CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

080701

The AOAC Research Institute hereby certifies the method known as:

RAPID'*Listeria* spp. Agar

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Signature for AOAC Research Institute

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METHOD NAME
RAPID'*Listeria* spp. Agar

CATALOG NUMBERS
356-4744, 356-4745, 356-4746

INDEPENDENT LABORATORY
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APPLICABILITY OF METHOD
Target organism – *Listeria* spp.

Matrixes – stainless steel, plastic, ceramic, and sealed concrete

Performance claims – RAPID'*Listeria* spp is a chromogenic medium for isolation and detection of *Listeria* spp from environmental surfaces.

REFERENCE METHOD
***Microbiology Laboratory Guidebook* (October 1, 2004) U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science, Chapter 8.05. (2)**

ORIGINAL CERTIFICATION DATE
August 15, 2007

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **January 2020 Level 1**
2. **January 2020 Level 1**
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4. **October 2021 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial/clerical changes and reformatting of insert.**
2. **Editorial/clerical changes.**
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4. **Editorial/clerical changes.**

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PRINCIPLE OF THE METHOD (1)
RAPID'*Listeria* spp is a selective chromogenic plating medium for the isolation and the presumptive identification of all species of *Listeria*. The cultural properties of the medium are based on a balance of carefully selected growth-promoting nutrients, enzymatic enhancers and a mixture of selective agents, including lithium chloride and nalidixic acid. The presumptive chromogenic identification system relies on a chromogenic substrate that allows the detection of the β-D-glucosidase activity, an enzyme common to all species of *Listeria*. The hydrolysis of the substrate leads to the formation of a colored precipitate and all the presumptive *Listeria* positive colonies are blue. Combined with this principle, RAPID'*Listeria* spp utilizes an original selective mixture which enables the inhibition of most background flora. The few non-*Listeria* bacteria that do grow on the agar will produce colorless colonies.

DISCUSSION OF THE VALIDATION STUDY (1)
Chromogenic media rely on the enzymatic activity of certain target organisms. The chromogens are selected based on the properties of these specific target organisms. These specific compounds, in addition to a selective mixture, make these media selective and specific. The use of chromogenic media has been demonstrated in previous studies (3,4). This highly selective media can reduce time to results. In this validation study, a modification of the reference method protocol was tested. After a primary enrichment in UVM for 24h, samples were plated to RAPID'*Listeria* spp, for results 24h sooner than the reference method. There was no significant difference between the 48h reference method and the 24h RAPID'*Listeria* spp method. Routine testing of the food processing environment for the presence of *Listeria* spp. as part of a Hazard Analysis of Critical Control Point (HACCP) program is a way to ensure the sanitation of the processing plant and of the food it produces. Results 24h sooner can be an early alert that conditions exist that can potentially support the growth of pathogenic organisms, like *Listeria monocytogenes*. In addition, since the RAPID'*Listeria* spp method only uses one broth, as opposed to the two broths that the reference method used, there is a cost savings as well.

Strain	Reference	Origin	Typical colonies
Listeria monocytogenes 1/2 a	L7	Munster (raw milk cheese)	+
Listeria monocytogenes 1/2 a	L10	Potted meat	+
Listeria monocytogenes 1/2 a	L12	Smoked salmon	+
Listeria monocytogenes 1/2 a	L128	Soy bean cattle cake	+
Listeria monocytogenes 1/2 b	L13	Pork ears in jelly	+
Listeria monocytogenes 1/2 b	L37	Maroille (raw milk cheese)	+
Listeria monocytogenes 1/2 b	L51	Germain (raw milk cheese)	+
Listeria monocytogenes 1/2 c	L17	Pork breast	+
Listeria monocytogenes 1/2 c	L18	Munster (raw milk cheese)	+
Listeria monocytogenes 1/2 c	L54	Beef Bourguignon	+
Listeria monocytogenes 1/2 c	L117	Montbéliard sausage	+
Listeria monocytogenes 3 a	L191	Fishery environment	+
Listeria monocytogenes 3 a	L192	Fishery environment	+
Listeria monocytogenes 3 b	L55	SLCC 2540 (human)	+
Listeria monocytogenes 3 b	L193	Fishery environment	+
Listeria monocytogenes 3 c	L56	SLCC 2479	+
Listeria monocytogenes 4 a	L57	ATCC 19114 (ruminant brain)	+
Listeria monocytogenes 4 b	L32	Munster (raw milk cheese)	+
Listeria monocytogenes 4 b	L58	Salad	+
Listeria monocytogenes 4 d	L60	ATCC 19117 (Sheep)	+
Listeria monocytogenes 4 d	L194	Fishery environment	+
Listeria monocytogenes 4 e	L62	Reblochon (raw milk cheese)	+
Listeria monocytogenes 4 e	L63	Munster (raw milk cheese)	+
Listeria monocytogenes 7	L67	SLCC 2482 (human feces)	+
Listeria innocua 6 a	L1	ATCC 33090 (cow brain)	+
Listeria innocua 6 a	L77	Toulouse sausage	+
Listeria innocua 6 b	L76	Ground meat	+
Listeria innocua 6 b	L144	Bin	+
Listeria innocua	L88	Pork sausage	+
Listeria innocua	L175	Process water	+
Listeria ivanovii 5	L151	Ground meat	+
Listeria ivanovii 5	L153	Environment	+
Listeria ivanovii 5	L154	Sausages with herbs	+
Listeria ivanovii 5	L182	Environment	+
Listeria ivanovii 5	L184	Birds trap	+
Listeria welshimeri 6 a	L89	Ground meat	+
Listeria welshimeri 6 b	L90	Ground meat	+
Listeria welshimeri 6 b	L86	ATCC 35897 (decaying plant material)	+
Listeria welshimeri	L91	Dried pork sausage	+
Listeria welshimeri	L101	Ham	+
Listeria welshimeri	L155	Raw salmon fillet	+
Listeria welshimeri	L174	Spinach	+
Listeria seeligeri 1/2 b	L82	ATCC 35897 (soil)	+
Listeria seeligeri 1/2 b	L83	Ox tongue	+
Listeria seeligeri 1/2 b	L84	Ground meat	+
Listeria seeligeri	L115	Lake water sampling	+
Listeria seeligeri	L140	Frozen french fries	+
Listeria seeligeri	L189	Frozen french fries	+
Listeria grayi	L81	ATCC 19120 (animal feces)	+
Listeria grayi	L143	Frozen french fries	-
Listeria grayi	L188	Environment	+

ATCC = American Type Culture Collection, USA
L = *Listeria* culture collection, Institut Pasteur de Lille, France
SLCC = Seeliger's Listeria Culture Collection, Würzburg, Germany

Table 2 – Exclusivity Results (1)

Strain	Reference	Origin	Colony color
Bacillus cereus	IPL-BA1	Raw egg	No growth
Bacillus cereus	IPL-BA2	Beet root	No growth
Bacillus cereus	IPL-BA3	Plant	No growth
Bacillus cereus	IPL-BA9	Dehydrated potatoes	No growth
Bacillus cereus	IPL-BA14	Custard	No growth
Bacillus cereus	IPL-BA15	Custard	No growth
Bacillus cereus	IPL-BA19	Environment	No growth
Bacillus cereus	IPL-BA21	Tabbouleh with poultry	No growth
Bacillus cereus	IPL-BA11778	ATCC 11778	No growth
Bacillus coagulans	IPL-BA7	Milk product	No growth
Bacillus mycoïdes	IPL-BA6	Environment	No growth
Bacillus mycoïdes	IPL-BA24	Soil	No growth
Bacillus pumilus	IPL-BA22	Tabbouleh with poultry	No growth
Bacillus sphaericus	IPL-BA5	Meat product	No growth
Bacillus sphaericus	IPL-BA23	Environment	No growth
Bacillus stearothermophilus	IPL-BA4	Milk product	No growth
Brochotrix thermosphacta	IPL-15	Ground meat	No growth
Carnobacterium divergens	IPL-46	Minced beef	No growth
Carnobacterium gallinarum	IPL-47	Ice slush of chicken carcasses	No growth
Carnobacterium pisicola	IPL-48	Raw milk	No growth
Citrobacter braakii	IPL-CIT86	Pork sausage	No growth
Citrobacter freundii	IPL-CIT24	Meat product	No growth
Corynebacterium flavescens	IPL-COR1	ATCC 10340 (cheese)	No growth
Corynebacterium variabile	IPL-COR2	ATCC 15753 (food)	No growth
Escherichia coli	IPL-EC20	Tomatoes	No growth
Escherichia coli	IPL-EC21	Celery with mayonnaise	No growth
Enterobacter cloacae	IPL-ENT76	Milk powder	No growth
Enterococcus faecalis	IPL-E1	Egg product	No growth
Enterococcus faecalis	IPL-E6	ATCC 19433	No growth
Enterococcus faecium	IPL-E2	ATCC 3286	No growth
Enterococcus faecium	IPL-E7	CIP 54.33 (Canned fish)	No growth
Enterococcus faecium	IPL-E9	Taramasalata	No growth
Enterococcus durans	IPL-E8	Meat product	No growth
Enterococcus durans	IPL-E10	Meat product	Light blue *
Enterococcus durans	IPL-E331	RDC 486	No growth
Enterococcus durans	IPL-E332	RDC 487	No growth
Enterococcus durans	IPL-E19432	ATCC 19432	No growth
Erysipelothrix rhusiopathiae	IPL-49	Spleen of pig with endocarditis	No growth
Jonesia denitrificans	IPL139	CIP 55134T	Colorless
Klebsiella pneumoniae	IPL-EN63	Celery	No growth
Klebsiella pneumoniae	IPL-EN68	Vegetable salad	No growth
Kurthia gibsonii	IPL-42	Meat product	No growth
Lactobacillus acidophilus	IPL-Lb2885	RDC 488	No growth
Lactobacillus casei	IPL-L33	Milk product	No growth
Lactobacillus casei	IPL-Lb9595	ATCC 9595	No growth
Lactobacillus bulgaricus	IPL-Lb120	RDC120	No growth
Lactobacillus fermentum	IPL-Lb9338	ATCC 9338	No growth
Lactobacillus lactis	IPL-L54	Emmental cheese	No growth
Lactobacillus paracasei	IPL-L35	Milk product	No growth
Lactobacillus plantarum	IPL-L34	Milk product	No growth
Lactobacillus spp	IPL-Lb11506	ATCC 11506	No growth
Lactococcus lactis	IPL-LL	Milk product	No growth
Lactococcus lactis	IPL-Lc7056	CIP 70.56	No growth
Micrococcus spp	IPL-M1	Environment	No growth
Pediococcus acidilactici	IPL-Pd240	RDC 240	No growth
Pediococcus damnosus	IPL-Pd29358	Beer	No growth
Pediococcus damnosus	IPL-Pd102264	Beer	No growth
Pediococcus pentosaceus	IPL-Pd119	Beer	No growth
Propionibacterium freundenreichii	IPL-43	Swiss cheese	No growth
Proteus mirabilis	IPL-EN45	Poultry	No growth
Pseudomonas putida	IPL-PS87	Fish	No growth
Pseudomonas putida	IPL-PS90	Fish	No growth
Rhodococcus equi	IPL-32	Meat product	Colorless
Rhodococcus equi	IPL-R2	Lung abscess of foal	No growth

Rhodotorula rubra	IPL-Le1	Pastry	Colorless
Saccharomyces cerevisiae	IPL-Le5	Coffee extract	No growth
Salmonella brandenburg	IPL-3	Pâté	No growth
Salmonella typhimurium	IPL-S31	Egg product	No growth
Salmonella virchow	IPL-S33	Cockle	No growth
Staphylococcus aureus	IPL-ST16	Meat product	No growth
Staphylococcus aureus	IPL-ST17	Frozen yogurt	No growth
Staphylococcus cohnii	IPL-ST21	Smoked salmon	No growth
Staphylococcus epidermidis	IPL-ST3	Yogurt	No growth
Staphylococcus epidermidis	IPL-ST20	Smoked salmon	No growth
Streptococcus anginosus	IPL-Str611	CIP 102921T	No growth
Streptococcus anginosus	IPL-Str1068	CIP 105031	No growth
Streptococcus bovis	IPL-E3	Meat product	No growth
Streptococcus bovis	IPL-Str44	RDC 44	No growth
Streptococcus bovis	IPL-Str5623	CIP 56.23	No growth
Streptococcus equinus	IPL-Str1074	CIP 102504T	No growth
Streptococcus intermedius	IPL-Str1201	CIP 103248T	No growth
Streptococcus salivarius	IPL-Str1075	CIP 102505	No growth
Streptococcus salivarius	IPL-Str1115	CIP 53.158	No growth

* Two colonies of *E. durans* (ref # L-E10) grew on the plate; they were pinpoint and not typical color of *Listeria* spp. This organism would not be confused for *Listeria* spp by the user.

ATCC = American Type Culture Collection, USA
CIP = Collection Institute Pasteur, France
IPL = Culture collection, Institut Pasteur de Lille, France
RDC = Culture collection, Bio-Rad Laboratories, France

Table 4 – Method Comparison Results (1)

Surface	Level	Inoculation	# samples	RAPID'Listeria spp positive	Reference positive	Method Agreement	X2
Stainless steel	Control	0	5	0	0	100%	-
(internal)	Low	$3.1x10^3$	20	19	19	100%	-
Stainless steel	Control	0	5	0	0	100%	-
(independent)	Low	$2.7x10^2$	20	19	19	100%	-
Plastic	Control	0	5	0	0	100%	-
	Low	$2.3x10^3$	20	8	8	100%	-
Ceramic	Control	0	5	0	0	100%	-
	Low	$2.3x10^3$	20	5	8	85%	1.33
Sealed concrete	Control	0	5	0	0	100%	-
	Low	$8.7x10^2$	20	17	19	90%	0.50

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