CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

030406

The AOAC Research Institute hereby certifies the method known as:

RAPID'*L.mono*

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Signature for AOAC Research Institute

Issue Date October 27, 2023

Expiration Date December 31, 2024

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METHOD NAME
RAPID'*L.mono*

CATALOG NUMBERS
355-5294, 356-3694, 356-4293, 356-4294, 356-4746

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

Matrixes – brie cheese, surimi, mixed salad, deli turkey

Performance claims
Sensitivity – 99.4%
Specificity – 100%
Overall Accuracy – 99.7%

REFERENCE METHOD

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Bacterial Analytical Manual 8th Edition (Revision A), Appendix 2 – Most Probable Number from Serial Dilutions. January 2001. (2)

ORIGINAL CERTIFICATION DATE
July 28, 2003

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **January 2020 Level 1**
2. **January 2021 Level 1**
3. **November 2021 Level 1**
4. **October 2023 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial/clerical changes and reformat of insert.**
2. **Editorial/clerical changes.**
3. **Editorial changes and addition of user information in French, German, Spanish, Portuguese, and Italian.**
4. **Editorial/clerical changes.**

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PRINCIPLE OF THE METHOD (1)
The principle of RAPID' L.Mono agar relies on the specific detection of phosphatidylinositol phospholipase C (PIPLC) activity of *L. monocytogenes* and the inability of this species to metabolize xylose. The only species of *Listeria* to demonstrate PIPLC activity are *L. monocytogenes* and *L. ivanovii*. The addition of xylose to the media allows for differentiation of these two species since *L. monocytogenes* does not metabolize xylose. *L. ivanovii* will produce colonies with a distinct yellow halo based on its ability to metabolize xylose where *L. monocytogenes* colonies will lack this halo. The other non-pathogenic species of *Listeria*, namely *L. innocua, L. welshimeri, L. seeligeri, and L. grayi* do not exhibit PIPLC activity and produce white colonies on RAPID' L.Mono agar. *L. welshimeri* will metabolize xylose and will therefore produce a white colony with a yellow halo. The selective solution contained in the medium permits more or less complete inhibition of the majority of interfering flora including, gram positive and gram negative bacteria, yeasts, and molds.

DISCUSSION OF THE VALIDATION STUDY (1)

Current methods for identification of *Listeria* are quite cumbersome. Differentiation of *L. monocytogenes* from other non-pathogenic species of *Listeria* requires additional tests and can take as many as 7 days to complete. The total time to results from enrichment broth when using RAPID' L.Mono is 48 hours. By taking a significant amount of time off of the procedure, RAPID' L.Mono is an efficient tool for food processors, when holding food to wait for results is not an option. Method agreement in this study is a measurement comparing the number of samples each "method" identified correctly. Since there were two enrichment schemes, the Bio-Rad using Demi Fraser broth for 24 hours and the reference method using Listeria Enrichment Broth for 48 hours, method agreement percentages can be misleading. This calculation is comparing two separate sets of samples. The food was inoculated in a batch but each batch was then subdivided and processed with each enrichment broth. To expect that exactly the same number of positives will be enriched by each individual method is unrealistic. The number of positive samples recovered is ultimately a function of the enrichment broth, not the plates on which the bacteria was isolated. Therefore, the method agreement calculations were made on sets of different samples. Every sample that was identified as positive by RAPID' L.Mono agar was confirmed by standard culture methods. This is 100% agreement, which is a more accurate depiction of the results. To prove this, for the independent laboratory portion of the study, all reference method enrichment broth samples were plated to RAPID' L.Mono. Agreement was 100% for this study. In addition, the Oxford and Palcam plate for each sample grew more background flora than the corresponding RAPID' L.Mono plate for that sample. The *L. monocytogenes* colony was also much easier to pick because it is blue. This is extremely useful in analysis of naturally contaminated samples where one or two *L. monocytogenes* colonies may be lost in a plate full of other species of *Listeria* and background flora, where differentiation is nearly impossible.

The statistical analysis of Most Probable Number (MPN) is a useful way to determine low numbers of cells in a food sample (6). As part of this validation procedure, food is allowed to equilibrate to room temperature for 24 hours after inoculation. By performing a 3-tube MPN analysis, the analyst can determine, within a range, how many cells are going to be enriched. Sometimes, however, problems arise where the MPN result does not seem logical, based on fractional positive results obtained. It is for this reason that, in addition to performing an MPN analysis on the day of enrichment, serial dilutions of the inoculum culture were plated and counted fresh, to get a more accurate count. The majority of the time, results were similar using these two procedures. For some foods, seafood for example, the results were not in the same range (i.e. low level by MPN was 11.5/25g and low level by plating was 1.78/25g). Based on the fractional positive results (15 of 20 samples were detected), it can be assumed that the inoculum fell somewhere in between the MPN result and the plating result. By performing both procedures, proper inoculation levels can be assured.

Table 1 – *L. monocytogenes* strains tested (Strain # assigned by Institut Pasteur de Lille, France - All strains tested are food isolates (1)

Strain #	Serotype	Result	Strain #	Serotype	Result	Strain #	Serotype	Result	Strain #	Serotype	Result
22573	4b	+	64233	1/2a	+	66439	4b	+	68603	4b	+
27993	4b	+	64235	1/2a	+	66462	1/2a	+	68605	1/2a	+
42388	4b	+	64239	1/2c	+	66490	1/2b	+	68630	1/2a	+
43985	4b	+	64262	1/2a	+	66512	1/2b	+	68643	1/2a	+
61419	1/2a	+	64419	1/2b	+	68554	1/2b	+	68733	1/2a	+
61523	1/2a	+	64467	1/2a	+	66572	4b	+	68915	1/2a	+
61833	4b	+	64549	4b	+	66573	4b	+	68934	1/2c	+
61634	1/2a	+	64571	1/2a	+	66603	1/2a	+	68942	1/2b	+
61635	1/2b	+	64572	4b	+	66638	1/2a	+	66967	1/2a	+
61657	1/2a	+	64875	1/2a	+	66880	1/2a	+	69115	1/2a	+
61674	4b	+	64704	1/2b	+	66943	1/2b	+	69241	4b	+
61763	1/2b	+	64716	4b	+	66980	1/2a	+	69293	4b	+
61764	1/2a	+	64732	4b	+	67086	1/2c	+	69300	4b	+
61841	1/2a	+	64836	1/2b	+	67129	1/2a	+	69301	4b	+
61842	1/2a	+	64846	1/2a	+	67143	1/2a	+	69307	1/2a	+
61868	1/2a	+	64920	1/2c	+	67189	1/2b	+	69310	1/2a	+
61970	1/2a	+	64963	1/2a	+	67224	1/2a	+	69373	3a	+
62125	1/2a	+	64964	1/2a	+	67324	4b	+	69400	1/2a	+
62177	1/2b	+	64966	4b	+	67325	1/2b	+	69413	1/2a	+
62416	4b	+	65031	1/2a	+	67458	1/2a	+	69509	1/2b	+
62574	1/2c	+	65035	1/2b	+	67479	4b	+	69703	1/2a	+
62624	1/2a	+	65038	1/2a	+	67590	1/2a	+	69842	1/2c	+
62645	1/2b	+	65048	1/2a	+	67846	1/2a	+	69871	1/2a	+
62733	1/2a	+	65102	1/2a	+	67674	1/2a	+	69873	1/2a	+
62890	1/2a	+	65118	1/2c	+	64677	1/2b	+	69875	1/2a	+
62966	1/2a	+	65160	1/2a	+	67757	1/2a	+	69881	1/2a	+
63159	4b	+	65334	1/2a	+	67887	1/2b	+	70112	4b	+
63389	1/2b	+	65426	1/2a	+	67893	1/2a	+	71405	4b	+
63401	1/2b	+	65430	1/2b	+	67982	1/2a	+	71777	4b	+
63561	1/2b	+	65505	4b	+	67984	1/2b	+	74548	4b	+
63563	4b	+	65509	1/2a	+	67989	4b	+	74711	4b	+
63591	1/2c	+	65588	1/2a	+	67990	1/2a	+	74902	1/2a	+
63599	1/2a	+	65672	1/2c	+	67995	1/2a	+	74903	1/2b	+
63704	1/2a	+	65674	1/2a	+	68091	4b	+	74904	1/2c	+
63713	4b	+	65678	1/2a	+	68148	1/2a	+	74905	3a	-
63669	4b	+	65704	1/2a	+	68237	1/2a	+	74906	3b	+
63892	1/2a	+	65749	1/2a	+	68308	1/2a	+	74907	3c	+
63883	1/2a	+	65763	4b	+	68514	4b	+	74908	4a	+
63926	4b	+	65766	4b	+	68454	4b	+	74910	4b	+
63933	4b	+	65979	1/2a	+	68585	1/2a	+	74911	4c	+
63976	1/2a	+	65991	1/2c	+	68595	1/2a	+	74912	4d	+
63979	1/2a	+	66320	1/2a	+	68601	1/2b	+	74913	4e	+
64043	1/2a	+	66412	1/2a	+	68602	1/2b	+	74917	7	+

Table 2 – Non-*L. monocytogenes* strains tested (Strain # designated by Institut Pasteur de Lille) (2)

Strain #	Species
B-Ba-ce-3	Bacillus cereus
B-Ba-ce-10	Bacillus cereus
B-Ba-ce-2	Bacillus cereus
B-Ba-ce-1	Bacillus cereus
B-Be-ce-4	Bacillus cereus
B-Be-ce-9	Bacillus cereus
B-Ba-su-1	Bacillus subtilis
B-Ba-me-2	Bacillus megaterium
B-Ba-me-1	Bacillus megaterium
15	Brochotrix
Le-Ca-pa-1	Candida parapsilosis
Le-Ca-pa-2	Clostridium perfringens
S-En-fa-1	Enterococcus faecalis
S-En-du-1	Enterococcus durans
ATCC 25922	Escherichia coli
RIVM WR1	Escherichia coli
4288	Escherichia coli O157:H7
L-La-ca-1	Lactobacillus casei
L-La-fe-1	Lactobacillus fermentum
L-La-la-21	Lactobacillus lactis lactis
L-La-pl-22	Lactobacillus plantarum
S-Le-me-1	Leuconostoc mesenteroides
763	Listeria innocua
7617	Listeria innocua
8445	Listeria innocua
8811	Listeria innocua
10984	Listeria innocua
14013	Listeria innocua
14232	Listeria innocua
74909	Listeria innocua
74915	Listeria innocua
74916	Listeria innocua
74947	Listeria innocua
75025	Listeria innocua
1347	Listeria invanovii
2300	Listeria invanovii
2737	Listeria invanovii

Strain #	Species
6645	Listeria invanovii
8181	Listeria invanovii
8457	Listeria invanovii
12068	Listeria invanovii
12229	Listeria invanovii
74750	Listeria invanovii
74914	Listeria invanovii
73019	Listeria grayi
7774	Listeria seeligeri
8565	Listeria seeligeri
9071	Listeria seeligeri
9180	Listeria seeligeri
9529	Listeria seeligeri
10408	Listeria seeligeri
10978	Listeria seeligeri
11070	Listeria seeligeri
73021	Listeria seeligeri
75267	Listeria seeligeri
81	Listeria welshimeri
82	Listeria welshimeri
10413	Listeria welshimeri
11448	Listeria welshimeri
73020	Listeria welshimeri
74405	Listeria welshimeri
75528	Listeria welshimeri
75530	Listeria welshimeri
32	Rhodococcus
Le-Rh-ru-1	Rhodotorula rubra
Le-Sa-ce-3	Saccharmyces cervisiae
Le-Sa-ce-4	Saccharmyces cervisiae
M-St-au-17	Staphylococcus aureus
M-St-au-1	Staphylococcus aureus
M-St-au-7	Staphylococcus aureus
M-St-au-2	Staphylococcus aureus
M-St-au-16	Staphylococcus aureus
M-St-ep-2	Staphylococcus epidermidis
M-St-ep-1	Staphylococcus epidermidis

Table 3 – Inclusivity and Exclusivity Results (1)

Organism	Number Tested	Positive	Negative	Sensitivity [a] /Specificity [b]
L. monocytogenes	172	171	1	99.4% [a]
non-*L. monocytogenes*	74	0	74	100% [b]

Table 6 – MPN Results – 3-Tube Method vs. Plating Inoculum (1)

Matrix	Inoc Level	MPN / 25g Tube method (95% conf. limits)	MPN / 25g Plating method
Brie cheese	low	1.075 (0.225 - 4.5)	1.88
	high	6.0 (1.05 – 25)	9.39
Surimi	low	11.5 (2.25 – 50)	1.78
	high	60.0 (10.5 – 250)	7.23
Mixed salad	low	0.09 (0.0043 – 0.45)	0.75
	high	0.23 (0.035 – 0.95)	3.0
Deli turkey	low	0.575 (0.115 – 2.35)	1.95
	high	6.0 (1.05 – 25)	4.42

REFERENCES CITED

1. Lauer, W., Facon, J.P., and Patel, A., Evaluation of RAPID'L. mono Agar: A Chromogenic Medium for Identification and Detection of *Listeria monocytogenes* in Selected Foods. AOAC *Performance Tested Methods*℠ certification number 030406.
2. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Bacterial Analytical Manual 8th Edition (Revision A), Chapter 10 – *Listeria monocytogenes.* January 2003.