	POS
5533	*Salmonella* Infantis I	Thyme	C1	POS
7111	*Salmonella* Infantis I		C1	POS
1693	*Salmonella* Javiana I		D1	POS
1695	*Salmonella* Johannesburg I		R	POS
3043	*Salmonella* Johannesburg I		R	POS
1251	*Salmonella* Kedougou I	Turkey	G2	POS
2628	*Salmonella* Kentucky I		C3	POS
12912	*Salmonella* Kentucky I	Poultry	C3	POS
12914	*Salmonella* Kentucky I	Poultry	C3	POS
12915	*Salmonella* Kentucky I	Poultry	C3	POS
12916	*Salmonella* Kentucky I	Poultry	C3	POS
12917	*Salmonella* Kentucky I	Poultry	C3	POS
12918	*Salmonella* Kentucky I	Poultry	C3	POS
12921	*Salmonella* Kentucky I	Poultry	C3	POS
12924	*Salmonella* Kentucky I	Poultry	C3	POS
12925	*Salmonella* Kentucky I	Poultry	C3	POS
12926	*Salmonella* Kentucky I	Poultry	C3	POS
12927	*Salmonella* Kentucky I	Poultry	C3	POS
12941	*Salmonella* Kentucky I	Poultry	C3	POS
12943	*Salmonella* Kentucky I	Poultry	C3	POS
12946	*Salmonella* Kentucky I	Poultry	C3	POS
12948	*Salmonella* Kentucky I	Poultry	C3	POS
12949	*Salmonella* Kentucky I	Poultry	C3	POS
12950	*Salmonella* Kentucky I	Poultry	C3	POS
12951	*Salmonella* Kentucky I	Poultry	C3	POS
12955	*Salmonella* Kentucky I	Poultry	C3	POS
12956	*Salmonella* Kentucky I	Poultry	C3	POS
12957	*Salmonella* Kentucky I	Poultry	C3	POS
12981	*Salmonella* Kentucky I	Poultry	C3	POS
12989	*Salmonella* Kentucky I	Poultry	C3	POS
12990	*Salmonella* Kentucky I	Poultry	C3	POS

12993	*Salmonella* Kentucky I	Poultry	C3	POS
12997	*Salmonella* Kentucky I	Poultry	C3	POS
13002	*Salmonella* Kentucky I	Poultry	C3	POS
13003	*Salmonella* Kentucky I	Poultry	C3	POS
13006	*Salmonella* Kentucky I	Poultry	C3	POS
13007	*Salmonella* Kentucky I	Poultry	C3	POS
13008	*Salmonella* Kentucky I	Poultry	C3	POS
13009	*Salmonella* Kentucky I	Poultry	C3	POS
13010	*Salmonella* Kentucky I	Poultry	C3	POS
13012	*Salmonella* Kentucky I	Poultry	C3	POS
13013	*Salmonella* Kentucky I	Poultry	C3	POS
13015	*Salmonella* Kentucky I	Poultry	C3	POS
13016	*Salmonella* Kentucky I	Poultry	C3	POS
2196	*Salmonella* Kiambu I		B	POS
2312	*Salmonella* Kottbus I		C2	POS
3038	*Salmonella* Krefeld I		E4	POS
2353	*Salmonella* Kristianstad I		E1	POS
7061	*Salmonella* Kubacha I		B	POS
7062	*Salmonella* Kubacha I		B	POS
2199	*Salmonella* Lexington I		E1	POS
13068	*Salmonella* Lexington I	Soy Plant Environmental	E1	POS
2263	*Salmonella* Lille I	Pancake	C1	POS
2868	*Salmonella* Lille I	Cocoa bean environment	C1	POS
2992	*Salmonella* Lille I		C1	POS
1650	*Salmonella* Livingstone I	Faeces	C1	POS
4036	*Salmonella* Livingstone I	Chicken	C1	POS
1652	*Salmonella* London I		E1	POS
1698	*Salmonella* Madelia I	Liver of hen	H	POS
2201	*Salmonella* Madelia I		H	POS
1424	*Salmonella* Manchester I	Autolysed yeast	C2	POS
1653	*Salmonella* Manhattan I		C2	POS
2673	*Salmonella* Manhattan I	Avian	C2	POS
6729	*Salmonella* Manila I	Sesame seeds	E2	POS
2309	*Salmonella* Maregrosso V		66:z35:-	POS
2755	*Salmonella* Mbandaka I	Swine tissue composite	C1	POS
13069	*Salmonella* Mbandaka I	Soy Plant Environmental	C1	POS
1701	*Salmonella* Miami I		D1	POS
2204	*Salmonella* Minnesota I		L	POS
1703	*Salmonella* Mississippi I	Faeces in 1942	G2	POS
2205	*Salmonella* Mississippi I		G2	POS
1255	*Salmonella* Montevideo I	Egg	C1	POS
1492	*Salmonella* Montevideo I		C1	POS
13071	*Salmonella* Montevideo I	Soy Plant Environmental	C1	POS
1704	*Salmonella* Muenchen I		C2	POS

3156	*Salmonella* Muenchen I	Cocoa bean environment	C2	POS
2748	*Salmonella* Muenster I	Chicken	E1	POS
966	*Salmonella* Napoli I		D1	POS
1476	*Salmonella* Napoli I		D1	POS
3898	*Salmonella* Neumuenster I	Poultry feed	B	POS
1707	*Salmonella* Newbrunswick I		E1	POS
2283	*Salmonella* Newbrunswick I	Malted barley flour	E1	POS
707	*Salmonella* Newport I	Fatal case of food poisoning	C2	POS
1261	*Salmonella* Newport I	Duck	C2	POS
13079	*Salmonella* Newport I	Basil	C2	POS
1710	*Salmonella* Oranienburg I		C1	POS
3863	*Salmonella* Othmarschen I	Poultry hatchery	C1	POS
1248	*Salmonella* Panama I	Pork sausages	D1	POS
918	*Salmonella* Paratyphi A I		A	POS
919	*Salmonella* Paratyphi A I		A	POS
1711	*Salmonella* Pomona I	Turkey intestine in 1941	M	POS
2215	*Salmonella* Poona I		G1	POS
1712	*Salmonella* Pretoria I	Pig	F	POS
1482	*Salmonella* Pullorum I	Chicks livers	D1	POS
1507	*Salmonella* Pullorum I	Chicks livers	D1	POS
1655	*Salmonella* Reading I		B	POS
4558	*Salmonella* Redlands I		I	POS
2289	*Salmonella* Rubislaw I	Barley malt berries	F	POS
1372	*Salmonella* Saintpaul I	Milk powder	B	POS
13080	*Salmonella* Saintpaul I	Basil	B	POS
1657	*Salmonella* Sandiego I		B	POS
2218	*Salmonella* Sandiego I		B	POS
2935	*Salmonella* Sandiego I		B	POS
6250	*Salmonella* Santiago I	Dried onion	C3	POS
6586	*Salmonella* Santiago I	Bourgignon powder	C3	POS
2352	*Salmonella* Saphra I		I	POS
1658	*Salmonella* Schwarzengrund I		B	POS
2637	*Salmonella* Schwarzengrund I	Chicken	B	POS
2641	*Salmonella* Schwarzengrund I	Chicken	B	POS
3184	*Salmonella* Sculcoates I	Cocoa bean environment	I	POS
1610	*Salmonella* Seminole IV	Lizard coelomic fluid	1,40:g,z51:-	POS
12960	*Salmonella* Senftenberg I	Poultry	E4	POS
12961	*Salmonella* Senftenberg I	Poultry	E4	POS
12962	*Salmonella* Senftenberg I	Poultry	E4	POS
12963	*Salmonella* Senftenberg I	Poultry	E4	POS
12964	*Salmonella* Senftenberg I	Poultry	E4	POS
12965	*Salmonella* Senftenberg I	Poultry	E4	POS
12966	*Salmonella* Senftenberg I	Poultry	E4	POS
12970	*Salmonella* Senftenberg I	Poultry	E4	POS

12971	*Salmonella* Senftenberg I	Poultry	E4	POS
12972	*Salmonella* Senftenberg I	Poultry	E4	POS
12973	*Salmonella* Senftenberg I	Poultry	E4	POS
12975	*Salmonella* Senftenberg I	Poultry	E4	POS
12978	*Salmonella* Senftenberg I	Poultry	E4	POS
12980	*Salmonella* Senftenberg I	Poultry	E4	POS
12982	*Salmonella* Senftenberg I	Poultry	E4	POS
12983	*Salmonella* Senftenberg I	Poultry	E4	POS
12984	*Salmonella* Senftenberg I	Poultry	E4	POS
12986	*Salmonella* Senftenberg I	Poultry	E4	POS
12987	*Salmonella* Senftenberg I	Poultry	E4	POS
12988	*Salmonella* Senftenberg I	Poultry	E4	POS
13056	*Salmonella* Senftenberg I	Soy Plant Environmental	E4	POS
13057	*Salmonella* Senftenberg I	Soy Plant Environmental	E4	POS
13058	*Salmonella* Senftenberg I	Soy Plant Environmental	E4	POS
13059	*Salmonella* Senftenberg I	Soy Plant Environmental	E4	POS
13060	*Salmonella* Senftenberg I	Soy Plant Environmental	E4	POS
1659	*Salmonella* Shangani I		E1	POS
739	*Salmonella* Stanley I		B	POS
1333	*Salmonella* Stanley I	Chicken	B	POS
3194	*Salmonella* Stanleyville I	Cocoa bean environment	B	POS
1660	*Salmonella* Sundsvall I		H	POS
2867	*Salmonella* Sya I	Cocoa bean environment	X	POS
3186	*Salmonella* Sya I	Cocoa bean environment	X	POS
1661	*Salmonella* Tennessee I		C1	POS
3536	*Salmonella* Tennessee I		C1	POS
13061	*Salmonella* Tennessee I	Soy Plant Environmental	C1	POS
13062	*Salmonella* Tennessee I	Soy Plant Environmental	C1	POS
13063	*Salmonella* Tennessee I	Soy Plant Environmental	C1	POS
13064	*Salmonella* Tennessee I	Soy Plant Environmental	C1	POS
13065	*Salmonella* Tennessee I	Soy Plant Environmental	C1	POS
13066	*Salmonella* Tennessee I	Soy Plant Environmental	C1	POS
2229	*Salmonella* Theilalle I		6,7,14:m,t:-	POS
2639	*Salmonella* Thomasville I	Turkey intestine	E3	POS
3924	*Salmonella* Thomasville I	Poultry feed	E3	POS
1336	*Salmonella* Thompson I	Chicken	C1	POS
1339	*Salmonella* Thompson I	Egg	C1	POS
12904	*Salmonella* Tranoroa II	ATCC 700148	55:k:z39	POS
1613	*Salmonella* Tuindorp IV	Zoo animal liver	43:z4,z32:-	POS
584	*Salmonella* Typhi I		D1	POS
585	*Salmonella* Typhi I		D1	POS
586	*Salmonella* Typhimurium I	Animal tissue	B	POS
1084	*Salmonella* Typhimurium I		B	POS
1467	*Salmonella* Typhimurium I		B	POS

13005	*Salmonella* Typhimurium I	Poultry	B	POS
13011	*Salmonella* Typhimurium I	Poultry	B	POS
2238	*Salmonella* Urbana I		N	POS
2239	*Salmonella* Uzaramo I		H	POS
2346	*Salmonella* Vietnam I		S	POS
738	*Salmonella* Virchow I		C1	POS
13081	*Salmonella* Virchow I	Basil	C1	POS
1614	*Salmonella* Volksdorf IV	Iguana bladder	43:z36,z38:-	POS
2313	*Salmonella* Wandsworth I		Q	POS
1609	*Salmonella* Wassennaar IV	Iguana swab	50:g,z51:-	POS
1714	*Salmonella* Wassennaar IV	Human	50:g,z51:-	POS
4035	*Salmonella* Waycross I		S	POS
1491	*Salmonella* Weltevreden I	Prawns	E1	POS
1560	*Salmonella* Westpark II	Tortoise intestine	3,10:l,z28:e,n,x	Neg at 10^5 cfu/ml, Pos at 10^6
4043	*Salmonella* Worthington I		G2	POS

Table 8. Exclusivity of the BAX ® Real Time *Salmonella* Test Kit (1)

DuPont Strain ID Number	ATCC Strain Number	Genus and Species	Isolate Source	BAX ® RT *Salmonella* Result
373	*13883*	*Klebsiella pneumoniae*		NEG
374	*29906*	*Proteus mirabilis*		NEG
383	*8090*	*Citrobacter freundii*		NEG
640	*43889*	*Escherichia coli O157:H7*	HUS Case Stool	NEG
641	*43890*	*Escherichia coli O157:H7*	Human Feces	NEG
657	*11296*	*Klebsiella ozaenae*		NEG
658	*13182*	*Klebsiella oxytoca*	Pharyngeal Tonsil	NEG
2389	*13337*	*Hafnia alvei*		NEG
2417		*Serratia liquefaciens*	Raw Mince	NEG
2558	*43864*	*Citrobacter freundii*		NEG
3064		*Morganella morganii*	Environmental Swab	NEG
3982	*27853*	*Pseudomonas aeruginosa*	Blood Culture	NEG
5588		*Hafnia alvei*	Ground Beef	NEG
6121		*Proteus mirabilis*	Herring Gull Cloacae	NEG
13142		*Morganella morganii*		NEG
13147		*Providencia rettgeri*		NEG
13148		*Pseudomonas aeruginosa*		NEG
13186		*Enterobacter amnigenus*		NEG
13187		*Enterobacter amnigenus*		NEG
ES9		*Enterobacter sakazakii*		NEG
ES14		*Enterobacter sakazakii*		NEG
ES53		*Enterobacter sakazakii*		NEG
ES1		*Enterobacter sakazakii*		NEG
ES20		*Enterobacter sakazakii*		NEG
ES34		*Enterobacter sakazakii*		NEG
ES35		*Enterobacter sakazakii*		NEG
ES38		*Enterobacter sakazakii*		NEG
700		*Shigella sonnei*		NEG
1083		*Shigella flexneri*		NEG
702		*Shigella sonnei*		NEG
846	29907	*Escherichia blattae*	Hindgut of Cockroach	NEG
847	35469	*Escherichia fergusonii*	Human Feces	NEG
848	33650	*Escherichia hermannii*	Human Toe	NEG
849	21073	*Escherichia intermedia*		NEG
850	33821	*Escherichia vulneris*	Human Wound	NEG
854	35539	*Staphylococcus gallinarum*	Chicken Nares	NEG
862	4698	*Micrococcus luteus*		NEG
863	12600	*Staphylococcus aureus*	Human Clinical	NEG

864	14990	*Staphylococcus epidermidis*	Nose	NEG
3354		*Listeria welshimeri*		NEG
1309		*Listeria monocytogenes*	Soft Cheese	NEG
1154		*Listeria innocua*	Pate	NEG
QC201	13048	*Enterobacter aerogenes*	Sputum	NEG
QC203	*51113*	*Citrobacter brakii*	Snake	NEG
QC204	*700814*	*Bacillus pumilus*		NEG
QC102	*51740*	*Staphylococcus aureus*	Margarine	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]		
***Salmonella* 2**	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	-0.19, 0.19

Table 3. BAX® System Results – DuPont™ Thermal Block vs. Analog Heating/Cooling Blocks (con't)

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]		
***Salmonella* 2 (con't)**	Beef trim	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	15	0.89	0.66, 0.97	0.1176	-0.085, 0.34
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.19, 0.19
	Spinach	High	17	17	1	0.82,1.0	17	1	0.82, 1.0	0	-0.18, 0.18
		Low	17	14	0.82	0.59, 0.94	16	0.94	0.73, 0.99	-0.12	-0.36, 0.12
		Control	17	0	0	0, 0.19	0	0	0, 0.19	0	-0.19, 0.19

DISCUSSION OF AUGUST 2015 MODIFICATION (6)

The alternative methods using the Actero™ Salmonella broth have been developed for a single-step recovery of *Salmonella* spp. from environmental and food samples followed by the detection using the BAX® System Real-Time PCR Assay for *Salmonella* or by direct plating. The internal and independent laboratory matrix studies were carried out to compare performance of the alternative methods against the reference method to detect *Salmonella* spp. in dry pet food, milk chocolate, chocolate liquor, cocoa powder, shell egg, and stainless steel and plastic environmental samples.

The comparison studies showed that the alternative methods were equivalent to the U.S. FDA reference method for dry pet food, milk chocolate, cocoa powder, shell egg, and stainless steel and plastic environmental samples. According to the POD statistical model, statistically significant superior performance was observed when chocolate liquor samples were tested using the Actero™ Salmonella method as compared to the reference method. Absence of false positive outcomes and a low rate of false negative outcomes (three out of 360 samples tested) indicated high accuracy and reliability of the proposed alternative method.

In conclusion, the data in these studies support the candidate method claims when testing dry pet food, milk chocolate, chocolate liquor, cocoa powder, shell egg and stainless steel and plastic environmental samples. The turnaround time for a result is as short as one day if the BAX® System method is used and two days if the direct plating is performed. Being shorter than for the reference method, this time, undoubtedly, presents an advantage for the proposed candidate methods.

Table 2. Actero™ Salmonella Enrichment with BAX® System Method —Presumptive *vs* Confirmed (6)

Matrix	Strain (stress)	Sample size	Enrichment time	MPN[a] or I[b]		N[e]	Candidate Method Presumptive			Candidate Method Confirmed			dPOD$_{CP}$[i]	95% CI[j]
				CFU/sample	(UCL[c], LCL[d])		X[f]	POD$_{CP}$[g]	95% CI	X	POD$_{CC}$[h]	95% CI		
Dry pet food	*S.* Anatum (lyophilized)	25 g	18 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				1.1	(0.7; 1.8)	20	13	0.65	(0.43; 0.82)	13	0.65	(0.43; 0.82)	0.00	(-0.28; 0.28)
				3.7	(1.6; 8.8)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Dry pet food[l] (Independent lab data)	*S.* Anatum (lyophilized)	375 g	18 ± 0.5 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				1.5	(1.0; 2.3)	20	14	0.70	(0.48; 0.86)	14	0.70	(0.48; 0.86)	0.00	(-0.28; 0.28)
				8.7	(2.8; 26.9)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Milk chocolate	*S.* Senftenberg (heated)	25 g	22 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.5	(0.3; 0.9)	20	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)
				8.7	(2.8; 26.9)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Milk chocolate[l] (Independent lab data)	*S.* Senftenberg (heated)	25 g	22 ± 0.5 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.3	(0.1; 0.6)	20	7	0.35	(0.18; 0.57)	10	0.50	(0.30; 0.70)	-0.15	(-0.41; 0.15)
				3.2	(1.4; 7.2)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Chocolate liquor	*S.* Virchow (heated)	25 g	26 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.1	(0.03; 0.3)	20	9	0.45	(0.26; 0.67)	9	0.45	(0.26; 0.67)	0.00	(-0.28; 0.28)
				2.7	(1.2; 6.1)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Cocoa powder	*S.* Orion (lyophilized)	25 g	16 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.6	(0.3; 1.0)	20	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)
				2.0	(0.9; 4.7)	5	3	0.60	(0.23; 0.88)	3	0.60	(0.23; 0.88)	0.00	(-0.47; 0.47)
Shell egg	*S.* Cerro	20 eggs	16h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.3	(0.1; 0.6)	20	7	0.35	(0.18; 0.57)	7	0.35	(0.18; 0.57)	0.00	(-0.28; 0.28)
				1.2	(0.9; 4.0)	5	4	0.80	(0.38; 0.96)	4	0.80	(0.38; 0.96)	0.00	(-0.46; 0.46)
Stainless steel	*S.* Braenderup (dried) + *C. freundii*	100 cm^2	14 h	0.0 + 0.0	N/A[k]	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				50.0 + 462.5	N/A	20	12	0.60	(0.39; 0.78)	12	0.60	(0.39; 0.78)	0.00	(-0.28; 0.28)
				437.0 + 1375.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
			18 h	0.0 + 0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				50.0 + 462.5	N/A	20	16	0.80	(0.58; 0.92)	16	0.80	(0.58; 0.92)	0.00	(-0.28; 0.28)
				437.0 + 1375.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

Stainless steel[l] (Independent lab data)	*S.* Braenderup (dried) + *C. freundii*	100 cm²	16 ± 2 h	0.0 + 0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				47.0 + 217.0	N/A	20	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)
				313.0 + 2217.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Plastic	*S.* Oranienburg (dried)	100 cm²	14 h	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				59.4	N/A	20	10	0.50	(0.30; 0.70)	10	0.50	(0.30; 0.70)	0.00	(-0.28; 0.28)
				400.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
			18 h	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				53.0	N/A	20	11	0.55	(0.34; 0.74)	11	0.55	(0.34; 0.74)	0.00	(-0.28; 0.28)
				120.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.0	(0.57; 1.00)	0.00	(-0.43; 0.43)

[a]MPN – Most Probable Number (16) is based on the POD of reference method test portions using the LCF MPN calculator (17), with 95% confidence interval. MPN has been calculated only for the food samples.
[b]I – Inoculum level which was determined only for the environmental samples.
[c]UCL – Upper Confidence Limit.
[d]LCL – Lower Confidence Limit.
[e]N – Number of test portions.
[f]X – Number of positive test portions.
[g]POD_{CP} – Candidate method presumptive positive outcomes divided by the total number of trials.
[h]POD_{CC} – Candidate method confirmed positive outcomes divided by the total number of trials.
[i]$dPOD_{CP}$ – Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[j]95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[k]N/A – Not applicable.
[l] Independent validation study

Table 3. Actero™ Salmonella Enrichment with BAX® System Method *vs* Reference Method (6)

Matrix	Strain (stress)	Sample size	Enrichment time	MPN[a] or I[b] CFU/ sample	(UCL[c], LCL[d])	N[e]	Candidate Method X[f]	POD_C[g]	95% CI	Reference Method X	POD_R[h]	95% CI	$dPOD_{CR}$[i]	95% CI[j]
Dry pet food	*S.* Anatum (lyophilized)	25 g	18 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				1.1	(0.7; 1.8)	20	13	0.65	(0.43; 0.82)	16	0.80	(0.58; 0.92)	-0.15	(-0.40; 0.12)
				3.7	(1.6; 8.8)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Dry pet food[l] (Independent lab data)	*S.* Anatum (lyophilized)	375 g	18 ± 0.5 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				1.5	(1.0; 2.3)	20	14	0.70	(0.48; 0.86)	15	0.75	(0.53; 0.89)	-0.05	(-0.31; 0.22)
				8.7	(2.8; 26.9)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Milk chocolate	*S.* Senftenberg (heated)	25 g	22 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.5	(0.3; 0.9)	20	10	0.50	(0.30; 0.70)	9	0.45	(0.26; 0.66)	0.05	(-0.24; 0.33)
				8.7	(2.8; 26.9)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Milk chocolate[l] (Independent lab data)	*S.* Senftenberg (heated)	25 g	22 ± 0.5 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.3	(0.1; 0.6)	20	7	0.35	(0.18; 0.57)	6	0.30	(0.15; 0.52)	0.05	(-0.23; 0.32)
				3.2	(1.4; 7.2)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Chocolate liquor	*S.* Virchow (heated)	25 g	26 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.1	(0.03; 0.3)	20	9	0.45	(0.26; 0.67)	2	0.10	(0.03; 0.30)	0.35	(0.07; 0.57)
				2.7	(1.2; 6.1)	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Cocoa powder	*S.* Orion (lyophilized)	25 g	16 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.6	(0.3; 1.0)	20	10	0.50	(0.30; 0.70)	9	0.45	(0.26; 0.67)	0.05	(-0.24; 0.33)
				2.0	(0.9; 4.7)	5	3	0.60	(0.23; 0.88)	5	1.00	(0.57; 1.00)	-0.40	(-0.77; 0.12)
Shell egg	*S.* Cerro	20 eggs	16 h	<0.075	(0.00; 0.00)	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				0.3	(0.1; 0.6)	20	7	0.35	(0.18; 0.57)	6	0.30	(0.15; 0.52)	0.05	(-0.23; 0.32)
				1.2	(0.9; 4.0)	5	4	0.80	(0.38; 0.96)	4	0.80	(0.38; 0.96)	0.00	(-0.46; 0.46)
Stainless steel	*S.* Braenderup (dried)+ *C. freundii*	100 cm²	14 h	0.0 + 0.0	N/A[k]	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				50.0 + 462.5	N/A	20	12	0.60	(0.39; 0.78)	12	0.60	(0.39; 0.78)	0.00	(-0.28; 0.28)
				437.0 + 1375.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
			18 h	0.0 + 0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				50.0 + 462.5	N/A	20	16	0.80	(0.58; 0.92)	12	0.60	(0.39; 0.78)	0.20	(-0.08; 0.45)
				437.0 + 1375.0	N/A	5	5	1.00	(0.57; 1.00)	4	0.80	(0.38; 0.96)	0.20	(-0.26; 0.62)

Stainless steel [I] (Independent lab data)	*S.* Braenderup (dried) + *C. freundii*	100 cm^2	16 ± 2 h	0.0 + 0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				47.0 + 217.0	N/A	20	10	0.50	(0.30; 0.70)	9	0.45	(0.26; 0.66)	0.05	(-0.24; 0.33)
				313.0 + 2217.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
Plastic	*S.* Oranienburg (dried)	100 cm^2	14 h	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				59.4	N/A	20	10	0.50	(0.30; 0.70)	14	0.70	(0.48; 0.85)	-0.20	(-0.45; 0.10)
				400.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)
			18 h	0.0	N/A	5	0	0.00	(0.00; 0.43)	0	0.00	(0.00; 0.43)	0.00	(-0.43; 0.43)
				53.0	N/A	20	11	0.55	(0.34; 0.74)	12	0.60	(0.39; 0.78)	-0.50	(-0.33; 0.24)
				120.0	N/A	5	5	1.00	(0.57; 1.00)	5	1.00	(0.57; 1.00)	0.00	(-0.43; 0.43)

[a]MPN – Most Probable Number (16) is based on the POD of reference method test portions using the LCF MPN calculator (17), with 95% confidence interval. MPN has been calculated only for the food samples.
[b]I – Inoculum level which was determined only for the environmental samples.
[c]UCL – Upper Confidence Limit.
[d]LCL – Lower Confidence Limit.
[e]N – Number of test portions.
[f]X – Number of positive test portions.
[g]POD_C – Candidate method positive outcomes divided by the total number of trials.
[h]POD_R – Reference method positive outcomes divided by the total number of trials.
[i]$dPOD_{CP}$ – Difference between the candidate method and candidate method result POD values.
[j]95% CI – If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[k]N/A – Not applicable.
[l] Independent validation study

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