



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

101801

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

Solus One *Salmonella*

manufactured by

Solus Scientific Ltd.

Unit 9 Mansfield Network Centre

Millennium Business Park

Concorde Way, Mansfield

Nottinghamshire, NG9 7JZ

This method has been evaluated in the AOAC[®] *Performance Tested MethodsSM* Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance TestedSM* certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (January 17, 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 17, 2020

Date

<p>METHOD AUTHORS ORIGINAL VALIDATION: David Higgins, Holly Urquhart, Siobhan Kelly, Simon Illingworth, Nevin Perera, Benjamin Bastin, Patrick Bird, M. James Agin, David Goins MODIFICATION AUGUST 2019: David Higgins, Siobhan Kelly, Simon Illingworth, Nevin Perera, Kateland Koch, Benjamin Bastin, M. Joseph Benzinger, Jr., James Agin MODIFICATION JANUARY 2020: Koch, K., Bastin, B., Benzinger, Jr., M.J., Agin, J.</p>	<p>SUBMITTING COMPANY Solus Scientific Ltd. Unit 9 Mansfield Network Centre Millennium Business Park Concorde Way, Mansfield Nottinghamshire, NG9 7JZ</p>	<p>CERTIFICATION MARK LICENESEE Solus Scientific Ltd. Unit 9 Mansfield Network Centre Millennium Business Park Concorde Way, Mansfield Nottinghamshire, NG9 7JZ</p>
<p>KIT NAME(S) Solus One <i>Salmonella</i></p>	<p>CATALOG NUMBERS SAL1-0480 (5 x 96 well), SAL1-0096 (1 x 96 well)</p>	
<p>INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Avenue Cincinnati, OH 45214 USA</p>	<p>AOAC EXPERTS AND PEER REVIEWERS Yi Chen^{1,4}, Michael Brodsky^{2,4}, Yvonne Salfinger^{3,4} ¹FDA CFSAN, College Park, MD, USA ² Brodsky Consultants, Thornhill, Ontario, Canada ³ Consultant, Denver, CO, USA ⁴ Modification August 2019 Level 3, January 2020 Level 2</p>	
<p>APPLICABILITY OF METHOD Target organisms – <i>Salmonella</i> species (<i>Salmonella enterica</i> and <i>Salmonella bongori</i>, and <i>Salmonella</i> subspecies)</p> <p>Matrices – FDA BAM Ch. 5 - raw salmon (fillet, 25 g), cheddar cheese (shredded, 25 g), Romaine lettuce (bagged, 25 g), non-fat dry milk powder (NFDM, 375 g), stainless steel (18 gauge: 304 food grade with a brushed finish) and plastic (polystyrene) environmental surface USDA MLG 4.09 - raw beef trim (>30% fat content, 375 g), pasteurized liquid egg (100 g) Modification August 2019 USDA BAM Ch 5: (375 g) honey mustard seasoning and flavored ranch seasoning (25 g) cinnamon powder, paprika powder, whole black peppercorns Modification January 2020: FDA Chapter 5 <i>Salmonella</i> and ISO 6579-1:2017: 375 g samples of cocoa powder, cocoa liquor, milk chocolate bar</p> <p>Performance claims - Performance equivalent to that of the reference methods.</p>	<p>REFERENCE METHOD U. S. Food and Drug Administration <i>Bacteriological Analytical Manual</i> Chapter 5: <i>Salmonella</i>. Revised: July 2018 (Accessed August 2018) https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm070149.htm (2) United States Department of Agriculture Food Safety and Inspection Service <i>Microbiology Laboratory Manual</i>, 4.09: <i>Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges</i>. Updated: January 2017 (Accessed August 2018) https://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES (3) ISO 6579-1:2017, <i>Microbiology of Food and Animal Feeding Stuffs- Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella – Part 1: Detection of Salmonella spp.</i> (12)</p>	
<p>ORIGINAL CERTIFICATION DATE October 01, 2018</p>	<p>CERTIFICATION RENEWAL RECORD Renewed annually through December 2020</p>	
<p>METHOD MODIFICATION RECORD</p> <ol style="list-style-type: none"> August 2019 Level 3 January 2020 Level 2 	<p>SUMMARY OF MODIFICATION</p> <ol style="list-style-type: none"> Matrix extension to include (375 g) honey mustard seasoning and flavored ranch seasoning, (25 g) cinnamon powder, paprika powder, whole black peppercorns Matrix extension to include 375 g samples of cocoa powder, cocoa liquor, milk chocolate bar 	
<p>Under this AOAC® <i>Performance Tested</i>SM License Number, 101801 this method is distributed by: NONE</p>	<p>Under this AOAC® <i>Performance Tested</i>SM License Number, 101801 this method is distributed as: NONE</p>	

PRINCIPLE OF THE METHOD (1)

Solus One *Salmonella* is an antibody-based high sensitivity ELISA method paired with media and our proprietary media supplement – Solus One *Salmonella* supplement; for the rapid and specific detection of *Salmonella* species in select foods and environmental samples. Solus One *Salmonella* relies on antibodies attached to the wells of microplate strips by non-covalent biological interactions that are highly 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-labelled antibody will bind to the captured 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 sulfuric acid changing any blue color present in the wells to yellow (4). Optical densities resulting from this color change are read within 10 minutes in a generic plate reader using a 450 nm filter (e.g. a microplate reader or a Dynex DS2 instrument plate reader), where a result of an OD₄₅₀ < 0.200 is considered to be negative for the target pathogen and OD₄₅₀ ≥ 0.200 is considered to be positive for the target pathogen.

DISCUSSION OF THE VALIDATION STUDY (1)

Solus One *Salmonella* methods successfully recovered *Salmonella* species from select food matrices and environmental surface analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate methods (both manual and automated) and the respective reference methods, BAM Ch. 5 and MLG 4.09, for food matrices and environmental surfaces tested.

POD analysis of Solus One *Salmonella* method Robustness indicated no statistically significant differences were observed between nominal and experimental combinations using the automated method.

POD analysis of Solus One *Salmonella* method Product consistency (lot-to-lot) and Stability using the automated method, indicated no statistically significant differences were observed between the lots of Solus One *Salmonella* test kits at neat and diluted *Salmonella* cultures at the LOD₅₀ of the method.

The results of the inclusivity and exclusivity evaluation demonstrated 100% agreement with expected results for the test panels and confirmed the high specificity and selectivity of the method to *Salmonella* species.

The method offers the benefit of the use of either a manual sample preparation or automated sample preparation to obtain results. Each method was quick and simple to perform, providing results in 2 h post incubation of the selective enrichment. 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. Additionally, the Dynex DS2 software and instrument also offer the ability to run multiple assays at one time and has an open platform. With the ability to conduct a direct streak from the primary enrichment, a secondary enrichment is eliminated thereby eliminating a day until a confirmed result can be obtained.

Table 1: Solus One *Salmonella* Inclusivity Results (1)

Organism species + subspecies	Serovar	Serogroup	Source	Origin	Result
<i>Salmonella bongori</i>	Not applicable	Group V 66:Z ₄₁ -	NCTC ^a 12419	Not Available	+ ^b
<i>Salmonella bongori</i>	Brookfield	Group V 66:Z ₄₁ -	NCTC 10946	Not Available	+
<i>Salmonella bongori</i> CIP 82.33	Not applicable	Group V 66:Z ₄₁ -	ATCC ^c 43975	Not Available	+
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa 51:Z ₄ ,Z ₂₃ -	ATCC 13314	Not Available	+
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa	ATCC BAA-1577	Not Available	+
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa	QL ^d 11007-4	Veterinary Isolate	+
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa	QL 011414.2	Environmental Isolate	+
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa	QL 024.114	Pet Food	+
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb	ATCC BAA-1579	Not Available	+
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb 35:i:z	ATCC BAA-216	Human Blood	+
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb	ATCC BAA-639	Human Feces	+
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb	QL 024.516	Pet Food	+
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb	QL 011414.1	Environmental Isolate	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Abaetetuba	Group I 11:k:1,5	ATCC 35640	Creek Water	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Abortusequi	Group I 4,12:-:e,n,x	FDA ^e 9842	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Abortusovis	Group I 4,12:c:1,6	NCTC 10241	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Abony	Group I 1,4,[5],12,[27]:b:e,n,x	NCTC 6017	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Adelaide	Group I 35:f,g:-:[z27]	STs ^f 2	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Agona	Group I 1,4,[5],12:f,g,s:[1,2]:[z27],[z45]	ATCC 51957	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Agama	Group I 4,12:i:1,6	STs 3	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Agoueve	Group I 13,22:z29:-	STs 5	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Alachua	Group I 35:z ₄ ,z ₂₃ -:[z37],[z45]	STs 6	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Albany	Group I 8,20:z ₄ ,z ₂₄ -:[z45]	STs 7	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Anatum	Group I 3,{10}{15}{15,34}:e,h:1,6:[z64]	ATCC 9270	Pork Liver	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Arkansas	Group I 3,{10}{15}{15,34}:e,h:1,5	STs 11	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Bareilly	Group I 6,7,14:y:1,5	FDA 1206H	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Berta	Group I 1,9,12:[f],g,[t]-	STs 13	Not Available	+

Organism species + subspecies	Serovar	Serogroup	Source	Origin	Result
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Binza	Group I 3,{10}{15}{15,34}:y:1,5	STs 14	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Bovismorbificans	Group I 6,8,20:r,[i]:1,5:[R1...]	STs 16	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Brandenburg	Group I 4,[5],12:l,v:e,n,z15	STs 18	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Bredeney	Group I 1,4,12,27:l,v:1,7:[Z40]	NCTC 5731	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	California	Group I 4,12:g,m,t:[z67]	NCTC 6018	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Cerro	Group I 6,14,18:z4,z23:[1,5]:[Z45],[Z82]	STs 22	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Choleraesuis	Group I 6,7:c:1,5	ATCC 10708	Equine Isolate	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Choleraesuis var Kunzendorf	Group I 6,7:[c]:1,5	ATCC 12011	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Cubana	Group I 1,13,23:z29:-:[Z37],[Z43]	STs 24	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Derby	Group I 1,4,[5],12:f,g:[1,2]	NCTC 5721	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Drypool	Group I 3,{10}{15}{15,34}:g,m,s:-	STs 26	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Dublin	Group I 1,9,12,[Vi]:g,p:-	STs 27	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Eastbourne	Group I 1,9,12:e,h:1,5	FDA 4017H	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Enteritidis	Group I 1,9,12:g,m:-	ATCC 13076	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Galiema	Group I 6,7,14:k:1,2	QL 024.2	Environmental Isolate	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Give	Group I 3,{10}{15}{15,34}:l,v:1,7:[d]	STs 42	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Haardt	Group I 8:k:1,5	STs 44	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Hadar	Group I 6,8:z10:e,n,x	ATCC 51956	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Havana	Group I 1,13,23:f,g,[s]:-[Z79]	STs 47	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Heidelberg	Group I 1,4,[5],12:r:1,2	ATCC 8326	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Indiana	Group I 1,4,12:z:1,7	NCTC 11304	Turkey	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Infantis	Group I 6,7,14:r:1,5:[R1...],[Z37],[Z45],[Z49]	ATCC 51741	Pasta	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Javiana	Group I 1,9,12,l,z28:1,5:[R1...]	ATCC 10721	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Jerusalem	Group I 6,7,14:z10:l,w	QL 024.12	Pet Food	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Johannesburg	Group I 1,40:b:e,n,x	STs 56	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Kahla	Group I 1,42:z35:1,6	ATCC 17980	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Kaitaan	Group I 1,6,14,25:m,t:-	QL 024.7	Pet Food	+
Organism species + subspecies	Serovar	Serogroup	Source	Origin	Result
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Kentucky	Group I 8,20:i:z6	ATCC 9263	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Krefeld	Group I 1,3,19:y:l,w	STs 58	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Lille	Group I 6,7,14:z38:-:[Z82]	STs 59	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Livingstone	Group I 6,7,14:d:l,w	STs 63	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	London	Group I 3,{10}{15}:l,v:1,6	STs 64	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Manhattan	Group I 6,8:d:1,5:[Z58]	STs 65	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Mbankaka	Group I 6,7,14:z10:e,n,z15:[Z37],[Z45]	FDA 37N	Low Moisture Ingredient	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Meleagridis	Group I 3,{10}{15}{15,34}:e,h:l,w	QL 12074-1	Environmental Isolate	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Menden	Group I 6,7:z10:1,2	ATCC 15992	Feces	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Menhaden	Group I	QL 024.20	Pet Food	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Montevideo	Group I 6,7,14,[54]:g,m,[p],s:[1,2,7]	ATCC 8387	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Muenchen	Group I 6,8:d:1,2:[Z67]	ATCC BAA- 1594	Human Stool	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Neasden	Group I 9,12:g,s,t:e,n,x	QL 024.4	Raw Material	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Newington	Group I 3,{10}{15}:e,h:1,6	QL 024.8	Fish Oil	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Newport	Group I 6,8,20:e,h:1,2:[Z67],[Z78]	ATCC 6962	Food Poisoning	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Ohio	Group I 6,7,14:b:l,w:[Z59]	STs 81	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Oranienburg	Group I 6,7,14:m,t:[Z57]	ATCC 9239	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Orthmarshen	Group I 6,7,14:g,m,[t]:-	QL 024.13	Pet Kibble	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Paratyphi A	Group I 1,2,12:a:[1,5]	ATCC 9150	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Paratyphi B	Group I 1,4,[5],12:b:1,2:[Z51],[Z33]	ATCC 10719	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Poona	Group I 1,13,22:z:1,6:[Z44],[Z59]	NCTC 4840	Infant Enteritis	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Preston	Group I 1,4,12:z:l,w	QL 024.16	Low Moisture Product	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Pullorum	Group I 9,12:-:-	ATCC 13036	Egg	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Rubislaw	Group I 11:r:e,n,x	STs 92	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Saintpaul	Group I 1,4,[5],12:e,h:1,2	ATCC 9712	Cystitis	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	San-Diego	Group I 1,4,[5],12:e,h,e,n,z15	STs 94	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Schalkwijk	Group I 6,14,[24]:i:e,n,z15	QL 024.10	Cat Food	+

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Organism species + subspecies	Serovar	Serogroup	Source	Origin	Result
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Schwarzengrund	Group I 1,4,12,27:d:1,7	STs 95	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Senftenberg	Group I 1,3,19:g,[s],t:- :[z ₂₇],[z ₃₄],[z ₃₇],[z ₄₃],[z ₄₅],[z ₄₆],[z ₈₂]	ATCC 43845	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Tallahassee	Group I 6,8:z ₄ ,z ₃₂ :-	ATCC 12002	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Tennessee	Group I 6,7,14:z ₂₉ :[1,2,7]	QL 024.6	Clinical Isolate	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Thompson	Group I 6,7,14:k:1,5:[R1...]	FDA 2051H	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Typhi	Group I 9,12[V]:d:-:[j],[z ₆₆]	ATCC 6539	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Typhimurium	Group I 1,4,[5],12:i:1,2	ATCC 14028	Animal Tissue	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Utrecht	Group I 52:d:1,5	NCTC 10077	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Vellore	Group I 1,4,12,27:z ₁₀ :z ₃₅	ATCC 15611	Rectal Swab	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Virchow	Group I 6,7,14:r:1,2	ATCC 51955	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Volta	Group I 11:r:[z ₁₃ ,z ₂₈	QL 024.9	Raw Material	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Westhampton	Group I 3,{10}{15}{15,34}:g,s,t:-:[z ₃₇]	QL 024.14	Dog Kibble	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Worthington	Group I 1,13,23:z:l,w:[z ₄₃]	STs 114	Not Available	+
<i>Salmonella enterica</i> subsp. <i>houtenae</i>	Halmstad	Group IV 3,15:g,m,s:-	QL 024.1	Clinical Isolate	+
<i>Salmonella enterica</i> subsp. <i>houtenae</i>	Harmelen	Group IV 51:z ₄ ,z ₂₃ :-	ATCC 15783	Boa Constrictor	+
<i>Salmonella enterica</i> subsp. <i>houtenae</i>	Ochsenzoll	Group IV 16:z ₄ ,z ₂₃ :-	ATCC 29932	Not Available	+
<i>Salmonella enterica</i> subsp. <i>houtenae</i>	Not applicable	Group IV	QL 18022.18	Beef	+
<i>Salmonella enterica</i> subsp. <i>indica</i>	Not applicable	Group VI	QL 024.62	Pet Food	+
<i>Salmonella enterica</i> subsp. <i>indica</i>	Not applicable	Group VI	QL 18022.6	Beef	+
<i>Salmonella enterica</i> subsp. <i>indica</i>	Ferlac	Group VI 1,6,14,25:a:e,n,x	ATCC 43976	Not Available	+
<i>Salmonella enterica</i> subsp. <i>indica</i>	Ferlac	Group VI 1,6,14,25:a:e,n,x	NCTC 10458	Ceylonese Dessicated Coconut	+
<i>Salmonella enterica</i> subsp. <i>indica</i>	Not applicable	Group VI 45:a:e,n,x	ATCC BAA- 1578	Not Available	+
<i>Salmonella enterica</i> subsp. <i>salamae</i>	Artis	Group II 56:b:-	ATCC 700149	Not Available	+
<i>Salmonella enterica</i> subsp. <i>salamae</i>	Basel	Group II 58:z ₁₃ ,z ₂₈ :1,5	ATCC 700151	Not Available	+
<i>Salmonella enterica</i> subsp. <i>salamae</i>	Not applicable	Group II	QL 024.15	Pet Food	+
<i>Salmonella enterica</i> subsp. <i>salamae</i>	Not applicable	Group II	QL 024.86	Pet Food	+
<i>Salmonella enterica</i> subsp. <i>salamae</i>	Not applicable	Group II	QL 011414.16	Pet Food	+

^aNCTC-National Collection of Type Cultures, Salisbury, U.K.

^b+ = The target analyte was detected by Solus One *Salmonella*.

^cATCC-American Type Culture Collection, Manassas, VA.

^dQL-Q Laboratories Inc. Culture Collection, Cincinnati, OH.

^eFDA-U.S. Food and Drug Administration Culture Collection, Silver Spring, MD.

^fSTs-University of Pennsylvania Culture Collection, Philadelphia, PA

Table 2: Solus One *Salmonella* Exclusivity Results (1)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Alcaligenes faecalis</i>	ATCC ^a 8750	Not Available	- ^b	<i>Lactobacillus acidophilus</i>	ATCC 314	Not Available	-
<i>Bacillus cereus</i>	ATCC 14579	Not Available	-	<i>Lactobacillus kefir</i>	ATCC 35411	Kefir	-
<i>Bacillus subtilis</i>	ATCC 6051	Not Available	-	<i>Lactobacillus lactis</i>	ATCC 4794	Not Available	-
<i>Campylobacter lari</i>	ATCC BAA-1060	Human Feces	-	<i>Listeria monocytogenes</i>	ATCC 7644	Human Isolate	-
<i>Campylobacter jejuni</i>	ATCC 33560	Bovine Feces	-	<i>Listeria innocua</i>	ATCC 33090	Cow Brain	-
<i>Candida albicans</i>	ATCC 24433	Nail Infection	-	<i>Klebsiella pneumoniae</i>	ATCC 13883	Not Available	-
<i>Carnobacterium maltaromaticum</i>	ATCC 43224	Vacuum-packed Beef	-	<i>Kocuria rhizophila</i>	ATCC 9341	Soil	-
<i>Citrobacter farmeri</i>	ATCC 51112	Human Feces	-	<i>Morganella morganii</i>	ATCC 25829	Human	-
<i>Citrobacter freundii</i>	ATCC 8090	Not Available	-	<i>Proteus mirabilis</i>	ATCC 7002	Urine	-
<i>Edwardsiella tarda</i>	ATCC 15947	Human Feces	-	<i>Proteus vulgaris</i>	ATCC 6380	Clinical Isolate	-
<i>Enterobacter aerogenes</i>	ATCC 13048	Sputum	-	<i>Pseudomonas aeruginosa</i>	ATCC 27853	Clinical Isolate	-
<i>Enterobacter cloacea</i>	ATCC 23355	Not Available	-	<i>Shigella sonnei</i>	ATCC 29930	Not Available	-
<i>Enterococcus faecium</i>	ATCC 19434	Not Available	-	<i>Staphylococcus aureus</i>	ATCC 10832	Not Available	-
<i>Enterococcus faecalis</i>	ATCC 29212	Urine	-	<i>Staphylococcus epidermidis</i>	ATCC 12228	Not Available	-
<i>Escherichia coli</i>	ATCC 25922	Clinical Isolate	-	<i>Staphylococcus haemolyticus</i>	ATCC 29970	Human Skin	-
<i>Escherichia coli</i>	QL ^c 4356.9	Not Available	-	<i>Staphylococcus hominis</i>	ATCC 27844	Human Skin	-
<i>Escherichia coli</i>	QL 1211.1	Not Available	-	<i>Streptococcus pneumoniae</i>	ATCC 6302	Not Available	-
<i>Hafnia alvei</i>	ATCC 51813	Milk	-				

^aATCC-American Type Culture Collection, Manassas, VA.

^b- = The target analyte was not detected by Solus One *Salmonella*.

^cQL-Q Laboratories Inc. Culture Collection, Cincinnati, OH

Table 3. Solus One *Salmonella* Results: Presumptive vs. Confirmed (1)

Matrix	Strain	ELISA method ^a	MPN ^b /test portion	Solus One <i>Salmonella</i> presumptive				Solus One <i>Salmonella</i> confirmed				
				N ^c	X ^d	POD _{CP} ^e	95% CI	x	POD _{CC} ^f	95% CI	dPOD _{CP} ^g	95% CI ^h
Raw Beef Trim	S. Agona, ATCC 51957	Automated	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.69 (0.40, 1.14)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
Liquid Pasteurized Egg	S. Enteritidis, ATCC 13076	Automated	2.57 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
			N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
Raw Salmon	S. Virchow, ATCC 51955	Automated	0.43 (0.21, 0.75)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			2.62 (1.20, 5.74)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cheddar Cheese	S. Heidelberg, ATCC 8326	Automated & Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.82 (0.49, 1.36)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
Bagged Romaine Lettuce	S. Muenchen, ATCC BAA-1594	Automated	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
			N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
Non-Fat Dry Milk	S. Senftenberg, ATCC 43845	Automated	0.35 (0.17, 0.63)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Stainless steel (4"x 4", sponge)	S. Typhimurium ^k , ATCC 14028/10X <i>C. freundii</i> ^l , ATCC 8090	Automated & Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
Plastic (1"x 1", swab)	S. Montevideo ^m , ATCC 8387	Automated	N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
			N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			N/A	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
			N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aThe Solus One *Salmonella* ELISA sample preparation method was manual and/or automated using the Dynex DS2. All were using the DS2, with the manual read using the instrument set on "Plate Read Only".

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

^cN = Number of test portions.

^dx = Number of positive test portions.

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

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

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

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱATCC = American Type Culture Collection, Manassas, VA.

^jN/A = Not applicable.

^kS. Typhimurium low inoculation level was 68 CFU/test area as determined by plate count prior to inoculation. The high inoculation level was 220 CFU/test area.

^lC. *freundii* low inoculation level was 890 CFU/test area as determined by plate count prior to inoculation. The high inoculation level was 3,700 CFU/test area.

^mS. Montevideo low inoculation level was 57 CFU/test area as determined by plate count prior to inoculation. The high inoculation level was 390 CFU/test area.

Table 4. Method Comparison Results: Solus One *Salmonella* vs. Reference (BAM Ch. 5 or MLG 4.09) (1)

Matrix	Strain	ELISA method ^a	Solus One <i>Salmonella</i> results					BAM Ch. 5 or MLG 4.09 results				
			MPN ^b /test portion	N ^c	X ^d	POD _c ^e	95% CI	x	POD _R ^f	95% CI	dPOD _c ^g	95% CI ^h
Raw Beef Trim	<i>S. Agona</i> , ATCC ⁱ 51957	Automated	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 1.14)	20	7	0.35	0.18, 0.57	9	0.45	0.26, 0.66	-0.10	-0.37, 0.19
			2.57 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Liquid Pasteurized Egg	<i>S. Enteritidis</i> , ATCC 13076	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.43 (0.21, 0.75)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			2.62 (1.20, 5.74)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Raw Salmon	<i>S. Virchow</i> , ATCC 51955	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.50 (0.25, 0.86)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
			3.70(1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cheddar Cheese	<i>S. Heidelberg</i> , ATCC 8326	Automated & Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.82 (0.49, 1.36)	20	11	0.55	0.34, 0.74	10	0.50	0.30, 0.70	0.05	-0.24, 0.33
		2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	
Bagged Romaine Lettuce	<i>S. Muenchen</i> , ATCC BAA-1594	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.35 (0.17, 0.63)	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.10	-0.18, 0.36
			1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Non-Fat Dry Milk	<i>S. Senftenberg</i> , ATCC 43845	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.44 (0.21, 0.76)	20	9	0.45	0.26, 0.66	6	0.30	0.15, 0.52	0.15	-0.14, 0.41
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Stainless steel (4" x 4" sponge)	<i>S. Typhimurium</i> ^k , ATCC 14028/10X <i>C. freundii</i> ^l , ATCC 8090	Automated & Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			N/A	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43	
Plastic (1" x 1" swab)	<i>S. Montevideo</i> ^m , ATCC 8387	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			N/A	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	-0.15, 0.41
			N/A	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aThe Solus One *Salmonella* ELISA sample preparation method was manual and/or automated using the Dynex DS2. All were using the Dynex DS2. All were using the DS2, with the manual read using the instrument set on "Plate Read Only".

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

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_c = Candidate method presumptive positive outcomes confirmed positive.

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

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

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱATCC = American Type Culture Collection, Manassas, VA.

^jN/A = Not applicable.

^k*S. Typhimurium* low inoculation level was 68 CFU/test area as determined by plate count prior to inoculation. The high inoculation level was 220 CFU/test area.

^l*C. freundii* low inoculation level was 890 CFU/test area as determined by plate count prior to inoculation. The high inoculation level was 3,700 CFU/test area.

^m*S. Montevideo* low inoculation level was 57 CFU/test area as determined by plate count prior to inoculation. The high inoculation level was 390 CFU/test area.

DISCUSSION MODIFICATION AUGUST 2019 (10)

Solus One *Salmonella* methods successfully recovered *Salmonella* species from spice and flavor blend matrices analyzed. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the candidate method and the FDA/BAM Ch. 5 reference method, for food matrices tested.

The results of the inclusivity and exclusivity evaluation with Solus mBPW supplemented with 2.22 mL Solus One *Salmonella* supplement per 1.0 L medium, demonstrated 100% agreement with expected results for the test panels and confirmed the high specificity and selectivity of the method to *Salmonella* species. The method offers the benefit of the use of either a manual sample preparation or automated sample preparation to obtain results. Each method was quick and simple to perform, providing results in 2 h post incubation of the selective enrichment. 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. Additionally, the Dynex DS2 software and instrument also offer the ability to run multiple assays at one time and has an open platform. With the ability to conduct a direct streak from the primary enrichment, a secondary enrichment is eliminated thereby eliminating a day until a confirmed result can be obtained.

This method modification recovered and detected *Salmonella* species from difficult spice and flavor blend matrices within a 24 h primary enrichment through an enhanced system comprising of additional neutralization properties of the Solus mBPW medium, enhanced selective properties of the Solus One *Salmonella* supplement and a sensitive Solus One *Salmonella* ELISA immunoassay method.

MODIFICATION DATA AUGUST 2019 (10)

Table 1: Solus One *Salmonella* Inclusivity Results

Organism species + subspecies	Serovar	Serogroup	Source	Origin	Result
<i>Salmonella bongori</i>	Brookfield	Group V 66:z ₄₁ :-	NCTC ^a 12419	Not Available	+ ^b
<i>Salmonella bongori</i>	Brookfield	Group V 66:z ₄₁ :-	NCTC 10946	Not Available	+
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa 16:22:21	NCTC 7349	Spray dried egg powder	+
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	Not applicable	Group IIIa 1,4:13,14	NCTC 7301	Spray dried egg powder	+
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb 61:i:z ₅₃	Nex ^c -1273	Not Available	+
<i>Salmonella enterica</i> subsp. <i>diarizonae</i>	Not applicable	Group IIIb 61:k:1,5,7	Nex-788	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Aberdeen	Group I 11:i:1,2	NCTC 5791	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Adelaide	Group I 35:f:g:-:[z ₂₇]	NCTC 6586	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Agona	Group I <u>1</u> ,4,[5],12:f,g,s:[1,2]:[z ₂₇],[z ₄₅]	NCTC 11377	Cattle, Ghana	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Aschersleben	Group I 30:b:1,5	NCTC 5779	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Bracknell	Group I 13,23:b:1,6	NCTC 9852	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Braenderup	Group I 6,7, <u>14</u> :e,h:e,n,z ₁₅	NCTC 5750	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Brandenburg	Group I 4,[5],12:l,v:e,n,z ₁₅	NCTC 5725	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Brazil	Group I 16:a:1,5	NCTC 18446	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Bredeney	Group I <u>1</u> ,4,12,27:l,v:1,7:[z ₄₀]	NCTC 5731	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Cambridge	Group I 3,15:e,h:l,w	NCTC 8256	Gastroenteritis	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Cerro	Group I <u>6</u> , <u>14</u> ,18:z ₄ ,z ₂₃ :[1,5]:[z ₄₅],[z ₈₂]	NCTC 5801	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Champaign	Group I 39:k:1,5:[z ₄₈]	ATCC ^d 10433	Equine Isolate	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Dahlem	Group I 48:k:e,n,z ₁₅	NCTC 9949	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Dublin	Group I <u>1</u> ,9,12,[Vi]:g,p:-	NCTC 12710	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Dugbe	Group I 45:d:1,6	NCTC 10347	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Duval	Group I <u>1</u> ,40:b:e,n,z ₁₅	NCTC 9875	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Ealing	Group 35:g,m,s:-	NCTC 11949	Dried baby milk outbreak strain UK 85/86	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Enteritidis	Group I <u>1</u> ,9,12:g,m:-	NCTC 4444	Human gastroenteritis	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Essen	Group I 4,12:g,m:-	NCTC 3045	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Florida	Group I [1],6,14,[25]:d:1,7	NCTC 6480	Human enteritis	+

Organism species + subspecies	Serovar	Serogroup	Source	Origin	Result
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Hadar	Group I 6,8:z ₁₀ :e,n,x	NCTC 9877	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Heves	Group I 6,14,24:d:1,5	NCTC 6755	Human	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Hvittingfoss	Group I 16:b:e,n,x	Nex-1467	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Infantis	Group I 6,7,14:r:1,5:[R1...],[z ₃₇],[z ₄₅],[z ₄₉]	NCTC 6703	Human, blood from baby with pyrexia bronchitis	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Inverness	Group I 38:k:1,6	NCTC 6591	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Kedougou	Group I 1,13,23:i:l,w	NCTC 12173	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Kentucky	Group I 8,20:i:z ₆	NCTC 5799	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Kirkee	Group I 17:b:1,2	NCTC 5798	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Livingstone	Group I 6,7,14:d:l,w	NCTC 9125	Faeces	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	London	Group I 3,{10}{15}:l,v:1,6	NCTC 5777	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Mbankaka	Group I 6,7,14:z ₁₀ :e,n,z ₁₅ :[z ₃₇],[z ₄₅]	FDA ^e 37N	Low Moisture Ingredient	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Minnesota	Group I 21:b:e,n,x:[z ₃₃],[z ₄₉]	NCTC 5800	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Montevideo	Group I 6,7,14,[54]:g,m,[p],s:[1,2,7]	ATCC 8387	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Newport	Group I 6,8,20:e,h:1,2:[z ₆₇],[z ₇₈]	Nex-816	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Nima	Group I 28:y:1,5	Nex-1881	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Nitra	Group I 2,12:g,m:-	NCTC 6297	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Nottingham	Group I 16:d:e,n,z ₁₅	NCTC 7832	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Ouakam	Group I 9,46:z ₂₉ :-:[z ₄₅]	Nex-837	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Paratyphi A	Group I 1,2,12:a:[1,5]	ATCC 9150	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Poona	Group I 1,13,22:z:1,6:[z ₄₄],[z ₅₉]	NCTC 5992	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Pullorum	Group I 9,12:-:-	ATCC 13036	Egg	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Senftenberg	Group I 1,3,19:g,[s],t:- :[z ₂₇],[z ₃₄],[z ₃₇],[z ₄₃],[z ₄₅],[z ₄₆],[z ₈₂]	NCTC 10081	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Tennessee	Group I 6,7,14:z ₂₉ :[1,2,7]	QL ^f 024.6	Clinical Isolate	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Typhimurium	Group I 1,4,[5],12:i:1,2	NCTC 5711	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Virchow	Group I 6,7,14:r:1,2	NCTC 5742	Not Available	+
<i>Salmonella enterica</i> subsp. <i>enterica</i>	Waycross	Group I 41:z ₄ ,z ₂₃ :[e,n,z ₁₅]	NCTC 7401	Not Available	+
<i>Salmonella enterica</i> subsp. <i>houtenae</i>	Halmstad	Group IV 3,15:g,m,s:-	QL 024.1	Clinical Isolate	+

Organism species + subspecies	Serovar	Serogroup	Source	Origin	Result
<i>Salmonella enterica</i> subsp. <i>houtenae</i>	Harmelen	Group IV 51:z ₄ ,z ₂₃ :-	ATCC 15783	Boa Constrictor	+
<i>Salmonella enterica</i> subsp. <i>indica</i>	Not applicable	Group VI	QL 18022.6	Beef	+
<i>Salmonella enterica</i> subsp. <i>indica</i>	Ferlac	Group VI 1,6,14,25:a:e,n,x	NCTC 10458	Ceylonese Desiccated Coconut	+
<i>Salmonella enterica</i> subsp. <i>salamae</i>	Basel	Group II 58:z ₁₃ ,z ₂₈ :1,5	ATCC 700151	Not Available	+
<i>Salmonella enterica</i> subsp. <i>salamae</i>	Tranaroa	Group II 55:k:z ₃₉	NCTC 10252	Not Available	+

^aNCTC-National Collection of Type Cultures, Salisbury, U.K.^b+ = The target analyte was detected by Solus One *Salmonella*.^cNex-Nexidia SAS, 15 Rue de Mayence, 21000 Dijon, France.^dATCC-American Type Culture Collection, Manassas, VA.^eFDA-U.S. Food and Drug Administration Culture Collection, Silver Spring, MD.^fQL-Q Laboratories Inc. Culture Collection, Cincinnati, OH

Table 2: Solus One *Salmonella* Exclusivity Results (10)

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Bacillus cereus</i>	NCTC ^a 10320	Not Available	- ^b	<i>Klebsiella pneumonia</i>	ATCC 13883	Not Available	-
<i>Citrobacter freundii</i>	NCTC 6272	Not Available	-	<i>Proteus mirabilis</i>	NCTC 11938	Not Available	-
<i>Enterobacter cloacae</i>	NCTC 10005	Human, cerebrospinal fluid	-	<i>Pseudomonas aeruginosa</i>	NCTC 10662	Human	-
<i>Escherichia coli</i>	NCTC 11560	Not Available	-	<i>Shigella sonnei</i>	NCTC 9774	Not Available	-
<i>Listeria monocytogenes</i> 4b	ATCC ^c 19115	Human Isolate	-	<i>Staphylococcus aureus</i>	NCTC 8530	Human fluid from septic arthritis	-

^aNCTC-National Collection of Type Cultures, Salisbury, U.K.

^b- = The target analyte was not detected by Solus One *Salmonella*.

^cATCC-American Type Culture Collection, Manassas, VA

Table 3. Solus One *Salmonella* Results: Presumptive vs. Confirmed (10)

Matrix	Strain	ELISA method ^a	MPN ^b /test portion	Solus One <i>Salmonella</i> presumptive				Solus One <i>Salmonella</i> confirmed				
				N ^c	X ^d	POD _{cp} ^e	95% CI	x	POD _{cc} ^f	95% CI	dPOD _{cp} ^g	95% CI ^h
Flavored Ranch Seasoning	S. Senftenberg, QL ⁱ 11031.2	Automated	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.75 (0.44, 1.23)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Honey Onion Mustard Seasoning	S. Typhimurium, ATCC ^k 14028	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.69 (0.40, 1.14)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cinnamon Powder	S. Mbandaka, QL 11031.1	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.61 (0.33, 1.01)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			3.70(1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Paprika Powder	S. Tennessee, QL 11031.3	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.56 (0.30, 0.95)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Black Peppercorn	S. Senftenberg, ATCC 43845	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.63 (0.35, 1.04)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aThe Solus One *Salmonella* ELISA sample preparation method was automated using the Dynex DS2. All were read using the DS2 plate reader, with the manual read using the instrument set on "Plate Read Only".

^bMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI (8), with 95% confidence interval.

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_{cp} = Candidate method presumptive positive outcomes divided by the total number of trials.

^fPOD_{cc} = Candidate method confirmed positive outcomes divided by the total number of trials.

^gdPOD_{cp} = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

ⁱQL-Q Laboratories Inc. Culture Collection, Cincinnati, OH.

^jN/A = Not applicable.

^kATCC = American Type Culture Collection, Manassas, VA

Table 4. Method Comparison Results: Solus One *Salmonella* vs. Reference (FDA/BAM Ch. 5) (10)

Matrix	Strain	ELISA method ^a	MPN ^b /test portion	N ^c	Solus One <i>Salmonella</i> results			FDA/BAM Ch. 5 results				
					X ^d	POD _C ^e	95% CI	x	POD _R ^f	95% CI	dPOD _C ^g	95% CI ^h
Flavored Ranch Seasoning	S. Senftenberg, QL ⁱ 11031.2	Automated	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.75 (0.44, 1.23)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Honey Onion Mustard Seasoning	S. Typhimurium, ATCC ^k 14028	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.69 (0.40, 1.14)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cinnamon Powder	S. Mbandaka, QL 11031.1	Automated	N/A	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, 1.01)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
			3.70(1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Paprika Powder	S. Tennessee, QL 11031.3	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.56 (0.30, 0.95)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	-0.18, 0.36
			2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Black Peppercorn	S. Senftenberg, ATCC 43845	Automated	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			0.63 (0.35, 1.04)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
			3.70 (1.52, 9.02)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aThe Solus One *Salmonella* ELISA sample preparation method was automated using the Dynex DS2. All were read using the DS2 plate reader, with the manual read using the instrument set on "Plate Read Only".

^bMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI (8), with 95% confidence interval.

^cN = Number of test portions.

^dx = Number of positive test portions.

^ePOD_C = Candidate method presumptive positive outcomes confirmed positive.

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

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

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

ⁱQL-Q Laboratories Inc. Culture Collection, Cincinnati, OH.

^jN/A = Not applicable.

^kATCC = American Type Culture Collection, Manassas, VA.

DISCUSSION MODIFICATION JANUARY 2020 (11)

The Solus One *Salmonella* ELISA assay successfully detected *Salmonella* species from cocoa powder, cocoa liquor, and milk chocolate bar. Using POD analysis, no statistically significant differences were observed between the number of positive samples detected by the reference methods and the Solus One *Salmonella* ELISA assay.

The method offers the benefit to the use of either a manual or automated preparation to obtain results. Each method is quick and simple to perform, providing results in almost 2 hours post incubation of the enrichment. The small footprint of both methods offers the ability to test in various laboratories. The Solus One *Salmonella* ELISA assay detected *Salmonella* spp. within a 20 hour primary enrichment.

MODIFICATION DATA JANUARY 2020 (11)

Table 2. Candidate vs. FDA/BAM Chapter 5 (Revised, July 2018) Reference Method – POD Results

Matrix	Strain	Inoculation Level	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		
Cocoa Powder (375 g)	<i>Salmonella</i> Montevideo ATCC 8387	Uninoculated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	0.49 (0.25, 0.85)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		High	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cocoa Liquor (375 g)	<i>Salmonella</i> Typhimurium ATCC 14028	Uninoculated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	0.40 (0.18, 0.69)	20	7	0.35	0.18, 0.57	6	0.30	0.15, 0.52	0.05	-0.23, 0.32
		High	1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Milk Chocolate Bar (375 g)	<i>Salmonella</i> Senftenberg ATCC 43845	Uninoculated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	0.69 (0.39, 1.14)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.28, 0.28
		High	1.97 (0.91, 4.27)	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 version 1.6 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 3. Candidate vs. ISO 6579-1:2017 Reference Method – POD Results (11)

Matrix	Strain	Inoculation Level	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		
Cocoa Powder (375 g)	<i>Salmonella</i> Montevideo ATCC 8387	Uninoculated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	0.57 (0.31, 0.96)	20	7	0.35	0.18, 0.57	8	0.40	0.22, 0.61	-0.05	-0.32, 0.23
		High	1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Cocoa Liquor (375 g)	<i>Salmonella</i> Typhimurium ATCC 14028	Uninoculated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.47
		Low	0.49 (0.25, 0.85)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.28, 0.28
		High	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
Milk Chocolate Bar (375 g)	<i>Salmonella</i> Senftenberg ATCC 43845	Uninoculated	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	0.55 (0.29, 0.92)	20	9	0.45	0.26, 0.66	8	0.40	0.22, 0.61	0.05	-0.24, 0.33
		High	1.97 (0.91, 4.27)	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 version 1.6 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 4: Presumptive vs. Confirmed – POD Results (11)

Matrix	Strain	Inoculation Level	FDA/BAM Chapter 5 MPN ^b / Test Portion	ISO 6579-1:2017 MPN ^b / Test Portion	N ^c	Presumptive ^a			Confirmed ^a		
						X ^d	POD _{CP} ^e	95% CI	X	POD _{CC} ^f	95% CI
Cocoa Powder (375 g)	<i>Salmonella</i> Montevideo ATCC 8387	Uninoculated	-	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		Low	0.49 (0.25, 0.85)	0.57 (0.31, 0.96)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57
		High	2.58 (1.15, 5.78)	1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00
Cocoa Liquor (375 g)	<i>Salmonella</i> Typhimurium ATCC 14028	Uninoculated	-	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		Low	0.40 (0.18, 0.69)	0.49 (0.25, 0.85)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57
		High	1.97 (0.91, 4.27)	2.58 (1.15, 5.78)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00
Milk Chocolate Bar (375 g)	<i>Salmonella</i> Senftenberg ATCC 43845	Uninoculated	-	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43
		Low	0.69 (0.39, 1.14)	0.55 (0.29, 0.92)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66
		High	1.97 (0.91, 4.27)	1.97 (0.91, 4.27)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00

^a Identical results obtained for confirmation with FDA/BAM Chapter 5 (Revised, July 2018) and ISO 6579-1:2017 reference methods

^b MPN = Most Probable Number is calculated using the LCF MPN calculator version 1.6 provided by AOAC RI, with 95% confidence interval

^c N = Number of test portions

^d x = Number of positive test portions

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

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

^g dPOD_C = Difference between the confirmed candidate method result and reference method confirmed result 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

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