CERTIFICATION

AOAC® *Performance Tested*^{SM}

Certificate No.

101102

The AOAC Research Institute hereby certifies that the method known as:

RapidChek® *Listeria* NextDay^{TM} Food and Environmental Test System

manufactured by

Romer Labs
130 Sandy Drive
Newark, DE 19713
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 (November 20, 2021 – December 31, 2022). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

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Scott Coates, Senior Director
Signature for AOAC Research Institute

November 20, 2021
_____
Date

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MODIFICATION MAY 2013: Mark Muldoon, Ann Christine Olsson-Allen, Vera Gonzalez, Kidist Asfaw, and Zheng Jiang
MODIFICATION OCTOBER 2014: Mark Muldoon, Ann-Christine Olsson Allen, and Kidist Asfaw

SUBMITTING COMPANY
Strategic Diagnostics, Inc.
 128 Sandy Drive
Newark, DE 19713

CURRENT SPONSOR
Romer Labs
130 Sandy Drive
Newark, DE 19713

KIT NAME(S)
RapidChek® *Listeria* NextDay™ Food and Environmental Test System

CATALOG NUMBERS
Original catalog numbers: 3000019, 7000171, 7000182, 7000238P, 7000243, 7000244, 7000246, 7000248, 7000248E, 7000248S, 7000249, 7000261, 7000262
Updated catalog numbers: 10001173, 10001361, 10001372, 10001718, 10001409, 10001410, 10001412, 10001413, 10001724, 10001725, 10001414, 10001420, 10001421

INDEPENDENT LABORATORY

Original Validation	**May 2013 Modification**
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APPLICABILITY OF METHOD
Target organism – *Listeria* species

Matrixes – (USDA FSIS Ch 8.07) - Stainless Steel (430 SS, Food Grade), plastic (polyurethane, Food Grade), Rubber (synthetic, Food Grade, Painted Concrete (Laytex-based white paint)

October 2014 Modification (USDA FDA BAM Ch 8.09): hot dogs (25 g, 125 g), roast beef (25 g), frozen breaded chicken (25 g, 125 g), frozen meatballs (25 g), whole milk (25 g), ice cream (25 g), ricotta cheese (25 g), shredded Mexican cheese (125 g), cheese powder (25 g)

Performance claims - RapidChek® *Listeria* NextDay™ Food and Environmental Test System (formerly SDIX RapidChek™ *Listeria* F.A.S.T.) was validated for the detection of *Listeria* and was found to be equivalent in performance to the reference method.

REFERENCE METHODS
US Department of Agriculture, Food Safety and Inspection Service. Microbiological Laboratory Guidelines. Chapter 8.07. Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Egg, and Environmental Samples*.* (9)

USDA FSIS MLG chapter 8.09 *"Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Egg, and Environmental Samples"* for meat and poultry and the Food and Drug Administration Bacteriological Analytical Manual chapter 10 *"Detection and Enumeration of Listeria monocytogenes"* for dairy products (11)

ORIGINAL CERTIFICATION DATE **October 07, 2011**	**CERTIFICATION RENEWAL RECORD** **Renewed Annually through December 2022.**

METHOD MODIFICATION RECORD	**SUMMARY OF MODIFICATION**
1. **December 2012 Level 1**	1. **Name change from Strategic Diagnostics to Romer**
2. **May 2013 Level 2**	2. **New Proprietary one-step enrichment**
3. **October 2014 Level 2**	3. **Matrix extension**
4. **December 2014 Level 1**	4. **Name change from F.A.S.T. to NextDay**
5. **May 2019 Level 1**	5. **Updated catalog numbers**
6. **November 2021 Level 1**	6. **Updated USDA/FDA information.**

Under this AOAC® *Performance Tested*[SM] License Number, 101102 this method is distributed by: **NONE**	**Under this AOAC® *Performance Tested*[SM] License Number, 101102 this method is distributed as:** **NONE**

PRINCIPLE OF THE METHOD (1)
The RapidChek® *Listeria* NextDay™ Food and Environmental Test System (formely SDIX RapidChek™ *Listeria* F.A.S.T.method for *Listeria* species) utilizes a proprietary enrichment system. After enrichment (24 to 40 h), an aliquot of the sample broth is dispensed into a test tube and boiled for 5 to 15 minutes. Once the sample has cooled, a test strip is added directly to the tube. The sample flows up the strip through a zone containing antibody-coated colloidal gold reagents specific to *Listeria* species. If antigens are present in the sample, they will bind to the antibody-conjugates to form an antigen/antibody complex. As this complex migrates through the nitrocellulose matrix, it passes a zone of anti-*Listeria* antibody. If antigen is present, the complex is captured in this zone forming an antibody-antigen "sandwich" and is visualized by the formation of a red line. A second zone on the membrane is designed to capture any antibody-gold complex not bound in the first zone. As a result, when *Listeria* antigen is present, the formation of 2 red lines is observed, whereas when no *Listeria* is present, only 1 line forms.

DISCUSSION OF THE VALIDATION STUDY (1)

The RapidChek *Listeria* NextDay Food and Environmental Test System was validated against the USDA-FSIS cultural reference method for the detection of *Listeria* species on stainless steel, plastic, rubber, and painted concrete. The method offers the flexibility of testing the enriched sample after a 24 to 40 enrichment time with no difference in results. The overall Chi square was 0.017 (p=0.104) and 0.611 (p=0.566) after a 24 h and 40 h enrichment, respectively, indicating that the test method was equivalent in performance to the reference method at both enrichment times. The test method gave a sensitivity of 100% and a specificity of 100% across all sample types. There were no false positives or false negatives found in the study. The new test method should provide the end user with a rapid and reliable tool for monitoring and controlling *Listeria* species in the food processing environment and by doing so, minimizing the contamination of food products by *Listeria monocytogenes*.

Table 1. Internal Validation Results from the Analysis of *Listeria monocytogenes* on Stainless Steel (Study 1). (1)

Surface	Inoculum	Surface Area, in^2	Inoculation Level, cfu/area	Method	Number of Samples Inoculated/ Neg. Control	Presumptive Positives	Confirmed Positives	Presumptive Negatives	Confirmed Negatives	Chi square	p-value	Sensitivity Rat
Stainless Steel	L. monocytogenes ATCC 19115 plus Bacillus subtilis ATCC 27370	4	400 + 4000	SDIX 24H	20	7	7	13	13	2.08	0.851	100
					5	0	0	5	5			
				SDIX 40H	20	8	8	12	12	3.06	0.92	100
					5	0	0	5	5			
				USDA	20	0	3	0	17	-	-	-
					5	-	0	-	5			

Table 2. Internal Validation Results from the Analysis of *Listeria innocua* on Plastic. (1)

Surface	Inoculum	Surface Area, in^2	Inoculation Level, cfu/area	Method	Number of Samples Inoculated/ Neg. Control	Presumptive Positives	Confirmed Positives	Presumptive Negatives	Confirmed Negatives	Chi square	p-value	Sensitivity Rat
Plastic	L. innocua ATCC 33090	4	50	SDIX 24H	20	7	7	13	13	0.104	0.253	100
					5	0	0	5	5			
				SDIX 40H	20	5	5	15	15	0.464	0.504	100
					5	0	0	5	5			
				USDA	20	-	8	-	12	-	-	-
					5	-	0	-	5			

Table 3. Internal Validation Results from the Analysis of *Listeria welshimeri* on Rubber. (1)

Surface	Inoculum	Surface Area, in^2	Inoculation Level, cfu/area	Method	Number of Samples Inoculated/ Neg. Control	Presumptive Positives	Confirmed Positives	Presumptive Negatives	Confirmed Negatives	Chi square	p-value	Sensitivity Rat
Rubber	L. welshimeri ATCC 35897	4	20	SDIX 24H	20	13	13	7	7	0.111	0.261	100
					5	0	0	5	5			
				SDIX 40H	20	17	17	3	3	1.258	0.74	100
					5	0	0	5	5			
				USDA	20	-	14	-	6	-	-	-
					5	-	0	-	5			

Table 4. Internal Validation Results from the Analysis of *Listeria seeligeri* on Painted Concrete. (1)

Surface	Inoculum	Surface Area, in^2	Inoculation Level, cfu/area	Method	Number of Samples Inoculated/ Neg. Control	Presumptive Positives	Confirmed Positives	Presumptive Negatives	Confirmed Negatives	Chi square	p-value	Sensitivity Rat
Painted Concrete	L. seeligeri ATCC 51334	1	100	SDIX 24H	20	8	8	12	12	1.56	0.788	100
					5	-	-	5	5			
				SDIX 40H	20	10	10	10	10	0.393	0.469	100
					5	0	0	5	5			
				USDA	20	-	12	-	8	-	-	-
					5	-	0	-	5			

Table 5. Internal Validation Results from the Analysis of *Listeria monocytogenes* on Stainless Steel (Study 2). (1)

Surface	Inoculum	Surface Area, in[2]	Inoculation Level, cfu/area	Method	Number of Samples Inoculated/ Neg. Control	Presumptive Positives	Confirmed Positives	Presumptive Negatives	Confirmed Negatives	Chi square	p-value	Sensitivity Rat
Stainless Steel	L. monocytogenes ATCC 19115 plus Bacillus subtilis ATCC 27370	4	6 + 62	SDIX 24H	20	7	7	13	13	0.111	0.261	100
					5	0	0	5	5			
				SDIX 40H	20	7	7	13	13	0.111	0.261	100
					5	0	0	5	5			
				USDA	20	0	6	0	14	-	-	-
					5	-	0	-	5			

NOTE: Inclusivity and Exclusivity data from the original report has been omitted but can be found in Evaluation of the SDIX RapidChek™ Listeria F.A.S.T.™ Environmental Test System for the Detection of *Listeria* species on Environmental Surfaces(1).

DISCUSSION OF THE MODIFICATION STUDY APPROVED MAY 2013 (10)

The RapidChek *Listeria* NextDay Food and Environmental Test System (formely SDIX RapidChek *Listeria* F.A.S.T.method for *Listeria* species) was validated against the USDA-FSIS cultural reference method for the detection of *Listeria* species on stainless steel, plastic, rubber, and painted concrete. The method offers the flexibility of testing the enriched sample after a 24 to 40 enrichment time. The number of positive results obtained with the RapidChek method at 24 h and 40 h were 58 and 62, respectively whereas the number obtained with the cultural reference method was 63. Probability of detection (POD) analysis showed that RapidChek test results at either 24 h or 40 h enrichment times were equivalent to the USDA-FSIS cultural reference method (Table 14 and 15) as the 95% confidence intervals for both $dPOD_C$ and $dPOD_{CP}$, determined at both test times, bracketed zero. The overall Mantel-Haenszel chi-square values were 0.524 and 0.084 after a 24 h and 40 h enrichment, respectively, also indicating that the RapidChek test method was equivalent in performance to the reference method at both enrichment times. Inclusivity testing showed that the method gave 100% sensitivity across the 50 *Listeria* spp. tested. In addition, the method showed 100% specificity against a panel of 30 non-*Listeria* bacterial strains.

The new test method should provide the end user with a rapid and reliable tool for monitoring and controlling *Listeria* species in the food processing environment and by doing so, minimize the contamination of food products by *Listeria monocytogenes*.

Table 2. POD Results from the Analysis of *Listeria monocytogenes* on Stainless Steel Surfaces after 24 h Enrichment Time (Internal Laboratory Study). (10)

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	5000	20	16	0.80	20	18	0.90	20	18	0.90	20	13	0.65	0.25	-0.10
LCL				0.58			0.70			0.70			0.43	-0.01	-0.33
UCL				0.92			0.97			0.97			0.82	0.48	0.13
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 3. POD Results from the Analysis of *Listeria monocytogenes* on Stainless Steel Surfaces after 40 h Enrichment Time (Internal Laboratory Study). (10)

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	5000	20	17	0.85	20	18	0.90	20	18	0.90	20	13	0.65	0.25	-0.05
LCL				0.64			0.70			0.70			0.43	-0.01	-0.27
UCL				0.95			0.97			0.97			0.82	0.48	0.17
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 4. POD Results from the Analysis of *Listeria innocua* on Plastic Surfaces after 24 h Enrichment Time. (10)

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	2500	20	12	0.60	20	12	0.60	20	12	0.60	20	14	0.70	-0.10	0.00
LCL				0.39			0.39			0.39			0.48	-0.36	-0.28
UCL				0.78			0.78			0.78			0.85	0.18	0.28
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 5. POD Results from the Analysis of *Listeria innocua* on Plastic Surfaces after 40 h Enrichment Time. (10)

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	2500	20	12	0.60	20	12	0.60	20	12	0.60	20	14	0.70	-0.10	0.00
LCL				0.39			0.39			0.39			0.48	-0.36	-0.28
UCL				0.78			0.78			0.78			0.85	0.18	0.28
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 6. POD Results from the Analysis of *Listeria welshimeri* on Rubber Surfaces after 24 h Enrichment Time. (10)

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	550	20	13	0.65	20	14	0.70	20	14	0.70	20	14	0.70	0.00	-0.05
LCL				0.43			0.48			0.48			0.48	-0.27	-0.32
UCL				0.57			0.85			0.85			0.85	0.27	0.23
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	550	20	14	0.70	20	14	0.70	20	14	0.70	20	14	0.70	0.00	0.00
LCL				0.48			0.48			0.48			0.48	-0.27	-0.27
UCL				0.85			0.85			0.85			0.85	0.27	0.27
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

Table 7. POD Results from the Analysis of *Listeria welshimeri* on Rubber Surfaces after 40 h Enrichment Time. (10)

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 8. POD Results from the Analysis of *Listeria seeligeri* on Painted Concrete Surfaces after 24 h Enrichment Time. (10)

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	50	20	7	0.35	20	8	0.40	20	8	0.40	20	9	0.45	-0.05	-0.05
LCL				0.18			0.22			0.22			0.26	-0.33	-0.32
UCL				0.57			0.61			0.61			0.66	0.23	0.23
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 9. POD Results from the Analysis of *Listeria seeligeri* on Painted Concrete Surfaces after 40 h Enrichment Time. (10)

Statistic	Concentration, CFU/surface	Candidate Presumptive (CP)			Candidate confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N	x	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL				0.00			0.00			0.00			0.00	-0.43	-0.43
UCL				0.43			0.43			0.43			0.43	0.43	0.43
Estimate	50	20	8	0.40	20	8	0.40	20	8	0.40	20	9	0.45	-0.05	0.00
LCL				0.22			0.22			0.22			0.26	-0.33	-0.28
UCL				0.61			0.61			0.61			0.66	0.23	0.28
Estimate	500000	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL				0.57			0.57			0.57			0.57	-0.43	-0.43
UCL				1.00			1.00			1.00			1.00	0.43	0.43

[1]If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 10. *Listeria* spp. Strains Tested in the Inclusivity Study. (10)

Sample #	Species	Sample #	Species
1	L. monocytogenes ATCC 51774	26	L. monocytogenes ATCC 19118
2	L. monocytogenes SDI 10-3b/c1	27	L. monocytogenes SDI 22-3b/e7
3	L. monocytogenes SDI 10a-3b/c2	28	L. monocytogenes SDI 23-3b/e9
4	L. monocytogenes SDI 11-3b/c3	29	L. monocytogenes ATCC 4428
5	L. monocytogenes USDA 472	30	L. monocytogenes NRRL B-33181
6	L. monocytogenes SDI 12-3b/c5	31	L. grayi ATCC 19120
7	L. monocytogenes SDI 13-3b/c7	32	L. grayi ATCC 25401
8	L. monocytogenes ATCC 7644	33	L. innocua USDA 15-666
9	L. monocytogenes ATCC 19112	34	L. innocua ATCC 33090
10	L. monocytogenes ATCC 19113	35	L. innocua ATCC 33091
11	L. monocytogenes SDI 14-3b/c	36	L. innocua SDI-3
12	L. monocytogenes SDI 15-3b/d2	37	L. innocua SDI-198
13	L. monocytogenes SDI 16-3b/d4	38	L. innocua #40
14	L. monocytogenes ATCC 19114	39	L. seeligeri ATCC 51334
15	L. monocytogenes SDI 17-3b/d6	40	L. seeligeri ATCC 51335
16	L. monocytogenes SDI 18-3b/d8	41	L. seeligeri ATCC 35967
17	L. monocytogenes ATCC 13932	42	L. seeligeri SDI 3BF1
18	L. monocytogenes ATCC 19115	43	L. seeligeri SDI 3BF2
19	L. monocytogenes ATCC 43256	44	L. welshimeri ATCC 35897
20	L. monocytogenes ATCC 51414	45	L. welshimeri ATCC 43548
21	L. monocytogenes SDI 19-3b/e1	46	L. welshimeri #58
22	L. monocytogenes U of G H7650	47	L. welshimeri SDI-50
23	L. monocytogenes ATCC 19116	48	L. welshimeri #83
24	L. monocytogenes SDI 20-3b/e3	49	L. ivanovii ATCC 700402
25	L. monocytogenes SDI 21-3b/e5	50	L. ivanovii ATCC 19119

Table 11. Non-*Listeria* spp. Strains Tested in the Exclusivity Study. (10)	
Sample #	Species
1	Acinetobacter baumannii ATCC 19606
2	Bacillus cereus ATCC 11778
3	Bacillus cereus ATCC 12826
4	Bacillus megaterium B4-10
5	Bacillus mycoides ATCC 6462
6	Bacillus subtilis ATCC 27370
7	Bacillus subtilis ATCC 6633
8	Bacillus thuringiensis ATCC 10792
9	Brochothrix thermosphacta ATCC 11509
10	Chryseobacterium meningosepticum ATCC 13253
11	Citrobacter freundii 7A12
12	Corynebacterium genitalium D4-9
13	Corynebacterium pseudogenitalium 13-2.3
14	Enterobacter cloacae ATCC 23355
15	Enterococcus durans ATCC 6056
16	Enterococcus faecalis ATCC 10741
17	Enterococcus faecalis ATCC 19433
18	Enterococcus faecium 349
19	Escherichia coli O157:H7 ATCC 35150
20	Lactobacillus acidophilus 314
21	Lactobacillus plantarum ATCC 8014
22	Lactococcus lactis ATCC 11454
23	Leuconostoc citreum DENCO 8
24	Micrococcus luteus 533
25	Proteus vulgaris ATCC 6380
26	Pseudomonas aeruginosa ATCC 10145
27	Rhodococcus equi ATCC 7698
28	Salmonella typhimurium ATCC 14028
29	Staphylococcus aureus ATCC 25923
30	Staphylococcus epidermidis ATCC 12228
31	Staphylococcus haemolyticus ATCC 29970
32	Staphylococcus haemolyticus G1(-6)-3
33	Staphylococcus hominis A1-2
34	Staphylococcus simulans A2-5
35	Streptococcus oralis ATCC 9811

DISCUSSION OF THE MODIFICATION STUDY APPROVED OCTOBER 2014 (11)

The Romer Labs Technology, Inc. RapidChek® *Listeria* NextDay™ Food and Environmental Test System was validated against the USDA-FSIS or FDA-BAM cultural reference method for the detection of *Listeria* species on various ready-to-eat foods. For most of the food types tested, the method offers the flexibility of testing the enriched sample after a 27 to 48 h enrichment time. For these (not including shredded Mexican cheese at 125 g sample size since only 48 h was tested), the number of positive results obtained with the RapidChek method at 27 h and 48 h were 160 and 169, respectively, whereas the number of positive samples identified by the respective cultural reference method was 158. For all sample types, Probability of Detection (POD) analysis showed that the number of positive test results obtained by the RapidChek test method at either 27 h or 48 h enrichment times were not significantly different than the number of positive test results obtained by the respective cultural reference method as the 95% confidence intervals for both $dPOD_C$ and $dPOD_{CP}$, determined at both test times, bracketed zero. The Mantel-Haenszel chi-square test also showed no significant difference in the number of presumptive positive results for the test method after either 27 h ($\chi^2 = 0.032$) or 48 h ($\chi^2 = 0.995$) enrichment times compared to the number of positives for the cultural reference method.

Based on these study results the following enrichment time recommendations are made: Hot dogs 25 g: 27 to 48 h; Hot dogs 125 g: 48 h; Roast beef 25 g: 27 to 48 h; Frozen breaded chicken 25 g: 27 to 48 h; Frozen breaded chicken 125 g: 27 to 48 h; Frozen meatballs 25 g: 27 to 48 h; Whole milk 25 g: 27 to 48 h; Ice cream 25 g: 27 to 48 h; Ricotta Cheese 25 g: 27 to 48 h; Shredded Mexican cheese 25 g: 48 h; Shredded Mexican cheese 125 g: 48 h; Cheese powder 25 g: 27 to 48 h.

The new test method should provide the end user with a rapid and reliable tool for monitoring and controlling *Listeria* species in ready-to-eat foods and by doing so minimize the introduction of *Listeria monocytogenes*-contaminated food products into commerce.

Table 2. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19114 in 25 g hot dogs after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.25	20	17	0.85	20	18	0.90	20	18	0.90	20	15	0.75	0.15	-0.05
LCL	2.679			0.64			0.70			0.70			0.53	-0.09	-0.27
UCL	7.272			0.95			0.97			0.97			0.89	0.38	0.17
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 3. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19114 in 25 g hot dogs after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.25	20	18	0.90	20	18	0.90	20	18	0.90	20	15	0.75	0.15	0.00
LCL	2.679			0.70			0.70			0.70			0.53	-0.09	-0.21
UCL	7.272			0.97			0.97			0.97			0.89	0.38	0.21
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 4. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19114 in 125 g hot dogs after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.25	20	14	0.70	20	18	0.90	20	18	0.90	20	15	0.75	0.15	-0.20
LCL	2.679			0.48			0.70			0.70			0.53	-0.09	-0.43
UCL	7.272			0.85			0.97			0.97			0.89	0.38	0.05
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 5. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19114 in 125 g hot dogs after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.25	20	18	0.90	20	18	0.90	20	18	0.90	20	15	0.75	0.15	0.00
LCL	2.679			0.70			0.70			0.70			0.53	-0.09	-0.21
UCL	7.272			0.97			0.97			0.97			0.89	0.38	0.21
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 6. POD Results from the Analysis of *Listeria monocytogenes* ATCC 51414 in 25 g roast beef after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.725	20	12	0.60	20	14	0.70	20	14	0.70	20	9	0.45	0.25	-0.10
LCL	0.425			0.39			0.48			0.48			0.26	-0.05	-0.36
UCL	1.225			0.78			0.85			0.85			0.66	0.50	0.18
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 7. POD Results from the Analysis of *Listeria monocytogenes* ATCC 51414 in 25 g roast beef after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.725	20	14	0.70	20	14	0.70	20	14	0.70	20	9	0.45	0.25	0.00
LCL	0.425			0.48			0.48			0.48			0.26	-0.05	-0.27
UCL	1.225			0.85			0.85			0.85			0.66	0.50	0.27
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 8. POD Results from the Analysis of *Listeria innocua* ATCC 51414 in 25 g frozen breaded chicken (containing 10-fold excess *Bacillus subtilis* ATCC 27370) after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate Method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.45	20	13	0.65	20	13	0.65	20	13	0.65	20	16	0.80	-0.15	0.00
LCL	0.925			0.43			0.43			0.43			0.58	-0.40	-0.28
UCL	2.325			0.82			0.82			0.82			0.92	0.12	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 9. POD Results from the Analysis of *Listeria innocua* ATCC 51414 in 25 g frozen breaded chicken (containing 10-fold excess *Bacillus subtilis* ATCC 27370) after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.45	20	13	0.65	20	13	0.65	20	13	0.65	20	16	0.80	-0.15	0.00
LCL	0.925			0.43			0.43			0.43			0.58	-0.40	-0.28
UCL	2.325			0.82			0.82			0.82			0.92	0.12	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 10. POD Results from the Analysis of *Listeria innocua* ATCC 51414 in 125 g frozen breaded chicken (containing 10-fold excess Bacillus subtilis ATCC 27370) after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.45	20	14	0.70	20	14	0.70	20	14	0.70	20	16	0.80	-0.10	0.00
LCL	0.925			0.48			0.48			0.48			0.58	-0.35	-0.27
UCL	2.325			0.85			0.85			0.85			0.92	0.17	0.27
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 11. POD Results from the Analysis of *Listeria innocua* ATCC 51414 in 125 g frozen breaded chicken (containing 10-fold excess Bacillus subtilis ATCC 27370) after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.45	20	14	0.70	20	14	0.70	20	14	0.70	20	16	0.80	-0.10	0.00
LCL	0.925			0.48			0.48			0.48			0.58	-0.35	-0.27
UCL	2.325			0.85			0.85			0.85			0.92	0.17	0.27
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 12. POD Results from the Analysis of *Listeria welshimeri* ATCC 43548 in 25 g frozen meatballs after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.625	20	14	0.70	20	14	0.70	20	14	0.70	20	12	0.60	0.10	0.00
LCL	1.025			0.48			0.48			0.48			0.39	-0.18	-0.27
UCL	2.5			0.85			0.85			0.85			0.78	0.36	0.27
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 13. POD Results from the Analysis of *Listeria welshimeri* ATCC 43548 in 25 g frozen meatballs after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.625	20	14	0.70	20	14	0.70	20	14	0.70	20	12	0.60	0.10	0.00
LCL	1.025			0.48			0.48			0.48			0.39	-0.18	-0.27
UCL	2.5			0.85			0.85			0.85			0.78	0.36	0.27
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 14. POD Results from the Analysis of *Listeria seeligeri* ATCC 51335 in 25 g whole milk after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.575	20	9	0.45	20	9	0.45	20	9	0.45	20	8	0.40	0.05	0.00
LCL	0.325			0.26			0.26			0.26			0.22	-0.24	-0.28
UCL	1			0.66			0.66			0.66			0.61	0.33	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 15. POD Results from the Analysis of *Listeria seeligeri* ATCC 51335 in 25 g whole milk after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.575	20	9	0.45	20	9	0.45	20	9	0.45	20	8	0.40	0.05	0.00
LCL	0.325			0.26			0.26			0.26			0.22	-0.24	-0.28
UCL	1			0.66			0.66			0.66			0.61	0.33	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 16. POD Results from the Analysis of *Listeria monocytogenes* ATCC 43256 in 25 g ice cream after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.875	20	17	0.85	20	17	0.85	20	17	0.85	20	13	0.65	0.20	0.00
LCL	0.55			0.64			0.64			0.64			0.43	-0.07	-0.23
UCL	1.45			0.95			0.95			0.95			0.82	0.44	0.23
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 17. POD Results from the Analysis of *Listeria monocytogenes* ATCC 43256 in 25 g ice cream after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.875	20	17	0.85	20	17	0.85	20	17	0.85	20	13	0.65	0.20	0.00
LCL	0.55			0.64			0.64			0.64			0.43	-0.07	-0.23
UCL	1.45			0.95			0.95			0.95			0.82	0.44	0.23
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 18. POD Results from the Analysis of *Listeria monocytogenes* M23 in 25 g ricotta cheese after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.125	20	13	0.65	20	13	0.65	20	13	0.65	20	12	0.60	0.05	0.00
LCL	0.7			0.43			0.43			0.43			0.39	-0.23	-0.28
UCL	1.8			0.82			0.82			0.82			0.78	0.32	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 19. POD Results from the Analysis of *Listeria monocytogenes* M23 in 25 g ricotta cheese after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.125	20	13	0.65	20	13	0.65	20	13	0.65	20	12	0.60	0.05	0.00
LCL	0.7			0.43			0.43			0.43			0.39	-0.23	-0.28
UCL	1.8			0.82			0.82			0.82			0.78	0.32	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 20. POD Results from the Analysis of *Listeria welshimeri* ATCC 35897 in 25 g shredded Mexican cheese after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.575	20	13	0.65	20	14	0.70	20	14	0.70	20	18	0.90	-0.20	-0.05
LCL	0.975			0.43			0.48			0.48			0.70	-0.43	-0.32
UCL	2.5			0.82			0.85			0.85			0.97	0.05	0.23
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 21. POD Results from the Analysis of *Listeria welshimeri* ATCC 35897 in 25 g shredded Mexican cheese after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	1.575	20	13	0.65	20	14	0.70	20	14	0.70	20	18	0.90	-0.20	-0.05
LCL	0.975			0.43			0.48			0.48			0.70	-0.43	-0.32
UCL	2.5			0.82			0.85			0.85			0.97	0.05	0.23
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 22. POD Results from the Analysis of *Listeria welshimeri* ATCC 35897 in 125 g shredded Mexican cheese after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.725	20	14	0.65	20	14	0.70	20	14	0.70	20	18	0.90	-0.20	-0.05
LCL	0.425			0.43			0.48			0.48			0.70	-0.43	-0.32
UCL	1.225			0.82			0.85			0.85			0.97	0.05	0.23
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 23. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19115 in 25 g cheese powder after 27 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.725	20	8	0.40	20	8	0.40	20	8	0.40	20	8	0.40	0.00	0.00
LCL	0.425			0.22			0.22			0.22			0.22	-0.28	-0.28
UCL	1.225			0.61			0.61			0.61			0.61	0.28	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 24. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19115 in 25 g cheese powder after 48 h enrichment time. (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.725	20	8	0.40	20	8	0.40	20	8	0.40	20	8	0.40	0.00	0.00
LCL	0.425			0.22			0.22			0.22			0.22	-0.28	-0.28
UCL	1.225			0.61			0.61			0.61			0.61	0.28	0.28
Estimate	>4.5	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	-			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	-			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 25. POD Results from the Analysis of *Listeria welshimeri* ATCC 35897 in 25 g shredded Mexican cheese after 27 h enrichment time (Independent Lab). (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.5	20	7	0.35	20	9	0.45	20	9	0.45	20	8	0.40	0.05	-0.10
LCL	0.25			0.18			0.26			0.26			0.22	-0.24	-0.37
UCL	0.86			0.57			0.66			0.66			0.61	0.33	0.19
Estimate	3.01	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	1.31			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	6.89			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 26. POD Results from the Analysis of *Listeria welshimeri* ATCC 35897 in 25 g shredded Mexican cheese after 48 h enrichment time (Independent Lab). (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.5	20	9	0.45	20	9	0.45	20	9	0.45	20	8	0.40	0.05	0.00
LCL	0.25			0.26			0.26			0.26			0.22	-0.24	-0.28
UCL	0.86			0.66			0.66			0.66			0.61	0.33	0.28
Estimate	3.01	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	1.31			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	6.89			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 27. POD Results from the Analysis of *Listeria welshimeri* ATCC 35897 in 125 g shredded Mexican cheese after 48 h enrichment time (Independent Lab). (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.5	20	4	0.20	20	4	0.20	20	4	0.20	20	8	0.40	-0.20	0.00
LCL	0.25			0.08			0.08			0.08			0.22	-0.44	-0.25
UCL	0.86			0.42			0.42			0.42			0.61	0.08	0.25
Estimate	3.01	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	1.31			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	6.89			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 28. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19114 in 125 g hot dogs after 27 h enrichment time (Independent Lab). (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.63	20	10	0.50	20	10	0.50	20	10	0.50	20	8	0.40	0.10	0.00
LCL	0.35			0.30			0.30			0.30			0.22	-0.19	-0.28
UCL	1.04			0.70			0.70			0.70			0.61	0.37	0.28
Estimate	4.38	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	1.72			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	11.15			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

Table 29. POD Results from the Analysis of *Listeria monocytogenes* ATCC 19114 in 125 g hot dogs after 48 h enrichment time (Independent Lab). (11)

Statistic	Concentration, MPN/sample	Candidate Presumptive (CP)			Candidate Confirmed (CC)			Candidate method (C)			Reference Method (R)			dPOD[1]	
		N[2]	x[3]	POD (CP)	N	x	POD (CC)	N	x	POD (C)	N	x	POD (R)	C vs R	CP vs CC
Estimate	0	5	0	0.00	5	0	0.00	5	0	0.00	5	0	0.00	0.00	0.00
LCL[4]	-			0.00			0.00			0.00			0.00	-0.43	-0.43
UCL[5]	-			0.43			0.43			0.43			0.43	0.43	0.43
Estimate	0.63	20	10	0.50	20	10	0.50	20	10	0.50	20	8	0.40	0.10	0.00
LCL	0.35			0.30			0.30			0.30			0.22	-0.19	-0.28
UCL	1.04			0.70			0.70			0.70			0.61	0.37	0.28
Estimate	4.38	5	5	1.00	5	5	1.00	5	5	1.00	5	5	1.00	0.00	0.00
LCL	1.72			0.57			0.57			0.57			0.57	-0.43	-0.43
UCL	11.15			1.00			1.00			1.00			1.00	0.43	0.43

[1]dPOD, difference in Probability of Detection. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level. [2]N, number of replicates. [3]x, number of positive test results. [4]LCL, lower 95% confidence limit. [5]UCL, upper 95% confidence limit.

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