CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

061203

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

ANSR® for *Salmonella*

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912

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 (November 23, 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

November 23, 2019
Date

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

METHOD AUTHORS
ORIGINAL VALIDATION: Mark Mozola, Paul Norton, Susan Alles, R. Lucas Gray, Jerry Tolan, Oscar Caballero, Lisa Pinkava, Edan Hosking, Karen Luplow, and Jennifer Rice
MODIFICATION JANUARY 2013: Oscar Caballero, Susan Alles, R. Lucas Gray, Jerry Tolan, Mark Mozola, and Jennifer Rice
MODIFICATION JUNE 2013: Oscar Caballero, Susan Alles, Kayla Walton, R. Lucas Gray, Mark Mozola, and Jennifer Rice
MODIFICATION NOVEMBER 2013: Debra Foti, Lei Zhang, Preetha Biswas, Mark Mozola, and Jennifer Rice
MODIFICIATION NOVEMBER 2014 Modification: Oscar Caballero, Susan Alles, Kayla Walton, R. Lucas Gray, Mark Mozola, and Jennifer Rice

SUBMITTING COMPANY
Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
USA

KIT NAME(S)
ANSR® for *Salmonella*

CATALOG NUMBERS
9843, 9870

INDEPENDENT LABORATORY
Q Laboratories, Inc[1]
1400 Harrison Avenue
Cincinnati, OH 45214
USA

[1] Original Validation, January 2013 Modification

AOAC EXPERTS AND PEER REVIEWERS
Thomas Hammack[1,4,9], Yi Chen[1,5,6], Michael Brodsky[2,4,7], Wayne Ziemer[3,4,7], Yvonne Salfinger[8]
[1] US Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA
[2] Brodsky Consultants, Thornhill, ON, Canada
[3] Consultant, Loganville, GA, USA
[4] Original Validation
[5] January 2013 Modification
[6] June 2013 Modification
[7] November 2013 Modification
[8] Consultant, Denver, CO, USA
[9] November 2014 Modification

APPLICABILITY OF METHOD
Target organism – *Salmonella enterica* and *Salmonella bongori*

Matrices
Original Validation:
(FDA BAM Ch. 5) - oat cereal (25g), stainless steel (1 x 1 in), plastic (1 x 1 in), sealed concrete (1 x 1 in), ceramic tile (1 x 1 in), rubber (1 x 1 in)
(USDA-FSIS/MLG Ch. 4.05) - hot dogs (25g, 325g), chicken carcass rinse (30mL), raw ground turkey (25 g), Raw ground beef (25g, 325g)
January 2013 Modification: (FDA BAM Ch. 5, 25 g) - ice cream, soy flour, almonds, peanut butter, spinach, black pepper, raw frozen shrimp, cocoa powder, pet food (25g and 375 g)
(USDA-FSIS/MLG Ch. 4.05, 100 g) – dried pasteurized egg
November 2013 Modification: (USDA-FSIS/MLG Ch. 4.05, 100 g) - dried pasteurized egg, dried egg white with sodium lauryl sulfate, frozen egg yolk w/10% sugar, liquid whole egg with citric acid

Performance claims – Method equivalent to reference methods.

REFERENCE METHODS
US FDA (2011) *Bacteriological Analytical Manual*, chapter 5
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm (4)
USDA-FSIS (2011) *Microbiology Laboratory Guidebook*, chapter 4.05
http://www.fsis.usda.gov/PDF/MLG_4_05.pdf (5)

ORIGINAL CERTIFICATION DATE
June 21, 2012

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2020

METHOD MODIFICATION RECORD
1. **January 2013 Level 3**
2. **June 2013**
3. **November 2013**
4. **November 2014**
5. **November 2018 Level 1**
6. **November 2019 Level 1**

SUMMARY OF MODIFICATION
1. **Matrix extension to include 10 foods**
2. **Modified lysis procedure**
3. **Egg claim extension**
4. **Reformulation of lyophilized pellet for increased solubility**
5. **Editorial/clerical changes for clarity**
6. **Editorial/clerical changes**

Under this AOAC® *Performance Tested*℠ License Number, 061203 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*℠ License Number, 061203 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)

ANSR *Salmonella* is a new isothermal nucleic acid amplification assay based on the nicking enzyme amplification reaction (NEAR™) technology [3]. The amplification mechanism involves binding of an oligonucleotide "template" to a specific sequence of target DNA. The template contains a recognition site for a specific endonuclease. The nicked strand is recognized as damaged and repaired by the action of a thermostable DNA polymerase, displacing the original strand with the newly-synthesized repaired portion. This displaced DNA "product" then binds to a second template and the same reactions lead to formation of a second product. The second product is homologous to the target sequence and is detected using a specific molecular beacon probe. Fluorescent signal is generated in real time, with amplification and detection complete within 10 minutes. The entire assay is conducted at a constant temperature of 56°C using a temperature-controlled fluorescence detection instrument. Assay software analyzes the fluorescent signal over time; a data interpretation algorithm interprets results as negative, positive, or invalid based on baseline, rate-of-change, and other criteria. Each tube of ANSR reagents also contains an internal positive control, signaling in a second fluorescence channel irrespective of the presence of target DNA, and indicating proper functioning of the amplification reagent**.**

DISCUSSION OF THE ORIGINAL VALIDATION STUDY (1)

Results of the internal and independent laboratory studies show that ANSR *Salmonella* is an effective procedure for detection of *Salmonella* spp. in raw meat products, hot dogs, oat cereal, and environmental sponge or swab samples from a variety of environmental surfaces. Inclusivity was 99.1% in testing of 113 strains belonging to *S. enterica* and *S. bongori*. Exclusivity was 100% in testing of 38 strains of non-salmonellae. Method sensitivity was comparable to that of the FDA/BAM and USDA-FSIS methods as determined by chi-square analysis. There were no statistically significant differences in the number of positive results obtained with the ANSR and reference culture methods in any trial, with the exception of the trial with stainless steel surface in which there were significantly more positives by the ANSR method. Considering all data, sensitivity of the ANSR assay relative to confirmation from ANSR-associated enrichment cultures was 98.7%, while sensitivity relative to the reference methods was 95.1%. There was only one unconfirmed positive result from uninoculated control test portions, for overall specificity of 98.3%.

In addition to high sensitivity and specificity, the ASNR *Salmonella* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results within 30 min following sample enrichment.

Table 1. Results of inclusivity testing for the ANSR *Salmonella* test. (1)							
					ANSR Result at ~ 1 x 10^5 cfu/mL		
Strain No.	***Salmonella* Serovar[a]**	**O Group**	**Source**	**Origin (if Known)**	**ANSR Broth 1**	**ANSR Broth 2**	**ANSR Broth 3**
GT2652	Arizonae (III)	51	CDC		+	+	+
GT799	Arizonae (III)	51	ATCC 13314		+	+	+
GT3136	Treforest	51	CDC		+	+	+
GT3089	Humber (II)	53	CDC		+	+	+
GT3090	Tranoroa (II)	55	CDC		+	+	+
GT3091	Artis (II)	56	CDC		+	+	+
GT3092	Tokai (II)	57	CDC		+	+	+
GT3093	Betioky (II)	59	CDC		+	+	+
GT3094	Luton (II)	60	CDC		+	+	+
GT2704	*S. bongori* ser. Brookfield	66	CDC		+	+	+
GT2705	*S. bongori* ser. Malawi	66	CDC		+	+	+
GT1614	*S. bongori* ser. Maregrosso	66	CDC		+	+	+
GT3199	Crossness	67	CDC		+	+	+
GT657	Kiel	A	Deibel Labs		+	+	+
GT2403	Paratyphi A	A	CDC		+	+	+
GT2284	Agona	B	CDC		+	+	+
GT2304	Heidelberg	B	CDC		+	+	+
GT2306	Java	B	CDC		+	+	+
GT2309	Paratyphi B	B	CDC		+	+	+
GT2360	Saint-Paul	B	CDC		+	+	+
GT546	Schwarzengrund	B	Mass. State Lab		+	+	+
GT2373	Typhimurium	B	ATCC 13311	Mutton	+	+	+
Neogen 190	Typhimurium	B	CDC		+	+	+
GT2365	Typhimurium var. Copenhagen	B	CDC		+	+	+
GT2378	Braenderup	C1	CDC		+	+	+
GT2886	Cholerasuis	C1	CDC		+	+	+
A144	Infantis	C1	ATCC 51741		+	+	+
GT2479	Mbandaka	C1	CDC		+	+	+
GT2483	Montevideo	C1	CDC		+	+	+
A149	Oranienberg	C1	ATCC 9239	Ill. State Hospital	+	+	+
GT2528	Paratyphi C	C1	CDC		+	+	+
GT2516	Tennessee	C1	CDC		+	+	+
GT2518	Thompson	C1	CDC		+	+	+
GT2524	Virchow	C1	CDC		+	+	+
GT2545	Bovismorbificans	C2	CDC		+	+	+
GT2547	Duesseldorf	C2	CDC		+	+	+
GT623	Hadar	C2	Mass. State Lab		+	+	+
GT2553	Muenchen	C2	CDC		+	+	+
GT2557	Newport	C2	CDC		+	+	+
GT2558	Newport var. Puerto Rico	C2	CDC		+	+	+
GT2579	Tulear (II)	C2	CDC		+	+	+
GT662	Albany	C3	U. Mass.		+	+	+
GT2549	Haardt	C3	CDC		+	+	+
GT2581	Kentucky	C3	CDC		+	+	+
GT2882	Virginia	C3	CDC		+	+	+
GT2674	Bornum	C4	CDC		+	+	+

GT2103	Eimsbuettel	C4	CDC		+	+	+
GT2884	Berta	D1	CDC		+	+	+
GT2583	Daressalaam	D1	CDC		+	+	+
GT2584	Dublin	D1	CDC		+	+	+
GT2881	Eastbourne	D1	CDC		+	+	+
Neogen 195	Enteritidis	D1	CDC		+	+	+
Neogen 207	Enteritidis	D1	CDC		+	+	+
GT2124	Enteritidis	D1	ATCC 13076		+	+	+
GT896	Gallinarum	D1	GENE-TRAK Systems		+	+	+
GT2589	Javiana	D1	CDC		+	+	+
GT2885	Pullorum	D1	CDC		+	+	+
GT2125	Typhi	D1	ATCC 6539		+	+	+
GT2620	Fresno	D2	CDC		+	+	+
GT2621	Gateshead	D2	CDC		+	+	+
GT2622	Strasbourg	D2	CDC		+	+	+
GT2626	Anatum	E1	CDC		+	+	+
GT2637	Butantan	E1	CDC		+	+	+
GT2638	Give	E1	CDC		+	+	+
GT2158	Lexington	E1	CDC		+	+	+
GT2641	Meleagridis	E1	CDC		+	+	+
GT2510	Muenster	E1	USDA, Athens, GA		+	+	+
Neogen 469	Uganda	E1	Ampcor	Pork Sausage	+	+	+
GT911	Weltervreden	E1	CDC		+	+	+
GT619	Binza	E2	U. Mass.		+	+	+
GT908	Kinshasa	E2	CDC		+	+	+
GT2101	Newbrunswick	E2	CDC		+	+	+
GT2643	Arkansas	E3	CDC		+	+	+
GT2645	Illinois	E3	CDC		+	+	+
GT2646	Minneapolis	E3	CDC		+	+	+
GT3233	Chittagong	E4	CDC		+	+	+
GT2650	Krefeld	E4	CDC		+	+	+
GT2883	Senftenberg	E4	CDC		+	+	+
GT3178	Simsbury	E4	CDC		+	+	+
GT2676	Westerstede	E4	CDC		+	+	+
GT1857	Pretoria	F	CDC		+	+	+
GT2703	Rubislaw	F	CDC		+	+	+
GT2680	Poona	G1	CDC		+	+	+
GT1858	Havana	G2	CDC		+	+	+
GT4701	Worthington	G2	GENE-TRAK Systems		+	+	+
GT5149	Ferlac	H	ATCC 43976		+	+	+
GT2711	Florida	H	CDC		+	+	+
GT913	Hvittingfoss	I	CDC		+	+	+
GT2716	Kirkee	J	CDC		+	+	+
GT2691	Cerro	K	CDC		+	+	+
GT2721	Minnesota	L	CDC		+	+	+
GT2723	Dakar	M	CDC		+	+	+
GT3025	Urbana	N	CDC		+	+	+
GT3028	Adelaide	O	CDC		+	+	+
GT3034	Inverness	P	CDC		+	+	+
GT3037	Champaign	Q	CDC		+	+	+
GT3039	Bern (IV)	R	CDC		+	+	+

GT3058	Springs (II)	R	CDC		+	+	+
GT3059	Waycross	S	CDC		+	+	+
GT3060	Weslaco	T	CDC		-	-	-
GT4467	Houten (IV)	U	Silliker		+	+	+
GT3066	Guinea	V	CDC		+	+	+
GT2707	*S. bongori* ser. Camdeni	V	CDC		+	+	+
GT3069	Dugbe	W	CDC		+	+	+
GT3073	Quimbamba	X	CDC		+	+	+
GT3083	Djakarta	Y	CDC		+	+	+
GT2706	*S. bongori* ser. Balboa	Y	CDC		+	+	+
GT2708	*S. bongori* ser. Bongor	Y	CDC		+	+	+
GT3084	Flint (IV)	Z	CDC		+	+	+
GT3085	Greenside (II)	Z	CDC		+	+	+
GT3086	Hooggraven (II)	Z	CDC		+	+	+
GT3087	Wassenaar (IV)	Z	CDC		+	+	+
GT1615	*S. enterica* subsp. indica (VI)		CDC		+	+	+

[a] All strains are serovars of *Salmonella enterica* unless otherwise indicated. Serovars of subspecies other than subsp. I are indicated in parentheses.

Table 2. Results of exclusivity testing for the ANSR *Salmonella* test. (1)				
Strain No.	**Organism**	**Source**	**Origin (if Known)**	**ANSR Result at 1 x 10^9 cfu/mL in TSB**
GT1485	*Citrobacter amalonaticus*	ATCC 25405	feces	-
GT1475	*Citrobacter diversus*	ATCC 27156		-
GT1477	*Citrobacter freundii*	ATCC33128		-
GT1476	*Citrobacter youngae*	ATCC 29935	meat	-
GT1483	*Cronobacter sakazakii*	ATCC 29544	human	-
GT1710	*Edwardsiella hoshinae*	ATCC 33379	bird	-
GT569	*Edwardsiella tarda*	ATCC 15947	feces	-
GT1487	*Enterobacter aerogenes*	ATCC 29940	human	-
GT1482	*Enterobacter amnigenus*	ATCC 33072	soil	-
GT1497	*Enterobacter cancerogenus*	ATCC 35317		-
GT1481	*Enterobacter cloacae*	ATCC 29941		-
GT2990	*Enterobacter cloacae*	GENE-TRAK	dairy plant	-
GT1486	*Enterobacter gergoviae*	ATCC 33028		-
GT1480	*Enterobacter intermedia*	ATCC 33110		-
GT1460	*Escherichia blattae*	CDC		-
GT1214	*Escherichia coli*	ATCC 12038		-
GT1459	*Escherichia fergusonii*	ATCC 35473	feces	-
GT1216	*Escherichia hermannii*	ATCC 33650	human	-
GT1217	*Escherichia vulneris*	ATCC 33821	human	-
GT241	*Hafnia alvei*	ATCC 29927	human	-
GT1503	*Klebsiella oxytoca*	ATCC 13182	human	-
GT1478	*Klebsiella planticola*	ATCC 33531	radish	-
GT1499	*Klebsiella pneumoniae* subsp. *ozaenae*	ATCC 11296		-
GT3600	*Kluyvera ascorbata*	ATCC 33433	human	-
GT303	*Morganella morganii*	ATCC 25830	human	-
GT1467	*Pantoea agglomerans*	ATCC 29917		-
GT358	*Pasteurella multocida*	ATCC 19427		-
GT1493	*Proteus mirabilis*	ATCC 25933	human	-
GT366	*Proteus myxofaciens*	ATCC 19692		-
GT367	*Proteus penneri*	ATCC 33519		-
GT368	*Proteus vulgaris*	ATCC 13315		-
GT371	*Providencia alcalifaciens*	ATCC 9886	feces	-
GT373	*Providencia rettgeri*	ATCC 29944		-
GT374	*Providencia rustigiani*	ATCC 33673		-
GT375	*Providencia stuartii*	ATCC 29914		-
GT1909	*Pseudomonas aeruginosa*	ATCC 27853	blood	-
GT392	*Serratia marcescens*	ATCC 29937	human	-
GT1713	*Serratia rubidae*	ATCC 15338		-

Table 3. Results of comparative testing of raw meats with the ANSR *Salmonella* and USDA-FSIS reference methods. (1)

Food Type	Inoculum Strain	Inoculation Level		No. Samples	Number of Positive Samples				USDA Ref. Method	Sensitivity (%)[e] (10 h/12 h)	Specificity (%)[f] (10 h/12 h)	χ^{2g} (10 h/12 h)
					ANSR Method							
		cfu/g[b]	cfu/portion[b]		Assay[c] 10 h	Conf.[d] 10 h	Assay[c] 12 h	Conf.[d] 12 h				
Chicken carcass rinse	Nat. Contam.[a]	-	-	20	not done	not done	7	6	8	100	-	0.43
Raw ground turkey	*S.* Heidelberg	0.036	0.90	20	not done	not done	15	15	19	100	-	3.06
	-	< 0.030	< 0.75	5			0	0	0	-	100	-
Raw ground turkey[h]	*S.* Heidelberg	0.027	0.67	20	not done	not done	7[i]	7[i]	10	100	-	0.90
	-	0	0	5			0	0	0	-	100	-
Raw ground beef	*S.* Newport	0.036	0.90	20	12	13	13	13 (14)[j]	14	86/93	-	0.43/0.11
	-	< 0.030	< 0.75	5	0	0	0	0	0	-	100/100	-

[a] *Salmonella* spp. of serogroups C_2, D_1, and E_4 were isolated from positive samples.
[b] Determined by most probable number analysis.
[c] Number of test portions positive by ANSR assay not considering subsequent culture confirmation.
[d] Number test portions positive by the ANSR assay and confirmed by culture from ANSR-associated enrichments.
[e] Sensitivity = ANSR confirmed positives divided by maximum number of culture positives from ANSR enrichments.
[f] Specificity = ANSR negatives divided by total number of negative test portions. Calculated only for uninoculated control samples.
[g] χ^2 by Mantel-Haenszel formula [4]; $\chi^2 > 3.84$ indicates a statistically significant difference at $p < 0.05$.
[h] Trial performed by independent laboratory.
[i] Also tested after 24 h enrichment with identical results.
[j] There was one ANSR assay-negative, culture-positive result.

Table 4. Results of comparative testing of processed foods with the ANSR *Salmonella* and USDA-FSIS and FDA/BAM reference methods. (1)

Food Type	Inoculum Strain	Inoculation Level		No. Samples	Number of Positive Samples						Sensitivity (%)[d] (16 h/24 h)	Specificity (%)[e] (16 h/24 h)	χ^{2f} (16 h/24 h)
					ANSR Method				USDA Ref. Method	FDA Ref. Method			
		cfu/g[a]	cfu/ portion[a]		Assay 16 h[b]	Conf. 16 h[c]	Assay 24 h[b]	Conf. 24 h[c]					
Hot dogs, 25 g	*S.* Oranienburg	0.036	0.90	20	8	8	9	9	13	-	89/100	-	2.44/1.58
	-	< 0.030	< 0.75	5	0	0	0	0	0	-	-	100	-
Hot dogs, 325 g	*S.* Oranienburg	0.036	0.90	20	11	11	11	11	13	-	100/100	-	0.41/0.41
	-	< 0.030	< 0.75	5	0	0	0	0	0	-	-	100/100	-
Oat cereal	*S.* Agona	0.092	2.3	20	13	13	15	15	-	13	87/100	-	0.00/0.46
	-	< 0.030	< 0.75	5	0	0	0	0	-	0	-	100/100	-

[a] Determined by most probable number analysis.
[b] Number of test portions positive by ANSR assay not considering subsequent culture confirmation.
[c] Number test portions positive by the ANSR assay and confirmed by culture from ANSR-associated enrichments.
[d] Sensitivity = ANSR confirmed positives divided by maximum number of culture positives from ANSR enrichments.
[e] Specificity = ANSR negatives divided by total number of negative test portions. Calculated only for uninoculated control samples.
[f] χ^2 by Mantel-Haenszel formula [4]; $\chi^2 > 3.84$ indicates a statistically significant difference at $p < 0.05$.

Table 5. Results of comparative testing of environmental samples with the ANSR *Salmonella* and FDA/BAM reference methods. (1)

Food Type	Inoculum Strain	Inoculation Level (cfu/surface)[a]	No. Samples	Number of Positive Samples				FDA Ref. Method	Sensitivity (%)[d] (10 h/12 h)	Specificity (%)[e] (10 h/12 h)	χ^{2f} (10 h/12 h)
				ANSR Method							
				Assay 16 h[b]	Conf. 16 h[c]	Assay 24 h[b]	Conf. 24 h[c]				
Stainless steel	*S.* Heidelberg + competitor cocktail	140/1,500	20	20	20	20	20	8	100/100	-	16.7/16.7
	-	-	5	0	0	0	0	0	-	100/100	-
Plastic	*S.* Javiana	210	30	12	12	13	13	13	92/100	-	0.07/0.00
	-	-	5	0	0	1	0	0	-	100/80	-
Sealed concrete	*S.* Infantis	21,700	20	11	11	11	11	16	100/100	-	2.78/2.78
	-	-	5	0	0	0	0	0	-	100/100	-
Ceramic tile	*S.* Meleagridis	110	30	19	19	19	19	24	100/100	-	2.02/2.02
	-	-	5	0	0	0	0	0	-	100/100	-
Ceramic tile[g]	*S.* Meleagridis	53	20	10	10	10	10	7	100/100	-	0.90/0.90
	-	-	5	0	0	0	0	0	-	100/100	-
Rubber	*S.* Arizonae	210	20	7	7	8	8	7	88/100	-	0.00/0.10
	-	-	5	0	0	0	0	0	-	100/100	-

[a] Determined by dilution plating of the inoculum cultures.
[b] Number of test portions positive by ANSR assay not considering subsequent culture confirmation.
[c] Number test portions positive by the ANSR assay and confirmed by culture from ANSR-associated enrichments.
[d] Sensitivity = ANSR confirmed positives divided by maximum number of culture positives from ANSR enrichments.
[e] Specificity = ANSR negatives divided by total number of negative test portions. Calculated only for uninoculated control samples.
[f] χ^2 by Mantel-Haenszel formula [4]; $\chi^2 > 3.84$ indicates a statistically significant difference at $p < 0.05$.
[g] Trial performed by independent laboratory.

DISCUSSION OF THE VALIDATION STUDY Modification Approved January 2013 (8)

Results of the internal and independent laboratory studies demonstrate that ANSR *Salmonella* is an effective platform for the definitive detection of *Salmonella* spp. in pet food, ice cream, dried pasteurized egg, soy flour, almonds, peanut butter, spinach, black pepper, raw frozen shrimp, and cocoa powder. With the exception of ice cream and dried pasteurized egg at 16 h, there were no statistically significant differences in the number of positive results obtained with the ANSR and reference culture methods in any other trial. In addition to ice cream and dried pasteurized egg, 24 h enrichment is indicated for black pepper and 375-g dry pet food samples, based on higher method sensitivity at this time point compared to 16 h. There were no unconfirmed positive results from uninoculated control test portions, for overall specificity of 100%.

In addition to high sensitivity and specificity, the ASNR *Salmonella* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results within 30 min following sample enrichment.

Table 3. Probability of detection calculations for ANSR *Salmonella* presumptive and confirmed results, 16 h results (8)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[a]	N[b]	ANSR Presumptive Result			ANSR Confirmed Result			$dPOD_{CP}$[f]	95% CI[g]
				x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI		
Ice Cream	*S.* Typhimurium	0.70 (0.35, 1.20)	20	3	0.1500	0.0524, 0.3604	8	0.4000	0.2188, 0.6134	-0.2500	-0.4847, 0.0277
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter	*S.* Tennessee	0.82 (0.45, 1.42)	25	19	0.7600	0.5657, 0.8850	19	0.7600	0.5657, 0.8850	0	-0.2310, 0.2310
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter [h]	*S.* Tennessee	1.01 (0.62, 1.69)	20	14	0.7000	0.4810, 0.8545	14	0.7000	0.4810, 0.8545	0	-0.2680, 0.2680
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Almonds	*S.* Montevideo	2.12 (1.07, 3.17)	25	18	0.7200	0.5242, 0.8572	18	0.7200	0.5242, 0.8572	0	-0.2391, 0.2391
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Black Pepper	*S.* Muenchen	1.10 (0.60, 1.79)	24	10	0.4167	0.2447, 0.6117	9	0.3750	0.2116, 0.5729	0.0417	-0.2205, 0.2961
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Cocoa Powder	*S.* Eastbourne	0.92 (0.50, 1.60)	25	12	0.4800	0.3003, 0.6650	12	0.4800	0.3003, 0.6650	0	-0.2579, 0.2579
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Soy Flour	*S.* Senftenberg	0.65 (0.32, 1.15)	25	10	0.4000	0.2340, 0.5926	12	0.4800	0.3003, 0.6650	-0.0800	-0.3286, 0.1834
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Frozen Raw Shrimp	*S.* Weltevreden	1.20 (0.70, 2.30)	20	13	0.6500	0.4328, 0.8188	13	0.6500	0.4328, 0.8188	0	-0.2751, 0.2751
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Spinach	*S.* Kentucky	0.80 (0.43, 1.38)	20	9	0.4500	0.2582, 0.6579	9	0.4500	0.2582, 0.6579	0	-0.2829, 0.2829
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 25 g	*S.* Schwarzengrund	0.38 (0.17, 0.72)	25	4	0.1600	0.0640, 0.3465	6	0.2400	0.1150, 0.4343	-0.0800	-0.2967, 0.1445
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 375 g	*S.* Schwarzengrund	0.56 (0.27, 1.01)	25	7	0.2800	0.1428, 0.4758	9	0.3600	0.2025, 0.5548	-0.0800	-0.3183, 0.1713
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 375 g [h]	*S.* Schwarzengrund	0.45 (0.15, 0.75)	20	2	0.1000	0.0279, 0.3010	7	0.35000	0.1812, 0.5672	-0.2500	-0.4789, 0.0125
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345-0.4345
Dried Past. Egg 100 g	*S.* Enteritidis	0.58 (0.27, 1.02)	25	0	0	0, 0.1332	11	0.4400	0.2667, 0.6293	-0.4400	-0.6293, -0.2214
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes.
[e] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[f] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed 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] Trial performed by independent laboratory

Table 4. Probability of detection calculations for ANSR *Salmonella* presumptive and confirmed results, 24 h results (8)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[a]	N[b]	ANSR Presumptive Result			ANSR Confirmed Result			dPOD$_{CP}$[f]	95% CI[g]
				x[c]	POD$_{CP}$[d]	95% CI	x	POD$_{CC}$[e]	95% CI		
Ice Cream	*S.* Typhimurium	0.70 (0.35, 1.20)	20	8	0.4000	0.2188, 0.6134	8	0.4000	0.2188, 0.6134	0.0000	-0.2800, 0.2800
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter	*S.* Tennessee	0.82 (0.45, 1.42)	25	18	0.7200	0.5242, 0.8572	19	0.7600	0.5657, 0.8850	-0.0400	-0.2723, 0.1979
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter [h]	*S.* Tennessee	1.01 (0.62, 1.69)	20	14	0.7000	0.4810, 0.8545	14	0.7000	0.4810, 0.8545	0	-0.2680, 0.2680
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Almonds	*S.* Montevideo	2.12 (1.07, 3.17)	25	19	0.7600	0.5657, 0.8850	18	0.7200	0.5242, 0.8572	0.0400	-0.1979, 0.2723
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Black Pepper	*S.* Muenchen	1.10 (0.60, 1.79)	24	14	0.5833	0.3883, 0.7553	13	0.5417	0.3507, 0.7211	0.0416	-0.2234, 0.2986
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Cocoa Powder	*S.* Eastbourne	0.92 (0.50, 1.60)	25	12	0.4800	0.3003, 0.6650	12	0.4800	0.3003, 0.6650	0	-0.2579, 0.2579
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Soy Flour	*S.* Senftenberg	0.65 (0.32, 1.15)	25	12	0.4800	0.3003, 0.6650	13	0.5200	0.3350, 0.6997	-0.0400	-0.2941, 0.2216
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Frozen Raw Shrimp	*S.* Weltevreden	1.20 (0.70, 2.30)	20	13	0.6500	0.4328, 0.8188	13	0.6500	0.4328, 0.8188	0	-0.2751, 0.2751
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Spinach	*S.* Kentucky	0.80 (0.43, 1.38)	20	9	0.4500	0.2582, 0.6579	9	0.4500	0.2582, 0.6579	0	-0.2829, 0.2829
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 25 g	*S.* Schwarzengrund	0.38 (0.17, 0.72)	25	5	0.2000	0.0886, 0.3913	6	0.2400	0.1150, 0.4343	-0.0400	-0.2640, 0.1885
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 375 g	*S.* Schwarzengrund	0.56 (0.27, 1.01)	25	8	0.3200	0.1720, 0.5159	9	0.3600	0.2025, 0.5548	-0.0400	-0.2846, 0.2114
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 375 g [h]	*S.* Schwarzengrund	0.45 (0.15, 0.75)	20	7	0.3500	0.1812, 0.5672	7	0.3500	0.1812, 0.5672	0	-0.2751, 0.2751
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dried Past. Egg 100 g	*S.* Enteritidis	0.58 (0.27, 1.02)	25	15	0.6000	0.4074, 0.7660	15	0.6000	0.4074, 0.7660	0	-0.2543, 0.2543
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD$_{CP}$ = Candidate method presumptive positive outcomes.
[e] POD$_{CC}$ = Candidate method presumptive positive outcomes confirmed positive.
[f] dPOD$_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed 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] Trial performed by independent laboratory.

Table 5. Probability of detection calculations for ANSR *Salmonella* confirmed and FDA/BAM or USDA-FSIS/MLG reference method results, 16 h results. (8)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[a]	N[b]	ANSR Result			FDA-BAM Reference Method Result			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Ice Cream	*S.* Typhimurium	0.70 (0.35, 1.20)	20	3	0.1500	0.0524, 0.3604	10	0.5000	0.2993, 0.7007	-0.3500	-0.5732, -0.0592
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter	*S.* Tennessee	0.82 (0.45, 1.42)	25	19	0.7600	0.5657, 0.8850	14	0.5600	0.3707, 0.7333	0.2000	-0.0604, 0.4268
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter [h]	*S.* Tennessee	1.01 (0.62, 1.69)	20	14	0.7000	0.4810, 0.8545	13	0.6500	0.4328, 0.8188	0.0500	-0.2265, 0.3165
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Almonds	*S.* Montevideo	2.12 (1.07, 3.17)	25	18	0.7200	0.5242, 0.8572	22	0.8800	0.7004, 0.9583	-0.1600	-0.3709, 0.0660
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Black Pepper	*S.* Muenchen	1.10 (0.60, 1.79)	24	9	0.3750	0.2116, 0.5729	16	0.6400	0.4452, 0.7975	-0.2650	-0.4919, 0.0127
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Cocoa Powder	*S.* Eastbourne	0.92 (0.50, 1.60)	25	12	0.4800	0.3003, 0.6650	15	0.6000	0.4074, 0.7660	-0.1200	-0.3646, 0.1471
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Soy Flour	*S.* Senftenberg	0.65 (0.32, 1.15)	25	10	0.4000	0.2340, 0.5926	12	0.4800	0.3003, 0.6650	-0.0800	-0.3286, 0.1834
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Frozen Raw Shrimp	*S.* Weltevreden	1.20 (0.70, 2.30)	20	13	0.6500	0.4328, 0.8188	14	0.7000	0.4810, 0.8545	-0.0500	-0.3165, 0.2265
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Spinach	*S.* Kentucky	0.80 (0.43, 1.38)	20	9	0.3600	0.2025, 0.5548	11	0.4400	0.2667, 0.6293	-0.0800	-0.3263, 0.1807
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 25 g	*S.* Schwarzengrund	0.38 (0.17, 0.72)	25	4	0.1600	0.0640, 0.3465	8	0.3200	0.1720, 0.5159	-0.1600	-0.3782, 0.0781
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 375 g	*S.* Schwarzengrund	0.56 (0.27, 1.01)	25	7	0.2800	0.1428, 0.4758	11	0.4400	0.2667, 0.6293	-0.1600	-0.3938, 0.1015
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 375 g [h]	*S.* Schwarzengrund	0.45 (0.15, 0.75)	20	2	0.1000	0.0279, 0.3010	7	0.3500	0.1812, 0.5672	-0.2500	-0.4789, 0.0125
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dried Past. Egg 100 g	*S.* Enteritidis	0.58 (0.27, 1.02)	25	0	0	0, 0.1332	11	0.4400	0.2667, 0.6293	-0.4400	-0.6293, -0.2214
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD$_C$ = Candidate method presumptive positive outcomes confirmed positive.
[e] POD$_R$ = Reference method confirmed 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] Trial performed by independent laboratory.

Table 6. Probability of detection calculations for ANSR *Salmonella* confirmed and FDA/BAM or USDA-FSIF/MLG reference method results, 24 h results (8)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[a]	N[b]	ANSR Result			FDA-BAM Reference Method Result			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Ice Cream	*S.* Typhimurium	0.70 (0.35, 1.20)	20	8	0.4000	0.2188, 0.6134	10	0.5000	0.2993, 0.7007	-0.1000	-0.3704, 0.1930
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter	*S.* Tennessee	0.82 (0.45, 1.42)	25	18	0.7200	0.5242, 0.8572	14	0.5600	0.3707, 0.7333	0.1600	-0.1015, 0.3938
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Peanut Butter [h]	*S.* Tennessee	1.01 (0.62, 1.69)	20	14	0.7000	0.4810, 0.8545	13	0.6500	0.4328, 0.8188	0.0500	-0.2265, 0.3165
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Almonds	*S.* Montevideo	2.12 (1.07, 3.17)	25	18	0.7200	0.5242, 0.8572	22	0.8800	0.7004, 0.9583	-0.1600	-0.3709, 0.0660
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Black Pepper	*S.* Muenchen	1.10 (0.60, 1.79)	24	13	0.5417	0.3507, 0.7211	16	0.6400	0.4452, 0.7975	-0.0983	-0.3459, 0.1665
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Cocoa Powder	*S.* Eastbourne	0.92 (0.50, 1.60)	25	12	0.4800	0.3003, 0.6650	15	0.6000	0.4074, 0.7660	-0.1200	-0.3646, 0.1471
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Soy Flour	*S.* Senftenberg	0.65 (0.32, 1.15)	25	12	0.4800	0.3003, 0.6650	12	0.4800	0.3003, 0.6650	0.0000	-0.2579, 0.2579
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Frozen Raw Shrimp	*S.* Weltevreden	1.20 (0.70, 2.30)	20	13	0.6500	0.4328, 0.8188	14	0.7000	0.4810, 0.8545	-0.0500	-0.3165, 0.2265
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Spinach	*S.* Kentucky	0.80 (0.43, 1.38)	20	9	0.4500	0.2582, 0.6579	11	0.5500	0.3421, 0.7418	-0.1000	-0.3712, 0.1940
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 25 g	*S.* Schwarzengrund	0.38 (0.17, 0.72)	25	5	0.2000	0.0886, 0.3913	8	0.3200	0.1720, 0.5159	-0.1200	-0.3454, 0.1219
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 37 5g	*S.* Schwarzengrund	0.56 (0.27, 1.01)	25	8	0.3200	0.1720, 0.5159	11	0.4400	0.2667, 0.6293	-0.1200	-0.3603, 0.1416
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dry Pet Food 375 g [h]	*S.* Schwarzengrund	0.45 (0.15, 0.75)	20	7	0.3500	0.1812, 0.5672	7	0.3500	0.1812, 0.5672	0.0000	-0.2751, 0.2751
		0	5	0	0	0, 0.4345	0	0	0, 0.4345	0	-0.4345, 0.4345
Dried Past. Egg 100g	*S.* Enteritidis	0.58 (0.27, 1.02)	25	15	0.6000	0.4074, 0.7660	11	0.4400	0.2667, 0.6293	0.1600	-0.1101, 0.4000
		0	5	0	0	0, 0.4345	0	0	0-0.4345	0	-0.4345, 0.4345

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD$_C$ = Candidate method presumptive positive outcomes confirmed positive.
[e] POD$_R$ = Reference method confirmed 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] Trial performed by independent laboratory.

DISCUSSION OF THE VALIDATION STUDY Modification Approved June 2013 (9)

The results presented here demonstrate that a modification to the lysis procedure for ANSR *Salmonella* does not affect the performance of the assay when compared to that of the reference methods. Of the 113 strains tested belonging to *S. enterica* and *S. bongori*, 112 strains were detected and one strain of *S*. Weslaco was not detected. Of the 38 strains of non-salmonellae tested, none were detected. Method sensitivity was comparable to that of the FDA/BAM and USDA-FSIS/MLG methods as determined by POD analysis. There were no statistically significant differences in the number of positive results obtained by the ANSR and reference culture methods in any of the trials.

The modified lysis procedure for ANSR *Salmonella* provides increased protection against possible deleterious matrix effects. In addition to high sensitivity and specificity, the ASNR *Salmonella* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results within 40 min following sample enrichment.

Table 1. Results of inclusivity testing for the ANSR *Salmonella* test. (9)

Strain No.	*Salmonella* Serovar[a]	O Group	Source	Origin (if Known)	ANSR Result at ~1x10^6 cfu/mL
GT2652	Arizonae (III)	51	CDC		Positive
GT799	Arizonae (III)	51	ATCC 13314		Positive
GT3136	Treforest	51	CDC		Positive
GT3089	Humber (II)	53	CDC		Positive
GT3090	Tranoroa (II)	55	CDC		Positive
GT3091	Artis (II)	56	CDC		Positive
GT3092	Tokai (II)	57	CDC		Positive
GT3093	Betioky (II)	59	CDC		Positive
GT3094	Luton (II)	60	CDC		Positive
GT2704	*S. bongori* ser. Brookfield	66	CDC		Positive
GT2705	*S. bongori* ser. Malawi	66	CDC		Positive
GT1614	*S. bongori* ser. Maregrosso	66	CDC		Positive
GT3199	Crossness	67	CDC		Positive
GT657	Kiel	A	Deibel Labs		Positive
GT2403	Paratyphi A	A	CDC		Positive
GT2284	Agona	B	CDC		Positive
GT2304	Heidelberg	B	CDC		Positive
GT2306	Java	B	CDC		Positive
GT2309	Paratyphi B	B	CDC		Positive
GT2360	Saint-Paul	B	CDC		Positive
GT546	Schwarzengrund	B	Mass. State Lab		Positive
GT2373	Typhimurium	B	ATCC 13311	Mutton	Positive
Neogen 190	Typhimurium	B	CDC		Positive
GT2365	Typhimurium var. Copenhagen	B	CDC		Positive
GT2378	Braenderup	C1	CDC		Positive
GT2436	Cholerasuis	C1	CDC		Positive
A144	Infantis	C1	ATCC 51741		Positive
GT2479	Mbandaka	C1	CDC		Positive
GT2483	Montevideo	C1	CDC		Positive
A149	Oranienberg	C1	ATCC 9239	Ill. State Hospital	Positive
GT2528	Paratyphi C	C1	CDC		Positive
GT2516	Tennessee	C1	CDC		Positive
GT2518	Thompson	C1	CDC		Positive
GT2524	Virchow	C1	CDC		Positive
GT2545	Bovismorbificans	C2	CDC		Positive
GT2547	Duesseldorf	C2	CDC		Positive
GT623	Hadar	C2	Mass. State Lab		Positive
GT2553	Muenchen	C2	CDC		Positive
GT2557	Newport	C2	CDC		Positive
GT2558	Newport var. Puerto Rico	C2	CDC		Positive
GT2579	Tulear (II)	C2	CDC		Positive
GT662	Albany	C3	U. Mass.		Positive
GT2549	Haardt	C3	CDC		Positive
GT2581	Kentucky	C3	CDC		Positive
GT2882	Virginia	C3	CDC		Positive
GT2674	Bornum	C4	CDC		Positive
GT2103	Eimsbuettel	C4	CDC		Positive
GT2884	Berta	D1	CDC		Positive
GT2583	Daressalaam	D1	CDC		Positive
GT2584	Dublin	D1	CDC		Positive
GT2881	Eastbourne	D1	CDC		Positive
Neogen 195	Enteritidis	D1	CDC		Positive

Neogen 207	Enteritidis	D1	CDC		Positive
GT2124	Enteritidis	D1	ATCC 13076		Positive
GT896	Gallinarum	D1	GENE-TRAK Systems		Positive
GT2589	Javiana	D1	CDC		Positive
GT2885	Pullorum	D1	CDC		Positive
GT2125	Typhi	D1	ATCC 6539		Positive
GT2620	Fresno	D2	CDC		Positive
GT2621	Gateshead	D2	CDC		Positive
GT2622	Strasbourg	D2	CDC		Positive
GT2626	Anatum	E1	CDC		Positive
GT2637	Butantan	E1	CDC		Positive
GT2638	Give	E1	CDC		Positive
GT2158	Lexington	E1	CDC		Positive
GT2641	Meleagridis	E1	CDC		Positive
GT2510	Muenster	E1	USDA, Athens, GA		Positive
Neogen 469	Uganda	E1	Ampcor	Pork Sausage	Positive
GT911	Weltervreden	E1	CDC		Positive
GT619	Binza	E2	U. Mass.		Positive
GT908	Kinshasa	E2	CDC		Positive
GT2101	Newbrunswick	E2	CDC		Positive
GT2643	Arkansas	E3	CDC		Positive
GT2645	Illinois	E3	CDC		Positive
GT2646	Minneapolis	E3	CDC		Positive
GT3233	Chittagong	E4	CDC		Positive
GT2650	Krefeld	E4	CDC		Positive
GT2883	Senftenberg	E4	CDC		Positive
GT3178	Simsbury	E4	CDC		Positive
GT2676	Westerstede	E4	CDC		Positive
GT1857	Pretoria	F	CDC		Positive
GT2703	Rubislaw	F	CDC		Positive
GT2680	Poona	G1	CDC		Positive
GT1858	Havana	G2	CDC		Positive
GT4701	Worthington	G2	GENE-TRAK Systems		Positive
GT5149	Ferlac	H	ATCC 43976		Positive
GT2711	Florida	H	CDC		Positive
GT913	Hvittingfoss	I	CDC		Positive
GT2716	Kirkee	J	CDC		Positive
GT2691	Cerro	K	CDC		Positive
GT2721	Minnesota	L	CDC		Positive
GT2723	Dakar	M	CDC		Positive
GT3025	Urbana	N	CDC		Positive
GT3028	Adelaide	O	CDC		Positive
GT3034	Inverness	P	CDC		Positive
GT3037	Champaign	Q	CDC		Positive
GT3039	Bern (IV)	R	CDC		Positive
GT3058	Springs (II)	R	CDC		Positive
GT3059	Waycross	S	CDC		Positive
100-1017	Weslaco	T	Silliker		Positive
GT3060	Weslaco	T	CDC		Negative
GT4467	Houten (IV)	U	Silliker		Positive
GT3066	Guinea	V	CDC		Positive
GT3069	Dugbe	W	CDC		Positive
GT3073	Quimbamba	X	CDC		Positive
GT3083	Djakarta	Y	CDC		Positive
GT2706	*S. bongori* ser. Balboa	Y	CDC		Positive
GT388	*S. bongori* ser. Bongor	Y	CDC		Positive
GT3084	Flint (IV)	Z	CDC		Positive
GT3085	Greenside (II)	Z	CDC		Positive
GT3086	Hooggraven (II)	Z	CDC		Positive
GT3087	Wassenaar (IV)	Z	CDC		Positive
GT1615	*S. enterica* subsp. indica (VI)		CDC		Positive

Table 2. Results of exclusivity testing for the ANSR *Salmonella* test. (9)

Strain No.	Organism	Source	Origin (if Known)	ANSR Result at 10^8-10^9 cfu/mL in TSB
GT1485	*Citrobacter amalonaticus*	ATCC 25405	feces	Negative
GT1475	*Citrobacter diversus*	ATCC 27156		Negative
GT1477	*Citrobacter freundii*	ATCC33128		Negative
GT1476	*Citrobacter youngae*	ATCC 29935	meat	Negative
GT1483	*Cronobacter sakazakii*	ATCC 29544	human	Negative
GT1710	*Edwardsiella hoshinae*	ATCC 33379	bird	Negative
GT569	*Edwardsiella tarda*	ATCC 15947	feces	Negative
GT1487	*Enterobacter aerogenes*	ATCC 29940	human	Negative
GT1482	*Enterobacter amnigenus*	ATCC 33072	soil	Negative
GT1497	*Enterobacter cancerogenus*	ATCC 35317		Negative
GT1481	*Enterobacter cloacae*	ATCC 29941		Negative
GT2990	*Enterobacter cloacae*	GENE-TRAK	dairy plant	Negative
GT1486	*Enterobacter gergoviae*	ATCC 33028		Negative
GT1480	*Enterobacter intermedia*	ATCC 33110		Negative
GT1460	*Escherichia blattae*	CDC		Negative
GT1214	*Escherichia coli*	ATCC 12038		Negative
GT1459	*Escherichia fergusonii*	ATCC 35473	feces	Negative
GT1216	*Escherichia hermannii*	ATCC 33650	human	Negative
GT1217	*Escherichia vulneris*	ATCC 33821	human	Negative
GT241	*Hafnia alvei*	ATCC 29927	human	Negative
GT1503	*Klebsiella oxytoca*	ATCC 13182	human	Negative
GT1478	*Klebsiella planticola*	ATCC 33531	radish	Negative
GT1499	*Klebsiella pneumoniae* subsp. *ozaenae*	ATCC 11296		Negative
GT3600	*Kluyvera ascorbata*	ATCC 33433	human	Negative
GT303	*Morganella morganii*	ATCC 25830	human	Negative
GT1467	*Pantoea agglomerans*	ATCC 29917		Negative
GT358	*Pasteurella multocida*	ATCC 19427		Negative
GT1493	*Proteus mirabilis*	ATCC 25933	human	Negative
GT366	*Proteus myxofaciens*	ATCC 19692		Negative
GT367	*Proteus penneri*	ATCC 33519		Negative
GT368	*Proteus vulgaris*	ATCC 13315		Negative
GT371	*Providencia alcalifaciens*	ATCC 9886	feces	Negative
GT373	*Providencia rettgeri*	ATCC 29944		Negative
GT374	*Providencia rustigiani*	ATCC 33673		Negative
GT375	*Providencia stuartii*	ATCC 29914		Negative
GT1909	*Pseudomonas aeruginosa*	ATCC 27853	blood	Negative
GT392	*Serratia marcescens*	ATCC 29937	human	Negative
GT1713	*Serratia rubidae*	ATCC 15338		Negative

Table 3. Probability of detection calculations for ANSR *Salmonella* presumptive and confirmed results, primary time point [a] (9)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Presumptive Result			ANSR Confirmed Result			$dPOD_{CP}$[g]	95% CI[h]
				X[d]	POD_{CP}[e]	95% CI	x	POD_{CC}[f]	95% CI		
Raw Ground Turkey	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		5.8 (1.1, 24)	20	13	0.65	0.43, 0.82	14	0.70	0.48, 0.85	-0.05	-0.32, 0.23
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Hot Dogs	*S.* Oranienburg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	14	0.70	0.48, 0.85	15	0.75	0.53, 0.89	-0.05	-0.31, 0.22
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Oat Cereal	*S.* Agona	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0 (0, 2.4)	20	3	0.15	0.05, 0.36	4	0.20	0.08, 0.42	-0.05	-0.29, -0.19
		11 (2.2, 46)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44

[a] Primary time point, 16 h for hot dogs and oat cereal, 10 h for raw ground turkey.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes
[f] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[g] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 4. Probability of detection calculations for ANSR *Salmonella* presumptive and confirmed results, secondary time point [a] (9)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Presumptive Result			ANSR Confirmed Result			$dPOD_{CP}$[g]	95% CI[h]
				X[d]	POD_{CP}[e]	95% CI	x	POD_{CC}[f]	95% CI		
Raw Ground Turkey	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		5.8 (1.1, 24)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	-0.27, 0.27
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Hot Dogs	*S.* Oranienburg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Oat Cereal	*S.* Agona	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0 (0, 2.4)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
		11 (2.2, 46)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44

[a] Secondary time point, 24 h for all three foods.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes.
[f] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[g] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 5. Probability of detection calculations for ANSR *Salmonella* confirmed and reference method results, primary time point [a] (9)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Confirmed Result			Reference Method Result			dPOD$_C$[g]	95% CI[h]
				X[d]	POD$_C$[e]	95% CI	x	POD$_R$[f]	95% CI		
Raw Ground Turkey	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		5.8 (1.1, 24)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.23, 0.32
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Hot Dogs	*S.* Oranienburg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	14	0.70	0.48, 0.85	12	0.60	0.39, 0.78	0.10	-0.18, 0.36
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Oat Cereal	*S.* Agona	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0 (0, 2.4)	20	3	0.15	0.05, 0.36	5	0.25	0.11, 0.47	-0.10	-0.34, 0.15
		11 (2.2, 46)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44

[a] Primary time point, 16 h for hot dogs and oat cereal, 10 h for raw ground turkey.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD$_{CP}$ = Candidate method presumptive positive outcomes.
[f] POD$_{CC}$ = Candidate method presumptive positive outcomes confirmed positive.
[g] dPOD$_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 6. Probability of detection calculations for ANSR *Salmonella* confirmed and reference method results, secondary time point [a] (9)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Confirmed Result			Reference Method Result			dPOD$_C$[g]	95% CI[h]
				X[d]	POD$_C$[e]	95% CI	x	POD$_R$[f]	95% CI		
Raw Ground Turkey	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		5.8 (1.1, 24)	20	14	0.70	0.48, 0.85	12	0.60	0.39, 0.78	0.10	-0.18, 0.36
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Hot Dogs	*S.* Oranienburg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.89 (0.04, 4.5)	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
		275 (45, 1025)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
Oat Cereal	*S.* Agona	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0 (0, 2.4)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
		11 (2.2, 46)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44

[a] Secondary time point, 24 h for all three foods.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD$_{CP}$ = Candidate method presumptive positive outcomes.
[f] POD$_{CC}$ = Candidate method presumptive positive outcomes confirmed positive.
[g] dPOD$_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

DISCUSSION OF THE VALIDATION STUDY Modification Approved November 2013 (10)

Results of this study show that the ANSR *Salmonella* method is an effective procedure for detection of *Salmonella* spp. in a variety of pasteurized egg product types. There were no significant differences in the number of positive test portions detected by the ANSR and USDA/MLG reference culture methods for any of the four product types examined. Results of independent laboratory testing of pasteurized liquid egg corroborate those of internal testing. Inclusivity from ANSR Enrichment Broth 3B, was 99.1%, with only a single strain of *S.* Weslaco not detected by the ANSR assay. Exclusivity was 100%, with all strains of non-salmonellae tested producing negative results. Application of the method to the analysis of pasteurized egg products offers the user high sensitivity and specificity, minimal labor and equipment requirements, and results within 24 h.

Modification Data Approved November 2013 (10)

Table 1. Inclusivity results for the ANSR *Salmonella* assay from ANSR Enrichment Broth 3B (10)

Strain No.	*Salmonella* Serovar	O Group	Source	Origin	Result at ~ 10^6 cfu/mL
GT2652	Arizonae (III)	51	CDC[a] stk 279	Unknown	Positive
GT799	Arizonae (III)	51	ATCC[b] 13314	Unknown	Positive
GT3136	Treforest	51	CDC stk 657	Unknown	Positive
GT3089	Humber (II)	53	CDC stk 752	Unknown	Positive
GT3090	Tranoroa (II)	55	CDC stk 857	Unknown	Positive
GT3091	Artis (II)	56	CDC stk 863	Unknown	Positive
GT3092	Tokai (II)	57	CDC stk 1182	Unknown	Positive
GT3093	Betioky (II)	59	CDC stk 946	Unknown	Positive
GT3094	Luton (II)	60	CDC stk 1009	Unknown	Positive
GT2704	*S. bongori* ser. Brookfield	66	CDC N56	Unknown	Positive
GT2705	*S. bongori* ser. Malawi	66	CDC N53	Unknown	Positive
GT1614	*S. bongori* ser. Maregrosso	66	CDC 1925	Unknown	Positive
GT3199	Crossness	67	CDC stk 1795	Unknown	Positive
GT657	Kiel	A	Deibel Laboratories, Madison, WI	Unknown	Positive
GT2403	Paratyphi A	A	CDC 567-79	Unknown	Positive
GT2284	Agona	B	CDC 1201-82	Unknown	Positive
GT2304	Heidelberg	B	CDC 1114-82	Unknown	Positive
GT2306	Java	B	CDC 791-82	Unknown	Positive
GT2309	Paratyphi B	B	CDC stk 8	Unknown	Positive
GT2360	Saint-Paul	B	CDC 239-82	Unknown	Positive
GT546	Schwarzengrund	B	Massachusetts State Laboratory, Jamaica Plain, MA	Unknown	Positive
Neogen 190	Typhimurium	B	ATCC 13311	Unknown	Positive
GT2373	Typhimurium	B	NCTC[c] 74	feces, human	Positive
GT2365	Typhimurium var. Copenhagen	B	CDC stk 12	Unknown	Positive
GT2378	Braenderup	C1	CDC 1662-82	Unknown	Positive
GT2886	Cholerasuis	C1	CDC 34	Unknown	Positive
A144	Infantis	C1	ATCC 51741	Pasta	Positive
GT2479	Mbandaka	C1	CDC 1726-82	Unknown	Positive
GT2483	Montevideo	C1	CDC 1384-82	Unknown	Positive
A149	Oranienberg	C1	ATCC 9239	Unknown, Illinois State Hospital	Positive
GT2528	Paratyphi C	C1	CDC stk 32	Unknown	Positive
GT2516	Tennessee	C1	CDC stk 155	Unknown	Positive
GT2518	Thompson	C1	CDC stk 152	Unknown	Positive
GT2524	Virchow	C1	CDC stk 41	Unknown	Positive
GT2545	Bovismorbificans	C2	CDC stk 53	Unknown	Positive

GT2547	Duesseldorf	C2	CDC stk 130	Unknown	Positive
GT623	Hadar	C2	Massachusetts State Laboratory, Jamaica Plain, MA	Unknown	Positive
GT2553	Muenchen	C2	CDC 1646-82	Unknown	Positive
GT2557	Newport	C2	CDC stk 50	Unknown	Positive
GT2558	Newport var. Puerto Rico	C2	CDC stk 51	Unknown	Positive
GT2579	Tulear (II)	C2	CDC stk 972	Unknown	Positive
GT662	Albany	C3	University of Massachusetts, Amherst, MA	Unknown	Positive
GT2549	Haardt	C3	CDC 1813-82	Unknown	Positive
GT2581	Kentucky	C3	CDC stk 98	Unknown	Positive
GT2882	Virginia	C3	CDC stk 189	Unknown	Positive
GT2674	Bornum	C4	CDC stk 783	Unknown	Positive
GT2103	Eimsbuettel	C4	CDC 21-85	Unknown	Positive
A105	Berta	D1	ATCC 8392	Unknown	Positive
GT2583	Daressalaam	D1	CDC stk 72	Unknown	Positive
GT2584	Dublin	D1	CDC stk 65	Unknown	Positive
GT2881	Eastbourne	D1	CDC STK 70	Unknown	Positive
GT2124	Enteritidis	D1	ATCC 13076	Unknown	Positive
Neogen 195	Enteritidis	D1	ATCC 13076	Unknown	Positive
Neogen 207	Enteritidis	D1	CDC	Unknown	Positive
Neogen 100-247	Gallinarum	D1	Neogen Corp., Lansing, MI	Unknown	Positive
GT896	Gallinarum	D1	Neogen Corp., Lansing, MI	Unknown	Positive
GT2589	Javiana	D1	CDC 620-82	Unknown	Positive
GT2885	Pullorum	D1	CDC stk 75	Unknown	Positive
GT2125	Typhi	D1	ATCC 6539	Unknown	Positive
GT2620	Fresno	D2	CDC stk 355	Unknown	Positive
GT2621	Gateshead	D2	CDC stk 1845-82	Unknown	Positive
GT2622	Strasbourg	D2	CDC stk 428	Unknown	Positive
GT2626	Anatum	E1	CDC 714-82	Unknown	Positive
GT2637	Butantan	E1	CDC 899-82	Unknown	Positive
GT2638	Give	E1	CDC stk 77	Unknown	Positive
GT2158	Lexington	E1	FDA	Unknown	Positive
GT2641	Meleagridis	E1	CDC 1692-82	Unknown	Positive
GT2510	Muenster	E1	USDA[d], Athens, GA	Pork Sausage	Positive
Neogen 469	Uganda	E1	Neogen Corp., Lansing, MI	Unknown	Positive
GT911	Weltervreden	E1	CDC 147	Unknown	Positive
GT619	Binza	E2	University of Massachusetts, Amherst, MA	Unknown	Positive
GT908	Kinshasa	E2	CDC 271	Unknown	Positive
GT2101	Newbrunswick	E2	CDC 419-88	Unknown	Positive
GT2643	Arkansas	E3	CDC 1072-82	Unknown	Positive
GT2645	Illinois	E3	CDC stk 121	Unknown	Positive
GT2646	Minneapolis	E3	CDC stk 378	Unknown	Positive
GT3233	Chittagong	E4	CDC stk 214	Unknown	Positive

GT2650	Krefeld	E4	CDC 1245-82	Unknown	Positive
GT2883	Senftenberg	E4	CDC stk 735	Unknown	Positive
GT3178	Simsbury	E4	CDC stk 151	Unknown	Positive
GT2676	Westerstede	E4	CDC stk 607	Unknown	Positive
GT1857	Pretoria	F	CDC 144	Unknown	Positive
GT2703	Rubislaw	F	CDC stk 304	Unknown	Positive
GT2680	Poona	G1	CDC stk 91	Unknown	Positive
GT1858	Havana	G2	CDC 119	Unknown	Positive
GT4701	Worthington	G2	Neogen Corp., Lansing, MI	Unknown	Positive
GT5149	*S. enterica* subsp. *indica* (VI)	H	ATCC 43976	Unknown	Positive
GT2711	Florida	H	CDC stk 162	Unknown	Positive
GT913	Hvittingfoss	I	CDC 95	Unknown	Positive
GT2716	Kirkee	J	CDC stk 97	Unknown	Positive
GT2691	Cerro	K	CDC stk 430	Unknown	Positive
GT2721	Minnesota	L	CDC 985-82	Unknown	Positive
GT2723	Dakar	M	CDC stk 444	Unknown	Positive
GT3025	Urbana	N	CDC stk 117	Unknown	Positive
GT3028	Adelaide	O	CDC 1238-82	Unknown	Positive
GT3034	Inverness	P	CDC stk 171	Unknown	Positive
GT3037	Champaign	Q	CDC 87-82	Unknown	Positive
GT3039	Bern (IV)	R	CDC 611-821	Unknown	Positive
GT3058	Springs (II)	R	CDC stk 424	Unknown	Positive
GT3059	Waycross	S	CDC stk 224	Unknown	Positive
GT3060	Weslaco	T	CDC stk 236	Unknown	Negative
Neogen 100-1017	Weslaco	T	Silliker, Crete, IL	Unknown	Positive
GT4467	Houten (IV)	U	Silliker, Crete, IL	Unknown	Positive
GT3066	Guinea	V	CDC 698-82	Unknown	Positive
GT3069	Dugbe	W	CDC stk 518	Unknown	Positive
GT3073	Quimbamba	X	CDC stk 611	Unknown	Positive
GT2706	*S. bongori* ser. Balboa	Y	CDC N52	Unknown	Positive
GT3083	Djakarta	Y	CDC stk 467	Unknown	Positive
GT3084	Flint (IV)	Z	CDC 1234-82	Unknown	Positive
GT3085	Greenside (II)	Z	CDC stk 551	Unknown	Positive
GT3086	Hooggraven (II)	Z	CDC stk 967	Unknown	Positive
GT3087	Wassenaar (IV)	Z	CDC 1039-82	Unknown	Positive
GT1615	*S. enterica* subsp. *indica* (VI)		CDC 2229 232-84	Unknown	Positive

[a] Centers for Disease Control and Prevention, Atlanta, GA
[b] American Type Culture Collection, Manassas, VA
[c] National Collection of Type Cultures, Public Health England, UK
[d] U.S. Department of Agriculture

Table 2. Exclusivity results for the ANSR *Salmonella* assay (10)

Strain No.	*Organism*	*Source*	*Origin*	*Result at ~ 10^9 cfu/mL*
GT1485	*Citrobacter amalonaticus*	ATCC[a] 25405	feces	Negative
GT1475	*Citrobacter koseri*	ATCC 27156	Unknown	Negative
GT1477	*Citrobacter freundii*	ATCC 33128	Urine	Negative
GT1476	*Citrobacter youngae*	ATCC 29935	Meat scraps	Negative
GT1483	*Cronobacter sakazakii*	ATCC 29544	Human throat	Negative
GT1710	*Edwardsiella hoshinae*	ATCC 33379	Dead female puffin	Negative
GT569	*Edwardsiella tarda*	ATCC 15947	Human feces	Negative
GT1487	*Enterobacter aerogenes*	ATCC 29940	human	Negative
GT1482	*Enterobacter amnigenus*	ATCC 33072	soil	Negative
GT1497	*Enterobacter cancerogenus*	ATCC 35317	Human arm wound	Negative
GT1481	*Enterobacter cloacae subsp cloacae*	ATCC 29941	Unknown	Negative
GT2990	*Enterobacter cloacae*	NeogenCorp., Lansing, MI	dairy plant	Negative
GT1486	*Enterobacter gergoviae*	ATCC 33028	Urine	Negative
GT1480	*Enterobacter intermedia*	ATCC 33110	Unknown	Negative
GT1460	*Escherichia blattae*	CDC[b]	Unknown	Negative
GT1214	*Escherichia coli*	ATCC 12038	Unknown	Negative
GT1459	*Escherichia fergusonii*	ATCC 35473	feces	Negative
GT1216	*Escherichia hermannii*	ATCC 33650	human	Negative
GT1217	*Escherichia vulneris*	ATCC 33821	human	Negative
GT241	*Hafnia paralvei*	ATCC 29927	human	Negative
GT1503	*Klebsiella oxytoca*	ATCC 13182	Pharyngeal tonsil	Negative
GT1478	*Raoultella planticola*	ATCC 33531	Radish root	Negative
GT1499	*Klebsiella pneumoniae* subsp. *ozaenae*	ATCC 11296	Unknown	Negative
GT3600	*Kluyvera ascorbata*	ATCC 33433	Human sputum	Negative
GT303	*Morganella morganii* subsp. *morganii*	ATCC 25830	Patient with summer diarrhea	Negative
GT1467	*Buttiauxella* group	ATCC 29917	Chicken liver	Negative
GT358	*Pasteurella multocida* subsp. *multocida*	ATCC 19427	Unknown	Negative
GT1493	*Proteus mirabilis*	ATCC 25933	Human vagina	Negative
GT366	*Proteus myxofaciens*	ATCC 19692	Insect	Positive
GT367	*Proteus penneri*	ATCC 33519	Urine	Negative
GT368	*Proteus hauseri*	ATCC 13315	Unknown	Negative
GT371	*Providencia alcalifaciens*	ATCC 9886	feces	Negative
GT373	*Providencia rettgeri*	ATCC 29944	Unknown	Negative
GT374	*Providencia rustigiani*	ATCC 33673	Human feces	Negative
GT375	*Providencia stuartii*	ATCC 29914	Human	Negative
GT1909	*Pseudomonas aeruginosa*	ATCC 27853	blood	Negative
GT392	*Serratia marcescens*	ATCC 29937	human	Negative

[a] American Type Culture Collection, Manassas, VA

[b] Centers for Disease Control and Prevention, Atlanta, GA

Table 3. Probability of detection calculations for ANSR *Salmonella* presumptive and confirmed results (10)

Food Type	Inoculum Strain	Inoculation Level (cfu/portion)[a]	N[b]	ANSR Presumptive Result			ANSR Confirmed Result			$dPOD_{CP}$[f]	95% CI[g]
				x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI		
Dried whole egg	*S*. Enteritidis A120	> 0.80	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		0.29 (0.12, 0.69)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0	-0.26, 0.26
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Dried egg white w/ sodium lauryl sulfate	*S*. Typhimurium GT2373	> 0.80	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		3.0 (1.6, 5.7)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Frozen egg yolk w/ sugar	*S*. Montevideo GT2483	> 0.80	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		1.1 (0.60, 1.8)	20	13	0.65	0.43, 0.82	14	0.70	0.48, 0.85	-0.05	-0.32, 0.23
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Liquid whole egg w/ citric acid	*S*. Senftenberg GT2883	24 (4.2, 100)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		1.5 (0.37, 4.2)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Liquid whole egg w/ citric acid[h]	*S*. Senftenberg ATCC 43845	1.9 (1.0, 3.7)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.26 (0.10, 0.48)	20	4	0.20	0.08, 0.42	4	0.20	0.08, 0.42	0	-0.25, 0.25
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes.
[e] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[f] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed 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] Trial performed by independent laboratory.

Table 4. Probability of detection calculations for ANSR *Salmonella* confirmed and reference method results (10)

Food Type	Inoculum Strain	Inoculation Level (cfu/portion)[a]	N[b]	ANSR Confirmed Result			Reference Method Result			dPOD$_C$[f]	95% CI[g]
				x[c]	POD$_C$[d]	95% CI	x	POD$_R$[e]	95% CI		
Dried whole egg	*S.* Enteritidis A120	> 0.80	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		0.29 (0.12, 0.69)	20	5	0.25	0.11, 0.47	5	0.25	0.11, 0.47	0	-0.26, 0.26
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Dried egg white w/ sodium lauryl sulfate	*S.* Typhimurium GT2373	> 0.80	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		3.0 (1.6, 5.7)	20	15	0.75	0.53, 0.89	19	0.95	0.76, 1	-0.20	-0.43, 0.04
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Frozen egg yolk w/ sugar	*S.* Montevideo GT2483	> 0.80	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		1.1 (0.60, 1.8)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0	-0.28, 0.28
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Liquid whole egg w/ citric acid	*S.* Senftenberg GT2883	24 (4.2, 100)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.44, 0.44
		1.5 (0.37, 4.2)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.23, 0.32
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
Liquid whole egg w/ citric acid[h]	*S.* Senftenberg ATCC 43845	1.9 (1.0, 3.7)	5	5	1	0.56, 1	5	1	0.56, 1	0	-0.43, 0.43
		0.26 (0.10, 0.48)	20	4	0.20	0.08, 0.42	5	0.25	0.11, 0.47	-0.05	-0.30, 0.21
		0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD$_C$ = Candidate method presumptive positive outcomes confirmed positive.
[e] POD$_R$ = Reference method confirmed 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] Trial performed by independent laboratory.

DISCUSSION OF THE VALIDATION STUDY Modification Approved November 2014 (11)

The results presented here demonstrate that a modification to the reagent formulation and a minor procedural change to the ANSR *Salmonella* method do not affect performance of the assay. In inclusivity and exclusivity testing, of the 113 strains tested belonging to *S. enterica* and *S. bongori*, 112 strains were detected and one strain of *Salmonella* Weslaco was not detected. Of the 38 strains of non-salmonellae tested, none were detected. These results are identical to those obtained in the original validation studies [1-3]. In comparative testing of foods and sponge sample from a stainless steel surface, performance of the ANSR method was comparable to that of the FDA/BAM or USDA-FSIS/MLG methods as determined by POD analysis. There were no statistically significant differences in the number of positive results obtained by the ANSR and reference culture methods in any of the trials. Results of robustness testing showed that the modified assay can withstand perturbations to multiple assay parameters and still yield accurate results.

The enhanced reagent formulation for ANSR *Salmonella* provides increased pellet solubility, thus eliminating the need for pipette mixing of the lysed sample and ANSR reagents. This improves operator ease of use and minimizes the opportunity for cross contamination or potential amplicon contamination of the work environment. In addition to high sensitivity and specificity, the ASNR *Salmonella* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and assay results within 40 min following sample enrichment.

Table 1. Results of inclusivity testing for the ANSR *Salmonella* test. (11)

Strain No.	*Salmonella* Serovar[a]	O Group	Source	Origin (if Known)	ANSR Result at ~1x10^6 cfu/mL
GT2652	Arizonae (III)	51	CDC		Positive
GT799	Arizonae (III)	51	ATCC 13314		Positive
GT3136	Treforest	51	CDC		Positive
GT3089	Humber (II)	53	CDC		Positive
GT3090	Tranoroa (II)	55	CDC		Positive
GT3091	Artis (II)	56	CDC		Positive
GT3092	Tokai (II)	57	CDC		Positive
GT3093	Betioky (II)	59	CDC		Positive
GT3094	Luton (II)	60	CDC		Positive
GT2704	*S. bongori* ser. Brookfield	66	CDC		Positive
GT2705	*S. bongori* ser. Malawi	66	CDC		Positive
GT1614	*S. bongori* ser. Maregrosso	66	CDC		Positive
GT3199	Crossness	67	CDC		Positive
GT657	Kiel	A	Deibel Labs		Positive
GT2403	Paratyphi A	A	CDC		Positive
GT2284	Agona	B	CDC		Positive
GT2304	Heidelberg	B	CDC		Positive
GT2306	Java	B	CDC		Positive
GT2309	Paratyphi B	B	CDC		Positive
GT2360	Saint-Paul	B	CDC		Positive
GT546	Schwarzengrund	B	Mass. State Lab		Positive
GT2373	Typhimurium	B	ATCC 13311	Mutton	Positive
Neogen 190	Typhimurium	B	CDC		Positive
GT2365	Typhimurium var. Copenhagen	B	CDC		Positive
GT2378	Braenderup	C1	CDC		Positive
GT2436	Cholerasuis	C1	CDC		Positive
A144	Infantis	C1	ATCC 51741		Positive
GT2479	Mbandaka	C1	CDC		Positive
GT2483	Montevideo	C1	CDC		Positive
A149	Oranienberg	C1	ATCC 9239	Ill. State Hospital	Positive
GT2528	Paratyphi C	C1	CDC		Positive
GT2516	Tennessee	C1	CDC		Positive
GT2518	Thompson	C1	CDC		Positive
GT2524	Virchow	C1	CDC		Positive
GT2545	Bovismorbificans	C2	CDC		Positive
GT2547	Duesseldorf	C2	CDC		Positive
GT623	Hadar	C2	Mass. State Lab		Positive
GT2553	Muenchen	C2	CDC		Positive
GT2557	Newport	C2	CDC		Positive
GT2558	Newport var. Puerto Rico	C2	CDC		Positive
GT2579	Tulear (II)	C2	CDC		Positive
GT662	Albany	C3	U. Mass.		Positive
GT2549	Haardt	C3	CDC		Positive
GT2581	Kentucky	C3	CDC		Positive
GT2882	Virginia	C3	CDC		Positive
GT2674	Bornum	C4	CDC		Positive

GT2103	Eimsbuettel	C4	CDC		Positive
GT2884	Berta	D1	CDC		Positive
GT2583	Daressalaam	D1	CDC		Positive
GT2584	Dublin	D1	CDC		Positive
GT2881	Eastbourne	D1	CDC		Positive
Neogen 195	Enteritidis	D1	CDC		Positive
Neogen 207	Enteritidis	D1	CDC		Positive
GT2124	Enteritidis	D1	ATCC 13076		Positive
GT896	Gallinarum	D1	GENE-TRAK Systems		Positive
GT2589	Javiana	D1	CDC		Positive
GT2885	Pullorum	D1	CDC		Positive
GT2125	Typhi	D1	ATCC 6539		Positive
GT2620	Fresno	D2	CDC		Positive
GT2621	Gateshead	D2	CDC		Positive
GT2622	Strasbourg	D2	CDC		Positive
GT2626	Anatum	E1	CDC		Positive
GT2637	Butantan	E1	CDC		Positive
GT2638	Give	E1	CDC		Positive
GT2158	Lexington	E1	CDC		Positive
GT2641	Meleagridis	E1	CDC		Positive
GT2510	Muenster	E1	USDA, Athens, GA		Positive
Neogen 469	Uganda	E1	Ampcor	Pork Sausage	Positive
GT911	Weltervreden	E1	CDC		Positive
GT619	Binza	E2	U. Mass.		Positive
GT908	Kinshasa	E2	CDC		Positive
GT2101	Newbrunswick	E2	CDC		Positive
GT2643	Arkansas	E3	CDC		Positive
GT2645	Illinois	E3	CDC		Positive
GT2646	Minneapolis	E3	CDC		Positive
GT3233	Chittagong	E4	CDC		Positive
GT2650	Krefeld	E4	CDC		Positive
GT2883	Senftenberg	E4	CDC		Positive
GT3178	Simsbury	E4	CDC		Positive
GT2676	Westerstede	E4	CDC		Positive
GT1857	Pretoria	F	CDC		Positive
GT2703	Rubislaw	F	CDC		Positive
GT2680	Poona	G1	CDC		Positive
GT1858	Havana	G2	CDC		Positive
GT4701	Worthington	G2	GENE-TRAK Systems		Positive
GT5149	Ferlac	H	ATCC 43976		Positive
GT2711	Florida	H	CDC		Positive
GT913	Hvittingfoss	I	CDC		Positive
GT2716	Kirkee	J	CDC		Positive
GT2691	Cerro	K	CDC		Positive
GT2721	Minnesota	L	CDC		Positive
GT2723	Dakar	M	CDC		Positive
GT3025	Urbana	N	CDC		Positive
GT3028	Adelaide	O	CDC		Positive
GT3034	Inverness	P	CDC		Positive
GT3037	Champaign	Q	CDC		Positive
GT3039	Bern (IV)	R	CDC		Positive
GT3058	Springs (II)	R	CDC		Positive
GT3059	Waycross	S	CDC		Positive
100-1017	Weslaco	T	Silliker		Positive
GT3060	Weslaco	T	CDC		Negative
GT4467	Houten (IV)	U	Silliker		Positive
GT3066	Guinea	V	CDC		Positive
GT3069	Dugbe	W	CDC		Positive
GT3073	Quimbamba	X	CDC		Positive
GT3083	Djakarta	Y	CDC		Positive

GT2706	*S. bongori* ser. Balboa	Y	CDC		Positive
GT388	*S. bongori* ser. Bongor	Y	CDC		Positive
GT3084	Flint (IV)	Z	CDC		Positive
GT3085	Greenside (II)	Z	CDC		Positive
GT3086	Hooggraven (II)	Z	CDC		Positive
GT3087	Wassenaar (IV)	Z	CDC		Positive
GT1615	*S. enterica* subsp. indica (VI)		CDC		Positive

[a] All strains are serovars of *Salmonella enterica* unless otherwise indicated. Serovars of subspecies other than subspecies I are indicated in parentheses.

Table 2. Results of exclusivity testing for the ANSR *Salmonella* test. (11)

Strain No.	Organism	Source	Origin (if Known)	ANSR Result at 10^8-10^9 cfu/mL in TSB
GT1485	*Citrobacter amalonaticus*	ATCC 25405	feces	Negative
GT1475	*Citrobacter diversus*	ATCC 27156		Negative
GT1477	*Citrobacter freundii*	ATCC33128		Negative
GT1476	*Citrobacter youngae*	ATCC 29935	meat	Negative
GT1483	*Cronobacter sakazakii*	ATCC 29544	human	Negative
GT1710	*Edwardsiella hoshinae*	ATCC 33379	bird	Negative
GT569	*Edwardsiella tarda*	ATCC 15947	feces	Negative
GT1487	*Enterobacter aerogenes*	ATCC 29940	human	Negative
GT1482	*Enterobacter amnigenus*	ATCC 33072	soil	Negative
GT1497	*Enterobacter cancerogenus*	ATCC 35317		Negative
GT1481	*Enterobacter cloacae*	ATCC 29941		Negative
GT2990	*Enterobacter cloacae*	GENE-TRAK	dairy plant	Negative
GT1486	*Enterobacter gergoviae*	ATCC 33028		Negative
GT1480	*Enterobacter intermedia*	ATCC 33110		Negative
GT1460	*Escherichia blattae*	CDC		Negative
GT1214	*Escherichia coli*	ATCC 12038		Negative
GT1459	*Escherichia fergusonii*	ATCC 35473	feces	Negative
GT1216	*Escherichia hermannii*	ATCC 33650	human	Negative
GT1217	*Escherichia vulneris*	ATCC 33821	human	Negative
GT241	*Hafnia alvei*	ATCC 29927	human	Negative
GT1503	*Klebsiella oxytoca*	ATCC 13182	human	Negative
GT1478	*Klebsiella planticola*	ATCC 33531	radish	Negative
GT1499	*Klebsiella pneumoniae* subsp. *ozaenae*	ATCC 11296		Negative
GT3600	*Kluyvera ascorbata*	ATCC 33433	human	Negative
GT303	*Morganella morganii*	ATCC 25830	human	Negative
GT1467	*Pantoea agglomerans*	ATCC 29917		Negative
GT358	*Pasteurella multocida*	ATCC 19427		Negative
GT1493	*Proteus mirabilis*	ATCC 25933	human	Negative
GT366	*Proteus myxofaciens*	ATCC 19692		Negative
GT367	*Proteus penneri*	ATCC 33519		Negative
GT368	*Proteus vulgaris*	ATCC 13315		Negative
GT371	*Providencia alcalifaciens*	ATCC 9886	feces	Negative
GT373	*Providencia rettgeri*	ATCC 29944		Negative
GT374	*Providencia rustigiani*	ATCC 33673		Negative
GT375	*Providencia stuartii*	ATCC 29914		Negative
GT1909	*Pseudomonas aeruginosa*	ATCC 27853	blood	Negative
GT392	*Serratia marcescens*	ATCC 29937	human	Negative
GT1713	*Serratia rubidae*	ATCC 15338		Negative

Table 3. Probability of detection calculations for ANSR *Salmonella* presumptive and confirmed results, primary time point [a] (11)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Presumptive Result			ANSR Confirmed Result			$dPOD_{CP}$[g]	95% CI[h]
				X[d]	POD_{CP}[e]	95% CI	x	POD_{CC}[f]	95% CI		
Raw Ground Turkey (7% fat)	*S.* Hadar	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.1 (0.23, 4.5)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0	-0.28, 0.28
		>27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef (10% fat)	*S.* Newport	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.58 (0.11, 2.4)	20	15	0.75	0.53, 0.89	14	0.70	0.48, 0.85	0.05	-0.22, 0.31
		>27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Dry Dog Food	*S.* Schwarzengrund	0 (0, 2.4)	5	1	0.20	0, 0.62	0	0	0, 0.43	0.20	-0.28, 0.62
		0.09 (0.0042, 0.45)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
		27.5 (4.5, 103)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Peanut Butter	*S.* Tennessee	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.92 (0.51, 1.6)	20	13	0.65	0.43, 0.82	14	0.70	0.48, 0.85	-0.05	-0.32, 0.23
		5.8 (1.2, 23.5)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Vanilla Ice Cream	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3.7 (0.92, 10.5)	20	15	0.75	0.53, 0.89	16	0.80	0.58, 0.92	-0.05	-0.3, 0.21
		60 (10.5, 250)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Stainless Steel	*S.* Heidelberg + 10X Competitor Cocktail	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1,425/15,000	20	12	0.60	0.39, 0.78	11	0.55	0.34, 0.74	0.05	-0.24, 0.33
		142,500/1,500,000	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43

[a] Primary time point, 12 h for raw ground turkey and raw ground beef, 16 h for all other commodities.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes
[f] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[g] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 4. Probability of detection calculations for ANSR *Salmonella* presumptive and confirmed results, secondary time point [a] (11)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Presumptive Result			ANSR Confirmed Result			$dPOD_{CP}$[g]	95% CI[h]
				X[d]	POD_{CP}[e]	95% CI	x	POD_{CC}[f]	95% CI		
Raw Ground Turkey (7% fat)	*S.* Hadar	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.1 (0.23, 4.5)	20	11	0.55	0.34, 0.74	12	0.60	0.39, 0.78	-0.05	-0.33, 0.24
		27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef (10% fat)	*S.* Newport	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.58 (0.11, 2.4)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	-0.27, 0.27
		27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Dry Dog Food	*S.* Schwarzengrund	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.09 (0.0042, 0.45)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	-0.28, 0.28
		27.5 (4.5, 103)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Peanut Butter	*S.* Tennessee	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.92 (0.51, 1.6)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	-0.27, 0.27
		5.8 (1.2, 23.5)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Vanilla Ice Cream	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3.7 (0.92, 10.5)	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0	-0.25, 0.25
		60 (10.5, 250)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Stainless Steel	*S.* Heidelberg + 10X Competitor Cocktail	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1,425/15,000	20	13	0.65	0.43, 0.82	11	0.55	0.34, 0.74	0.10	-0.19, 0.37
		142,500/1,500,000	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43

[a] Secondary time point, 24 h for all commodities.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes.
[f] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[g] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 5. Probability of detection calculations for ANSR *Salmonella* confirmed and reference method results, primary time point [a] (11)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Confirmed Result			Reference Method Result			dPOD$_C$[g]	95% CI[h]
				X[d]	POD$_C$[e]	95% CI	x	POD$_R$[f]	95% CI		
Raw Ground Turkey (7% fat)	*S.* Hadar	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.1 (0.23, 4.5)	20	12	0.60	0.39, 0.78	14	0.70	0.48, 0.85	-0.10	-0.36, 0.18
		27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef (10% fat)	*S.* Newport	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.58 (0.11, 2.4)	20	14	0.70	0.48, 0.85	18	0.90	0.7, 0.97	-0.20	-0.43, 0.05
		27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Dry Dog Food	*S.* Schwarzengrund	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.09 (0.0042, 0.45)	20	8	0.40	0.22, 0.61	11	0.55	0.34, 0.74	-0.15	-0.41, 0.15
		27.5 (4.5, 103)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Peanut Butter	*S.* Tennessee	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.92 (0.51, 1.6)	20	13	0.65	0.43, 0.82	12	0.60	0.39, 0.78	0.05	-0.23, 0.32
		5.8 (1.2, 23.5)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Vanilla Ice Cream	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3.7 (0.92, 10.5)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		60 (10.5, 250)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Stainless Steel	*S.* Heidelberg + 10X Competitor Cocktail	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1,425/15,000	20	11	0.55	0.34, 0.74	10	0.50	0.3, 0.7	0.05	-0.24, 0.33
		142,500/1,500,000	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43

[a] Primary time point, 12 h for raw ground turkey and raw ground beef, 16 h for all other commodities.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes.
[f] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[g] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 6. Probability of detection calculations for ANSR *Salmonella* confirmed and reference method results, secondary time point [a] (11)

Food Type	Inoculum Strain	Inoc. Level (cfu/portion)[b]	N[c]	ANSR Confirmed Result			Reference Method Result			dPOD$_C$[g]	95% CI[h]
				X[d]	POD$_C$[e]	95% CI	x	POD$_R$[f]	95% CI		
Raw Ground Turkey (7% fat)	*S.* Hadar	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.1 (0.23, 4.5)	20	11	0.55	0.34, 0.74	14	0.07	0.48, 0.85	-0.15	-0.41, 0.14
		27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Raw Ground Beef (10% fat)	*S.* Newport	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.58 (0.11, 2.4)	20	14	0.70	0.48, 0.85	18	0.90	0.7, 0.97	-0.20	-0.43, 0.05
		27.5 (10.5, >210)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Dry Dog Food	*S.* Schwarzengrund	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.09 (0.0042, 0.45)	20	9	0.45	0.26, 0.66	11	0.55	0.34, 0.74	-0.10	-0.37, 0.19
		27.5 (4.5, 103)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Peanut Butter	*S.* Tennessee	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		0.92 (0.51, 1.6)	20	14	0.70	0.48, 0.85	12	0.60	0.39, 0.78	0.10	-0.18, 0.36
		5.8 (1.2, 23.5)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Vanilla Ice Cream	*S.* Senftenberg	0 (0, 2.4)	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		3.7 (0.92, 10.5)	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.3
		60 (10.5, 250)	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43
Stainless Steel	*S.* Heidelberg + 10X Competitor Cocktail	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1,425/15,000	20	11	0.55	0.34, 0.74	10	0.50	0.3, 0.7	0.05	-0.24, 0.33
		142,500/1,500,000	5	5	1.0	0.57, 1.0	5	1.0	0.57, 1.0	0	-0.43, 0.43

[a] Secondary time point, 24 h for all commodities.
[b] Determined by most probable number analysis.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes.
[f] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[g] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

REFERENCES CITED

1. Mozola, Mark, Norton, Paul, Alles, Susan, Gray, R. Lucas, Tolan, Jerry, Caballero, Oscar, Pinkava, Lisa, Hosking, Edan, Luplow, Karen, and Rice, Jennifer., Validation of the ANSR™ for *Salmonella* Method for Detection of *Salmonella* spp. in Selected Foods and Environmental Samples, AOAC® *Performance Tested℠* certification number 061203.
2. AOAC Research Institute Validation Outline for ANSR™ for *Salmonella* , Approved – June 2012.
3. Van Ness, J., Van Ness, L.K., & Galas, D. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 4504-4509
4. US FDA (2011) *Bacteriological Analytical Manual*, chapter 5 http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm
5. USDA-FSIS (2011) *Microbiology Laboratory Guidebook*, chapter 4.05 http://www.fsis.usda.gov/PDF/MLG_4_05.pdf
6. Mantel, N. & Haenszel, W. (1959) *J. Nat. Cancer Inst.* **22**,719-748
7. Anderson, G. & Scott, M. (1991) *Clin. Chem*. **37**, 398-402
8. Caballero, O., Alles, S., Gray. R.L., Tolan, J., Mozola, M., and Rice, J., Validation of the ANSR™ for *Salmonella* Method for Detection of *Salmonella* spp. in a Variety of Foods, AOAC® *Performance Tested℠* certification number 061203. Modification approved January 2013.
9. Caballero, O., Alles, S., Gray. R.L., Walton, K., Mozola, M., and Rice, J., Validation of a New Lysis Procedure for the ANSR™ for *Salmonella* Method, *Modification to AOAC® Performance Tested℠* certification number 061203. Modification approved June 2013.
10. Foti, D., Zhang, L., Biswas, P., Mozola, M., and Rice. J., Matrix Extension Study: Validation of the ANSR™ *Salmonella* Method for Detection of *Salmonella* spp. In Pasteurized Egg Products, Modification *to AOAC® Performance Tested℠* certification number 061203. Modification approved November 2013.
11. Caballero, O., Alles, S., Gray. R.L., Walton, K., Mozola, M., and Rice, J., Validation of Modifications to the ANSR™ for *Salmonella* Method for Improved Ease of Use, *Modification to AOAC® Performance Tested℠* certification number 061203. Modification approved November 2014.