



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

051601

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

Solus Scientific *Salmonella* ELISA

manufactured by

Solus Scientific Ltd.

Unit 9 Mansfield Networkcentre

Millennium Business Park

Concorde Way, Mansfield

Nottinghamshire, NG9 7JZ

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 (January 3, 2020 – 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

January 3, 2020

Date

METHOD AUTHORS

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SUBMITTING COMPANY

Solus Scientific Ltd.
Millennium Business Park
Concorde Way, Mansfield
Nottinghamshire, NG9 7JZ United Kingdom

KIT NAME(S)

Solus Scientific *Salmonella* ELISA

CATALOG NUMBERS

SAL0096S, SAL0480S

INDEPENDENT LABORATORY

Q Laboratories, Inc.
1400 Harrison Ave
Cincinnati, OH
USA

AOAC EXPERTS AND PEER REVIEWERS

Yi Chen¹, Michael Brodsky², Yvonne Salfinger³
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APPLICABILITY OF METHOD

Target organism – *Salmonella* species

Matrixes – (25g) – raw chicken breast, raw salmon filet, bagged romaine lettuce, shredded cheddar cheese, instant non-fat dry milk, shell eggs
(375 g) raw beef trim
stainless steel environmental surface (swab, 1 x 1 in),
polystyrene environmental surface (sponge, 4 x 4 in)

Performance claims - The Solus Scientific *Salmonella* ELISA method is equivalent to the reference methods.

REFERENCE METHODS

United States Department of Agriculture Microbiological Laboratory Guidelines 4.08: *Isolation and Identification of Salmonella from Red Meat, Poultry, Pasteurized Egg, Catfish Products, and Environmental Sponges*. June 29th, 2014. (Accessed October 2015)
<http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES> (2)
Food and Drug Administration Bacteriological Analytical Manual Chapter 5: *Salmonella*. February, 2014. (Accessed October 2015)
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm> (3)

ORIGINAL CERTIFICATION DATE

May 04, 2016

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2020

METHOD MODIFICATION RECORD

1. December 2018 Level 1

SUMMARY OF MODIFICATION

1. Editorial/clerical changes to text to bring the language and style in line with more recently approved products.

Under this AOAC® *Performance Tested*SM License Number, 051601 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*SM License Number, 051601 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)

The Solus Scientific *Salmonella* ELISA method is used for the rapid and specific detection of *Salmonella* species in enriched food and environmental samples. The Solus Scientific *Salmonella* ELISA method relies on antibodies attaches to the wells of the microplate strips that are specific to *Salmonella* antigens. Samples are heat treated and an aliquot is added to the antibody coated wells. *Salmonella* specific antigens present in the samples will bind immunologically to the antibody. After washing to remove unbound material, an enzyme-labeled antibody will bind to the proteins and thus to the well. After a second wash step to remove any unbound enzyme-antibody, the enzyme substrate is added. The substrate reacts in the presence of the enzyme producing a blue color change in the sample well. The substrate reaction is stopped after 30 minutes with the addition of dilute sulphuric acid changing any blue color present in the wells to yellow. [4]

DISCUSSION OF THE VALIDATION STUDY (1)

The inclusivity study showed the Solus *Salmonella* ELISA had a high specificity for the *Salmonella* species but one limitation was noted for *Salmonella arizonae*, since the assay did not detect all the strains from this subspecies.

The exclusivity study showed the Solus *Salmonella* ELISA had a high specificity and the few crossreactions observed with a small number of strains after a non-selective enrichment in BPW were all eliminated after the subculture in the selective broth, RVS. Since the enrichment protocol for food matrixes and environmental surfaces always includes a second enrichment step in RVS, the crossreactions remain limited.

The Solus Scientific *Salmonella* ELISA methods successfully recovered *Salmonella* species from all of the matrixes and environmental surfaces analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and each of the reference methods for all matrixes and environmental surfaces tested.

After results of the original analysis of the raw chicken breast were obtained, an investigation into the cause of the discrepant results was performed. The samples that produced the false positive results in the first round of testing, displayed previously in Table D, had notably higher absorbance values (OD_{450} of ~0.250-0.425) than what would be expected for a typical negative sample ($OD_{450} < 0.050$). These results suggested two potential sources for the issues: a cross reaction with background flora or an issue with the lot of the testing media, RVS (i.e. an issue with the specificity of this lot of media). For all of the matrixes evaluated, the raw chicken breast had the second highest background microflora (APC of $>10^5$ CFU/g) and further identification of the background flora was conducted. Several colonies recovered from the selective agar plates were struck for isolation onto non-selective agar for further biochemical analysis. Two species were predominately isolated, *Pseudomonas aeruginosa* and *Hafnia alvei*.

When tested with the Somatic Polyvalent O antisera, these two strains cross reacted strongly with the antisera indicating the potential for cross reaction with *Salmonella* antibodies. The combination of the high absorbance readings, may indicate that these strains were able to grow in the lot of RVS and produce a cross reaction with the assay. Discussions with the manufacturer of the RVS indicated that the different sources for the raw materials, including ingredients designed to make the media select, were being used and could be one of the factors that lead to the discrepant results. To determine if the issue was specific to that lot of media, or that lot of chicken breast, a second set of raw chicken breast samples were analyzed. For the retest of the raw chicken breast, two lots of Solus Scientific RVS (the original lot and a newly manufactured lot) and a third lot of Accumedica brand RVS were evaluated. Based on the results obtained from the retest, summarized in Table E, it does not appear that the lot or brand of the media was the definitive cause, nor does it appear that the matrix itself is the issue. No definitive cause of the issues was identified, but for matrixes with high microbial backgrounds, special note should be taken with all presumptive positive samples.

The manual and the automated methods for the Solus Scientific *Salmonella* ELISA method are quick and simple to perform, providing results in less than 2 hours post incubation of the selective enrichment for 30 sample replicates. The method offers the benefit of the use of both the manual method and the automated method to obtain results. The small footprint of both methods offers the ability to test in various laboratories. The Dynex DS2 software is user friendly with the ability to track lot information and sample identification quickly and with ease. The Dynex DS2 software and instrument also offer the ability to run multiple assays at one time and has an open platform.

Table 19: Solus Scientific *Salmonella* ELISA, Candidate* vs. Reference – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Raw Beef Trim (375 g)	<i>Salmonella</i> Typhimurium ATCC 14028	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.17, 0.69)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			3.76 (1.55, 9.10)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Breast (25 g)	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Breast (25 g)	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Salmon Filet (25 g)	<i>Salmonella</i> Senftenberg ATCC 43845	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	-0.05	-0.33, 0.24
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*The alternative and traditional confirmation both produced identical results

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 20: Solus Scientific *Salmonella* ELISA, Candidate* vs. Reference – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Bagged Romaine Lettuce (25 g)	<i>Salmonella</i> Hadar ATCC 51956	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.38 (0.17, 0.67)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shredded Cheddar Cheese (25 g)	<i>Salmonella</i> Heidelberg NCTC 5717	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.46 (0.23, 0.78)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			2.29 (1.04, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Instant Non-Fat Dry Milk (25 g)	<i>Salmonella</i> Abony NCTC 6017	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	7	0.35	0.18, 0.57	-0.05	-0.32, 0.23
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shell Eggs (25 g)	<i>Salmonella</i> Jerusalem QL 024.12	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.98 (0.60, 1.69)	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
			4.38 (1.72, 11.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shell Eggs (25 g)	<i>Salmonella</i> Jerusalem QL 024.12	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.98 (0.60, 1.69)	20	15	0.75	0.53, 0.89	12	0.60	0.39, 0.78	0.15	-0.13, 0.40
			4.38 (1.72, 11.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*The alternative and traditional confirmation both produced identical results.

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 21: Solus Scientific *Salmonella* ELISA, Candidate* vs. Reference – POD Results (1)

Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Typhimurium ATCC BAA-215 & <i>Citrobacter freundii</i> ATCC 8090	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 210	20	9	0.45	0.26, 0.66	8	0.40	0.34, 0.74	0.05	-0.24, 0.33
			440 & 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI							
Stainless Steel	<i>Salmonella</i> Typhimurium ATCC BAA-215 & <i>Citrobacter freundii</i> ATCC 8090	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			32 & 510	20	9	0.45	0.26, 0.66	8	0.40	0.43, 0.82	0.05	-0.24, 0.33
			400 & 3700	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI							
Polystyrene	<i>Salmonella</i> Montevideo ATCC 8387 & <i>E. coli</i> ATCC 8090	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			40 & 390	20	6	0.30	0.15, 0.52	7	0.35	0.26, 0.66	-0.05	-0.32, 0.23
			320 & 2200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*The alternative and traditional confirmation produced identical results.

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c= Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 22: Solus Scientific *Salmonella* ELISA, Presumptive vs. Alternative Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Raw Beef Trim (375 g)	<i>Salmonella</i> Typhimurium ATCC 14028	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.17, 0.69)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			3.76 (1.55, 9.10)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Breast (25 g)	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	2	0.40	0.12, 0.77	0	0.00	0.00, 0.43	0.40	-0.12, 0.77
			0.61 (0.33, 1.02)	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Breast (25 g)	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	2	0.40	0.12, 0.77	0	0.00	0.00, 0.43	0.40	-0.12, 0.77
			0.61 (0.33, 1.02)	20	13	0.65	0.43, 0.82	11	0.55	0.34, 0.74	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Salmon Filet (25 g)	<i>Salmonella</i> Senftenberg ATCC 43845	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 23: Solus Scientific *Salmonella* ELISA, Presumptive vs. Alternative Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Bagged Romaine Lettuce (25 g)	<i>Salmonella</i> Hadar ATCC 51956	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.38 (0.17, 0.67)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shredded Cheddar Cheese (25 g)	<i>Salmonella</i> Heidelberg NCTC 5717	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.46 (0.23, 0.78)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.29 (1.04, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Instant Non-Fat Dry Milk (25 g)	<i>Salmonella</i> Abony NCTC 6017	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shell Eggs (25 g)	<i>Salmonella</i> Jerusalem QL 024.12	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.98 (0.60, 1.69)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			4.38 (1.72, 11.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shell Eggs (25 g)	<i>Salmonella</i> Jerusalem QL 024.12	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.98 (0.60, 1.69)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			4.38 (1.72, 11.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 24: Solus Scientific *Salmonella* ELISA, Presumptive vs. Alternative Confirmed – POD Results (1)

Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Typhimurium ATCC BAA-215 & <i>Citrobacter freundii</i> ATCC 8090	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 210	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			440 & 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
Stainless Steel	<i>Salmonella</i> Typhimurium ATCC BAA-215 & <i>Citrobacter freundii</i> ATCC 8090	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 210	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			440 & 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
Polystyrene	<i>Salmonella</i> Montevideo ATCC 8387 & <i>E. coli</i> ATCC 8090	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			40 & 390	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			320 & 2200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 25: Solus Scientific *Salmonella* ELISA, Presumptive vs. Traditional Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Raw Beef Trim (375 g)	<i>Salmonella</i> Typhimurium ATCC 14028	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.17, 0.69)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			3.76 (1.55, 9.10)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Breast (25 g)	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	2	0.40	0.12, 0.77	0	0.00	0.00, 0.43	0.40	-0.12, 0.77
			0.61 (0.33, 1.02)	20	15	0.75	0.53, 0.89	11	0.55	0.34, 0.74	0.20	-0.09, 0.45
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Chicken Breast (25 g)	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	2	0.40	0.12, 0.77	0	0.00	0.00, 0.43	0.40	-0.12, 0.77
			0.61 (0.33, 1.02)	20	13	0.65	0.43, 0.82	11	0.55	0.34, 0.74	0.10	-0.19, 0.37
			4.38 (1.72, 11.15)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Salmon Filet (25 g)	<i>Salmonella</i> Senftenberg ATCC 43845	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.61 (0.33, 1.02)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 26: Solus Scientific *Salmonella* ELISA, Presumptive vs. Traditional Confirmed – POD Results (1)

Matrix/Test Portion	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Bagged Romaine Lettuce (25 g)	<i>Salmonella</i> Hadar ATCC 51956	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.38 (0.17, 0.67)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shredded Cheddar Cheese (25 g)	<i>Salmonella</i> Heidelberg NCTC 5717	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.46 (0.23, 0.78)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.29 (1.04, 5.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Instant Non-Fat Dry Milk (25 g)	<i>Salmonella</i> Abony NCTC 6017	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.49 (0.25, 0.85)	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shell Eggs (25 g)	<i>Salmonella</i> Jerusalem QL 024.12	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.98 (0.60, 1.69)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			4.38 (1.72, 11.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Shell Eggs (25 g)	<i>Salmonella</i> Jerusalem QL 024.12	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.98 (0.60, 1.69)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0.00	-0.26, 0.26
			4.38 (1.72, 11.2)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 27: Solus Scientific *Salmonella* ELISA, Presumptive vs. Traditional Confirmed – POD Results (1)

Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					X ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Typhimurium ATCC BAA-215 & <i>Citrobacter</i> <i>freundii</i> ATCC 8090	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 210	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			440 & 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
X ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI							
Stainless Steel	<i>Salmonella</i> Typhimurium ATCC BAA-215 & <i>Citrobacter</i> <i>freundii</i> ATCC 8090	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			35 & 210	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			440 & 1200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Matrix	Strain	ELISA Method	CFU ^a / Test Area	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
X ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI							
Polystyrene	<i>Salmonella</i> Montevideo ATCC 8387 & <i>E. coli</i> ATCC 8090	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			40 & 390	20	6	0.30	0.15, 0.52	6	0.30	0.15, 0.52	0.00	-0.27, 0.27
			320 & 2200	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aCFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

^bN = Number of test portions

^cX = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 28: Solus Scientific *Salmonella* ELISA Raw Chicken Breast Retest, Candidate* vs. Reference – POD Results (1)

Media Formulation	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS New Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Accumedia RVS	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
RV-R10	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*The alternative and traditional confirmation both produced identical results

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 29: Solus Scientific *Salmonella* ELISA Raw Chicken Breast Retest, Candidate* vs. Reference – POD Results (1)

Media Formulation	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
					x ^c	POD _c ^d	95% CI	X	POD _R ^e	95% CI		
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS New Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Accumedia RVS	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
RV-R10	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*The alternative and traditional confirmation both produced identical results

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials

^fdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 30: Solus Scientific *Salmonella* ELISA Raw Chicken Breast Retest, Presumptive vs. Alternative Confirmed – POD Results (1)

Media Formulation	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 31: Solus Scientific *Salmonella* ELISA Raw Chicken Breast Retest, Presumptive vs. Alternative Confirmed – POD Results (1)

Media Formulation	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 32: Solus Scientific *Salmonella* ELISA Raw Chicken Breast Retest, Presumptive vs. Traditional Confirmed – POD Results (1)

Media Formulation	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Manual	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 33: Solus Scientific *Salmonella* ELISA Raw Chicken Breast Retest, Presumptive vs. Traditional Confirmed – POD Results (1)

Media Formulation	Strain	ELISA Method	MPN ^a / Test Portion	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
					x ^c	POD _{CP} ^d	95% CI	X	POD _{CC} ^e	95% CI		
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Solus Scientific RVS Original Lot	<i>Salmonella</i> Enteritidis ATCC 13076	Automated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.45 (0.22, 0.78)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cx = Number of positive test portions

^dPOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^ePOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^fdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

Table 34: Inclusivity Results – First Testing done by Adria Développement Expert Laboratory – 55 strains (1)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Salmonella</i> Agona	A00 ¹ V38	Feedstuff	+	<i>Salmonella</i> Kedougou	Ad ¹ 929	Environmental sample	+
<i>Salmonella</i> Anatum	Ad ¹ 6140	Bœuf Bourguignon	+	<i>Salmonella</i> Kottbus	ADRIA ¹ 1	Environmental sample	+
<i>Salmonella arizonae</i> S IIIa 51:z4,z23:-	CIP ³ 5523	Turkey	-	<i>Salmonella</i> Livingstone	ADRIA ¹ E1	Egg white powder	+
<i>Salmonella arizonae</i> S IIIa 44:z4,z23:z32:-	CIP ³ 5522	Not available	-	<i>Salmonella</i> London	ADRIA ¹ 326	Ham	+
<i>Salmonella arizonae</i> S IIIa 50 ;z4 ;z23	CIP ³ 5526	Egg powder	+	<i>Salmonella</i> Manhattan	ADRIA ¹ 900	Dairy environmental sample	+
<i>Salmonella diarizonae</i> S IIIb 38:IV:z53	Ad ¹ 451	Raw milk cheese	+	<i>Salmonella</i> Mbandaka	Ad ¹ 914	Mayonnaise	+
<i>Salmonella diarizonae</i> S IIIb 61:-,1,5,7	Ad ¹ 1280	Raw milk cheese	+	<i>Salmonella</i> Montevideo	Ad ¹ 912	Raw milk	+
<i>Salmonella</i> Blockley	Ad ¹² 923	Chicken	+	<i>Salmonella</i> Napoli	Ad ¹ 928	Bovine	+
<i>Salmonella</i> Bovismorbificans	ADRIA ¹ 728	Agar	+	<i>Salmonella</i> Newport	ADRIA ¹ 540	Toulouse sausage	+
<i>Salmonella</i> Braenderup	ADRIA ¹ 178	Food product	+	<i>Salmonella</i> Panama	ADRIA ¹ 195	Ground beef	+
<i>Salmonella</i> Brandenburg	Ad ¹² 351	Seafood	+	<i>Salmonella</i> Paratyphi A	ATCC ² 9150	Not available	+
<i>Salmonella</i> Bredeney	ADRIA ¹ 396	Ground beef	+	<i>Salmonella</i> Paratyphi B	Ad ¹ 301	Clinical	+
<i>Salmonella</i> Cerro	Ad ¹² 689	Dehydrated proteins	+	<i>Salmonella</i> Paratyphi C	ATCC ² 13428	Not available	+
<i>Salmonella</i> Cremieu	ADRIA ¹ 230	Hare	+	<i>Salmonella</i> Regent	ADRIA ¹ 328	Duck	+
<i>Salmonella</i> Derby	Ad ¹ 1093	Frozen fish fillet	+	<i>Salmonella</i> Rissen	ADRIA ¹ 39	Poultry	+
<i>Salmonella</i> Dublin	Ad ¹ 528	Beef meat	+	<i>Salmonella</i> Saintpaul	ADRIA ¹ F31	Pilchard fillet	+
<i>Salmonella</i> Enteritidis	Ad ¹ 926	Raw veal meat	+	<i>Salmonella salamae</i> S II 42:b:enz	Ad ¹ 593	Cereals	+
<i>Salmonella</i> Gallinarum	Ad ¹ 300	Poultry slaughterhouse	-	<i>Salmonella</i> Senftenberg	Ad ¹ 355	Seafood	+
<i>Salmonella</i> GIVE	ADRIA ¹ 436	Ground beef	+	<i>Salmonella</i> Typhi	Ad ¹ 302	Clinical	+
<i>Salmonella</i> Hadar	ADRIA ¹ 35	Poultry	+	<i>Salmonella</i> Typhimurium	ADRIA ¹ 305	Paella	+
<i>Salmonella</i> Havana	Ad ¹ 930	Poultry	+	<i>Salmonella</i> Typhimurium SI 1,4 [5], 12 :- :-	Ad ¹ 1333	Tiramisu	+
<i>Salmonella</i> Heidelberg	A00 ¹ E005	Dairy industry environmental sample	+	<i>Salmonella</i> Typhimurium SI 1,4 [5], 12 : i :-	Ad ¹ 1334	Ready-to-eat meal	+
<i>Salmonella houtenae</i> S IV 43:z4z32	Ad ¹² 597	Fish	+	<i>Salmonella</i> Typhimurium	Ad ¹ 1335	Environmental sample	+
<i>Salmonella</i> Indiana	ADRIA ¹ 2	Fish flour	+	<i>Salmonella</i> Urbana	Ad ¹ 501	Food product	+
<i>Salmonella indica</i> S VI 1,26,14,25:a:enx	Ad ¹² 600	Environmental sample	+	<i>Salmonella</i> Virchow	ADRIA ¹ F276	Curry	+
<i>Salmonella</i> Infantis	ADRIA ¹ 12	Ready-to-eat	+	<i>Salmonella</i> Gallinarum	ADRIA ¹ 3778	Not available	+
<i>Salmonella</i> Gallinarum	ADRIA 11006	Not available	+	<i>Salmonella</i> Gallinarum	ADRIA ¹ 8202	Not available	+
<i>Salmonella</i> Gallinarum	ADRIA 154	Not available	+				

¹ADRIA culture collection - ²ATCC-American Type Culture Collection - ³CIP- Collection of Institut Pasteur

Table 35: Inclusivity Results – Second Testing done by Solus Scientific – 54 strains (1)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Salmonella</i> Abaetatuba	NCTC ¹ 8244	Not available	+	<i>Salmonella</i> Goldcoast	NCTC ¹ 13175	Not available	+
<i>Salmonella</i> Aberdeen	NCTC ¹ 5791	Not available	+	<i>Salmonella</i> Heves	NCTC ¹ 6755	Not available	+
<i>Salmonella</i> Abortusequi	NEX 1624	Food product	+	<i>Salmonella</i> Hvittingfoss	NEX ² 1467	Not available	+
<i>Salmonella</i> Adelaide	NCTC ¹ 6586	Not available	+	<i>Salmonella</i> Inverness	NCTC ¹ 6591	Not available	+
<i>Salmonella</i> Anatum	NEX ² 1724	Canola	+	<i>Salmonella</i> Kirkee	NCTC ¹ 5798	Not available	+
<i>Salmonella arizonae</i>	NCTC ¹ 7301	Not available	+	<i>Salmonella</i> Lagos	NEX ² 703	Beef meat	+
<i>Salmonella arizonae</i>	NCTC ¹ 7349	Not available	+	<i>Salmonella</i> Lille	NEX ² 296	Not available	+
<i>Salmonella arizonae</i>	SOL ² 0369	Food product	-	<i>Salmonella</i> Meleagridis	NEX ² 742	Not available	+
<i>Salmonella arizonae</i>	SOL ² 0370	Food product	+	<i>Salmonella</i> Minnesota	NCTC ¹ 5800	Not available	+
<i>Salmonella</i> Aschersleben	NEX ² 1906	Not available	+	<i>Salmonella</i> Mississippi	Solus ² WT	Not available	+
<i>Salmonella</i> Bergen	NEX ² 1644	Not available	+	<i>Salmonella</i> Muenchen	NEX ² 1326	Not available	+
<i>Salmonella</i> Bracknell	NCTC ¹ 9852	Not available	+	<i>Salmonella</i> Newport	SOL ² 0368	Beef meat	+
<i>Salmonella</i> Brazil	NCTC ¹ 18446	Not available	+	<i>Salmonella</i> Nima	NEX ² 1881	Not available	+
<i>Salmonella</i> Brookfield	NCTC ¹ 10946	Not available	+	<i>Salmonella</i> Nitra	NCTC ¹ 6297	Not available	+
<i>Salmonella</i> Champaign	NCTC ¹ 10433	Not available	+	<i>Salmonella</i> Nottingham	NCTC ¹ 7832	Not available	+
<i>Salmonella</i> Caracas	NEX ² 1785	Food product	+	<i>Salmonella</i> Oukam	NEX ² 837	Not available	+
<i>Salmonella</i> Chester	NEX ² 862	Duck liver	+	<i>Salmonella</i> Oranienburg	NCTC ¹ 5743	Not available	+
<i>Salmonella</i> Cubana	NEX ² 1829	Not available	+	<i>Salmonella</i> Poona	NCTC ¹ 5792	Not available	+
<i>Salmonella</i> Dahlem	NCTC ¹ 9949	Not available	+	<i>Salmonella</i> Ramatgan	NEX ² 311	Not available	+
<i>Salmonella diarizonae</i>	SOL ² 0371	Food product	+	<i>Salmonella</i> Reading	NEX ² 919	River water	+
<i>Salmonella diarizonae</i>	SOL ² 0372	Food product	+	<i>Salmonella</i> Salford	NEX ² 1698	Food product	+
<i>Salmonella</i> Dugbe	NCTC ¹ 10347	Not available	+	<i>Salmonella</i> Stuivenberg	NEX ² 702	Beef meat	+
<i>Salmonella</i> Duval	NCTC ¹ 9875	Not available	+	<i>Salmonella</i> Thompson	NEX ² 1569	Food product	+
<i>Salmonella</i> Eilbeck	NCTC ¹ 10381	Not available	+	<i>Salmonella</i> Tranoroa	NCTC ¹ 10252	Not available	+
<i>Salmonella</i> Essen	NCTC ¹ 3045	Not available	+	<i>Salmonella</i> Typhimurium non motile	Solus ² WT	Not available	+
<i>Salmonella</i> Ferlac	NCTC ¹ 10458	Not available	+	<i>Salmonella</i> Umbilo	NEX ² 1413	Not available	+
<i>Salmonella</i> Gallinarum	Solus ² EQA	Not available	+	<i>Salmonella</i> Worthington	NEX ² 1880	Not available	+

¹NCTC: National Collection of Type Cultures – ²NEX & SOL; SOLUS collections

Table 36: Exclusivity Results – First Testing done by Adria Développement Expert Laboratory – 30 strains (1)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Citrobacter braakii</i>	Ad ¹ 833	Raw beef	-	<i>Hafnia alvei</i>	Adria ¹ 167	Raw pork sausage	-
<i>Citrobacter diversus</i>	Adria ¹ 140	Raw milk	-	<i>Klebsiella oxytoca</i>	Adria ¹ 57	Food product	-
<i>Citrobacter freundii</i>	Adria ¹ 23	Raw pork sausage	-	<i>Klebsiella pneumoniae</i>	Adria ¹ 47	Raw turkey meat	-
<i>Citrobacter freundii</i>	Adria ¹ 175	Raw duck meat	-	<i>Kluyvera spp</i>	Adria ¹ 41	Raw milk	-
<i>Citrobacter koseri</i>	Adria ¹ 71	Frozen vegetables	-	<i>Morganella morganii</i>	CIP ² A236	Not available	-
<i>Enterobacter agglomerans</i>	Adria ¹ 11	Cheese	-	<i>Pantoea agglomerans</i>	Adria ¹ 86	Frozen vegetables	-
<i>Enterobacter amnigenus</i>	A00 ¹ C068	Raw poultry meat	-	<i>Proteus mirabilis</i>	Ad ¹ 639	Mayonnaise	-
<i>Enterobacter cloacae</i>	Adria ¹ 10	Raw milk	-	<i>Proteus vulgaris</i>	Adria ¹ 43	Sliced ham	-
<i>Enterobacter intermedius</i>	Adria ¹ 60	Green beans	-	<i>Providencia rettgeri</i>	Adria ¹ 112	White liquid egg	-
<i>Enterobacter kobei</i>	Ad ¹ 342	Ham	-	<i>Rhanelia aquatilis</i>	Adria ¹ 69	Molluscs	-
<i>Enterobacter sakazakii</i>	Adria ¹ 95	Fermented milk	-	<i>Serratia liquefaciens</i>	Adria ¹ 26	Egg product	-
<i>Erwinia carotovora</i>	CIP ² 8283	Potatoes	-	<i>Serratia proteomaculans</i>	A00 ¹ C056	Ham	-
<i>Escherichia coli</i>	Adria ¹ 19	Greated carrots	-	<i>Shigella flexneri</i>	CIP ² 8248	Not available	-
<i>Escherichia hermanii</i>	Ad ¹ 461	Dessert	-	<i>Shigella sonnei</i>	CIP ² 8249	Not available	-
<i>Escherichia vulneris</i>	Adria ¹ 127	Raw milk	-	<i>Yersinia enterocolitica</i>	Adria ¹ 32	Bacon	-

¹Adria Culture Collection – ²CIP- Collection of Institut Pasteur

Table 37: Exclusivity Results – Second Testing done by Solus Scientific – 30 strains

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Bacillus cereus</i>	ATCC ³ 14579	Not available	-	<i>Kluyvera ascorbata</i>	NCTC ¹ 9737	Not available	-
<i>Bacillus coagulans</i>	NCTC ¹ 10334	Not available	-	<i>Kurthia zopfii</i>	NCTC ¹ 405	Not available	-
<i>Bacillus licheniformis</i>	NCTC ¹ 10341	Not available	-	<i>Listeria ivanovii</i>	ATCC ³ 19119	Not available	-
<i>Brochothrix thermosphacta</i>	NCTC ¹ 10822	Not available	-	<i>Listeria monocytogenes</i>	ATCC ³ 13932	Not available	-
<i>Citrobacter farmer</i>	SOLUS ² WT	Not available	-	<i>Micrococcus luteus</i>	ATCC ³ 4698	Not available	-
<i>Citrobacter freundii</i>	NCTC ¹ 6272	Not available	-	<i>Proteus vulgaris</i>	NCTC ¹ 4175	Not available	-
<i>Citrobacter freundii</i>	NCTC ¹ 9737	Not available	-	<i>Pseudomonas aeruginosa</i>	NCTC ¹ 10662	Not available	+/- [†]
<i>Corynebacterium parvum</i>	NCTC ¹ 10387	Not available	-	<i>Proteus mirabilis</i>	NCTC ¹ 11938	Not available	-
<i>Enterobacter aerogenes</i>	NCTC ¹ 55	Not available	-	<i>Serratia liquifaciens</i>	Solus ¹ WT	Not available	-
<i>Enterobacter cloacae</i>	NCTC ¹ 10005	Not available	-	<i>Serratia marcescens</i>	SOLUS ² WT	Not available	-
<i>Enterococcus faecalis</i>	NCTC ¹ 12697	Not available	+/- [†]	<i>Shigella sonnei</i>	NCTC ¹ 9774	Not available	-
<i>Escherichia coli</i>	NCTC ¹ 11560	Not available	-	<i>Shigella sonnei</i>	NCTC ¹ 9779	Not available	-
<i>Escherichia coli</i>	SOLUS ² WT	Not available	-	<i>Staphylococcus aureus</i>	NCTC ¹ 4135	Not available	+/- [†]
<i>E. coli</i> O157	NCTC ¹ 12900	Not available	-	<i>Staphylococcus aureus</i>	NCTC ¹ 8530	Not available	+/- [†]
<i>Klebsiella pneumoniae</i>	SOLUS ² WT	Not available	-	<i>Streptococcus bovis</i>	ATCC ³ 33317	Not available	-

¹NCTC: National Collection of Type Cultures – ²SOLUS collections – ³ATCC-American Type Culture Collection

[†]: positive ELISA result after BPW/negative ELISA result after subculture in RVS.

REFERENCES CITED

1. Bastin, B., DeSheilds, A., Bird, P., Benzinger, Jr., M.J., Crowley, E., Agin, J., Goins, D., Wakefield, R., Illingworth, S., and Wells, P., Independent Evaluation of the Solus Scientific *Salmonella* ELISA Method for the Detection of *Salmonella* Species in Selected Foods and Environmental Surfaces, AOAC® *Performance Tested*SM certification number 051601
2. United States Department of Agriculture Microbiological Laboratory Guidelines 4.08: *Isolation and Identification of Salmonella from Red Meat, Poultry, Pasteurized Egg, Catfish Products, and Environmental Sponges*. June 29th, 2014. (Accessed October 2015)
<http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES>
3. Food and Drug Administration Bacteriological Analytical Manual Chapter 5: *Salmonella*. February, 2014. (Accessed October 2015)
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm>
4. Solus *Salmonella* ELISA Package Insert: ELISA-BASED TEST SYSTEM FOR THE DETCTION OF *SALMONELLA IN FOODS AND FEEDSTUFFS* – QCF 16 – AOAC Draft – 08/15 (Accessed October 2015)