



# CERTIFICATION

**AOAC® Performance Tested<sup>SM</sup>**

Certificate No.

**060402**

The AOAC Research Institute hereby certifies the test kit known as:

**Microgen Listeria-ID**

manufactured by

**Microgen Bioproducts Ltd.  
Unit 1, Watchmoor Point  
Camberley, Surrey GU15 3AD  
United Kingdom**

This method has been evaluated in the AOAC® *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC® Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (December 27, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

*Scott Coates*

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

December 27, 2019

\_\_\_\_\_  
Date

**METHOD AUTHORS**

**ORIGINAL VALIDATION:** Stuart Clark  
**MODIFICATION FEBRUARY 2018:** Microgen Bioproducts Ltd.

**SUBMITTING COMPANY**

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

**CURRENT LOCATION**

Microgen Bioproducts Ltd.  
 Unit 1, Watchmoor Point  
 Camberley, Surrey GU15 3AD  
 United Kingdom

**KIT NAME(S)**

Microgen Listeria ID

**CATALOG NUMBERS**

MID-67

**INDEPENDENT LABORATORY**

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 Station Road  
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**AOAC EXPERTS AND PEER REVIEWERS**

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<sup>4</sup> February 2018 Modification

**APPLICABILITY OF METHOD**

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

**Matrices – Pure Culture**

**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**

Hitchins A.D. (1998) *Listeria monocytogenes* US FDA Bacteriological Analytical Manual 8<sup>th</sup> Edition, AOAC, Arlington, VA (3)

**ORIGINAL CERTIFICATION DATE**

September 27, 2004

**CERTIFICATION RENEWAL RECORD**

Renewed Annually through December 2020

**METHOD MODIFICATION RECORD**

1. February 2018 Level 2

**SUMMARY OF MODIFICATION**

1. Location change from 1 Admiralty Way, Camberley to Unit 1, Watchmoor Point, Camberley.

**Under this AOAC® Performance Tested<sup>SM</sup> License Number, 060402 this method is distributed by:**  
**NONE**

**Under this AOAC® Performance Tested<sup>SM</sup> License Number, 060402 this method is distributed as:**  
**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 metabolise certain chemical substrates. The pattern of substrate metabolism produced in the MID 67 multiwell strip is characteristic for each species. The results from the multiwell plate are converted into four digit codes which are analysed by a dedicated computer software programme 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 multiwell strip which should be metabolized by all *Listeria* species so non-*Listeria*s 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 haemolysis 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          |
|--------------------------------|----------------------|------------------|-------------------|
| <b><i>L. monocytogenes</i></b> | 55                   | 55               | All 1/1           |
| <i>L. innocua</i>              | 17                   | 17               | All 1/1           |
| <i>L. seeligeri</i>            | 8                    | 8                | All 1/1           |
| <i>L. welshimeri</i>           | 5                    | 5                | 3 (1/1) / 2 (1/8) |
| <i>L. ivanovii</i>             | 3                    | 3                | All 1/3           |
| <i>L. grayi</i>                | 3                    | 3                | All 1/2           |
| <i>Other</i>                   | 0                    | 0                | N/A               |
| <i>Total</i>                   | 91                   | 91               |                   |

Table 9: Summary of Exclusivity study results (1)

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

Table 11: Summary of Method Comparison Results (1)

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

Note: Probability = Prob.

#### REFERENCES CITED

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