CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

041101

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

Reveal® 2.0 *Listeria* Test System

manufactured by

Neogen Corportation
620 Lesher Place
Lansing, Michigan 48912

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 23, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

November 23, 2019
Date

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SUBMITTING COMPANY
Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
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KIT NAME(S)
Reveal® 2.0 *Listeria* Test System

CATALOG NUMBERS
9707

INDEPENDENT LABORATORY
Q Laboratories
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Cincinnati, OH
USA

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APPLICABILITY OF METHOD
Target organism – *Listeria* spp. Excluding *L. grayi*

Matrices – (25 g) - Pasteurized liquid egg, ice cream, parmesan cheese, 2% milk, deli turkey, hot dogs, frozen cooked hamburgers, pepperoni, pasteurized crab meat, smoked salmon, (sponge, 4 x 4 in) - stainless steel, concrete; (swab, 1 x 1 in) - ceramic tile, plastic

Performance claims -
Sensitivity relative to the reference methods: foods ≥ 98%, environmental samples ≥ 100%; specificity ≥ 99%.

REFERENCE METHODS
US FDA (2003) *Bacteriological Analytical Manual*, chapter 10
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM071400 (2)
USDA-FSIS (2009) *Microbiology Laboratory Guidebook*, chapter 8.07
http://www.fsis.usda.gov/PDF/MLG_8_07.pdf (3)

ORIGINAL CERTIFICATION DATE
April 01, 2011

CERTIFICATION RENEWAL RECORD
Renewed Annually through December 2020

METHOD MODIFICATION RECORD
1. **November 2018 Level 1**
2. **November 2019 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial/clerical updates to inserts**
2. **Editorial/clerical updates to inserts**

Under this AOAC® *Performance Tested*℠ License Number, 041101 this method is distributed by:
NONE

Under this AOAC® *Performance Tested*℠ License Number, 041101 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The Reveal *Listeria* 2.0 test is a lateral-flow format, immunodiagnostic test that facilitates rapid and accurate detection of *Listeria* spp. in food and environmental samples. A portion (200 µL) of the final enrichment culture is introduced to the Reveal *Listeria* 2.0 device. The sample is wicked through a reagent zone, which contains specific antibodies conjugated to colloidal gold particles. If *Listeria* antigens are present in the sample, they will bind to the colloidal gold conjugated antibodies. This antigen-antibody complex then leaves the reagent zone and travels through the nitrocellulose membrane, which contains a zone of anti-*Listeria* antibodies. The immune complex with colloidal gold conjugate is captured and aggregates in this zone, displaying a visible line. The remainder of the sample continues to migrate to the end of the membrane, where it will eventually be deposited into a waste pad. The reagent zone also contains a colloidal gold conjugate of a second antigen, which is also eluted by the sample. The colloidal gold-conjugated control indicator migrates through the membrane to the negative control capture zone (containing antibody to the second antigen), where it is captured and aggregated to form a visible line. Regardless of the presence of *Listeria* antigen, the control line will form in the control zone, ensuring that the test is working properly. Positive assay results must be confirmed by standard culture methods.

DISCUSSION OF THE VALIDATION STUDY (1)
Results of the internal and independent laboratory validation studies show that the Reveal *Listeria* 2.0 method is an effective procedure for detection of *Listeria* spp. in a variety of foods and environmental samples. Use of the single-step LESS broth enrichment procedure in conjunction with the Reveal assay provides results in 27-30 h with minimal labor. While the method was specifically validated using an enrichment period of 27-30 h, extending the incubation period up to 48 h does not have an adverse effect on results. In the internal trial with hot dogs, enrichment cultures were tested at 27, 30 and 48 h, and Reveal results were identical at each time point (48 h data not shown).
Considering all data from both internal and independent laboratory trials, for foods tested in parallel with the FDA/BAM reference culture procedure, relative sensitivity of the Reveal 2.0 method was 98.3% at 27 h and 101% at 30 h. For foods tested in parallel with the USDA-FSIS reference method, relative sensitivity of Reveal 2.0 was 91.2% at 27 h and 98.2% at 30 h. There were no statistically significant differences in the number of positive results by the Reveal and reference culture procedures in any food trials. For environmental surface samples tested in comparison to the USDA-FSIS method, relative sensitivity of the Reveal method was 119% at 27 h and 127% at 30 h. For plastic and sealed concrete surfaces, there were statistically significant
differences in the number of positives by the Reveal and reference procedures, with the Reveal method detecting more positives in both cases. The Reveal 2.0 assay produced no unconfirmed positive results in any phase of the study, for specificity of 100%.

Table 1. Inclusivity testing results for Reveal *Listeria* 2.0 test (1)

					Reveal 2.0 Result	
Organism	***Serotype***	***Strain***	***Source***	***Origin (if known)***	***1:10 Dilution ~ 10^8 cfu/mL***	***1:100 Dilution ~ 10^7 cfu/mL***
L. grayi	-	GT4800	GENE-TRAK	Environmental	Neg	Neg
L. grayi	-	A203	ATCC19120	Chinchilla feces	Neg	Neg
L. grayi subsp. murrayi	-	A198	GENE-TRAK	-	Neg	Neg
L. innocua	6a	GT3627	H. Seeliger	Cheese	Pos	Pos
L. innocua	6a	GT3631	H. Seeliger	Cheese	Pos	Pos
L. innocua	6a	A102	ATCC33090	Cow brain	Pos	Pos
L. innocua	6b	GT1026	H. Seeliger	Cheese	Pos	Pos
L. innocua	6b	GT1042	H. Seeliger	Cheese	Pos	Pos
L. innocua	6b	GT1044	H. Seeliger	Cheese	Pos	Pos
L. innocua	6b	GT1050	H. Seelifer	Cheese	Pos	Pos
L. innocua	-	GT3785	CDC	-	Pos	Pos
L. innocua	-	GT1052	J. Farber	Raw milk	Pos	Pos
L. ivanovii	5	GT1028	H. Seeliger	Mouse	Pos	Pos
L. ivanovii	5	GT1040	H. Seeliger	Human	Pos	Pos
L. ivanovii	5	GT3699	H. Seeliger	Watercress	Pos	Pos
L. ivanovii	-	A140	ATCC19119	Sheep	Pos	Neg
L. monocytogenes	1/2a	GT3727	H. Seeliger	Human blood	Pos	Pos
L. monocytogenes	1/2a	GT4340	CDC	Fish	Pos	Pos
L. monocytogenes	1/2a	GT1038	H. Seeliger	Human blood	Pos	Pos
L. monocytogenes	1/2b	GT3635	H. Seeliger	Human blood	Pos	Pos
L. monocytogenes	1/2b	GT3728	H. Seeliger	Cheese	Pos	Pos
L. monocytogenes	1/2b	GT3856	H. Seeliger	Cheese	Pos	Pos
L. monocytogenes	1/2c	GT3698	H. Seeliger	Cheese	Pos	Pos
L. monocytogenes	1/2c	GT3648	H. Seeliger	Cheese	Pos	Pos
L. monocytogenes	1/2c	GT3730	H. Seeliger	-	Pos	Pos
L. monocytogenes	1/2c	GT3741	H. Seeliger	-	Pos	Pos
L. monocytogenes	1a	GT3829	C. Donnelly	Raw milk	Pos	Pos
L. monocytogenes	1a	GT1072	C. Donnelly	Raw milk	Pos	Pos
L. monocytogenes	1a	GT1880	J. Lovett	Brie cheese	Pos	Pos

L. monocytogenes	1a	GT3812	J. Lovett	Chocolate milk	Pos	Pos
L. monocytogenes	2	A169	ATCC19112	Human CSF	Pos	Pos
L. monocytogenes	3a	GT3720	H. Seeliger	Cheese	Pos	Pos
L. monocytogenes	3a	GT1035	H. Seeliger	-	Pos	Pos
L. monocytogenes	3b	GT1057	J. Lovett	Brie cheese	Pos	Pos
L. monocytogenes	3b	GT3715	H. Seeliger	Human blood	Pos	Pos
L. monocytogenes	3b	GT3817	H. Seeliger	Cheese	Pos	Pos
L. monocytogenes	3b	GT3857	J. Lovett	Brie cheese	Pos	Pos
L. monocytogenes	4a	A170	ATCC19114	Ruminant brain	Pos	Pos
L. monocytogenes	4b	A207	ATCC13932	Human CSF	Pos	Pos
L. monocytogenes	4b	GT1019	GENE-TRAK	-	Pos	Pos
L. monocytogenes	4b	GT1081	CDC	-	Pos	Pos
L. monocytogenes	4c	GT3819	H. Seeliger	Human	Pos	Pos
L. seeligeri	1/2b	GT3693	H. Seeliger	Sewage	Pos	Pos
L. seeligeri	4a	GT289	H. Seeliger	Cheese	Pos	Pos
L. seeligeri	-	A201	ATCC51334	Vole	Pos	Pos
L. seeligeri	6b	GT3708	H. Seeliger	Cheese	Pos	Pos
L. welshimeri	6a	GT293	H. Seeliger	Cheese	Pos	Pos
L. welshimeri	-	A199	ATCC35897	Plant material	Pos	Pos
L. welshimeri	-	A200	ATCC43550	Soil	Pos	Pos
L. welshimeri	-	GT1773	GENE-TRAK	Environmental isolate	Pos	Pos
L. welshimeri	-	GT1729	GENE-TRAK	Dairy plant	Pos	Pos

Table 2. Exclusivity testing results for Reveal *Listeria* 2.0 test (1)

				Reveal Result			
Organism	***Strain #***	***ATCC #***	***Origin (if known)***	***TSB Undiluted (~ 10^9 cfu/mL)***	***TSB 1:10 (~ 10^8 cfu/mL)***	***LESS Broth***	***Culture Conditions[a]***
Bacillus cereus	A208	25621	Cow dung	Neg	Neg		
Bacillus coagulans	GT811	7050	Milk	Neg	Neg		
Bacillus megaterium	GT2128	14581	-	Neg	Neg		
Bacillus subtilis	GT4402	21556	-	Neg	Neg		
Brevibacillus parabrevis	GT803	8186	Cheese	Neg	Neg		
Brocothrix thermosphacta	GT664	11509	Pork sausage	Neg	Neg		48 hr, BHI broth, CO_2, 25°C
Enterococcus durans	GT407	6056	Human feces	Neg	Neg		
Enterococcus faecalis	GT3242	27275	-	Neg	Neg		
Enterococcus faecium	GT919	6057	Cheese	Neg	Neg		
Enterococcus hirae	GT923	35220	Cow dung	Neg	Neg		
Geobacillus stearothermophilus	GT4373	12980	-	Neg	Neg		
Gordonia sputi	GT3474	29627	Human	Neg	Neg		48 hr, Nutrient broth, CO_2, 37°C
Kocuria rosea	GT1944	185	-	Neg	Neg		48 hr, BHI broth, 26°C
Kocuria varians	GT4404	15306	Milk	Neg	Neg		
Kurthia gibsonii	GT2129	43195	Meat	Neg	Neg		
Kurthia zopfii	GT1941	33403	Turkey cecum	Neg	Neg		
Lactobacillus acidophilus	GT256	ATCC 4356	Human	Neg	Neg		
Lactobacillus buchneri	GT4082	11307	Beer	Pos	Pos	Neg	48 hr, MRS broth, 30°C
Lactobacillus casei	GT805	393	Cheese	Pos	Neg	Neg	
Lactobacillus fermentum	GT4063	9338	-	Pos	Neg	Neg	
Lactococcus lactis	GT3516	11454	-	Neg	Neg		
Micrococcus luteus	GT1943	381	Water	Neg	Neg		
Rhodococcus equi	GT665	6939	Horse	Neg	Neg		
Rhodococcus fascians	GT3524	12974	-	Neg	Neg		48 hr, BHI broth, 26°C
Staphylococcus aureus	A179	12600	Human pleural fluid	Neg	Neg		
Staphylococcus epidermidis	A183	14990	Human	Neg	Neg		
Staphylococcus saprophyticus	A185	15305	Human urine	Neg	Neg		
Streptococcus equi	GT3596	33398	-	Neg	Neg		
Streptococcus gallolyticus	GT668	9809	-	Neg	Neg		
Streptococcus mutans	GT412	25175	Human mouth	Neg	Neg		
Streptococcus pneumoniae	GT408	6303	-	Neg	Neg		
Streptococcus sanguinis	GT411	10556	Human	Neg	Neg		

[a] If other than TSB, 35°C, 24 hr.

Table 3. Results of food sample testing using the Reveal *Listeria* 2.0 test with LESS broth enrichment (1)

Food Type	Inoculum Strain	cfu/g	cfu/ Portion	No. Samples	Reveal Results			Reference Method		Sens (%)[d] 27 h	Sens (%)[d] 30 h	Spec (%)[e]	χ^{2f} 27 h	χ^{2f} 30 h
					27 h[a]	30 h[b]	Confirmed[c]	FDA	USDA					
Deli turkey	*L. seeligeri* GT3693	0.092	2.3	20	18	18	18		14	129	129	-	2.44	2.44
		< 0.03	< 0.75	5	0	0	0		0	-	-	100	-	-
Hot dogs	*L. ivanovii* GT3699	0.036	0.9	20	13	16	16		16	81	100	-	1.10	-
		< 0.03	< 0.75	5	0	0	0		0	-	-	100	-	-
Frozen hamburgers	*L. innocua* A102	0.036	0.9	20	12	12	12		13	92	92	-	0.10	0.10
		< 0.03	< 0.75	5	0	0	0		0	-	-	100	-	-
Frozen hamburgers[g]	*L. innocua* ATCC BAA-680	0.021	0.53	20	5	5	5		10	50	50	-	2.60	2.60
		< 0.03	< 0.75	5	0	0	0		0	-	-	100	-	-
Pepperoni	*L. mono*. 4b GT1081	< 0.03	< 0.75	20	4	5	5		4	100	125	-	0.00	0.14
		< 0.03	< 0.75	5	0	0	0		0	-	-	100	-	-
Pasteurized liquid egg	*L. mono*. 4b A207	0.036	0.9	20	5	5	5	6		83	83	-	0.12	0.12
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-
Vanilla ice cream	*L. mono*. 1/2a GT3727	0.036	0.9	20	13	13	13	16		81	81	-	1.10	1.10
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-
Parmesan cheese	*L. mono*. 1/2b GT4341	0.092	2.3	20	17	17	17	14		121	121	-	1.26	1.26
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-
2% milk	*L. welshimeri* GT293	0.092	2.3	20	10	10	10	11		91	91	-	0.10	0.10
		0.092	2.3	20	17	18	18	15		113	120	-	0.61	1.52
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-
2% milk[g]	*L. welshimeri* ATCC35897	0.093	2.3	20	11	11	11	12		92	92	-	0.10	0.10
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-
Pasteurized crab meat #1	*L. mono*. 1/2a GT4340	0.036	0.9	20	16	17	17	16		100	106	-	0.00	0.17
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-
Pasteurized crab meat #2	*L. mono*. 1/2a GT4340	0.036	0.9	20	16	16	16	13		123	123	-	1.10	1.10
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-
Smoked salmon	*L. mono*. 4b GT1081	0.074	1.8	20	14	15	15	18		78	83	-	2.44	1.52
		< 0.03	< 0.75	5	0	0	0	0		-	-	100	-	-

[a] Number of samples positive by Reveal assay after 27 h enrichment not considering subsequent confirmation.
[b] Number of samples positive by Reveal assay after 30 h enrichment not considering subsequent confirmation.
[c] Number of samples confirmed positive by plating from Reveal method LESS broth enrichment cultures.
[d] Sensitivity of Reveal method relative to that of reference method ([Reveal +/reference +] x 100).
[e] Specificity of Reveal assay ([Reveal -/no. control samples] x 10). Calculated only for uninoculated control samples.
[f] χ^2 value by Mantel-Haenszel formula [5]. $\chi^2 > 3.84$ indicates a statistically significant difference at $\rho < 0.05$.
[g] Trial performed at independent laboratory.

Table 4. Results of environmental sample testing using the Reveal *Listeria* 2.0 test with LESS broth enrichment (1)

Sample	*Inoculum Strain*	*cfu/Surface*	*N*	*Reveal Results*			*USDA Method*	Sens (%)[d] 27 h	Sens (%)[d] 30 h	Spec (%)[e]	χ^{2f}	χ^{2f}
				27 hr[a]	*30 hr*[b]	*Confirmed*[c]						
Ceramic tile surface	*L. innocua* GT3627	1,200	20	6	7	7	7	86	100	-	0.11	0.00
		-	5	0	0	0	0	-	-	100	-	-
Plastic surface	*L. mono*. 4a A170	14,000	20	17	18	18	12	142	150	-	3.06	4.68
		-	5	0	0	0	0	-	-	100	-	-
Stainless steel surface	*L. mono*. 1/2c GT3646 *Enterococcus faecium*	70	20	7	9	9	7	100	129	-	0.00	0.41
		250	5	0	0	0	0	-	-	100	-	-
Stainless steel surface[g]	*L. mono*. 1/2c ATCC7644 *E. faecium*	125	20	7	7	7	7	100	100	-	0.00	0.00
		1,100	5	0	0	0	0	-	-	100	-	-
Sealed concrete surface	*L. welshimeri* A199	26,000	20	20	20	20	15	133	133	-	5.57	5.57
		-	5	0	0	0	0	-	-	100	-	-

[a] Number of samples positive by Reveal assay after 27 h enrichment not considering subsequent confirmation.

[b] Number of samples positive by Reveal assay after 30 h enrichment not considering subsequent confirmation.

[c] Number of samples confirmed positive by plating from Reveal method LESS broth enrichment cultures.

[d] Sensitivity of Reveal method relative to that of reference method ([Reveal +/reference +] x 100).

[e] Specificity of Reveal assay ([Reveal -/no. control samples] x 10). Calculated only for uninoculated control samples.

[f] χ^2 value by Mantel-Haenszel formula [5]. $\chi^2 > 3.84$ indicates a statistically significant difference at $\rho < 0.05$.

[g] Trial performed at independent laboratory.

REFERENCES CITED

1. Alles, Susan, Curry, Stephanie, Almy, Dave, Jagadeesan, Balamurgan, Rice, Jennifer, and Mozola, Mark., Evaluation of Reveal® 2.0 *Listeria* Test for Detection of *Listeria* spp. In Foods and Environmental Samples, AOAC® *Performance Tested*℠ certification number 041101.
2. US FDA (2003) *Bacteriological Analytical Manual*, chapter 10 http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM071400
3. USDA-FSIS (2009) *Microbiology Laboratory Guidebook*, chapter 8.07 http://www.fsis.usda.gov/PDF/MLG_8_07.pdf