CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

041404

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

DNAble® Molecular Detection Kit for *Salmonella*

manufactured by

EnviroLogix Inc.
500 Riverside Industrial Parkway
Portland, ME 04103
USA

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 1, 2016 – December 31, 2016). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Deborah McKenzie

Deborah McKenzie, Senior Director
Signature for AOAC Research Institute

December 15, 2015
Date

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SUBMITTING COMPANY
EnviroLogix Inc.
500 Riverside Industrial Parkway
Portland, ME 04103
USA

KIT NAME(S)
DNAble® Molecular Detection Kit for *Salmonella*

CATALOG NUMBERS
DF-026, ACC-085, 1622 (XSALMD550-300)

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Ave.
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AOAC EXPERTS AND PEER REVIEWERS
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APPLICABILITY OF METHOD
Target analyte – Salmonella
Matrices – Dry pet food [25 g (9:1, v/w), 375g (9:1 and 3:1, v/w), stainless steel (swab or sponge enriched in 225 mL), and poultry environmental drag swabs (swab enriched in 100 mL)
Performance claims – Qualitative detection of Salmonella in above matrixes with sensitivity comparable to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) Chapter 5, Salmonella (3), and to the FDA Environmental Sampling and Detection of Salmonella in Poultry Houses (4)

REFERENCE METHODS
U. S. Food and Drug Administration *Bacteriological Analytical Manual*, Chapter 5, *Salmonella* (2007)
http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM309839.pdf (3)
U. S. Food and Drug Administration Environmental Sampling and Detection of *Salmonella* in Poultry Houses (2013)
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114716.htm (4)

PRINCIPLE OF THE METHOD
Three products are used to enable qualitative *Salmonella* DNA detection. DNAble *Salmonella* Media Supplement provides a selective agent and components that facilitate growth of stressed *Salmonella* organisms. DNAble Sample Extraction Set 2 includes an Extraction Buffer. The DNAble *Salmonella* Detection Kit contains pre-dispensed Reaction Buffer that promotes isothermal DNA amplification. This kit also includes lyophilized isothermal amplification and detection master mix reagents arrayed in amplification tubes.
Supplement is added to Buffered Peptone Water (BPW) to construct modified BPW (mBPW) a medium that selects for and promotes *Salmonella* growth. A sample is cultured in mBPW for 20-22 h at 37±1°C. Cultured organisms are concentrated by centrifugation of a sample of the culture media. The pellet is suspended in the Extraction Buffer and heated at 95±1.5°C to enable access to *Salmonella* DNA. Particulates are removed by a second centrifugation and a portion of the supernatant is diluted in Extraction Buffer. An aliquot of this dilution is added to Reaction Buffer; Reaction Buffer-sample dilution is then transferred to lyophilized master mix. The master mix tube is placed at 56° ±1°C in the DNAble Reader for isothermal DNA amplification and fluorescent detection. Algorithms in the DNAble Reader software inform the user of the presence or absence of *Salmonella* DNA.

DISCUSSION OF THE VALIDATION STUDY
Studies performed at the sponsor's laboratory included inclusivity, exclusivity, robustness, instrument comparison, and product consistency. The inclusivity study showed successful detection of 119 of 120 various *Salmonella* isolates representing 100 serovars including all six subspecies of S*. enterica* and four strains of serogroup V (now classified as *S. bongori*). Although one *S. bongori* strain, Malawi, produced negative results, overall these data indicate broad detection of *Salmonella* serovars and strains. The exclusivity challenge examined 35 strains of Gram positive and negative organisms representing 32 species. The assay did not cross react with any challenge strains.

Many of the DNAble *Salmonella* Detection assay steps are not likely to be subject to inadvertent modification by the user since once properly set up the associated equipment and instrumentation control the respective procedure. Several user interface parameters, including buffer volumes used for sample preparation and dilution and lysis timing, were examined in the Robustness Study. The assay tolerated variation in these parameters and false positives were not observed with the non-*Salmonella* challenge organism (*P. vulgaris*). The POD comparisons showed no statistical difference between the combinations of varied parameters and the nominal assay conditions. Overall, no weakness in the user interface was identified. The independent laboratory mentioned difficulty sampling dry pet food cultures when the smaller culture volume (3:1, v/w) was used, which likely reflects the sample absorbency. The laboratory suggested squeezing the culture bag contents before sampling to facilitate this step. This suggestion has been incorporated in the Sample Extraction Set 2 instructions.

DISCUSSION OF THE VALIDATION STUDY Cont.
The Instrument Variation and Product Consistency studies demonstrate reliability for the equipment and assay components. False positive results were not observed with the non-*Salmonella* challenge strain and the PODs for *Salmonella* detection were not statistically different among instruments or kit lots of varied ages. These results indicate consistent manufacture of the assay and instruments.
Multiple matrixes were evaluated at the independent laboratory (Q Laboratories) in the Matrix Studies. Two sample sizes of dry pet food (25 g, 375 g) and 2 mBPW culture volume matrix ratios, 3:1 and 9:1 (v/w) were tested. Two types of environmental samples were included; drag swabs taken from poultry production facilities and swabs taken from stainless steel surfaces. The matrix studies evaluated assay performance against accepted reference culture methods. The studies were designed to challenge the assay under stringent conditions including use of stressed organisms spiked at the limit of detection. Moreover, the
environmental samples added the complexity of potential bacterial growth competitors. *P. vulgaris* was included in the stainless steel surface study. Drag swabs were pretreated by exposure to a poultry production facility to sample endogenous flora. The partial positive results obtained for all matrixes closely paralleled the results obtained with the respective reference methods. Moreover, the dPOD analyses indicated there were no statistically significant differences between the DNAble and reference methods results.

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Table 1. Inclusivity

ELIX _ID[a]	Genus	Species	Information provided by source	Sero-group[b]	Alternate ID from source	Source	Origin if Available	DNAble
9V1	*Salmonella*	*enterica*	(Salamae) II 30:I, z28:z6	II	NA [c]	NVSL[d]	Unknown	Positive
9Y1	*Salmonella*	*enterica*	(Salamae) II 48:d:z6	II	NA	NVSL	Unknown	Positive
9S1	*Salmonella*	*enterica*	(Salamae) II 55:k:z39	II	NA	NVSL	Unknown	Positive
9W1	*Salmonella*	*enterica*	(Salamae) II 58:c:z6	II	NA	NVSL	Unknown	Positive
9X1	*Salmonella*	*enterica*	(Salamae) II 6,7:m,t:-	II	NA	NVSL	Unknown	Positive
10C1	*Salmonella*	*enterica*	II 47:d:z39	II	Safe 11 (00-0324)	CFSAN[e]	Unknown	Positive
10D1	*Salmonella*	*enterica*	II 48:d:z6	II	Safe 12 (01-0227)	CFSAN	Unknown	Positive
10 E 1	*Salmonella*	*enterica*	II 50:b:z6	II	Safe 13 (01-0249)	CFSAN	Unknown	Positive
10F1	*Salmonella*	*enterica*	II 53:1z28:z39	II	Safe 14 (CNM-169)	CFSAN	Unknown	Positive
6F1	*Salmonella*	*enterica*	Phoenix	II 47:b:1,5	SGSC 2565	SGSC[f]	Unknown	Positive
10B1	*Salmonella*	*enterica*	II 58:1, z13, z28:z6	II	Safe 10 (00-0163)	CFSAN	Unknown	Positive
4B-1	*Salmonella*	*enterica*	Arizonae	IIIa	SGSC4693	SGSC	Unknown	Positive
1G-1	*Salmonella*	*enterica*	Arizonae	IIIa	SGSC-3061	SGSC	Cornsnake	Positive
10V1	*Salmonella*	*enterica*	51:gz51:- IIIa	IIIa	Safe 23 (CNM-247)	CFSAN	Unknown	Positive
10W1	*Salmonella*	*enterica*	62:g,z51: IIIa	IIIa	Safe 24 (CNM-259)	CFSAN	Unknown	Positive
10T1	*Salmonella*	*enterica*	IIIa 40:z4, z23	IIIa	Safe20 (01-0204)	CFSAN	Unknown	Positive
10S1	*Salmonella*	*enterica*	IIIa 41:z4, z23	IIIa	Safe19 (01-0089)	CFSAN	Unknown	Positive
10U1	*Salmonella*	*enterica*	IIIa 48:g,z51	IIIa	Safe21 (01-0324)	CFSAN	Unknown	Positive
9Q1	*Salmonella*	*enterica*	(Diarizonae) IIIb 16:z10:e, n, x, z15	IIIb	Safe21 (01-0324)	CFSAN	Unknown	Positive
9Z1	*Salmonella*	*enterica*	(Diarizonae) IIIb 38:k:z35	IIIb	N/A	NVSL	Unknown	Positive
9P1	*Salmonella*	*enterica*	(Diarizonae) IIIb 42:1, v:1, 5, 7	IIIb	N/A	NVSL	Unknown	Positive
10G1	*Salmonella*	*enterica*	IIIb 60:r:e,n,x,z15	IIIb	Safe 27 (01-0170)	CFSAN	Unknown	Positive
10H1	*Salmonella*	*enterica*	IIIb 48:i:z	IIIb	Safe 28 (01-0221)	CFSAN	Unknown	Positive
10 I1	*Salmonella*	*enterica*	IIIb 61:k:1,5,(7)	IIIb	Safe 29 (01-0248)	CFSAN	Unknown	Positive
9M1	*Salmonella*	*enterica*	(Houtenae) IV 40:z4, z24:-	IV	N/A	NVSL	Unknown	Positive
9R1	*Salmonella*	*enterica*	(Houtenae) IV 50:g,z51:-	IV	N/A	NVSL	Unknown	Positive
9N1	*Salmonella*	*enterica*	(Houtenae) IV: 44:z4, z24:-	IV	N/A	NVSL	Unknown	Positive
9O1	*Salmonella*	*enterica*	(Houtenae) IV: 50:z4, z23:-	IV	N/A	NVSL	Unknown	Positive
10 L1	*Salmonella*	*enterica*	IV 44:z4,z23:-	IV	Safe 37 (01-0149)	CFSAN	Unknown	Positive
10 M1	*Salmonella*	*enterica*	IV 45:g,z51:-	IV	Safe 38 (01-0276)	CFSAN	Unknown	Positive

10 K1	*Salmonella*	*enterica*	IV 48:g,z51:-	IV	Safe 36 (01-0147)	CFSAN	Unknown	Positive
6X1	*Salmonella*	*enterica*	Argentina	IV 6,7:z36:-	SGSC 2555	SGSC	Unknown	Positive
8J1	*Salmonella*	*enterica*	Wassenaar	IV 50:g,z51:-	SGSC 2576	SGSC	Unknown	Positive
6G1	*Salmonella*	*enterica*	Flint	IV 50:z4,z23:-	SGSC 2477	SGSC	Unknown	Positive
10 J1	*Salmonella*	*enterica*	IV 50:g,z51:-	IV	Safe 35 (01-0133)	CFSAN	Unknown	Positive
9U1	*Salmonella*	*enterica*	(Indica) VI 45:a:e, n, x	VI	NA	NVSL	Unknown	Positive
10A2	*Salmonella*	*enterica*	(Indica) VI RoughO:a:e,n, x	VI	N/A	NVSL	Unknown	Positive
10 Q1	*Salmonella*	*enterica*	VI 11:a:1,5	VI	Safe 54 (2229)	CFSAN	Unknown	Positive
10 O1	*Salmonella*	*enterica*	VI 11:b:1,7	VI	Safe 52 (1415)	CFSAN	Unknown	Positive
10 P1	*Salmonella*	*enterica*	VI 6,7:z41:1,7	VI	Safe 53 (1937)	CFSAN	Unknown	Positive
6W1	*Salmonella*	*enterica*	Ferlac	VI 1,6,14,25:a:e,n,x	SGSC 2581	SGSC	Unknown	Positive
6I1	*Salmonella*	*enterica*	Vrindaban	VI 45:a:e,n,x	SGSC 2230	SGSC	Unknown	Positive
10 N1	*Salmonella*	*enterica*	VI I 6, 14, 25:z10:1,(2)	VI	Safe 51 (1121)	CFSAN	Unknown	Positive
1B-1	*Salmonella*	*bongori*		V	ATCC-43975	ATCC[g]	Unknown	Positive
1L-1	*Salmonella*	*bongori*		V	SGSC-3100	SGSC	Frog	Positive
8H1	*Salmonella*	*enterica (bongori)*	Maregrosso	V 66:z35:-	SGSC 2578	SGSC	Unknown	Positive
7R	*Salmonella*	*enterica (bongori)*	Malawi	V	SGSC 2577	SGSC	Unknown	Negative
3X-1	*Salmonella*	*enterica*	1,4,(5),12:i:-	B	SGSC4913	SGSC	Unknown	Positive
6D1	*Salmonella*	*enterica*	Abony	B	SGSC 113	SGSC	Unknown	Positive
6M1	*Salmonella*	*enterica*	Abony	B	SGSC 2459	SGSC	Unknown	Positive
6J1	*Salmonella*	*enterica*	Anatum	E1	SGSC 114	SGSC	Unknown	Positive
13D	*Salmonella*	*enterica*	Blockley	C2-C3	FSLS5648	ILSI[h]	Human	Positive
13B	*Salmonella*	*enterica*	Braenderup	C1	FSLS5 373	ILSI	Human	Positive
7O1	*Salmonella*	*enterica*	Brandenburg	B	SGSC 2460	SGSC	Unknown	Positive
13A	*Salmonella*	*enterica*	Cerro	K	FSLR8370	ILSI	Bovine	Positive
1J-1	*Salmonella*	*enterica*	Choleraesuis	C1	SGSC-4770	SGSC	Human	Positive
6R1	*Salmonella*	*enterica*	Decatur	C1	SGSC 2465	SGSC	Unknown	Positive
12W	*Salmonella*	*enterica*	Dessau	E4	FSLR87229	ILSI	Peanuts	Positive
1H-1	*Salmonella*	*enterica*	Dublin	D1	SGSC-4916	SGSC	Unknown	Positive
6P1	*Salmonella*	*enterica*	Emek	C2-C3	SGSC 2554	SGSC	Unknown	Positive
1R-1	*Salmonella*	*enterica*	Enteritidis	D1	SGSC-4901	SGSC	Unknown	Positive
6T1	*Salmonella*	*enterica*	Enteritidis	D1	SGSC 2275	SGSC	Unknown	Positive

7U1	*Salmonella*	*enterica*	Enteritidis	D1	SGSC 2282	SGSC	Unknown	Positive
13E	*Salmonella*	*enterica*	G (1):b,-	G	FSLR85224	ILSI	Peanuts	Positive
1I-1	*Salmonella*	*enterica*	Gallinarum	D1	SGSC-4691	SGSC	Unknown	Positive
3Z-1	*Salmonella*	*enterica*	Hadar	C2-C3	SGSC4906	SGSC	Unknown	Positive
6L1	*Salmonella*	*enterica*	Haifa	B	SGSC 2482	SGSC	Unknown	Positive
12T	*Salmonella*	*enterica*	Hartford	C1	FSLR85223	ILSI	Peanuts	Positive
9A1	*Salmonella*	*enterica*	Heidelberg	B	SGSC 2217	SGSC	Unknown	Positive
1U-1	*Salmonella*	*enterica*	Heidelberg	B	SGSC-4915	SGSC	Unknown	Positive
6H1	*Salmonella*	*enterica*	Heidelberg	B	SGSC 2527	SGSC	Unknown	Positive
8P1	*Salmonella*	*enterica*	Heidelberg	B	SGSC 2211	SGSC	Unknown	Positive
8X1	*Salmonella*	*enterica*	Heidelberg	B	SGSC 2218	SGSC	Unknown	Positive
6K1	*Salmonella*	*enterica*	Indiana	B	SGSC 2479	SGSC	Unknown	Positive
3V-1	*Salmonella*	*enterica*	infantis	C1	SGSC4905	SGSC	Unknown	Positive
4A-1	*Salmonella*	*enterica*	Javiana	D1	SGSC4917	SGSC	Unknown	Positive
1E-1	*Salmonella*	*enterica*	Kentucky	C2-C3	SGSC-4914	SGSC	Unknown	Positive
13C	*Salmonella*	*enterica*	Mbandaka	C1	FSLS5451	ILSI	Human	Positive
12Q	*Salmonella*	*enterica*	Meleagridis	E1	FSLR6938	ILSI	Bovine	Positive
12P	*Salmonella*	*enterica*	Mississippi	G	FSLA4633	ILSI	Human	Positive
6Q1	*Salmonella*	*enterica*	Montevideo	C1	SGSC 2488	SGSC	Unknown	Positive
6V1	*Salmonella*	*enterica*	Montevideo	C1	SGSC 2487	SGSC	Unknown	Positive
7T1	*Salmonella*	*enterica*	Muenchen	C2-C3	SGSC 2490	SGSC	Unknown	Positive
12M	*Salmonella*	*enterica*	Muenster	E1	FSLS5432	ILSI	Bovine	Positive
1S-1	*Salmonella*	*enterica*	Newport	C2-C3	SGSC-4910	SGSC	Human	Positive
8T1	*Salmonella*	*enterica*	Newport	C2-C3	SGSC 2494	SGSC	Unknown	Positive
6Z1	*Salmonella*	*enterica*	Newport	C2-C3	SGSC 2493	SGSC	Unknown	Positive
12N	*Salmonella*	*enterica*	Oranienburg	C1	FSLS5 642	ILSI	Human	Positive
12R	*Salmonella*	*enterica*	Orion va. 15+,34+	E1	FSLR83408	ILSI	Bovine	Positive
7V1	*Salmonella*	*enterica*	Panama	D1	SGSC 2496	SGSC	Unknown	Positive
1O-1	*Salmonella*	*enterica*	Paratyphi A	A	SGSC-2499	SGSC	ATCC9150	Positive
8F1	*Salmonella*	*enterica*	Paratyphi B	B	SGSC 2501	SGSC	Unknown	Positive
1M-1	*Salmonella*	*enterica*	Paratyphi B	B	SGSC-4150	SGSC	Human	Positive
6N1	*Salmonella*	*enterica*	Paratyphi B	B	SGSC 2582	SGSC	Unknown	Positive
8O1	*Salmonella*	*enterica*	Paratyphi C	C1	SGSC 2506	SGSC	Unknown	Positive
3Y-1	*Salmonella*	*enterica*	Paratyphi C	C1	SGSC2290	SGSC	Unknown	Positive

6S1	*Salmonella*	*enterica*	Paratyphi C	C1	SGSC 2507	SGSC	Unknown	Positive
1K-1	*Salmonella*	*enterica*	Pullorum	D1	SGSC-2508	SGSC	Unknown	Positive
8I1	*Salmonella*	*enterica*	Pullorum	D1	SA 1689	SGSC	Unknown	Positive
6U1	*Salmonella*	*enterica*	Reading	B	SGSC 2510	SGSC	Unknown	Positive
6Y1	*Salmonella*	*enterica*	Rubislaw	F	SGSC 2511	SGSC	Unknown	Positive
8Q1	*Salmonella*	*enterica*	Saintpaul	B	SGSC 2203	SGSC	Unknown	Positive
8Z1	*Salmonella*	*enterica*	Saintpaul	B	SGSC 2202	SGSC	Unknown	Positive
1F-1	*Salmonella*	*enterica*	Saintpaul	B	SGSC-4920	SGSC	Human	Positive
1N-1	*Salmonella*	*enterica*	Schwarzengrund	B	SGSC-4919	SGSC	Unknown	Positive
7W1	*Salmonella*	*enterica*	Sendai	D1	SGSC 2515	SGSC	Unknown	Positive
12Y	*Salmonella*	*enterica*	Senftenberg	E4	FSLS5658	ILSI	Human	Positive
8G1	*Salmonella*	*enterica*	Stanley	B	SGSC 2517	SGSC	Unknown	Positive
8N1	*Salmonella*	*enterica*	Stanyville		SGSC2518	SGSC	Unknown	Positive
12X	*Salmonella*	*enterica*	Tyhimurium var Copenhagen	B	FSLS5786	ILSI	Bovine	Positive
12S	*Salmonella*	*enterica*	Tennessee	C1	FS8R81965	ILSI	Bovine	Positive
6C1	*Salmonella*	*enterica*	Thompson	C1	SGSC 2519	SGSC	Unknown	Positive
12L	*Salmonella*	*enterica*	Tornow	W	FSLR85222	ILSI	Peanuts	Positive
1D-1	*Salmonella*	*enterica*	Typhi	D1	SGSC-4072	SGSC	Human	Positive
1T-1	*Salmonella*	*enterica*	Typhimurium	B	SGSC-1412 (Strain LT2)	SGSC	Unknown	Positive
12V	*Salmonella*	*enterica*	Uganda	E1	FSLR83404	ILSI	Bovine	Positive
12U	*Salmonella*	*enterica*	Virchow	C1	FSLS5961	ILSI	Human	Positive
12O	*Salmonella*	*enterica*	Weltevreden	E1	FSLS5438	ILSI	Human	Positive
9B1	*Salmonella*	*enterica*	Wien	B	SGSC 2528	SGSC	Unknown	Positive
12K	*Salmonella*	enterica	Worthington	G	FSLS5490	ILSI	Human	Positive

[a] Assay Sponsor, EnviroLogix, accession number.
[b] Based on Antigenic Formulae of the *Salmonella* Serovars (2007), 9th Edition (17).
[c] NA = Not applicable.
[d] U.S. Department of Agriculture, National Veterinary Services Laboratory, Ames, IA.
[e] U.S. Food and Drug Administration, Center For Food Safety and Applied Nutrition, College Park, MD.
[f] *Salmonella* Genetic Stock Center, University of Calgary, Canada.
[g] American Type Culture Collection, Manassas, VA.
[h] International Life Sciences Institute, Cornell University, Ithaca, NY.

Table 2. Exclusivity

ELIX _ID	Genus	Species	Information provided by source	Alternate ID	Source	Origin	DNAble
2Y-1	*Campylobacter*	*coli*	strain SC1732	NA[b]	NCSU[c]	Unknown	Negative
2W-1	*Campylobacter*	*jejuni*	strain-10882	NA	NCSU	Unknown	Negative
5I-1	*Citrobacter*	*braakii*	NA	ATCC-6750	ATCC[d]	Unknown	Negative
2T-1	*Citrobacter*	*freundii*	NA	ATCC-6879	ATCC	Unknown	Negative
3F1	*Cronobacter*	*sakazakii*	BAA-894	ATCC, VA	ATCC	Unknown	Negative
2P-1	*Enterobacter*	*aerogenes*	NA	ATCC-15038	ATCC	Unknown	Negative
3R-1	*Enterobacter*	*cloacae*	NA	NRRL-B-411	USDA-ARS[e]	Unknown	Negative
12G1	*Enterobacter*	*hormaechei*	NA	ATCC 700323	ATCC	Unknown	Negative
7Z1	*Escherichia*	*fergusonii*	SA 5718	NA	SGSC[f]	Unknown	Negative
8M1	*Escherichia*	*hermannii*	NA	SA 5715	SGSC	Unknown	Negative
8A1	*Escherichia*	*vulneris*	SA 5716	NA	SGSC	Unknown	Negative
3A-1	*Escherichia*	*coli*	strain O103:H2	MI-80-TW08101	STEC Ctr.[g]	Unknown	Negative
5J-1	*Hafnia*	*alvei*	NA	ATCC9760	ATCC	Unknown	Negative
7C1	*Hafnia*	*alvei*	NA	SA5940	SGSC	Unknown	Negative
7H1	*Klebsiella*	*oxytoca*	NA	SGSC3356	SGSC	Unknown	Negative
5G-1	*Klebsiella*	*pneumoniae*	NA	ATCC12658	ATCC	Unknown	Negative
2F-1	*Listeria*	*grayi*	NA	NA	NCSU	Food Processing plant	Negative

2D-1	*Listeria*	*innocua*	NA	NA	NCSU	Food Processing plant	Negative
2C-1	*Listeria*	*ivanovii*	NA	NA	NCSU	Food Processing plant	Negative
2I-1	*Listeria*	*monocytogenes*	206a-5 (1/2a)	NA	NCSU	Food Processing plant	Negative
1V-1	*Listeria*	*monocytogenes*	Strain- F2365	NA	NCSU	Jalisco Cheese, 1985	Negative
2E-1	*Listeria*	*seeligeri*	NA	NA	NCSU	Food Processing plant	Negative
2B-1	*Listeria*	*welshimeri*	NA	NA	NCSU	Food Processing plant	Negative
3T-1	*Morganella*	*morganii*	NA	NRRL-B-1663	USDA-ARS	Unknown	Negative
7F1	*Morganella*	*morganii*	*siboni*	SA5705	SGSC	Unknown	Negative
7L1	*Proteus*	*mirabilis*	NA	SGSC 3360	SGSC	Unknown	Negative
3E-1	*Proteus*	*vulgaris*	NA	ATCC- 6380	ATCC	Unknown	Negative
3Q-1	*Serratia*	*marcescens*	NA	NRRL-B-159	USDA-ARS	Unknown	Negative
3U-1	*Serratia*	*liquifaciens*	NA	NRRL-B-41184	USDA-ARS	Unknown	Negative
2Q-1	*Shigella*	*boydii*	NA	ATCC-9210	ATCC	Unknown	Negative
2V-1	*Shigella*	*dysenteriae*	NA	ATCC-9750	ATCC	Unknown	Negative
5H-1	*Shigella*	*flexneri*	NA	ATCC9199	ATCC	Unknown	Negative
7E1	*Shigella*	*sonnei*	NA	SA5575	SGSC	Unknown	Negative
2R-1	*Staphylococcus*	*aureus*	NA	ATCC-11371	ATCC	Unknown	Negative
2S-1	*Yersinia*	*enterocolitica*	NA	ATCC-23715	ATCC	Unknown	Negative

[a] Assay Sponsor, EnviroLogix, accession number.
[b] NA = Not applicable.
[c] North Carolina State University, Raleigh, NC.
[d] American Type Culture Collection, Manassas, VA.
[e] U.S. Department of Agriculture, Agricultural Research Services, Culture Collection (NRRL), Peoria, IL.
[f] *Salmonella* Genetic Stock Center, University Calgary, Canada.
[g] Shiga-toxin Producing *E. coli* Center, Michigan State University, East Lansing, MI.

Table 4. POD analyses DNAble *Salmonella* presumptive and confirmed

Matrix	Strain	Test area/test portion	CFU per test area[a] or MPN/test portion[b] (95% CI)	N[c]	Presumptive			Confirmed			dPOD$_{CP}$[g]	95% CI[h]
					x[d]	POD$_{CP}$[e]	95% CI	x	POD$_{CC}$[f]	95% CI		
Stainless steel	*Salmonella diarizonae* 1116	4 × 4 "	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			224	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
			690	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
Dry pet food	*Salmonella* Typhimurium SGSC[i] 1412	25 g (9:1,v/w)	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			0.45 (0.22, 0.78)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
		375 g (3:1,v/w)	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			0.45 (0.22, 0.78)	20	8	0.4	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	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
		375 g (9:1,v/w)	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			0.45 (0.22, 0.78)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
Drag swabs	*Salmonella* Enteritidis (ATCC[j]# BAA-1587)	NA[k]	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		NA	4	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0	-0.28, 0.28
		NA	70	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43

[a] CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate.
[b] MPN = Most Probable Number is based on the POD of reference method test portions across labs using the LCF MPN calculator, with 95% confidence interval.
[c] N = Number of test portions.
[d] x = Number of positive test portions.
[e] POD$_{CP}$ = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] POD$_{CC}$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[g] dPOD$_{CP}$ = Difference between the candidate method presumptive result and candidate 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.
[i] *Salmonella* Genetic Stock Center, University of Calgary, Canada
[j] American Type Culture Collection, Manassas, VA
[k] NA, Not applicable

Table 5. POD analyses candidate and reference

Matrix	Strain	Test area/test portion	CFU per test area[a] or MPN/test portion[b] (95% CI)	N[c]	DNAble *Salmonella*			Reference method			$dPOD_C$[g]	95% CI[h]
					x[d]	POD_C[e]	95% CI	x	POD_R[f]	95% CI		
Stainless steel	*Salmonella diarizonae* 1116	4 × 4 "	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			224	20	15	0.75	0.53, 0.89	13	0.65	0.43, 0.82	0.1	-0.18, 0.36
			690	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
Dry pet food	*Salmonella* Typhimurium SGSC[i] 1412	25 g (9:1,v/w)	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			0.45 (0.22, 0.78)	20	11	0.55	0.34, 0.74	7	0.35	0.18, 0.57	0.2	-0.10, 0.46
			3.01 (1.31, 6.89)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43
		375 g (3:1,v/w)	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-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	-0.28, 0.28
			3.01 (1.31, 6.89)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43,0.43
		375 g (9:1,v/w)	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
			0.45 (0.22, 0.78)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.1	-0.19, 0.37
			3.01 (1.31, 6.89)	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43,0.43
Drag swabs	*Salmonella* Enteritidis (ATCC[j] # BAA-1587)	NA[k]	0	5	0	0	0.00, 0.43	0	0	0.00, 0.43	0	-0.43, 0.43
		NA	4	20	7	0.35	0.18, 0.57	5	0.25	0.11, 0.47	0.1	-0.18, 0.36
		NA	70	5	5	1	0.57, 1.00	5	1	0.57, 1.00	0	-0.43, 0.43

[a] CFU/Test Area = Results of the CFU/test area were determined by plating the inoculum for each matrix in triplicate.
[b] MPN = Most Probable Number is based on the POD of reference method test portions across labs using the LCF MPN calculator, with 95% confidence interval.
[c] N = Number of test portions.
[d] x = Number of confirmed positive test portions.
[e] POD_{CP} = 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] $dPOD_{CP}$ = Difference between the candidate method confirmed 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.
[i] *Salmonella* Genetic Stock Center, University of Calgary, Canada
[j] American Type Culture Collection, Manassas, VA. .
[k] NA, Not applicable

ORIGINAL CERTIFICATION DATE	**CERTIFICATION RENEWAL RECORD**
April 30, 2014	**Renewed Annually through December 2016**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
1. August 2015	**1. Change of manufacturing location of supplement (Part No. 12035) and an increase of shelf life to 12 months.**

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