Instruction for Use Microgen Listeria ID

Cat. No. – MID67

GOLD STANDARD DIAGNOSTICS

MICROGEN BIOPRODUCTS

v04 06 SEPT 2023

Contents

1 INTRODUCTION

The Microgen Listeria-ID system is intended for use by qualified laboratory personnel using aseptic technique and appropriate microbiological precautions. This kit is not intended for Clinical/ Medical purposes.

The Microgen Listeria-ID system employs 12 standardised micro-well substrates combined with the Microgen Identification System Software to identify members of the genus *Listeria*:

- *Listeria monocytogenes*
- *Listeria welshimeri*
- *Listeria ivanovii*
- *Listeria innocua*
- *Listeria grayi*
- *Listeria seeligeri*

The above organisms can be identified from selective or non-selective agar using Microgen Listeria-ID. Identification is achieved using all the tests recommended in international standard methods for the identification of *Listeria spp.* without the need for additional confirmatory tests (1; 2; 3; 4)

2 PRINCIPLE

Each Microgen Listeria-ID microwell test strip contains 11 dehydrated substrates for the performance of carbohydrate utilisation tests, and one empty round bottomed well for the performance of a haemolysin reaction (5). The selection of the substrates included in the test panel is based on a combination of those substrates recommended in international standard methods (1; 2; 3; 4) plus additional tests which either confirm the isolate being tested as belonging to the genus *Listeria* (Esculin Hydrolysis, Trehalose and Arabitol Fermentation (6; 7) and/ or further enhance the differentiation of the various species comprising the genus.

Identification of isolates is achieved by recording the results visualised by a colour change after 18-24 hours incubation (there are no reagents to be added on Day 2). For more information regarding the colour changes, please refer to *Table 1* or *Section 10*. These results are then analysed using the Microgen Identification System Software (MID60).

3 REAGENTS

Kit Contents (20 tests)

- Holding frame for test microwell test strips
- Result forms
- 20 microwell test strips in individual foil pouches
- 20 bottles of *Listeria* Suspending Medium
- 1 bottle of Haemolysin Reagent

Additional Materials Required (not supplied in the kit)

- Microgen Identification System Software (MID60)
- Sterile bacteriological loops
- Sterile Pasteur pipettes
- Non selective media (purity plate)
- Incubator (35 - 37°C), not fan assisted.

- Refrigerator (2 - 8°C)
- Marking Pen
- Oxidase strips (MID61G) – for previous characterization of *Listeria*
- Hydrogen Peroxide, use at 3% (w/w), for catalase test see Reference (2)- for previous characterization of *Listeria*
- Gram stain reagents - for previous characterization of *Listeria*
- Microscope and Microscope slides - for previous characterization of *Listeria*
- 25°C Incubator, not fan assisted - for previous characterization of *Listeria*

Table 1. Microgen Listeria-ID microwell test strip consists of twelve wells containing the substrates for the following 11 biochemical reactions. Well number 12 is empty and is used for an in-well haemolysis reaction when haemolysin reagent is added to a bacterial suspension.

Well	Substrate	Reaction	Positive	Negative
1	Esculin	Esculin hydrolysis	Black	Yellow colour
2	Mannitol	Fermentation of specific sugars producing acid end products changes the Bromocresol Purple indicator from purple to yellow.	Yellow	Purple
3	Xylose			
4	Arabitol			
5	Ribose			
6	Rhamnose			
7	Trehalose			
8	Tagatose			
9	Glucose-1-Phosphate			
10	Methyl-D-Glucose			
11	Methyl-D-Mannose			
12	Haemolysin	Haemolysis of sheep red blood cells.	Straw – Brown coloured homogeneous liquid. No button of red blood cells in bottom of the well. Refer to section Interpretation (4.3) for further information.	Button of red blood cells in bottom of well.

4 STORAGE

The microwell test strips are stable in the unopened foil pouches at 2 - 8°C until the expiry date stated.
The *Listeria* suspending broth and haemolysin reagent should be stored at 2 – 8°C.
The haemolysin reagent should be returned to 2 – 8°C immediately after use.

5 TEST STEPS

Before using this product, refer to Precautions and Limitations.

1. Selection of colonies for identification

1.1. Isolates can be tested from any *Listeria* selective or non-selective media.

1.2. Prior to inoculation into the Microgen Listeria ID, isolates should be checked to ensure they are members of the genus *Listeria* (short Gram positive bacillus, oxidase negative, catalase positive, motile at 25°C but non motile at 37°C -motility can be determined by the microscopy method described in Reference **Hiba! A hivatkozási forrás nem található.**).

2. Inoculum preparation

2.1. Bring the suspending broth to room temperature before inoculation of microwell test strips.

2.2. Select a single well-isolated large colony from an 18–24-hour culture and emulsify it in a vial of *Listeria* Suspending medium (2.5ml). Suspension must appear slightly cloudy.

2.3. Mix thoroughly (optionally vortex) to produce a homogenous suspension.

3. Inoculation and Incubation

3.1. Remove a microwell test strip from the foil pouch, place it in the holding frame and remove the lid.

3.2. Using a sterile Pasteur pipette transfer 4 drops (approximately 100µl) of the bacterial suspension to each well of the microwell test strip.

3.3. As a purity check, transfer 1 drop of the organism suspension onto an appropriate non-selective agar plate. Incubate the plate aerobically at 35 - 37°C for 18 - 24 hours.

3.4. Add 1 drop of the haemolysin reagent to well 12.

3.5. Put the lid back onto the microwell test strip and incubate at 35 - 37°C for 18- 24 hours.

4. Interpretation

4.1. After incubation remove the lid from the microwell test strip and record results on the report forms provided.

4.2. Refer to the table of tests (Table 1) for guidelines in the interpretation of the results.

4.3. The haemolysin reaction should be interpreted as follows:

4.3.1. Examine the inoculum in the well.

4.3.2. The presence of a CLEAR straw/very pale pink solution with a large button of intact red blood cells in the bottom of the microwell, should be interpreted as a NEGATIVE haemolysis reaction.

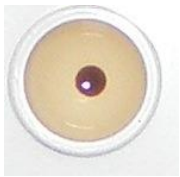


Figure 1.: Example of no haemolysis

4.3.3. The presence of a CLOUDY straw-brown solution/suspension either in the absence of a button of intact red blood cells (TOTAL HAEMOLYSIS) or in the presence of a much-reduced button of intact red blood cells (PARTIAL HAEMOLYSIS) should be interpreted as a POSITIVE HAEMOLYSIS REACTION

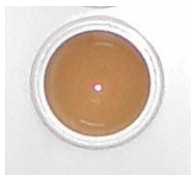


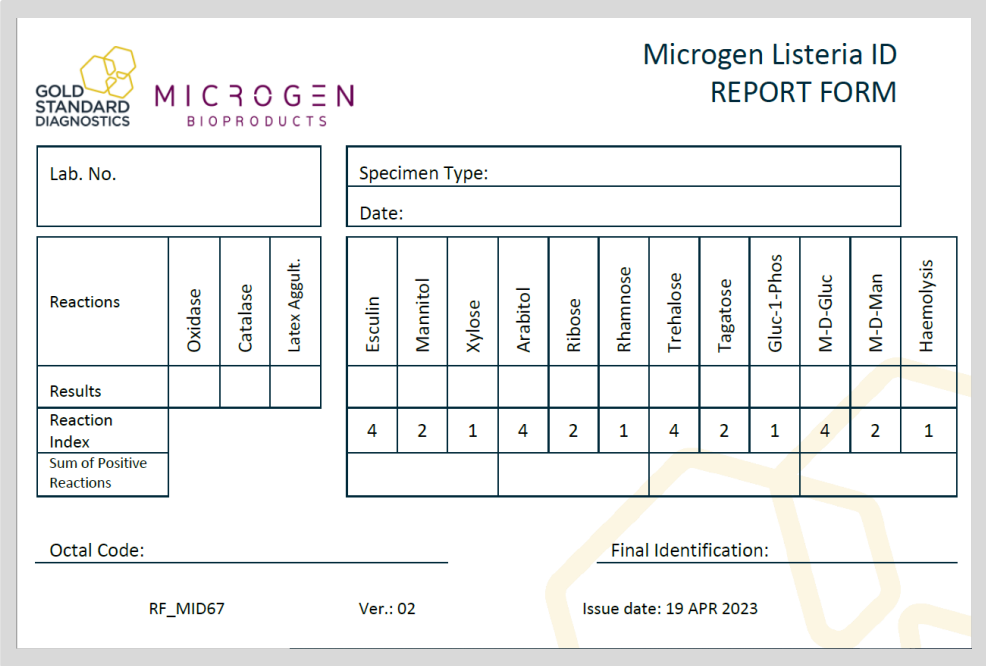
Figure 2.: Example of total haemolysis (score positive)

Figure 3.: Example of partial haemolysis (score positive)

4.4. Examine the purity plate for viability of the test organism and purity.

4.5. The tests on the report form have been organised into triplets (sets of 3 reactions), with each test assigned a numerical value (1, 2 or 4). The sum of the positive reactions for each triplet forms a single digit of the Octal Code (Octal Code) that is used to determine the identity of the *Listeria spp.* being identified. The Octal Code (Octal Code) is entered into the Microgen Identification System Software, which generates a report of the five most likely organisms based on the selected database (8).

6 REPORT FORM



Microgen Listeria ID
REPORT FORM

Lab. No.	Specimen Type:
	Date:

Reactions	Oxidase	Catalase	Latex Aggut.	Esculin	Mannitol	Xylose	Arabitol	Ribose	Rhamnose	Trehalose	Tagatose	Gluc-1-Phos	M-D-Gluc	M-D-Man	Haemolysis
Results															
Reaction Index				4	2	1	4	2	1	4	2	1	4	2	1
Sum of Positive Reactions															

Octal Code: Final Identification:

RF_MID67 Ver.: 02 Issue date: 19 APR 2023

7 PRECAUTIONS

1. The Microgen Listeria-ID system is intended for use by qualified laboratory personnel (professional use only) using aseptic technique and appropriate microbiological precautions and this kit is not intended for Clinical/ Medical purposes.
2. Used materials must be disposed of safely by autoclaving, incineration, or immersion into an appropriate disinfectant prior to disposal.
3. The microwell test strip lids do not seal the microwells completely so the strips **must not** be incubated in either a CO_2 incubator (due to erroneous pH effects) or fan assisted incubator (potential for excess evaporation).
4. The haemolysin reagent contains live sheep red blood cells which may deteriorate if not handled correctly.
5. Always store at 2 - 8°C. Exposure of the haemolysin reagent at temperatures below 0°C for any period will result in immediate haemolysis of the red blood cells. Exposure to elevated temperatures i.e., >37°C for prolonged periods my significantly reduce the shelf life of the haemolysin reagent.
6. In addition, contamination of the haemolysin reagent will result in haemolysis of the red blood cells. Avoid contact of the dropper with the microwell strip, skin or other surfaces which will result in contamination.
7. The haemolysin reagent may not perform properly if it has deteriorated. The most common indications of the deterioration of the haemolysin reagent include significant haemolysis or a change in the colour of the reagent to a wine – brown colour.
8. If the result of the haemolysis test is unclear, the isolate should be inoculated onto a sheep blood agar plate and checked for haemolysis after incubation at 35 - 37°C for 18 – 24 hours or perform a CAMP test.

9. On rare occasions, non-haemolytic *L. monocytogenes* may be isolated. The pathogenicity of these strains is currently unclear. If typical *L. monocytogenes* colonies are isolated on chromogenic agar but produce a negative haemolysis reaction in the MID67 Listeria ID, further investigations should be undertaken e.g., CAMP Test.

8 LIMITATIONS

1. Although selective media for the isolation of *Listeria spp.* are formulated to inhibit the growth of a wide range of contaminating normal microbiome, organisms which resemble *Listeria* spp. on these media may grow through (*Bacillus spp.*, *Enterococcus spp.* and *Staphylococcus spp.*).
2. The Microgen Listeria ID system has been designed to identify organisms belonging to the genus *Listeria* and no other genera. If the isolate being identified does not hydrolyse esculin or ferment Trehalose or Arabitol; the gram stain, motility, oxidase and catalase should be re checked.
3. Specimens or samples may contain a mixture of species therefore the selection of a single well-isolated colony is critical to obtaining the most accurate result.
4. Inoculation of a purity plate is recommended as it will confirm that a single species was inoculated into the microwell test strips.

9 QUALITY CONTROL

The performance of the Microgen Listeria ID system should be monitored using appropriate control strains. The following are recommended for independent laboratory assessment. It is on the users' responsibility to ensure that the Quality control is performed in accordance with any applicable local regulation:

Table 2 Quality control test references

Microorganism strain	ESC	MAN	XYL	ARL	RIB	RHA	TRE	TAG	GIP	MDG	MDM	HEM
L. monocytogenes (ATCC 13932)	+	-	-	+	-	+	+	-	-	+	+	+
L. inoccua (ATCC 33090)	+	-	-	+	-	+	+	-	-	+	+	-
L. seeligeri (ATCC 35967)	+	-	+	+	-	-	+	-	-	+	-	+

Note: Profiles obtained after culture on Columbia sheep blood agar. Please, refer to the official taxonomy codes for the latest updates on strain names.

10 COLOUR CHART

Read microwell test strips at 24 hours.

Table 3 Reaction colours for positive/negative results

WELL	1	2 to 11	12
Reaction	Esculin Hydrolysis	Carbohydrate Fermentation	Haemolysin
Negative			
Positive			

11 WASTE DISPOSAL

Dispose of according to any local, national, or regional regulations.

12 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

Gold Standard Diagnostics Budapest ("GSDB") warrants that the products manufactured by it will be free of defects in materials and workmanship, when used in accordance with the applicable instructions before the expiration date marked on the product packaging, and when stored under the storage conditions recommended in the instructions and/or on the package.

GSDB makes no other warranty, expressed or implied.

GSDB's sole obligation shall be, at its option, to either replace or to refund the purchase price of the product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies GSDB promptly of any such defect within a reasonable time and with solid proof of the defect. GSDB shall investigate the defect locally and will justify the approval or disapproval of the complaint.

GSDB shall not be liable for any direct, indirect or consequential damages resulting from economic loss or property damages sustained by buyer or any customer from the use of the product(s).

A copy of the terms and conditions can be obtained on request and is also provided in our price lists.

13 REFERENCES

1. ISO 11290-1:2017 - Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 1: Detection. .

2. Online Bacteriological Analytical - BAM Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods. [Online] 22 04 2022. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration.

3. Confirmation of Listeria species Method 11.3:1995 CCRFA Microbiological Methods Manual.

4. AS/NZS 1766.2.15:1998 - Examination for specific organisms – Listeria monocytogenes in dairy products.

5. A Microplate Technique to Determine Hemolytic Activity for Routine Typing of Listeria Strains. 24:99-103. Rodriguez L.D., J.A. Vazquez Boland, j.f. Fernandez Garayzabal, P. Echalecu Tranchant, E. Gomez-Lucia, E.F. Rodriguez Ferri and G. Suarez Fernandez. 1986.

6. Identification of species of the genus Listeria by fermentation of carbohydrates and enzymatic patterns. Acta Microbiologica Hungarica 37:123 – 129. 1990., Mira-Gutierrez J. and C.Perz De Lara and M.A. Rodriguez-Igesias.

7. A Numerical Taxanomic Survey of Listeria and Related Bacteria. J.Gen. Microbiol. 98: 399 – 421. 1977., Wilkinson B.J. and D.Jones.

8. Identification of Bacteria by Computer: General Apects and Perspectives J.Gen. Microbiol. 77: 273 -290. Lapage S.P, S.Bascombe, W.R. Willcox and M.A.Curtis. 1973.

TECHNICAL SUPPORT SERVICE

For technical assistance and more information please contact Gold Standard Diagnostics Budapest's Customer Service or your local distributor.

Gold Standard Diagnostics Budapest Kft.
Fóti út 56 A. ép.
1047 Budapest, Hungary

www.goldstandarddiagnostics.com

v04 06 SEPT 2023