CERTIFICATION

AOAC Research Institute

*Performance Tested Methods*SM

Certificate No.

081201

The AOAC Research Institute hereby certifies the method 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 Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods* SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date	December 19, 2023
Expiration Date	December 31, 2024

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MODIFICATION AUGUST 2015: Sergiy Olishevskyy, Melissa Buzinhani, Cathy St-Laurent, Benoit Crevier, Renaud Tremblay, and F. Morgan Wallace
MODIFICATION JANUARY 2021: Nisha Corrigan, April Englishbey, Tyler Stephens, Savannah Forgey
MODIFICATION JANUARY 12, 2022: Nisha Corrigan, Tyler P. Stephens, PhD., Savannah F. Applegate, PhD., April Englishbey, PhD., Rossy Bueno, M.S.
MODIFICATION JANUARY 13, 2022: Nisha Corrigan, Casey Simmons, Leo Horine, and Alex Tudor
MODIFICATION APRIL 2023: Nisha Corrigan, Julie Weller, Deja Latney, Margaret Morris, and Stoltenberg

SUBMITTING COMPANY
DuPont Nutrition & Health
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200 Powder Mill Road
Wilmington, DE 19803
USA

CURRENT SPONSOR
Hygiena
2 Boulden Circle
New Castle, DE 19720
USA

METHOD NAME
BAX® System Real-Time PCR Assay for *Salmonella*
Formerly DuPont™ BAX® System Real-Time PCR Assay for *Salmonella*

CATALOG NUMBERS
BAX® System Assay KIT2006, MP Media MED2003

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[a] ORIGINAL VALIDATION
[b] MODIFICATION AUGUST 2015
[c] MODIFICATION JANUARY 2021, JANUARY 12, 2022
[d] MODIFICATION JANUARY 13, 2022

APPLICABILITY OF METHOD
Target organism – *Salmonella*

Matrixes – 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
MODIFICATION AUGUST 2015 (USDA BAM Ch 5): dry pet food, milk chocolate, chocolate liquor, cocoa powder, shell egg, stainless steel, plastic
MODIFICATION JANUARY 2021 (USDA MLG 2.05 and USDA MLG 4.10) – Raw comminuted chicken (325 g), turkey (325g).
MODIFICATION JANUARY 12, 2022
BAX System SalQuant - USDA MLG 2.05 and USDA MLG 4.10 – Whole Carcass Poultry Rinses (30 mL), Fresh Raw Ground Beef (375 g), Fresh Raw Beef Trim (375 g), MicroTally on Fresh Raw Beef Trim (1 cloth), Fresh Raw Ground Pork (375 g), Fresh Raw Pork Trim (375 g) and MicroTally on Fresh Raw Pork Trim (1 cloth)
BAX MPN - USDA MLG 2.05 - Whole Carcass Poultry Rinses (30 mL), Fresh Raw Beef Trim (375 g)
MODIFICATION JANUARY 13, 2022 – dried cannabis flower [10 g, >0.3% delta 9-tetrahydrocannabinol (THC)] and dried hemp flower (10 g, ≤0.3% THC).
MODIFICATION APRIL 2023 – sampling cloths swabbed from 375 g beef trim portions.

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*. (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. (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. (4)

U.S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG), 2.05, *Most Probable Number Procedure and Tables*. (8)

U. S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG), 4.10, *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges*. (9)

***Standard Method Performance Requirements* (SMPRs®) for Detection of *Salmonella* species in Cannabis and Cannabis Products (AOAC SMPR 2020.002). (12)**

U. S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook*, 4.12 (2022), Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges (14)

Performance claims – Results were comparable to the reference methods.

MODIFICATION APRIL 2023 – The study data were unable to find a statistically detectable difference in results between the BAX System Real-Time PCR Assay for *Salmonella* test kit and the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG) 4.12 (2022), Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges (14) from sampling cloths swabbed from 375 g beef trim test portions in 8-24 h using modified Tryptic Soy Broth with casamino acids (mTSB +caa) enrichment media and 10-24 h using MP Media.

ORIGINAL CERTIFICATION DATE **August 07, 2012**	**CERTIFICATION RENEWAL RECORD** **Renewed annually through December 2024.**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
1. July 2013	**1. Addition of Thermal Block for automated sample lysis.**
2. August 2015 Level 2	**2. Matrix Extension in collaboration with FoodChek PTM 041303.**
3. March 2017 Level 1	**3. Name change from DuPont Nutrition & Health to Qualicon Diagnostics LLC., a Hygiena company.**
4. January 2018 Level 1	**4. Inserts, labels, manuals updated to Hygiena.**
5. May 2019 Level 1	**5. Editorial updates to insert and corporate address.**
6. December 2019 Level 1	**6. Editorial/clerical changes.**
7. January 2021 Level 3	**7. Certification of BAX System SalQuant (SalQuant) method for Salmonella Quantification and BAX MPN in comminuted turkey and comminuted chicken.**
8. December 2021 Level 1	**8. Editorial changes.**
9. January 12, 2022 Level 2	**9. Matrix extension of SalQuant method for Salmonella quantification to include whole Carcass Poultry Rinses, Fresh Raw Ground Beef, Fresh Raw Beef Trim, MicroTally on Fresh Raw Beef Trim, Fresh Raw Ground Pork, Fresh Raw Pork Trim and MicroTally on Fresh Raw Pork Trim. Matrix extension for BAX MPN method to include whole carcass poultry rinses and fresh raw beef trim.**
10. January 13, 2022 Level 2	**10. Matrix extension to include dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC).**
11. November 2022 Level 1	**11. Editorial changes.**
12. April 2023 Level 2	**12. Matrix extension to include sampling cloths swabbed from 375 g beef trim portions.**
13. December 2023 Level 1	**13. Editorial/clerical changes.**

Under this AOAC *Performance Tested Methods*SM License Number, 081201 this method is distributed by: **NONE**	**Under this AOAC *Performance Tested Methods*SM License Number, 081201 this method is distributed as:** **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. braakii* 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. braakii* 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 MODIFICATION APPROVED JULY 2013 (5)

The results of the method comparison between the digital DuPont™ Thermal Block and the analog heating/cooling blocks are provided in Table 3 below. For all sample types and BAX System assays evaluated, the results for samples processed with the DuPont™ Thermal Block and the original heating/cooling blocks demonstrated no significant statistical difference as indicated by POD analysis (the 95% confidence interval of the dPOD included 0 in all cases). For additional figures illustrating the target responses of the individual BAX System assays, see Appendix B.

All 544 samples inoculated with high levels of the target organism returned positive results with the BAX System using both sample preparation methods, and all 544 samples tested as unspiked negative controls returned negative results with the BAX System using both sample preparation methods with the exception of the non-inoculated poultry rinse samples that gave positive results *for Campylobacter 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 MODIFICATION APPROVED AUGUST 2015 (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 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)	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]		N[e]	Candidate Method			Reference Method			$dPOD_{CR}$[i]	95% CI[j]
				CFU/ sample	(UCL[c], LCL[d])		X[f]	POD_C[g]	95% CI	X	POD_R[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)	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[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)	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 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)	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

DISCUSSION OF MODIFICATION APPROVED JANUARY 2021 (7)

For all comparisons, SalQuant vs. USDA/FSIS MLG 2.05 and BAX MPN vs USDA/FSIS MLG 2.05, all mean differences between methods were <0.5 Log_{10} for both comminuted chicken and turkey. However, not all confidence intervals fell within the -0.5, 0.5 acceptance criterion for statistical equivalence at the 90% level.

For SalQuant, the middle and high contamination levels for both comminuted chicken and turkey showed CIs outside of this criterion (the high level for the chicken just barely so at -0.526, 0.273. Grubb's outlier test was conducted on all results to determine if any outliers were present. In the SalQuant vs. USDA/FSIS MLG 2.05 comparison, outliers were found in the high contamination level for the SalQuant method for comminuted chicken, and in the middle level for USDA/MLG 2.05 for comminuted turkey.

In the BAX MPN vs USDA/FSIS MLG 2.05 comparison, outliers were found in the middle level for both the BAX® MPN and USDA/FSIS MLG 2.05 methods. There was no justifiable cause to remove any of the outliers from the statistical analysis, and thus all data were included in the calculations. The fairly high variability between the replicates for both the candidate and reference methods (s_r > than 0.4, for example), along with the low contamination levels, are likely contributing to the CIs outside the -0.5, 0.5 range.

In addition, the SalQuant PCR method and the USDA/FSIS MLG 2.05 MPN methods are vastly different technologies. Both methods estimate the concentration of Salmonella, as opposed to direct plate count methods that determine concentration. The current recommended acceptance criteria are based on statistics for plate count methods and thus different technologies may require different consideration. For practical purposes, all mean differences were <0.5 Log_{10}, which was the acceptance criteria when this project was proposed.

The BAX MPN and the USDA/FSIS MLG 2.05 MPN showed statistical equivalence, except for the middle level for turkey, where the CI was -0.614, 0.299. In this the mean difference between the methods was -0.157. Outliers were seen in in both methods for this level and are likely contributing to the wider interval. The comparison between the BAX MPN and the USDA/FSIS MLG 2.05 MPN was a paired analysis.

The SalQuant method allows the user to obtain results in 10 h (8 h enrichment and 2 h of process time) and get an estimation of the amount of *Salmonella* in raw comminuted chicken and turkey; this in contrast to completing the full cultural MPN reference method which takes 5, more labor-intensive, days. The SalQuant sample setup is simple, and the PCR run provides results in two hours, including sample lysing and processing. The procedure is easy to follow, allowing for a technician at any level of training to perform the method and obtain accurate results. The BAX System Q7 software is easy to use and sample creation and analysis only require a few steps, allowing for quick preparation and interpretation of samples. Results are displayed after the run is completed and are clearly differentiated between positive and negative. The CT values are easy to find and is done so by printing a detailed view of the report or exporting as a .csv file extension, allowing for the user to easy input data into the SalQuant calculator via excel spreadsheet (provided by Hygiena, LLC).

Table 1: Raw Comminuted Chicken Method Comparison Results of BAX System SalQuant vs. USDA/FSIS MLG 2.05 (7)

Inoculation Level	Sample Replicate	SalQuant					USDA/FSIS MLG 2.05[a]					Mean Difference[d]	90% CI[e]	95% CI
		CFU/g	Log_{10}	Log_{10} Mean	SD[b] (S_r)	RSD_r[c]	MPN/g	Log_{10}	Log_{10} Mean	SD (S_r)	RSD_r			
Low	1	3.62	0.571	0.706	0.413	58.500	4.3	0.643	0.757	0.159	21.004	-0.051	-0.450, 0.348	-0.560, 0.458
	2	1.02	0.049				4.3	0.643						
	3	10.73	1.035				7.5	0.881						
	4	10.73	1.035				9.3	0.973						
	5	6.83	0.841				4.3	0.643						
Medium	1	38.1	1.582	1.418	0.355	25.035	46	1.664	1.823	0.323	17.718	-0.404	-0.811, 0.002	-0.912, 0.103
	2	29.04	1.464				110	2.042						
	3	49.99	1.700				110	2.042						
	4	34.81	1.543				110	2.042						
	5	6.23	0.801				21	1.324						
High	1	254.95	2.407	2.415	0.295	12.215	110	2.042	2.541	0.368	14.482	-0.127	-0.526, 0.273	-0.601, 0.359
	2	177.51	2.249				380	2.580						
	3	212.73	2.328				1100	3.041						
	4	148.11	2.171				240	2.380						
	5	826.9	2.918				460	2.663						

[a]Unpaired analysis following the USDA/FSIS-MLG 2.05 reference method.

[b]SD = Standard deviation.

[c]RSD_r = Relative Standard deviation.

[d]Mean Difference = Candidate Log Mean - Reference Log Mean.

[e]90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

Table 2: Raw Comminuted Chicken Method Comparison Results of BAX MPN vs. USDA/FSIS MLG 2.05 (7)

		BAX MPN[a]					USDA/FSIS MLG 2.05[a]							
Inoculation Level	Sample Replicate	MPN/g	Log_{10}	Log_{10} Mean	SD[b] (S_r)	RSD_r[c]	MPN/g	Log_{10}	Log_{10} Mean	SD (S_r)	RSD_r	Mean Difference[d]	90% CI[e]	95% CI
	1	2.3	0.380				4.3	0.643						
	2	9.3	0.973				4.3	0.643						
Low	3	7.5	0.881	0.717	0.310	43.240	7.5	0.881	0.757	0.159	21.004	-0.039	-0.273, 0.194	-0.343, 0.264
	4	9.3	0.973				9.3	0.973						
	5	2.3	0.380				4.3	0.643						
	1	110	2.042				46	1.664						
	2	110	2.042				110	2.042						
Medium	3	110	2.042	1.794	0.381	21.237	110	2.042	1.823	0.323	17.718	-0.029	-0.291, 0.233	-0.371, 0.313
	4	46	1.664				110	2.042						
	5	15	1.179				21	1.324						
	1	110	2.042				110	2.042						
	2	750	2.875				380	2.580						
High	3	1100	3.041	2.544	0.407	16.000	1100	3.041	2.541	0.368	14.482	0.003	-0.192, 0.197	-0.251, 0.256
	4	240	2.380				240	2.380						
	5	240	2.380				460	2.663						

[a]Paired analysis following the USDA/FSIS-MLG 2.05 reference method.
[b]SD = Standard deviation.
[c]RSD_r = Relative Standard deviation.
[d]Mean Difference = Candidate Log Mean – Reference Log Mean.
[e]90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

Table 3: Raw Comminuted Turkey Method Comparison Results of BAX System SalQuant vs. USDA/FSIS MLG 2.05 (7)

		SalQuant					USDA/FSIS MLG 2.05[a]							
Inoculation Level	Sample Replicate	CFU/g	Log_{10}	Log_{10} Mean	SD[b] (S_r)	RSD_r[c]	MPN/g	Log_{10}	Log_{10} Mean	SD (S_r)	RSD_r	Mean Difference[d]	90% CI[e]	95% CI
	1	1.09	0.076				0.43	-0.276						
	2	0.93	0.013				1.5	0.204						
Low	3	3.05	0.500	0.136	0.209	153.680	4.6	0.672	0.169	0.343	202.960	-0.033	-0.382, 0.316	-0.448, 0.381
	4	0.86	-0.018				1.0	0.041						
	5	1.18	0.108				1.5	0.204						
	1	33.05	1.520				15.0	1.179						
	2	26.05	1.417				27.0	1.433						
Medium	3	18.97	1.280	1.289	0.448	34.756	240.0	2.380	1.526	0.544	35.650	-0.237	-0.834, 0.360	-0.983, 0.508
	4	49.13	1.692				46.0	1.664						
	5	3.31	0.533				9.3	0.973						
	1	49.13	1.692				240.0	2.380						
	2	621.82	2.794				150.0	2.176						
High	3	356.89	2.553	2.518	0.473	18.785	460.0	2.663	2.156	0.476	22.080	0.363	-0.206, 0.931	-0.347, 1.072
	4	574.40	2.759				24.0	1.382						
	5	621.82	2.794				150.0	2.176						

[a]Unpaired analysis following the USDA/FSIS-MLG 2.05 reference method.
[b]SD = Standard deviation.
[c]RSD_r = Relative Standard deviation.
[d]Mean Difference = Candidate Log Mean - Reference Log Mean.
[d]90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

Table 4: Raw Comminuted Turkey Method Comparison Results of BAX MPN vs. USDA/FSIS MLG 2.05 (7)

Inoculation Level	Sample Replicate	BAX MPN[a]					USDA/FSIS MLG 2.05[a]					Mean Difference[d]	90% CI[e]	95% CI
		MPN/g	Log_{10}	Log_{10} Mean	SD[b] (S_r)	RSD_r[c]	MPN/g	Log_{10}	Log_{10} Mean	SD (S_r)	RSD_r			
Low	1	0.23	-0.481	0.026	0.447	1719.200	0.43	-0.276	0.169	0.343	202.960	-0.143	-0.276, 0.010	-0.316, 0.030
	2	0.93	0.013				1.5	0.204						
	3	4.6	0.672				4.6	0.672						
	4	0.43	-0.276				1.0	0.041						
	5	1.5	0.204				1.5	0.204						
Medium	1	15.0	1.179	1.369	0.337	24.617	15	1.179	1.526	0.544	35.650	-0.157	-0.614, 0.299	-0.752, 0.438
	2	27.0	1.433				27	1.433						
	3	24.0	1.382				240	2.380						
	4	75.0	1.876				46	1.664						
	5	9.3	0.973				9.3	0.973						
High	1	240.0	2.380	2.314	0.214	9.248	240	2.380	2.156	0.476	22.080	0.159	-0.180, 0.498	-0.282, 0.600
	2	150.0	2.176				150	2.176						
	3	460.0	2.663				460	2.663						
	4	150.0	2.176				24	1.382						
	5	150.0	2.176				150	2.176						

[a]Paired analysis following the USDA/FSIS-MLG 2.05 reference method.
[b]SD = Standard deviation.
[c]RSD_r = Relative Standard deviation.
[d]Mean Difference = Candidate Log Mean – Reference Log Mean.
[e]90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent.

Table 5: Inclusivity Panel (7)

No.	Hygiena Culture Collection No.	Name	Origin	Source	Serogroup	BAX® Real-Time PCR Assay for *Salmonella* Result
1	SAFE-45	*Salmonella bongori*	Unknown	USDA-FSIS[a] 94-0708	V48:i:-	POS
2	SAFE-46	*Salmonella bongori*	Unknown	USDA-FSIS 95-0123	V 40:z35:-	POS
3	SAFE-47	*Salmonella bongori*	Unknown	USDA-FSIS 96-0233	V 44:z39:-	POS
4	SAFE-48	*Salmonella bongori*	Unknown	USDA-FSIS CNM-256	V 60:z41:-	POS
5	1773	*Salmonella bongori*	ATCC	ATCC[b] 43975	66:z41:-	POS
6	1535	*Salmonella bongori* ser. *Brookfield*	Frog	HCC[c]	66:z41:-	POS
7	SAFE-27	*Salmonella enterica* subsp. *diarizonae*	Unknown	USDA-FSIS 01-0170	IIIb 60:r:e,n,x,zl5	POS
8	SAFE-28	*Salmonella enterica* subsp. *diarizonae*	Unknown	USDA-FSIS 01-0221	IHb 48:i:z	POS
9	SAFE-29	*Salmonella enterica* subsp. *diarizonae*	Unknown	USDA-FSIS 01-0248	IIIb 6 l:k: 1,5,(7)	POS
10	SAFE-30	*Salmonella enterica* subsp. *diarizonae*	Unknown	USDA-FSIS 02-0188	IIlb 61 :l,v: 1,5,7	POS
11	SAFE-31	*Salmonella enterica* subsp. *diarizonae*	Unknown	USDA-FSIS CNM-3511/02	IIlb 48: zlO: e,n,x,zl5	POS
12	SAFE-32	*Salmonella enterica* subsp. *diarizonae*	Unknown	USDA-FSIS CNM-4190/02	IIlb 38:zl0:z53	POS
13	1774	*Salmonella enterica* subsp. *diarizonae*	Unknown	ATCC 43973	6,7:l,v:z53	POS
14	SAFE-51	*Salmonella enterica* subsp. *indica*	Unknown	USDA-FSIS 1121	VI 6,14,25:zl0:l,(2),7	POS
15	SAFE-52	*Salmonella enterica* subsp. *indica*	Unknown	USDA-FSIS 1415	VI ll:b:l,7	POS
16	SAFE-53	*Salmonella enterica* subsp. *indica*	Unknown	USDA-FSIS 1937	VI 6,7:z41:l,7	POS
17	SAFE-54	*Salmonella enterica* subsp. *indica*	Unknown	USDA-FSIS 2229	VI ll:a:l,5	POS
18	SAFE-55	*Salmonella enterica* subsp. *indica*	Unknown	USDA-FSIS 811	VI 6,14,25:a:e,n,x	POS
19	13739	*Salmonella* ser. 4,12:i:-	Unknown	GPLN[d]	B	POS
20	13777	*Salmonella* ser. 4,5,12:i:-	Unknown	GPLN	B	POS
21	13641	*Salmonella* ser. Abaetetuba I	Creek Water	ATCC 35640	F	POS
22	3218	*Salmonella* ser. Agama I	Cocoa Bean Environment	HCC	B	POS
23	13743	*Salmonella* ser. Agona I	Unknown	GPLN	B	POS
24	13731	*Salmonella* ser. Alabama I	Unknown	GPLN	D1	POS
25	1556	*Salmonella* ser. Alachua I	Soil, abattoir	HCC	O	POS
26	6735	*Salmonella* ser. Albany I	Sesame seeds	HCC	C3	POS
27	13725	*Salmonella* ser. Anatum I	Unknown	GPLN	E	POS
28	1429	*Salmonella* ser. Anfo	African meat box (1967)	HCC	Q	POS
29	725	*Salmonella* ser. Arizonae IIIa	Unknown	ATCC 13314	IIIa 51:z4,z23:-	POS
30	726	*Salmonella* ser. Arizonae IIIa	Unknown	ATCC 12324	40:z4,z23:- Ar. 10:1,2,5	POS
31	6177	*Salmonella* ser. Arkansas I	Chicken giblets	HCC	E3	POS
32	1523	*Salmonella* ser. Berkeley I	Diseased turkey	HCC	U	POS
33	1606	*Salmonella* ser. Bern	Opossum	HCC	1,40:z4,z32:-	POS
34	13730	*Salmonella* ser. Berta I	Unknown	GPLN	D1	POS
35	737	*Salmonella* ser. Blegdam	Unknown	HCC	D1	POS
36	1509	*Salmonella* ser. Bovismobificans I	Unknown	HCC	C2	POS
37	13746	*Salmonella* ser. Brandenburg I	Unknown	GPLN	B	POS
38	964	*Salmonella* ser. Bredeney I	Fresh chicken	HCC	B	POS
39	3882	*Salmonella* ser. Broughton I	Poultry feed	HCC	E4	POS
40	1558	*Salmonella* ser. Canastel II	Feed	HCC	D1	POS
41	1620	*Salmonella* ser. Carmel I	Unknown	HCC	O17	POS
42	2629	*Salmonella* ser. Cerro I	Unknown	ATCC 10723	18:z4,z23: --	POS
43	13729	*Salmonella* ser. Cerro I	Unknown	GPLN	K	POS
44	1615	*Salmonella* ser. Chameleon IV	Lizard Liver	HCC	I	POS
45	1623	*Salmonella* ser. Champaign I	Liver of hen	HCC	Q	POS
46	1625	*Salmonella* ser. Chester I	Unknown	HCC	B	POS
47	13035	*Salmonella* ser. Choleraesuis I	Unknown	ATCC 10708	C1	POS
48	13828	*Salmonella* ser. Cubana I	Unknown	GPLN	G	POS
49	13916	*Salmonella* ser. Diarizonae	Human blood	ATCC BAA-216	IIIb 35:i:z	POS
50	1641	*Salmonella* ser. Durban I	Faeces	HCC	D1	POS
51	1644	*Salmonella* ser. Ealing I	Dried baby milk (1985-1986)	HCC	O	POS
52	13759	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
53	13760	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
54	13761	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS

55	13762	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
56	13763	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
57	13764	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
58	13784	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
59	13785	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
60	13786	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
61	13794	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
62	13795	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
63	13797	*Salmonella* ser. Enteritidis I	Unknown	GPLN	D1	POS
64	1428	*Salmonella* ser. Frintrop	Animal Feed	HCC	D1	POS
65	13813	*Salmonella* ser. Give	Unknown	GPLN	E	POS
66	3915	*Salmonella* ser. Haardt	Broiler Breeders	HCC	C3	POS
67	13717	*Salmonella* ser. Hadar	Unknown	USDA-ARS[e]	C3	POS
68	2290	*Salmonella* ser. Hartford	Cheesecake	HCC	C1	POS
69	R-SAL-38	*Salmonella* ser. Heidelberg	Turkey-Arizona	NVSL[f] 4960	B	POS
70	R-SAL-39	*Salmonella* ser. Heidelberg	Human-N. Carolina	CDC[g] B2487	B	POS
71	13742	*Salmonella* ser. Heidelberg	Unknown	GPLN	B	POS
72	13772	*Salmonella* ser. Heidelberg	Unknown	GPLN	B	POS
73	13847	*Salmonella* ser. Hillingdon	Unknown	ATCC 9184	D2	POS
74	1776	*Salmonella enterica* subsp. *houtenae*	Unknown	ATCC 43974	45:g,z51:-	POS
75	3699	*Salmonella* ser. Hvittingfoss	Herbs/Spices	HCC	I	POS
76	13915	*Salmonella* ser. Indiana	Ground turkey	HCC	B	POS
77	13845	*Salmonella* ser. Infantis	Unknown	ATCC BAA-1675	C1	POS
78	13723	*Salmonella* ser. Javiana	Unknown	GPLN	D1	POS
79	1251	*Salmonella* ser. Kedougou I	Turkey	HCC	G2	POS
80	2628	*Salmonella* ser. Kentucky	Unknown	ATCC 9263	(8),20:i:z6	POS
81	13859	*Salmonella* ser. Kentucky	Drag Swab	HCC	C3	POS
82	13555	*Salmonella* ser. Kentucky	Raw Chicken Wings	HCC	C3	POS
83	13747	*Salmonella* ser. Kiambu	Unknown	GPLN	B	POS
84	6729	*Salmonella* ser. Lexington	Sesame seeds	HCC	E1	POS
85	2263	*Salmonella* ser. Lille	Pancake	HCC	C1	POS
86	13810	*Salmonella* ser. Liverpool	Unknown	GPLN	E	POS
87	1650	*Salmonella* ser. Livingstone I	Faeces	HCC	C1	POS
88	13910	*Salmonella* ser. Maastricht	Fishmeal	ATCC 15789	F	POS
89	1698	*Salmonella* ser. Madelia I	Liver of hen	HCC	H	POS
90	1424	*Salmonella* ser. Manchester	Autolysed yeast	HCC	C2	POS
91	2673	*Salmonella* ser. Manhattan	Avian	HCC	C3	POS
92	13738	*Salmonella* ser. Mbandaka	Unknown	GPLN	C1	POS
93	13734	*Salmonella* ser. Miami	Unknown	GPLN	D1	POS
94	1703	*Salmonella* ser. Mississippi	Faeces from 1942	HCC	G	POS
95	13724	*Salmonella* ser. Montevideo	Unknown	GPLN	C1	POS
96	1562	*Salmonella* ser. Montgomery	Unknown	HCC	F	POS
97	R-SAL-65	*Salmonella* ser. Muenchen	Chicken-Florida	NVSL 2817	C2	POS
98	R-SAL-66	*Salmonella* ser. Muenchen	Human-Massachusetts	CDC B2026	C2	POS
99	13783	*Salmonella* ser. Muenchen	Unknown	GPLN	C2	POS
100	2748	*Salmonella* ser. Muenster	Chicken	HCC	E1	POS
101	707	*Salmonella* ser. Newport	Fatal food Poisoning	ATCC 6962	C2	POS
102	2735	*Salmonella* ser. Ohio	Protien supplement for feed	HCC	C1	POS
103	13721	*Salmonella* ser. Ouakam	Unknown	GPLN	D2	POS
104	1248	*Salmonella* ser. Panama	Pork Sausages	HCC	D1	POS
105	918	*Salmonella* ser. Paratyphi A	Unknown	ATCC 9150	A	POS
106	R-SAL-41	*Salmonella* ser. Paratyphi B	Human-France	FDA[h] DMS 155/76	B	POS
107	R-SAL-42	*Salmonella* ser. Paratyphi B	Human-Scotland	FDA DMS 724/74	B	POS
108	3984	*Salmonella* ser. Paratyphi B	Gall bladder	ATCC 8759	B	POS
109	3988	*Salmonella* ser. Paratyphi C	Unknown	ATCC 13428	C1	POS
110	1711	*Salmonella* ser. Pomona I	Turkey intestine in 1941	HCC	M	POS
111	1712	*Salmonella* ser. Pretoria	Pig	HCC	F	POS
112	1482	*Salmonella* ser. Pullorum I	Chicks livers	HCC	D1	POS
113	13694	*Salmonella* ser. Reading	Unknown	USDA-ARS	B	POS
114	13848	*Salmonella* ser. Rubislaw	Unknown	ATCC 10717	F	POS
115	13812	*Salmonella* ser. Ruiru	Unknown	GPLN	L	POS
116	R-SAL-23	*Salmonella* ser. Saintpaul	Human- Pennsylvania	CDC B1722	B	POS
117	R-SAL-24	*Salmonella* ser. Saintpaul	Human-Texas	CDC B2076	B	POS
118	1777	*Salmonella enterica* subsp. *salamae*	Unknown	ATCC 43972	1,9,12:l,w:e,n,x	POS
119	6586	*Salmonella* ser. Santiago	Bourguignon powder	HCC	C2	POS
120	8008	*Salmonella* ser. Schleissheim	Cheese	HCC	B	POS
121	13741	*Salmonella* ser. Schwarzengrund	Unknown	GPLN	B	POS

122	3184	*Salmonella* ser. Sculcoates	Cocoa Bean Environment	HCC	I	POS
123	1610	*Salmonella* ser. Seminole	Lizard coelomic fluid	HCC	R	POS
124	13356	*Salmonella* ser. Senftenberg	Cilantro	HCC	E4	POS
125	13846	*Salmonella* ser. Sloterdijk	Netherland outbreak	ATCC 15791	B	POS
126	13814	*Salmonella* ser. Soerenga	Unknown	GPLN	N	POS
127	1333	*Salmonella* ser. Stanley I	Chicken	HCC	B	POS
128	2372	*Salmonella* ser. Stanleyville	Cocoa Bean Environment	HCC	B	POS
129	3186	*Salmonella* ser. Sya	Cocoa Bean Environment	HCC	X	POS
130	13835	*Salmonella* ser. Tennessee	Unknown	GPLN	C1	POS
131	1339	*Salmonella* ser. Thompson I	Egg	HCC	C1	POS
132	1613	*Salmonella* ser. Tuindorp	Zoo animal liver	HCC	U	POS
133	585	*Salmonella* ser. Typhi	Unknown	ATCC 19430	D1	POS
134	586	*Salmonella* ser. Typhimurium	Chicken Animal tissue	ATCC 14028	B	POS
135	1775	*Salmonella* ser. Typhimurium	Derived from the hydrogen sulfide producing wild strain LT2, New York, United States, 1960	ATCC 43971	4,5,12:i:1,2	POS
136	13752	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
137	13768	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
138	13769	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
139	13790	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
140	13791	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
141	13796	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
142	13799	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
143	13801	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
144	13808	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
145	13818	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
146	13819	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
147	13823	*Salmonella* ser. Typhimurium	Unknown	GPLN	B	POS
148	R-SAL-03	*Salmonella* ser. Typhimurium	Horse- Rhode Island	NVSL 7095	B	POS
149	R-SAL-04	*Salmonella* ser. Typhimurium	Rabbit-Indiana	NVSL 5820	B	POS
150	1431	*Salmonella* ser. Virchow	Meat Powder	HCC	C1	POS
151	1614	*Salmonella* ser. Volksdorf	Iguana Bladder	HCC	U	POS
152	1714	*Salmonella* ser. Wassennaar	Human	HCC	Z	POS
153	13619	*Salmonella* ser. Weltevreden	Human Stool 1977 Connecticut	Taxonometrics[i]	E1	POS
154	1560	*Salmonella* ser. Westpark	Tortoise Intestine	HCC	E1	POS
155	13809	*Salmonella* ser. Widemarsh	Unknown	GPLN	O	POS

[a]United States Department of Agriculture-Food Safety and Inspection Services, Athens, GA.
[b]American Type Culture Collection, Manassas, VA.
[c]HCC- Hygiena Culture Collection, New Castle, DE.
[d]Georgia Poultry Lab Network, Gainesville, GA.
[e]United States Department of Agriculture-Agricultural Research Service, Wyndmoor, PA.
[f]National Veterinary Services Laboratories, Ames, IA.
[g]Centers for Disease Control and Prevention, Atlanta, GA.
[h]United States Food and Drug Administration, College Park, MD.
[i]Taxonometrics, West Chester, PA.

Table 6: Exclusivity Panel (7)

No.	Hygiena Culture Collection No.	Name	Source	Origin	BAX Real-Time PCR Assay for *Salmonella* Result
1	373	*Klebsiella pneumoniae*	ATCC[a] 13883	Unknown	NEG
2	374	*Proteus mirabilis*	ATCC 29906	Unknown	NEG
3	383	*Citrobacter freundii*	ATCC 8090	Unknown	NEG
4	610	*Staphylococcus aureus*	ATCC 13565	Ham	NEG
5	640	*Escherichia coli* O157:H7	ATCC 43889	HUS Case Stool	NEG
6	641	*Escherichia coli* O157:H7	ATCC 43890	Human Feces	NEG
7	657	*Klebsiella ozaenae*	ATCC 11296	Unknown	NEG
8	658	*Klebsiella oxytoca*	ATCC 13182	Pharyngeal Tonsil	NEG
9	700	*Shigella sonnei*	ATCC 9290	Unknown	NEG
10	702	*Shigella sonnei*	ATCC 25931	Human feces	NEG
11	846	*Shimwellia blattae*	ATCC 29907	Hindgut of Cockroach	NEG
12	847	*Escherichia fergusonii*	ATCC 35469	Human Feces	NEG
13	848	*Escherichia hermannii*	ATCC 33650	Human Toe	NEG
14	849	*Raoultella species*	ATCC 21073	Unknown	NEG
15	850	*Escherichia vulneris*	ATCC 33821	Human Wound	NEG
16	854	*Staphylococcus gallinarum*	ATCC 35539	Chicken Nares	NEG
17	862	*Micrococcus luteus*	ATCC 4698	Unknown	NEG
18	863	*Staphylococcus aureus*	ATCC 12600	Human Clinical	NEG
19	864	*Staphylococcus epidermidis*	ATCC 14990	Nose	NEG
20	1083	*Shigella flexneri*	ATCC 29903	Unknown	NEG
21	1154	*Listeria innocua*	HCC[b]	Pate	NEG
22	1309	*Listeria monocytogenes*	HCC	Soft Cheese	NEG
23	2389	*Hafnia alvei*	ATCC 13337	Unknown	NEG
24	2417	*Serratia liquefaciens*	HCC	Raw Mince	NEG
25	2558	*Citrobacter freundii*	ATCC 43864	Unknown	NEG
26	2847	*Cronobacter sakazakii*	HCC	Environmental Swabbing	NEG
27	2850	*Cronobacter sakazakii*	HCC	Environmental Swabbing	NEG
28	3064	*Morganella morganii*	HCC	Environmental Swab	NEG
29	3354	*Listeria welshimeri*	HCC	Unknown	NEG
30	3982	*Pseudomonas aeruginosa*	ATCC 27853	Blood Culture	NEG
31	5588	*Hafnia alvei*	HCC	Ground Beef	NEG
32	6121	*Proteus mirabilis*	HCC	Herring Gull Cloacae	NEG
33	10011	*Cronobacter sakazakii*	HCC	Unknown	NEG
34	10014	*Cronobacter sakazakii*	HCC	Unknown	NEG
35	13135	*Enterobacter cloacae*	ATCC 13047	Spinal Fluid	NEG
36	13136	*Enterobacter aerogenes*	ATCC 13048	Sputum	NEG
37	13142	*Morganella morganii*	ATCC 25830	Summer Diarrhea patient	NEG
38	13145	*Pantoea agglomerans*	ATCC 27982	IV Fluid	NEG
39	13147	*Providencia rettgeri*	ATCC 29944	Unknown	NEG
40	13152	*Alcaligenes faecalis*	ATCC 15246	Unknown	NEG
41	13477	*Citrobacter brakii*	ATCC 51113	Snake	NEG
42	13478	*Bacillus pumilus*	ATCC 700814	Unknown	NEG
43	13512	*Cronobacter sakazakii*	University of Zurich[c]	Unknown	NEG
44	13513	*Cronobacter sakazakii*	University of Zurich	Unknown	NEG
45	13514	*Cronobacter sakazakii*	University of Zurich	Unknown	NEG
46	13515	*Cronobacter sakazakii*	University of Zurich	Unknown	NEG

[a]American Type Culture Collection, Manassas, VA.
[b]HCC- Hygiena Culture Collection, New Castle, DE.
[c]University of Zurich, Zurich, Switzerland.

DISCUSSION OF MODIFICATION APPROVED JANUARY 12, 2022 (10)

The data presented in this study support the product claim that the BAX System SalQuant method can estimate pre-enrichment levels of *Salmonella* spp. in whole carcass poultry rinses, raw ground beef, raw beef trim, MicroTally on raw beef trim, raw ground pork, raw pork trim and MicroTally on raw pork trim in a short amount of time (8–9 h total time to result) when using the BAX System Q7 instrument and BAX System Real-Time Assay for *Salmonella*. This allows laboratories to process more samples in a shorter amount of time than BAX MPN (1 day) or the traditional USDA/FSIS MLG 2.05 MPN method (5 to 7 days). Additionally, the BAX MPN can be used to detect *Salmonella* from USDA/FSIS MLG 2.05 whole carcass poultry rinses and beef trim enrichments.

Table 1. Matrix study summary results: BAX SalQuant vs MLG 2.05/4.10 reference method procedure for *Salmonella* (10)

Matrix/Inoculating strain	Cont. level[a]	BAX SalQuant results		MLG 2.05/4.10 results[d]			90% CI[f]		95% CI	
		Mean[b]	s_r[c]	Mean	s_r	DOM[e]	LCL[g]	UCL[h]	LCL	UCL
Whole carcass poultry rinses, 30 mL/*S*. Infantis C_1 (DD[i]1279)	Non	0.000	NA[j]	0.000	NA	NA	NA	NA	NA	NA
	Low	0.993	0.296	0.711	0.357	0.282	-0.111	0.675	-0.208	0.773
	Med	2.280	0.297	2.194	0.289	0.086	-0.265	0.438	-0.325	0.525
	High	3.315	0.441	3.614	0.286	-0.299	-0.755	0.158	-0.874	0.277
Fresh raw ground beef, 375 g/*S*. Newport C_2 (DD1261)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	0.862	0.157	0.971	0.267	-0.109	-0.377	0.160	-0.448	0.230
	Med	2.103	0.156	2.370	0.304	-0.267	-0.575	0.040	-0.660	0.126
	High	3.229	0.094	3.592	0.417	-0.363	-0.770	0.043	-0.894	0.164
Fresh raw beef trim, 375 g/*S*. Kentucky C_3 (ATCC[k] 9263)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	0.698	0.487	0.476	0.363	0.222	-0.292	0.736	-0.420	0.894
	Med	2.115	0.491	2.179	0.427	-0.064	-0.616	-0.487	-0.752	0.623
	High	3.510	0.295	3.123	0.401	0.387	-0.035	0.809	-0.140	0.914
MicroTally on fresh raw beef trim, 1 cloth/*S*. Montevideo C_1 (64TT[l])	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	0.755	0.321	0.639	0.225	0.116	-0.216	0.448	-0.298	0.530
	Med	2.006	0.296	2.252	0.427	-0.246	-0.686	0.194	-0.795	0.303
	High	3.260	0.180	3.614	0.286	-0.354	-0.647	-0.060	-0.723	0.060
Fresh raw ground pork, 375 g/*S*. Cerro K (ATCC 10723)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	0.700	0.056	0.714	0.420	-0.014	-0.417	0.390	-0.539	0.511
	Med	1.588	0.184	1.725	0.465	-0.137	-0.588	0.314	-0.712	0.438
	High	3.390	0.186	3.528	0.335	-0.138	-0.471	0.195	-0.558	0.281
Fresh raw pork trim, 375 g/*S*. Heidelberg B (TI[m]-480)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	0.532	0.343	0.693	0.156	-0.161	-0.500	0.179	-0.594	0.272
	Med	2.208	0.099	2.482	0.275	-0.274	-0.537	-0.011	-0.610	0.062
	High	3.413	0.155	3.493	0.156	-0.080	-0.266	0.106	-0.313	0.153
MicroTally on fresh raw pork trim, 1 cloth/S. Hadar C_2 (T1-231)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	0.652	0.229	0.601	0.605	0.050	-0.532	0.633	-0.693	0.763
	Med	2.127	0.175	1.797	0.460	0.330	-0.114	0.773	-0.236	0.895
	High	3.223	0.229	3.162	0.838	0.061	-0.767	0.889	-1.017	1.139

[a]All matrices were artificially contaminated. Non=non-inoculated.
[b]Mean of five replicate portions, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f], where f is the reported CFU/unit corresponding to the smallest reportable result and unit is the reported unit of measure.
[c]Repeatability standard deviation.
[d]USDA FSIS Microbiology Laboratory Guidebook (MLG) 2.05, Most Probable Number Procedure and Tables, and MLG 4.11, Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges.
[e]Difference of means between the candidate and reference methods, analyzed using an unpaired statistical analysis.
[f]Confidence interval.
[g]Lower confidence limit for difference of means.
[h]Upper confidence limit for difference of means.
[i]Hygiena Culture Collection, New Castle, DE.
[k]American Type Culture Collection, Manassas, VA.
[l]Texas Tech University Culture Collection, Lubbock, TX.
[m]Texas Tech University Culture Collection, Lubbock, TX.

Table 2. Matrix study summary results: SalQuant vs MLG 2.05/4.10 reference method procedure for *Salmonella* (10)

Matrix/Inoculating strain	Cont. level[a]	BAX MPN results		MLG 2.05/4.10 results[d]				90% CI[f]		95% CI	
		Mean[b]	s_r[c]	Mean	s_r	DOM[e]	SE	LCL[g]	UCL[h]	LCL	UCL
Whole carcass poultry rinses, 30 mL/*S*. Infantis C_1 (DD [i]1279)	Non	0.000	NA[j]	0.000	NA	NA	NA	NA	NA	NA	NA
	Low	0.645	0.332	0.711	0.357	-0.066	0.124	-0.330	0.198	-0.410	0.278
	Med	2.033	0.818	2.194	0.289	-0.161	0.374	-0.958	0.636	-1.199	0.877
	High	3.509	0.223	3.614	0.286	-0.105	0.074	-0.263	0.053	-0.311	0.101
Fresh raw beef trim, 375 g/*S*. Kentucky C_3 (ATCC[k] 9263)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA	NA
	Low	0.293	0.466	0.476	0.363	-0.183	0.132	-0.465	0.099	-0.551	0.184
	Med	1.994	0.546	2.179	0.427	-0.185	0.133	-0.468	0.098	-0.554	0.184
	High	2.938	0.552	3.123	0.531	-0.185	0.120	-0.440	0.071	-0.518	0.148

[a]All matrices were artificially contaminated. Non=non-inoculated.
[b]Mean of five replicate portions, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f], where f is the reported CFU/unit corresponding to the smallest reportable result and unit is the reported unit of measure.
[c]Repeatability standard deviation.
[d]USDA FSIS Microbiology Laboratory Guidebook 2.05, Most Probable Number Procedure and Tables, 4.11, Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges.
[e]Difference of means between the candidate and reference methods, analyzed using paired statistical analysis.
[f]Confidence interval.
[g]Lower confidence limit for difference of means.
[h]Upper confidence limit for difference of means.
[i]American Type Culture Collection, Manassas, VA.

DISCUSSION OF MODIFICATION APPROVED JANUARY 13, 2022 (11)

The BAX System Real-time PCR Assay successfully detected the target *Salmonella* species in dried cannabis flower and dried hemp flower at a 10 g test portion size. Difference in POD analysis for the presumptive versus confirmed results showed no statistically significant differences, with all ranges of the 95% confidence intervals containing the zero. There was one BAX presumptive positive result for *Salmonella* in the low level of the dried hemp flower that could not be confirmed. It is possible that *Salmonella* was present in the test portion but below the detectable level of the culture procedure.

Feedback from the independent laboratory conducting this study stated that processing samples for these assays was very user friendly with a standard heat dependent lysis step and transfer into pre-aliquoted lyophilized pellets in PCR wells. Short run times on the instrument helped improve throughput for processing samples.

The BAX Real-time PCR Assay for detecting *Salmonella* species allow users to obtain presumptive positive results in as little as 26 h of incubation, processing and PCR run for *Salmonella* analysis. Presumptive results are easily visualized, denoted by a plus or minus sign within the software.

Table 1. Matrix study: BAX Real-time PCR Assay for *Salmonella* presumptive vs. confirmed results in dried cannabis flower (>0.3% THC) and dried hemp flower (≤0.3% THC) (11)

	MPN[a] / Test			Presumptive			Confirmed			
Matrix and Inoculum	Portion	N[b]	x[c]	POD_{cp}[d]	95% CI	x	POD_{cc}[e]	95% CI	$dPOD_{cp}$[f]	95% CI[g]
Dried cannabis flower	NA[i]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
10 g (*S.* Typhimurium	1.74 (0.91, 8.08)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	(-0.13, 0.13)
ATCC[h] 14028)	4.90 (2.50, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Dried hemp Flower 10 g	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
(*S.* Enteritidis ATCC	1.03 (0.46, 2.53)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	(-0.11, 0.21)
13076)	4.03 (2.10, 16.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

[a]MPN = Most Probable Number is based on the POD of reference method test portions using the Least Cost Formulations MPN calculator, with 95% confidence interval.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result 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]American Type Culture Collection, Manassas, VA.
[i]Not applicable.

DISCUSSION OF MODIFICATION APPROVED APRIL 2023 (13)

The BAX System Real-time PCR Assay for *Salmonella* successfully detected the target *Salmonella* species in beef trim sampling cloths at a 375 g test portion size. The study data were unable to find a statistical difference between the BAX *Salmonella* method presumptive and confirmed results, nor between the BAX *Salmonella* and the MLG 4.12 reference method results with 95% confidence.

The BAX Real-time PCR Assay for detecting *Salmonella* species allow users to obtain presumptive positive results in as little as 8 h (in mTSB + caa) or 10 h (in MP media) of incubation, processing and PCR run for *Salmonella* analysis allowing users to have more media options. Presumptive results are easily visualized and denoted by a plus or minus sign within the software.

Table 1. Matrix study: BAX Real-time PCR Assay for *Salmonella* presumptive vs. confirmed results in beef trim (375 g size) sampling cloths (13)

	cfu[a]/ Test			Presumptive			Confirmed			
Matrix and Inoculum	Portion	N[b]	x[c]	POD_{cp}[d]	95% CI	x	POD_{cc}[e]	95% CI	$dPOD_{cp}$[f]	95% CI[g]
Beef trim Sampling cloth	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
(S. Typhimurium DD	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
13557[h]) 10 h, MP media	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
(*S.* Typhimurium DD	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
13557[h]) 24 h, MP media	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

[a]cfu/test portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.
[b]N = Number of test potions.
[c]x = Number of positive test portions.
[d]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[f]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result 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]Hygiena Culture Collection, New Castle, DE.
[i]Not applicable.

Table 2. BAX Real-time PCR Assay for *Salmonella* method vs. reference method results in beef trim (375 g size) sampling cloths (13)

Matrix and Inoculum	cfu[a]/ Test Portion	N[b]	x[c]	BAX Method POD$_c$[d]	BAX Method 95% CI	x	Reference Method POD$_g$[e]	Reference Method 95% CI	dPOD$_c$[f]	95% CI[g]
Beef trim Sampling cloth	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
(S. Typhimurium DD	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.28, 0.28)
13557) 10 h, MP media	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
Beef trim Sampling cloth	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.43, 0.43)
(*S.* Typhimurium DD	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.28, 0.28)
13557) 24 h, MP media	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.43, 0.43)
Beef trim Sampling cloth	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
(S. Typhimurium DD	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
13557) 8 h, mTSB+caa[k]	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
(*S.* Typhimurium DD	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
13557) 10 h, mTSB+caa	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)
Beef trim Sampling cloth	NA	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	(-0.47, 0.47)
(S. Typhimurium DD	0.57	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	(-0.13, 0.13)
13557) 24 h, mTSB+caa	4.68	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	(-0.47, 0.47)

[a]cfu/portion = Inoculating strain was grown overnight, then serially diluted and plated in triplicate to determine appropriate concentration for inoculation.
[b]N = Number of test potions.
[c]X = Number of positive test portions.
[d]POD$_C$ = Confirmed candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f]POD$_R$ = Confirmed reference method positive outcomes divided by the total number of trials.
[e]dPOD$_C$ = Difference between the candidate method and reference method POD values.
[f]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h]Hygiena Culture Collection, New Castle, DE.
[i]Results calculated using unpaired POD statistical analysis.
[i]Not applicable.
[k]Results calculated using paired POD statistical analysis.

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