CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

060402

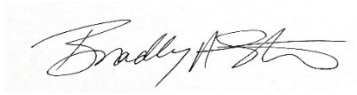
The AOAC Research Institute hereby certifies the method known as:

Microgen Listeria ID

manufactured by

Gold Standard Diagnostics Budapest Kft.
Fóti út 56
1047 Budapest
Hungary

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.



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

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2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

METHOD AUTHORS
ORIGINAL VALIDATION: Stuart Clark
MODIFICATION FEBRUARY 2018: Microgen Bioproducts Ltd.
MODIFICATION DECEMBER 2023: Gold Standard Diagnostics (Veronika Bodnar and Cristina Sánchez Conde)

SUBMITTING COMPANY
Microgen Bioproducts Limited
1 Admiralty Way
Camberley, Surrey GU15 3DT
United Kingdom

CURRENT LOCATION
Gold Standard Diagnostics Budapest Ktf., formerly Microgen Bioproducts Ltd.
Fóti út 56 A ép.
1047 Budapest, Hungary

METHOD NAME
Microgen Listeria ID

CATALOG NUMBER
MID67

INDEPENDENT LABORATORY
Campden and Chorleywood Food Research Association
Station Road
Chipping Campden
Gloucestershire GL55 6LD

APPLICABILITY OF METHOD
Target organism – *Listeria* species (*monocytogenes, innocua, welshimeri, seeligeri, ivanovii, grayi*)

Matrixes – Isolated colonies on Columbia Sheep Blood Agar.

Performance claims – The Microgen Listeria ID product has been validated to differentiate accurately between members of the genus Listeria, identify individual Listeria species from a single colony on a selective agar plate, produce an accurate result within 18-24 hours, match the performance of the FDA/BAM method, match the performance of a leading competitor product which has AOAC performance tested status (API Listeria).

REFERENCE METHOD

Hitchens A.D. (1998) *Listeria monocytogenes* US FDA Bacteriological Analytical Manual 8th Edition, AOAC, Arlington, VA 2004 (3)

USDA/BAM Chapter 10. Online Bacteriological Analytical - Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods. [Online] 04 22, 2022. (12)

ORIGINAL CERTIFICATION DATE
September 27, 2004

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **February 2018 Level 2**
2. **January 2023 Level 1**
3. **December 2023 Level 2**
4. **January 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Location change from 1 Admiralty Way, Camberley, Surrey UK to Unit 1, Watchmoor Point, Camberley, Surrey UK.**
2. **Company rebranded to Gold Standard Diagnostics Budapest, Kft.**
3. **Location change from Camberley, Surrey UK to Budapest, Hungary with catalog number change.**
4. **Editorial/clerical changes.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The genus *Listeria* represents a group of species including *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii* and *L. grayi.* The product under test Microgen Listeria ID (MID 67) has been designed to enable laboratories to properly identify / differentiate these species when sampled from a single colony isolated on selective agar plates. The product utilizes the ability of each of these species to metabolize certain chemical substrates. The pattern of substrate metabolism produced in the MID 67 multi-well strip is characteristic for each species. The results from the multi-well plate are converted into four-digit codes which are analyzed by a dedicated computer software program which calculates the percentage probability that the isolate being tested is one of the *Listeria* species. The most probable species is identified, and this is taken as the result. If non-*Listeria* species colonies are introduced into the test system (which should not happen if the proper pre-testing has been correctly performed as per the manufacturers' insert) there are a number of tests included in the multi-well strip which should be metabolized by all *Listeria* species so non-*Listeria* organisms can be identified. Many of the substrates used in the MID 67 product are taken directly from the FDA BAM method including an in-well hemolysis test to differentiate *L. monocytogenes* from *L. innocua*.

DISCUSSION OF THE VALIDATION STUDY (1)
The validation studies performed to assess the equivalence of the Microgen Listeria ID kit (Product Code MID 67) to the FDA / BAM approved *Listeria* Identification procedure have generated strong evidence that both methods agree with each other 100%. Furthermore, both methods successfully identified all of the individual *Listeria* species isolates and can discriminate between *Listeria* species and all of the non-*Listeria* species isolates even those which can be considered closely related.

Table 8: Summary of results of inclusivity study (1)

Organism	Microgen Listeria ID	Confirmed Status	Comments
L. monocytogenes	55	55	All 1/1
L. innocua	17	17	All 1/1
L. seeligeri	8	8	All 1/1
L. welshimeri	5	5	3 (1/1) / 2 (1/8)
L. ivanovii	3	3	All 1/3
L. grayi	3	3	All 1/2
Other	0	0	N/A
Total	91	91	

Table 9: Summary of Exclusivity study results (1)

No.	Listeria ID Result	Software Result	Probability	Organism Tested	Comment
1	4241	*L. ivanovii*	1/14,660,184	*B. cereus*	ARL Negative
2	4001	*L. ivanovii*	1/79,076,143	*B. cereus*	ARL Negative
3	4001	*L. ivanovii*	1/79,076,143	*B. mycoides*	ARL Negative
4	4001	*L. ivanovii*	1/79,076,143	*B. subtilis*	ARL Negative
5	6260	*L. grayi*	1/25,197,646	*Brochothrix*	ARL Negative
6	4240	*L. grayi*	1/815,539	*C. divergens*	ARL Negative
7	4260	*L. grayi*	1/814/723/887	*C. gallinarum*	ARL Negative
8	6244	*L. grayi*	1/58,853	*C. pisicola*	ARL Negative
9	3341	*L. ivanovii*	1/100,100,100	*C. freundii*	ARL Negative
10	5605	*L. ivanovii*	1/329	*E. aerogenes*	TRE Negative
11	0000	No. ID	N/A	*E. rhusiopathiae*	No ID
12	0000	No. ID	N/A	*Kurthia*	No ID
13	6060	*L. grayi*	1/100,100,100	*L. casei*	ARL Negative
14	6041	*L. ivanovii*	1/100,100,100	*L. lactis*	ARL Negative
15	6240	*L. grayi*	1/25,223	*L. plantarum*	ARL Negative
16	0000	No. ID	N/A	*Micrococcus sp.*	No ID
17	0000	No. ID	N/A	*P. freundenreichii*	No ID
18	0201	*L. ivanovii*	1/100,100,100	*P. mirabilis*	ESC Negative
19	1201	*L. ivanovii*	1/100,100,100	*P. vulgaris*	ESC Negative
20	0000	No. ID	N/A	*R. equi*	No ID
21	3340	*L. grayi*	1/100,100,100	*S. typhimurium*	ESC Negative
22	3240	*L. grayi*	1/100,100,100	*S. enteritidis*	ESC Negative
23	2041	*L. ivanovii*	1/100,100,100	*S. aureus*	ESC Negative
24	2041	*L. ivanovii*	1/100,100,100	*S. aureus*	ESC Negative
25	2240	*L. grayi*	1/25,197,646	*S. aureus*	ESC Negative
26	2041	*L. ivanovii*	1/100,100,100	*S. xylosis*	ESC Negative
27	4041	*L. ivanovii*	1/9,773,456	*Streptococcus sp.*	ARL Negative
28	0230	*L. ivanovii*	1/100,100,100	*Streptococcus sp.*	ARL Negative
29	4241	*L. ivanovii*	1/14,660,184	*Streptococcus sp.*	ARL Negative
30	6260	*L. grayi*	1/25,197,646	*Streptococcus sp.*	ARL Negative
31	4240	*L. grayi*	1/815,539	*E. durans*	ARL Negative
32	0000	No. ID	N/A	*C. renale*	No ID

Table 11: Summary of Method Comparison Results (1)

Listeria Species	Sero-Type	ID Code	Microgen ID Result	Prob	FDA BAM Result
L. monocytogenes	1/2A	**4547**	*L. monocytogenes*	**1/1**	*L. monocytogenes*
L. monocytogenes	1/2B	**4547**	*L. monocytogenes*	**1/1**	*L. monocytogenes*
L. monocytogenes	1/2C	**4547**	*L. monocytogenes*	**1/1**	*L. monocytogenes*
L. monocytogenes	4B	**4547**	*L. monocytogenes*	**1/1**	*L. monocytogenes*
L. monocytogenes	4C	**4547**	*L. monocytogenes*	**1/1**	*L. monocytogenes*
L. innocua	6A	**4546**	*L. innocua*	**1/1**	*L. innocua*
L. seeligeri	1/2B	**5445**	*L.seeligeri*	**1/1**	*L. seeligeri*
L. welshimeri	6B	**5566**	*L. welshimeri*	**1/1**	*L. welshimeri*
L. ivanovii	N/A	**5455**	*L. ivanovii*	**1/3**	*L. ivanovii*
L. grayi	N/A	**6642**	*L. grayi*	**1/2**	*L. grayi*

Note: Probability = Prob.

DISCUSSION OF MODIFICATION APPROVED DECEMBER 2023 (10)

Results were determined at three levels: *(1)* the final identification of the *Listeria* species (Level 3); *(2)* the octal code obtained for the identification of the species (Level 2); and *(3)* the detailed results of the chemical substrates per *Listeria* species (Level 1). The results obtained at level 3, for the identification of the *Listeria* species with the computer software, show no difference between the lots analysed. It is important to note that the biochemical profile is generally described in classical literature per *Listeria* species. Some of the substrate results might vary (V) among the *Listeria* species group. The discussed variability encountered at level 1 or level 2 results for the identification show variability against the expected identification of the pathogen, defining the probability of identification of each lot or production site methods. As per the scope of this study, the probability of identification of each manufactured lot has been statistically compared. In all of the discussions analyzed, the study data indicate with 95% confidence that the method produced at Budapest and method produced at Surrey are equivalent for the identification of *Listeria* spp.

REFERENCES CITED

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