



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
030601

The AOAC Research Institute hereby certifies the method known as:

Petrifilm® Environmental *Listeria* (EL) Plate

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
USA

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A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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METHOD NAME Neogen® Petrifilm® Environmental <i>Listeria</i> (EL) Plate Formerly 3M™ Petrifilm™ Environmental <i>Listeria</i> (EL) Plate		CATALOG NUMBERS 6447, 6448	
INDEPENDENT LABORATORY rtech laboratories 1200 W. County Road Arden Hills, MN 55112 USA			
APPLICABILITY OF METHOD Target organism – <i>Listeria monocytogenes</i> , <i>Listeria innocua</i> , and <i>Listeria welshimeri</i> . Matrixes – (enriched in 5 mL of buffered peptone water) Stainless steel (1 in x 1 in), plastic (polypropylene, 1 in x 1 in), ceramic tile (4 in x 4 in), sealed concrete (4 in x 4 in). Performance claims – The Environmental Listeria Plate proves equivalent or better than the reference method.		REFERENCE METHOD USDA-FSIS MLG 8.04; Isolation and Identification of <i>Listeria monocytogenes</i> from Red Meat, Poultry, Egg, and Environmental Samples (3)	
ORIGINAL CERTIFICATION DATE March 06, 2006		CERTIFICATION RENEWAL RECORD Renewed through December 2026.	
METHOD MODIFICATION RECORD 1. November 2017 Level 2 2. November 2018 Level 1 3. January 2024 Level 1		SUMMARY OF MODIFICATION 1. Manufacturing location change from Brookings, SD, USA to Wroclaw, Poland 2. Edits to insert to include PTM Mark, matrixes, clarification of information. 3. Editorial changes to rebrand method from 3M to Neogen Corporation.	
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PRINCIPLE OF THE METHOD (1)

The Petrifilm EL Plate is a dry rehydratable sample-ready-culture medium system containing selective agents, nutrients, a cold-water-soluble gelling agent, and a chromogenic indicator that facilitates *Listeria* detection and enumeration but does not differentiate species from one another. Petrifilm EL Plates are used for testing environmental samples and can increase the efficiency of monitoring processing plant sanitation.

Many organisms can be stressed by environmental conditions or sanitizers; buffered peptone water is used as a repair broth in conjunction with the Petrifilm EL Plate to facilitate resuscitation of stressed *Listeria*. The environmental sample is collected using a swab, moistened sponge or other sampling device. After sample collection, five milliliters of repair broth (buffered peptone water) is added to the sample, and the sample is mixed and then allowed to remain at room temperature for a minimum of 1 hour and up to a maximum of 1.5 hours. The sample is remixed and 3 milliliters is plated onto the Petrifilm EL Plate. The gel is allowed to form and the plate is then incubated for 28 ± 2 hours at 35 ± 1°C or 37 ± 1°C (temperature based on validated references). Red-violet colonies on the plate are *Listeria*.

DISCUSSION OF THE VALIDATION STUDY (1)

The Neogen Petrifilm Environmental *Listeria* Plate detected and enumerated *Listeria* on a variety of environmental surfaces. Four surfaces – stainless steel, ceramic tile, plastic and sealed concrete – were inoculated with *Listeria* low, medium and high levels or *Listeria* at low, medium and high levels plus background flora. *Listeria* was recovered from all surfaces and the log counts from the Petrifilm Environmental *Listeria* Plates were compared with log counts from USDA method.

Two-sample t-tests showed there were no differences between the Petrifilm Environmental *Listeria* Plate method and the USDA method on the plastic and concrete surfaces tested, but there were differences detected on the ceramic and stainless steel, with the Petrifilm trending high. The reasons for the differences between the Petrifilm and the USDA methods are not fully understood but could be due to: 1) testing or 2) stress and/or injury incurred on the stainless steel and ceramic surfaces that was mitigated during the repair step of the Petrifilm method. A study⁷ evaluated the survival of *Listeria monocytogenes* under various conditions on different surfaces. Stainless steel was found to be less supportive of survival than either acetal resin or fiberglass-reinforced plastic wall paneling. Speculation could be made that material characteristics such as hydrophobicity, pH or surface charge influence attachment of cells, and thus, the state of the surviving cells. It may be speculated that a repair step permits greater recovery from injury and thus, higher counts in the completed tests, but this has not been examined.

In the independent laboratory validation study, there was no significant difference in the ability to detect and enumerate a low level of *Listeria* spp. from stainless steel, but as shown with internal testing, the Petrifilm method yielded higher results than the USDA reference method at the medium level. No significant differences in repeatability were detected between the two methods. In strain studies, of the 59 *Listeria* isolates that should have been detected on the Petrifilm Environmental *Listeria* Plate, 57 were detected giving an inclusivity of 97%. Of the 53 bacterial isolates that should not have been detected on the Petrifilm Environmental *Listeria* Plate, 53 were not detected giving an exclusivity of 100%. The Petrifilm Environmental *Listeria* Plate has a shelf life of 18 months when stored at 2-8°C. Assay ruggedness was demonstrated for nine test parameters including sample volume, incubation temperature and plate position in stacks, sample pH, time in the repair broth, the volume of repair broth and the temperature of the repair step on *Listeria* recovery, various repair broths and their effect on *Listeria* recovery, replication of organisms in repair broth, and various times of freezer storage.

These studies have demonstrated that the Petrifilm Environmental *Listeria* Plate is an accurate, specific, sensitive and rugged method that detects and enumerates *Listeria* on a variety of environmental surfaces yielding equivalent or higher values than the USDA-MPN method.

Table 1. Bacteria tested for Petrifilm Environmental *Listeria* Plate inclusivity (1)

#	Genus	species	Reference Number	Serotype	Ribotype	Source
1	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19111	1	unknown	poultry, England
2	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19112	2	unknown	spinal fluid
3	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19113	3	unknown	human
4	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19114	4a	unknown	unknown
5	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19115	4b	unknown	human
6	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19116	4c	unknown	chicken
7	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19117	4d	unknown	sheep
8	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 19118	4e	unknown	chicken
9	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 43256	unknown	unknown	Mexican-style cheese
10	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 43257	unknown	unknown	Mexican-style cheese
11	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 49594	unknown	unknown	derived from Scott A
12	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 51414	unknown	unknown	raw milk
13	<i>Listeria</i>	<i>monocytogenes</i>	ATCC 51772	½a	unknown	cheese, Belgium
14	<i>Listeria</i>	<i>monocytogenes</i>	C1-056	½a	DUP-1030	human
15	<i>Listeria</i>	<i>monocytogenes</i>	C1-115	3a	DUP-1039C	human
16	<i>Listeria</i>	<i>monocytogenes</i>	C1-122	4b	DUP-1038B	human
17	<i>Listeria</i>	<i>monocytogenes</i>	J1-031	4a	DUP-1059A	human
18	<i>Listeria</i>	<i>monocytogenes</i>	J1-049	3c	DUP-1042	human
19	<i>Listeria</i>	<i>monocytogenes</i>	J1-094	½c	DUP-1030	human
20	<i>Listeria</i>	<i>monocytogenes</i>	J1-110	4b	DUP-1038	food epidemic
21	<i>Listeria</i>	<i>monocytogenes</i>	J1-158	4b	DUP-10142	goat
22	<i>Listeria</i>	<i>monocytogenes</i>	J1-168	4a	DUP-1061	human
23	<i>Listeria</i>	<i>monocytogenes</i>	J1-169	3b	DUP-1052	human
24	<i>Listeria</i>	<i>monocytogenes</i>	J1-177	½b	DUP-1024	human
25	<i>Listeria</i>	<i>monocytogenes</i>	J1-225	4b	DUP-1042	human
26	<i>Listeria</i>	<i>monocytogenes</i>	J2-020	½a	DUP- 1044/dd1049	cow
27	<i>Listeria</i>	<i>monocytogenes</i>	J2-031	½a	DUP- 1039/dd6362	bovine
28	<i>Listeria</i>	<i>monocytogenes</i>	J2-035	½b	dd3581	goat
29	<i>Listeria</i>	<i>monocytogenes</i>	J2-054	½a	DUP- 1045/dd1067	sheep
30	<i>Listeria</i>	<i>monocytogenes</i>	J2-063	½a	DUP- 1047/dd1153	sheep
31	<i>Listeria</i>	<i>monocytogenes</i>	J2-064	½b	DUP- 1052/dd1962	cow
32	<i>Listeria</i>	<i>monocytogenes</i>	J2-066	½a	DUP- 1054/dd3075	Sheep
33	<i>Listeria</i>	<i>monocytogenes</i>	M1-004	unknown	DUP-1039B	human
34	<i>Listeria</i>	<i>monocytogenes</i>	N1-225	4b	DUP-1044A	human
35	<i>Listeria</i>	<i>monocytogenes</i>	W1-110	4c	dd 3823	unknown
36	<i>Listeria</i>	<i>monocytogenes</i>	W1-111	4c	dd 6821	unknown
37	<i>Listeria</i>	<i>monocytogenes</i>	X1-010	4a	dd 6824	unknown
38	<i>Listeria</i>	<i>monocytogenes</i>	RT-54	½a	unknown	beer
39	<i>Listeria</i>	<i>monocytogenes</i>	RT-1637	4b	unknown	poultry

40	<i>Listeria</i>	<i>monocytogenes</i>	RT-472	½b	unknown	unknown
41	<i>Listeria</i>	<i>monocytogenes</i>	A-1	unknown	unknown	food plant
42	<i>Listeria</i>	<i>innocua</i>	ATCC 33090	unknown	unknown	cow brain
43	<i>Listeria</i>	<i>innocua</i>	ATCC 33091	unknown	unknown	human feces
44	<i>Listeria</i>	<i>innocua</i>	ATCC 49595	unknown	unknown	derived from 33090
45	<i>Listeria</i>	<i>innocua</i>	ATCC 51742	unknown	unknown	cabbage
46	<i>Listeria</i>	<i>innocua</i>	Li2236	unknown	unknown	unknown
47	<i>Listeria</i>	<i>innocua</i>	Li2248	unknown	unknown	unknown
48	<i>Listeria</i>	<i>innocua</i>	19B	unknown	unknown	cheese plant
49	<i>Listeria</i>	<i>innocua</i>	21A	unknown	unknown	cheese plant
50	<i>Listeria</i>	<i>welshimeri</i>	ATCC 35897	6b	unknown	decaying plant matter
51	<i>Listeria</i>	<i>welshimeri</i>	RT-7233	unknown	unknown	unknown
52	<i>Listeria</i>	<i>welshimeri</i>	A-7	unknown	unknown	food plant
53	<i>Listeria</i>	<i>welshimeri</i>	H6-105	unknown	unknown	Food
54	<i>Listeria</i>	<i>welshimeri</i>	H6-017	unknown	unknown	Food environment
55	<i>Listeria</i>	<i>welshimeri</i>	ATCC 43548	6a	unknown	unknown
56	<i>Listeria</i>	<i>welshimeri</i>	ATCC 43549	6b	unknown	unknown
57	<i>Listeria</i>	<i>welshimeri</i>	ATCC 43550	1 / 2b	unknown	Cornfield soil
58	<i>Listeria</i>	<i>welshimeri</i>	ATCC 49591	unknown	unknown	unknown
59	<i>Listeria</i>	<i>welshimeri</i>	ATCC 43551	6a	unknown	Feces from healthy human male

Table 2. Bacteria tested for Petrifilm Environmental *Listeria* Plate exclusivity (1)

#	Genus	species	Reference Number	Source
1	<i>Bacillus</i>	<i>cereus</i>	ATCC 13061	unknown
2	<i>Bacillus</i>	<i>circulans</i>	ATCC 61	unknown
3	<i>Bacillus</i>	<i>coagulans</i>	ATCC 7050	evaporated milk
4	<i>Bacillus</i>	<i>coagulans</i>	ATCC 23498	rhizosphere
5	<i>Bacillus</i>	<i>pumilus</i>	ATCC 72	unknown
6	<i>Bacillus</i>	<i>pumilus</i>	A-6	food plant environment
7	<i>Bacillus</i>	<i>subtilis</i>	ATCC 6051	unknown
8	<i>Bacillus</i>	<i>subtilis</i>	ATCC 23856	unknown
9	<i>Bacillus</i>	<i>subtilis</i>	ATCC 29056	unknown
10	<i>Brevibacterium</i>	<i>linens</i>	ATCC 9172	unknown
11	<i>Enterococcus</i>	<i>faecalis</i>	ATCC 6055	pasteurized milk
12	<i>Enterococcus</i>	<i>faecalis</i>	ATCC 7080	meat
13	<i>Enterococcus</i>	<i>faecalis</i>	ATCC 14506	unknown
14	<i>Enterococcus</i>	<i>faecalis</i>	ATCC 29212	human
15	<i>Enterococcus</i>	<i>faecium</i>	ATCC 882	cheese
16	<i>Enterococcus</i>	<i>faecium</i>	ATCC 12952	citrus juice
17	<i>Enterococcus</i>	<i>faecium</i>	ATCC 35667	unknown
18	<i>Enterococcus</i>	<i>faecium</i>	ATCC 49624	cheese
19	<i>Erysipelothrix</i>	<i>rhusiopathiae</i>	ATCC 19414	spleen of pig
20	<i>Escherichia</i>	<i>coli</i>	ATCC 33456	water
21	<i>Kurthia</i>	<i>zopfii</i>	ATCC 6900	unknown
22	<i>Lactobacillus</i>	<i>alimentarius</i>	ATCC 29643	marinated fish product
23	<i>Lactobacillus</i>	<i>brevis</i>	Bbr	food plant
24	<i>Lactobacillus</i>	<i>faracinis</i>	ATCC 29644	sausage
25	<i>Lactobacillus</i>	<i>fermentum</i>	ATCC 9338	unknown
26	<i>Lactobacillus</i>	<i>johnsonii</i>	ATCC 11506	unknown
27	<i>Lactobacillus</i>	<i>plantarum</i>	ATCC 49445	ground pork
28	<i>Lactococcus</i>	<i>lactis subsp. cremoris</i>	ATCC 9596	unknown
29	<i>Lactococcus</i>	<i>lactis subsp. lactis</i>	ATCC 19435	unknown
30	<i>Listeria</i>	<i>grayi</i>	ATCC 19120	chinchilla feces
31	<i>Listeria</i>	<i>grayi</i>	25401	Standing corn stalks
32	<i>Listeria</i>	<i>grayi</i>	ATCC 25402	Standing corn stalks
33	<i>Listeria</i>	<i>grayi</i>	ATCC 25403	Standing corn stalks
34	<i>Listeria</i>	<i>grayi</i>	ATCC 700545	unknown
35	<i>Listeria</i>	<i>grayi</i>	RT7358	unknown
36	<i>Listeria</i>	<i>ivanovii</i>	ATCC 19119	sheep, Bulgaria
37	<i>Listeria</i>	<i>ivanovii</i>	ATCC 49954	Food, France

38	<i>Listeria</i>	<i>ivanovii</i>	C2-010	Animal
39	<i>Listeria</i>	<i>ivanovii</i>	C2-011	Animal
40	<i>Listeria</i>	<i>ivanovii</i>	ATCC 49953	Goat, Belgium
41	<i>Listeria</i>	<i>seeligeri</i>	ATCC 35967	soil, Germany
42	<i>Listeria</i>	<i>seeligeri</i>	H6-011	Food
43	<i>Listeria</i>	<i>seeligeri</i>	H6-169	Food environment
44	<i>Listeria</i>	<i>seeligeri</i>	ATCC 51334	Vole intestinal contents
45	<i>Listeria</i>	<i>seeligeri</i>	ATCC 51335	unknown
46	<i>Pediococcus</i>	<i>acidilacris</i>	PA	unknown
47	<i>Pediococcus</i>	<i>pentosaceus</i>	PP	unknown
48	<i>Pseudomonas</i>	<i>fragi</i>	ATCC 51821	milk
49	<i>Staphylococcus</i>	<i>aureus</i>	ATCC 25923	clinical
50	<i>Staphylococcus</i>	<i>sciuri</i>	P89	clinical
51	<i>Streptococcus</i>	<i>mutans</i>	ATCC 25175	carious dentine
52	<i>Streptococcus</i>	<i>sanguis</i>	ATCC 10556	clinical
53	<i>Streptococcus</i>	<i>viridans</i>	M1040	clinical

Table 12. MEAN log₁₀ counts, repeatability [standard deviation] and relative repeatability [relative standard deviation] for Petrifilm method and USDA method; *p* value shown for each level [low, medium & high]. (1)

Surface <i>p</i> value	Level	Mean log ₁₀ Petrifilm	Sr Petrifilm	RSDr, % Petrifilm	Mean log ₁₀ USDA	Sr USDA	RSDr, % USDA
Plastic	Non	-0.48			-0.52		
<i>p</i> = 0.33	Low	0.31	0.48	152	0.59	0.41	69.4
<i>p</i> = 0.49	Med	0.96	0.41	42.7	1.26	0.76	0.60
<i>p</i> = 0.48	High	1.52	0.41	26.9	1.47	0.69	46.9
Concrete	Non	-0.48			-0.52		
<i>p</i> = 0.18	Low	-0.13	0.29	126	-0.005	0.42	4200
<i>p</i> = 0.11	Med	0.67	0.53	79.1	0.96	0.49	51.0
<i>p</i> = 0.05	High	1.98	0.29	14.6	1.64	0.55	33.5
Stainless steel	Non	-0.48			-0.52		
<i>p</i> = 0.13	Low	1.36	0.17	12.5	1.17	0.33	28.2
<i>p</i> = 0.00	Med	2.92	0.30	10.2	1.57	0.33	21.0
<i>p</i> = 0.00	High	3.78	0.17	4.4	2.48	0.55	22.1
Ceramic tile	Non	-0.48			-0.52		
<i>p</i> = 0.00	Low	0.92	0.32	34.7	0.17	0.44	258
<i>p</i> = 0.00	Med	1.27	0.37	29.1	0.42	0.44	105
<i>p</i> = 0.00	High	1.49	0.25	16.7	0.19	0.38	200

Table 13. MEAN arithmetic counts for Petrifilm method and USDA method. Confidence intervals for USDA-MPN are estimated based on the mean USDA-MPN values. (1)

Surface	Level	Mean Petrifilm CFU/mL	LOWER 95% Confidence Interval USDA MPN/mL	Mean USDA MPN/mL	UPPER 95% Confidence Interval USDA MPN/mL
Plastic	Non	<0.33	--	<0.30	0.95
	Low	2.0	0.87	3.90	11.0
	Med	9.1	4.0	18.2	42.0
	High	33.1	9.0	29.5	100
Concrete	Non	<0.33	--	<0.30	0.95
	Low	0.7	1.36	1.00	1.8
	Med	4.7	1.8	9.10	42.0
	High	95.5	9.0	43.6	200
Stainless steel	Non	<0.33	--	<0.30	0.95
	Low	23.0	3.7	14.8	42.0
	Med	832.0	8.7	37.1	110.0
	High	6025.0	90.0	302.0	1000.0
Ceramic tile	Non	<0.33	--	<0.30	0.95
	Low	8.3	0.45	1.5	4.2
	Med	18.6	0.87	2.6	9.4
	High	31.0	0.45	1.5	4.2

Independent Laboratory Results: Table 14. Statistical analysis of the low and medium inoculation levels. (1)

Statistic	LOW		MEDIUM	
	Neogen	USDA	Neogen	USDA
Mean log CFU or MPN index	2.15	1.87	3.51	2.76
Standard deviation	0.22	0.17	0.06	0.08
Relative standard deviation	10.23	9.09	1.71	2.89
P value	0.18		0.00	

Independent Laboratory Results: Table 15. Internal stainless steel testing and independent stainless steel testing (1)

Inoculum level	LOW		MEDIUM		HIGH	
Mean log CFU or MPN index	Neogen	USDA	Neogen	USDA	Neogen	USDA
Internal mean	1.36	1.17	2.92	1.57	3.78	2.48
Independent mean	2.15	1.87	3.51	2.76	>3.04	>3.04
Internal Sr	0.17	0.33	0.30	0.33	0.33	0.55
Independent Sr	0.22	0.17	0.06	0.08	-	-
Internal RSDr, %	12.5	28.2	10.2	21.0	4.4	22.1
Independent RSDr, %	10.23	9.09	1.71	2.89	-	-

REFERENCES CITED

1. Zook, C. and Horter, B., Evaluation of the 3M™ Petrifilm™ Environmental *Listeria* (EL) Plate , AOAC Performance Tested MethodsSM certification number 030601.
2. AOAC Research Institute Validation Outline for 3M™ Petrifilm™ Environmental *Listeria* (EL) Plate, Approved – March 2006.
3. USDA-FSIS MLG 8.04; Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg, and Environmental Samples; http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp