



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

Bradley A. Stawick, AOAC Research Institute Senior Director

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| METHOD NAME RAPID' <i>Listeria</i> spp. Agar | | CATALOG NUMBERS 356-4744, 356-4745, 356-4746 | |
| INDEPENDENT LABORATORY rtech Laboratories 4001 Lexington Ave. North Arden Hills, MN 55112 | | | |
| APPLICABILITY OF METHOD Target organism – <i>Listeria</i> spp. Matrixes – stainless steel, plastic, ceramic, and sealed concrete Performance claims – RAPID' <i>Listeria</i> spp is a chromogenic medium for isolation and detection of <i>Listeria</i> spp from environmental surfaces. | | REFERENCE METHOD <i>Microbiology Laboratory Guidebook</i> (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 2026. | |
| METHOD MODIFICATION RECORD 1. January 2020 Level 1 2. January 2020 Level 1 3. October 2021 Level 1 4. October 2021 Level 1 5. November 2024 Level 1 | | SUMMARY OF MODIFICATION 1. Editorial changes and reformatting of insert. 2. Editorial changes. 3. Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian. 4. Editorial changes. 5. Insert version change for editorial 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 |
|-------------------------------------|-----------|--------------------------------------|------------------|
| <i>Listeria monocytogenes</i> 1/2 a | L7 | Munster (raw milk cheese) | + |
| <i>Listeria monocytogenes</i> 1/2 a | L10 | Potted meat | + |
| <i>Listeria monocytogenes</i> 1/2 a | L12 | Smoked salmon | + |
| <i>Listeria monocytogenes</i> 1/2 a | L128 | Soy bean cattle cake | + |
| <i>Listeria monocytogenes</i> 1/2 b | L13 | Pork ears in jelly | + |
| <i>Listeria monocytogenes</i> 1/2 b | L37 | Maroille (raw milk cheese) | + |
| <i>Listeria monocytogenes</i> 1/2 b | L51 | Germain (raw milk cheese) | + |
| <i>Listeria monocytogenes</i> 1/2 c | L17 | Pork breast | + |
| <i>Listeria monocytogenes</i> 1/2 c | L18 | Munster (raw milk cheese) | + |
| <i>Listeria monocytogenes</i> 1/2 c | L54 | Beef Bourguignon | + |
| <i>Listeria monocytogenes</i> 1/2 c | L117 | Montbéliard sausage | + |
| <i>Listeria monocytogenes</i> 3 a | L191 | Fishery environment | + |
| <i>Listeria monocytogenes</i> 3 a | L192 | Fishery environment | + |
| <i>Listeria monocytogenes</i> 3 b | L55 | SLCC 2540 (human) | + |
| <i>Listeria monocytogenes</i> 3 b | L193 | Fishery environment | + |
| <i>Listeria monocytogenes</i> 3 c | L56 | SLCC 2479 | + |
| <i>Listeria monocytogenes</i> 4 a | L57 | ATCC 19114 (ruminant brain) | + |
| <i>Listeria monocytogenes</i> 4 b | L32 | Munster (raw milk cheese) | + |
| <i>Listeria monocytogenes</i> 4 b | L58 | Salad | + |
| <i>Listeria monocytogenes</i> 4 d | L60 | ATCC 19117 (Sheep) | + |
| <i>Listeria monocytogenes</i> 4 d | L194 | Fishery environment | + |
| <i>Listeria monocytogenes</i> 4 e | L62 | Reblochon (raw milk cheese) | + |
| <i>Listeria monocytogenes</i> 4 e | L63 | Munster (raw milk cheese) | + |
| <i>Listeria monocytogenes</i> 7 | L67 | SLCC 2482 (human feces) | + |
| <i>Listeria innocua</i> 6 a | L1 | ATCC 33090 (cow brain) | + |
| <i>Listeria innocua</i> 6 a | L77 | Toulouse sausage | + |
| <i>Listeria innocua</i> 6 b | L76 | Ground meat | + |
| <i>Listeria innocua</i> 6 b | L144 | Bin | + |
| <i>Listeria innocua</i> | L88 | Pork sausage | + |
| <i>Listeria innocua</i> | L175 | Process water | + |
| <i>Listeria ivanovii</i> 5 | L151 | Ground meat | + |
| <i>Listeria ivanovii</i> 5 | L153 | Environment | + |
| <i>Listeria ivanovii</i> 5 | L154 | Sausages with herbs | + |
| <i>Listeria ivanovii</i> 5 | L182 | Environment | + |
| <i>Listeria ivanovii</i> 5 | L184 | Birds trap | + |
| <i>Listeria welshimeri</i> 6 a | L89 | Ground meat | + |
| <i>Listeria welshimeri</i> 6 b | L90 | Ground meat | + |
| <i>Listeria welshimeri</i> 6 b | L86 | ATCC 35897 (decaying plant material) | + |
| <i>Listeria welshimeri</i> | L91 | Dried pork sausage | + |
| <i>Listeria welshimeri</i> | L101 | Ham | + |
| <i>Listeria welshimeri</i> | L155 | Raw salmon fillet | + |
| <i>Listeria welshimeri</i> | L174 | Spinach | + |
| <i>Listeria seeligeri</i> 1/2 b | L82 | ATCC 35897 (soil) | + |
| <i>Listeria seeligeri</i> 1/2 b | L83 | Ox tongue | + |
| <i>Listeria seeligeri</i> 1/2 b | L84 | Ground meat | + |
| <i>Listeria seeligeri</i> | L115 | Lake water sampling | + |
| <i>Listeria seeligeri</i> | L140 | Frozen french fries | + |
| <i>Listeria seeligeri</i> | L189 | Frozen french fries | + |
| <i>Listeria grayi</i> | L81 | ATCC 19120 (animal feces) | + |
| <i>Listeria grayi</i> | L143 | Frozen french fries | - |
| <i>Listeria grayi</i> | L188 | Environment | + |

ATCC = American Type Culture Collection, USA

L = *Listeria* culture collection, Institut Pasteur de Lille, FranceSLCC = Seeliger's *Listeria* Culture Collection, Würzburg, Germany

Table 2 – Exclusivity Results (1)

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

| | | | |
|-----------------------------------|-------------|----------------|-----------|
| <i>Rhodotorula rubra</i> | IPL-Le1 | Pastry | Colorless |
| <i>Saccharomyces cerevisiae</i> | IPL-Le5 | Coffee extract | No growth |
| <i>Salmonella brandenburg</i> | IPL-3 | Pâté | No growth |
| <i>Salmonella typhimurium</i> | IPL-S31 | Egg product | No growth |
| <i>Salmonella virchow</i> | IPL-S33 | Cockle | No growth |
| <i>Staphylococcus aureus</i> | IPL-ST16 | Meat product | No growth |
| <i>Staphylococcus aureus</i> | IPL-ST17 | Frozen yogurt | No growth |
| <i>Staphylococcus cohnii</i> | IPL-ST21 | Smoked salmon | No growth |
| <i>Staphylococcus epidermidis</i> | IPL-ST3 | Yogurt | No growth |
| <i>Staphylococcus epidermidis</i> | IPL-ST20 | Smoked salmon | No growth |
| <i>Streptococcus anginosus</i> | IPL-Str611 | CIP 102921T | No growth |
| <i>Streptococcus anginosus</i> | IPL-Str1068 | CIP 105031 | No growth |
| <i>Streptococcus bovis</i> | IPL-E3 | Meat product | No growth |
| <i>Streptococcus bovis</i> | IPL-Str44 | RDC 44 | No growth |
| <i>Streptococcus bovis</i> | IPL-Str5623 | CIP 56.23 | No growth |
| <i>Streptococcus equinus</i> | IPL-Str1074 | CIP 102504T | No growth |
| <i>Streptococcus intermedius</i> | IPL-Str1201 | CIP 103248T | No growth |
| <i>Streptococcus salivarius</i> | IPL-Str1075 | CIP 102505 | No growth |
| <i>Streptococcus salivarius</i> | 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' <i>Listeria</i> 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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