



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

051802

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

Solus One *Listeria*

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 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 (November 30, 2020 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads 'Scott Coates'.

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 01, 2020

Date

METHOD AUTHORS ORIGINAL VALIDATION: Elizabeth Tonner, Siobhan Kelly, Simon Illingworth, Nevin Perera, Benjamin Bastin, Patrick Bird, M. Joseph Benzinger, Jr., James Agin, David Goins MODIFICATION NOVEMBER 2020: Kirsty Roberts, Paul Wells, and Simon Illingworth	SUBMITTING COMPANY Solus Scientific Ltd. Millennium Business Park Concorde Way, Mansfield Nottinghamshire, NG9 7JZ United Kingdom
---	---

KIT NAME(S) Solus One <i>Listeria</i>	CATALOG NUMBERS LIS1-0480 (5x96 well microplate kit), LIS1-0096 (1x96 well microplate kit)
---	--

INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Avenue Cincinnati, OH 45214 USA	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Michael Brodsky ² , Yvonne Salfinger ³ ¹ US FDA CFSAN, College Park, MD, USA ² Brodsky Consultants, Thornhill, Ontario, Canada ³ Consultant, Denver, CO, USA
--	---

APPLICABILITY OF METHOD Target organism – <i>Listeria</i> species (<i>L. monocytogenes</i> , <i>L. innocua</i> , <i>L. ivanovii</i> , <i>L. seeligeri</i> , <i>L. welshimeri</i> , <i>L. grayi</i> , <i>L. marthii</i>)	REFERENCE METHOD Food and Drug Administration Bacteriological Analytical Manual Chapter 10: <i>Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods</i> . March 2017 (2)
---	--

Matrixes –stainless steel (18 gauge: 304 food grade with a brushed finish; 4x4 in sponge), plastic (polystyrene; 1x1 in swab)

Performance claims - The results obtained by the POD analysis of the method study demonstrated that there were no statistically significant differences between the number of positive samples detected by the candidate and the reference methods for both environmental surfaces analyzed.

ORIGINAL CERTIFICATION DATE May 23, 2018	CERTIFICATION RENEWAL RECORD Renewed annually through December 2021
--	---

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. December 2018 Level 1	1. Editorial/clerical changes to text to bring the language and style in line with more recently approved products.
2. February 2020 Level 1	2. Rebranding from Solus Scientific to PerkinElmer. Combined 1 and 5 plate kits into single IFU.
3. November 2020 Level 2	3. Shelf life extension from 12 to 18 months.

Under this AOAC® <i>Performance Tested</i> SM License Number, 051802 this method is distributed by: NONE	Under this AOAC® <i>Performance Tested</i> SM License Number, 051802 this method is distributed as: NONE
--	--

PRINCIPLE OF THE METHOD (1)

Solus One *Listeria* is a proprietary SOLO+ enrichment media coupled with an ELISA method for the rapid and specific detection of *Listeria* species in environmental samples. Solus One *Listeria* relies on antibodies attached to the wells of microplate strips by non-covalent biological interactions that are highly specific to *Listeria* antigens. Samples are heat treated and an aliquot is added to the antibody coated wells.

Listeria 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 450nm filter, 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 *Listeria* methods successfully recovered *Listeria* species from both environmental surfaces 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 reference method for both environmental surfaces tested.

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 *Listeria* species.

The method offers the benefit of the use of either a manual preparation or automated preparation to obtain results. Each method was quick and simple to perform, providing results in 2 h and 45 min 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.

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

Organism + serotype	Source	Origin	Result	Organism + serotype	Source	Origin	Result
<i>Listeria grayi</i>	NCTC ^a 19120	Animal Feces	+ ^b	<i>Listeria monocytogenes</i> 1/2c	CWD ^f 1552	Not Available	+
<i>Listeria grayi</i>	ATCC ^c 25401	Corn Stalks	+	<i>Listeria monocytogenes</i> 1/2c	CWD 1553	Not Available	+
<i>Listeria grayi</i>	ATCC 700545	Not Available	+	<i>Listeria monocytogenes</i> 1/2a	CWD 1554	Not Available	+
<i>Listeria innocua</i>	QL ^d 030911-12	Environmental	+	<i>Listeria monocytogenes</i> 1/2a	CWD 1555	Not Available	+
<i>Listeria innocua</i>	QL 051111-1	Environmental	+	<i>Listeria monocytogenes</i> 4b	CWD1561	Human Placenta	+
<i>Listeria innocua</i>	QL 32811.2	Seasoning Powder	+	<i>Listeria monocytogenes</i> 4b	CWD 1563	Not Available	+
<i>Listeria innocua</i>	ATCC 33091	Human Feces	+	<i>Listeria monocytogenes</i> 4b	CWD 1590	Not Available	+
<i>Listeria innocua</i>	QL 32911.1	Environmental	+	<i>Listeria monocytogenes</i> 1/2a	CWD 1611	Turkey	+
<i>Listeria innocua</i>	CSU ^e W1-301	Not Available	+	<i>Listeria monocytogenes</i> 1/2a	CWD 1613	Turkey	+
<i>Listeria innocua</i>	CSU W1-305	Not Available	+	<i>Listeria monocytogenes</i> 1/2a	CWD 1614	Not Available	+
<i>Listeria ivanovii</i>	ATCC 49954	Food, France	+	<i>Listeria monocytogenes</i> 1/2b	CWD 1626	Not Available	+
<i>Listeria ivanovii</i>	ATCC BAA-678	Sheep Fetus	+	<i>Listeria monocytogenes</i> 1/2b	CWD 1627	Mother/Baby	+
<i>Listeria ivanovii</i>	ATCC Liv004	Not Available	+	<i>Listeria monocytogenes</i> 1/2a	CWD 1629	Not Available	+
<i>Listeria ivanovii</i>	ATCC Liv005	Not Available	+	<i>Listeria monocytogenes</i> 1/2a	CWD 1630	Turkey	+
<i>Listeria ivanovii</i>	QL 030911-9	Clinical Isolate	+	<i>Listeria monocytogenes</i>	QL 030911-10	Shellfish	+
<i>Listeria monocytogenes</i> 1/2c	ATCC 7644	Human Isolate	+	<i>Listeria seeligeri</i> 6b	ATCC 11289	Human Feces	+
<i>Listeria monocytogenes</i> 4b	ATCC 13932	Spinal Fluid	+	<i>Listeria seeligeri</i>	ATCC 11856	Not Available	+
<i>Listeria monocytogenes</i> 1/2a	ATCC 15313	Rabbit	+	<i>Listeria seeligeri</i> 1/2b	ATCC 35967	Soil	+
<i>Listeria monocytogenes</i> 4a	ATCC 19114	Animal Tissue	+	<i>Listeria seeligeri</i>	FSL ^g -S4-035	Not Available	+
<i>Listeria monocytogenes</i> 4b	ATCC 19115	Human Isolate	+	<i>Listeria seeligeri</i>	QL 030911-2	Creamer	+
<i>Listeria monocytogenes</i> 4d	ATCC 19117	Sheep	+	<i>Listeria welshimeri</i>	ATCC 35897	Not Available	+
<i>Listeria monocytogenes</i> 1/2a	ATCC 49594	Not Available	+	<i>Listeria welshimeri</i> 6a	ATCC 43548	Not Available	+
<i>Listeria monocytogenes</i> 4b	ATCC 51778	Dairy Products	+	<i>Listeria welshimeri</i> 6b	ATCC 43549	Soil	+
<i>Listeria monocytogenes</i> 1/2b	ATCC 51780	Dairy Products	+	<i>Listeria welshimeri</i> 1/2b	ATCC 43550	Human Feces	+
<i>Listeria monocytogenes</i> 4b	ATCC Li2	Human Isolate	+	<i>Listeria welshimeri</i>	LW ^h 003	Not Available	+

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

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

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

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

^eCSU-Colorado State University Culture Collection, Fort Collins, CO.

^fCWD-University of Vermont Culture Collection, Burlington, VT.

^gFSL-Cornell University Culture Collection, Ithaca, NY.

^hLW-University of Vermont Culture Collection, Burlington, VT

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

Organism	Source	Origin	Result	Organism	Source	Origin	Result
<i>Bacillus mycoides</i>	ATCC ^a 6462	Soil	- ^b	<i>Lactobacillus fermentum</i>	ATCC 9338	Not Available	-
<i>Brochothrix thermosphacta</i>	ATCC 11509	Pork Sausage	-	<i>Lactobacillus lactis</i>	ATCC 4797	Not Available	-
<i>Bacillus cereus</i>	ATCC 14579	Not Available	-	<i>Lactobacillus plantarum</i>	ATCC 8014	Not Available	-
<i>Geobacillus stearothermophilus</i>	ATCC 12980	Not Available	-	<i>Micrococcus luteus</i>	ATCC 7468	Not Available	-
<i>Rhodococcus fascians</i>	ATCC 12974	Not Available	-	<i>Proteus mirabilis</i>	ATCC 7002	Urine	-
<i>Enterococcus hirae</i>	ATCC 8043	Not Available	-	<i>Streptococcus mutans</i>	ATCC 25715	Not Available	-
<i>Enterococcus faecium</i>	ATCC 19434	Not Available	-	<i>Rhodococcus equi</i>	ATCC 6939	Lung Abscess	-
<i>Enterococcus durans</i>	ATCC 19432	Not Available	-	<i>Salmonella</i> Typhimurium	ATCC 14028	Chicken Hearts and Livers	-
<i>Enterococcus faecalis</i>	ATCC 29212	Urine	-	<i>Bacillus subtilis</i>	ATCC 6051	Not Available	-
<i>Kurthia gibsonii</i>	ATCC 43195	Not Available	-	<i>Staphylococcus aureus</i>	ATCC 29247	Not Available	-
<i>Escherichia coli</i>	ATCC 8739	Feces	-	<i>Staphylococcus epidermidis</i>	ATCC 12228	Not Available	-
<i>Klebsiella oxytoca</i>	ATCC 43165	Clinical Isolate	-	<i>Staphylococcus haemolyticus</i>	ATCC 29970	Human Skin	-
<i>Klebsiella pneumoniae</i>	ATCC 13883	Not Available	-	<i>Staphylococcus warneri</i>	ATCC 29885	Not Available	-
<i>Kurthia zopfii</i>	ATCC 10538	Not Available	-	<i>Streptococcus pneumoniae</i>	ATCC 6302	Not Available	-
<i>Lactobacillus casei</i>	ATCC 11578	Oral Cavity	-	<i>Streptococcus pyogenes</i>	ATCC 19615	Pharynx of Child	-

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

^b- = The target analyte was not detected.

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

Matrix	Strain	ELISA method ^a	CFU/test area	N ^b	Solus One <i>Listeria</i> presumptive			Solus One <i>Listeria</i> confirmed				
					x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Stainless steel (4"x 4", sponge)	<i>L. monocytogenes</i> 4b, ATCC ^h 19115/10X <i>E. faecalis</i> , ATCC 29212	Automatic	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			53 Lm ^j & 860 Ef ^k	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
			270 Lm & 4700 Ef	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)	<i>L. monocytogenes</i> 4b, ATCC 19115/10X <i>E. faecalis</i> , ATCC 29212	Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			53 Lm & 860 Ef	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
			270 Lm & 4700 Ef	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Plastic (1"x 1", swab)	<i>L. innocua</i> ATCC BAA-680	Automatic	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			43	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
			480	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Plastic (1"x 1", swab)	<i>L. innocua</i> ATCC BAA-680	Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
			43	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.13, 0.13
			480	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

^aThe Solus One *Listeria* ELISA method was evaluated automatically on the Dynex DS2 and manually.

^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.

^hAmerican Type Culture Collection, Manassas, VA.

ⁱNot applicable.

^j*Listeria monocytogenes*.

^k*Enterococcus faecalis*

Table 4. Method Comparison Results: Solus One *Listeria* vs. BAM Ch. 10 (1)

Matrix	Strain	ELISA method ^a	CFU/test area	N ^b	Solus One <i>Listeria</i> results				BAM Ch. 10 results			
					x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI	dPOD _{CP} ^f	95% CI ^g
Stainless steel (4"x 4", sponge)	<i>L. monocytogenes</i> 4b, ATCC ^h 19115/10X <i>E. faecalis</i> , ATCC 29212	Automatic	N/A ⁱ	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			53 Lm ^j & 860 Ef ^k	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
			270 Lm & 4700 Ef	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>L. monocytogenes</i> 4b, ATCC 19115/10X <i>E. faecalis</i> , ATCC 29212	Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			53 Lm & 860 Ef	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	-0.24, 0.33
			270 Lm & 4700 Ef	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>L. innocua</i> ATCC BAA-680	Automatic	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			43	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
			480	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>L. innocua</i> ATCC BAA-680	Manual	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			43	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	-0.15, 0.41
			480	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aThe Solus One *Listeria* ELISA method was evaluated automatically on the Dynex DS2 and manually.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_C = Candidate method presumptive positive outcomes confirmed positive.

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

^fdPOD_C = Difference between the candidate method and reference method 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.

^hAmerican Type Culture Collection, Manassas, VA.

ⁱNot applicable.

^j*Listeria monocytogenes*.

^k*Enterococcus faecalis*

REFERENCES CITED

1. Tonner, E., Kelly, S., Illingworth, S., Perera, N., Bastin, B., Bird, P., Benzinger, Jr., M.J., Agin, J., Goins, D., Evaluation of the Solus One *Listeria* Method for the Detection of *Listeria* Species on Environmental Surfaces, AOAC® *Performance Tested*SM certification number 051802.
2. Food and Drug Administration Bacteriological Analytical Manual Chapter 10: *Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods*. March 2017 (Accessed March 2018) <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm>
3. *Official Methods of Analysis* (2016), 20th Edition, Appendix J, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j (Accessed March 2018)
4. Solus *Listeria* ELISA Package Insert: Immunoassay Based Test System for the Detection of *Listeria* in Environmental Samples (Version 1.2, December 2017)
5. Botsaris, G., Nikolaou, K., Liapi, M., Pipis, C. *Prevalence of Listeria Spp. and Listeria Monocytogenes in Cattle Farms in Cyprus using Bulk Tank Milk Samples*. *Journal of Food Safety*, Vol. 36, Issue 4, Pages: 482 – 488, November 2016. (Accessed April 2018). <https://doi.org/10.1111/jfs.12265>
6. Chen, Yi Ph.D. *Listeria monocytogenes*. *Bad Bug Book – Foodborne Pathogenic Microorganisms and Natural Toxins*, 2nd Ed. 2012. <https://www.fda.gov/downloads/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/UCM297627.pdf> (Accessed March 2018)
7. Roberts, K., Wells, P., and Illingworth, S., Real-time Stability evaluation to Extend the shelf-life of Solus *Listeria* method from 12 to 18 months for the Detection of *Listeria* species on environmental surfaces, AOAC® *Performance Tested*SM certification number 051802. Approved November 2020.