Microgen™ Listeria -ID System

An identification system for Listeria species

Instructions for Use

REF **MID-67**

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MICROGEN Listeria-ID

Quick Reference

STEP 1	**SELECT A SINGLE, WELL - ISOLATED COLONY**
STEP 2	**EMULSIFY IN LISTERIA SUSPENDING BROTH**
STEP 3	**TRANSFER 4 DROPS TO EACH MICROWELL**
STEP 4	**ADD 1 DROP OF HAEMOLYSIN REAGENT (Well 12)**
STEP 5	**INCUBATE 35 –37°C FOR 18 – 24 HOURS**
STEP 6	**READ AND RECORD RESULTS**
STEP 7	**INTERPRET USING MICROGEN IDENTIFICATION SYSTEM SOFTWARE**

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 innocua***
Listeria welshimeri	***Listeria grayi***
Listeria ivanovii	***Listeria seeligeri***

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

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 (4). 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) plus additional tests which either confirm the isolate being tested as belonging to the genus ***Listeria*** (Esculin Hydrolysis, Trehalose and Arabitol Fermentation(5,6)) 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). These results are then analysed using the Microgen Identification System Software (MID-60)

Each Microgen Listeria-ID microwell test strip consists of twelve wells containing the substrates for the following 11 biochemical reactions:

		Reaction	Positive	Negative
1	Esculin	Esculin hydrolysis	Black	Straw 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.	Button of red blood cells in bottom of well.

Well number 12 is empty and is used for an in-well haemolysis reaction when haemolysin reagent is added to a bacterial suspension.

REAGENTS

Kit Contents (20 tests)

Holding frame for test microwell test strips
Result forms
Instructions for use
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 (MID-60)
Sterile bacteriological loops
Sterile pasteur pipettes
Incubator (35 - 37°C), not fan assisted
Refrigerator (2 - 8°C)
Marking Pen
Oxidase strips (MID61G)
Hydrogen Peroxide, use at 3% (w/w), for catalase test see Reference 1
Gram stain reagents
Microscope and Microscope slides
25°C Incubator, not fan assisted
Non selective media (purity plate)

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.

INSTRUCTIONS FOR USE
(Before using this product, refer to Precautions and Limitations)

1. Selection of colonies for identification

1.1. Isolates can be tested from any 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 (we recommend that motility be determined by the microscopy method described in Reference 1) Alternatively the Microgen Listeria Latex test (F48) may be employed.

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

4. Interpretation

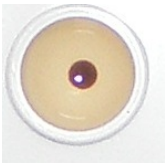
4.1 After incubation remove the lid from the microwell test strip and record results on the report forms provided.

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

4.3 The haemolysin reaction should be interpreted as follows:

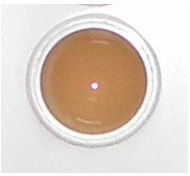
4.3.1 Examine the inoculum in the well.

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



EXAMPLE OF NO HAEMOLYSIS
(SCORE NEGATIVE)

4.2.3 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



EXAMPLE OF TOTAL HAEMOLYSIS
(SCORE POSITVE)

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 (7).

Report Form

LISTERIA – ID

MICROGEN REPORT FORM

MICROGEN BIOPRODUCTS

Lab. No.

Specimen Type:

Date:

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

Octal Code:

Final Identification:

PRECAUTIONS

1. The Microgen Listeria-ID system is intended for use by qualified laboratory personnel 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.

   Always store at 2 - 8°C. Exposure of the haemolysin reagent at temperatures below 0°C for any period of time 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.

   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.

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.

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.

5. 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 MID Listeria, further investigations should be undertaken e.g. CAMP Test.

LIMITATIONS

1. Although selective media for the isolation of ***Listeria spp***. are formulated to inhibit the growth of a wide range of contaminating normal flora, 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.

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:

	ESC	MAN	XYL	ARL	RIB	RHA	TRE	TAG	GIP	MDG	MDM	HEM
L.monocytogenes (ATCC 35152, NCTC 7973)	+	-	-	+	-	+	+	-	-	+	+	+
L.inoccua (ATCC 33090, NCTC11288)	+	-	-	+	-	+	+	-	-	+	+	-
L..grayi (ATCC 19120, NCTC 10815)	+	+	-	+	+	-	+	-	-	-	+	-

DATABASE

	ESC	MAN	XYL	ARL	RIB	RHA	TRE	TAG	GIP	MDG	MDM	HEM
L.monocytogenes	100	0	0	97	0	98	97	0	2	99	98	99
L.inoccua	100	0	1	100	0	70	100	0	0	100	100	0

L.welshimeri	100	0	95	100	0	87	100	94	0	98	94	0
L.seeligeri	100	0	100	100	0	0	97	0	0	100	5	93
L.ivanovii	100	0	97	100	42	5	86	0	92	95	0	90
L.grayi	100	97	0	100	100	0	98	0	0	30	94	0

Figures denote percentage positive strains
Highlighted reactions are confirmatory for *Listeria spp*.

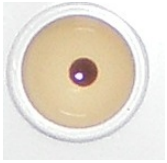
REFERENCES

1. On Line *Bacteriological Analytical Manual* - www.FDA/CFSAN Bacteriological Analytical Manual Online, Chapter 10 - Detection and Enumeration of *Listeria monocytogenes* in Foods.
2. Confirmation of Listeria species Method 11.3:1995 CCRFA Microbiological Methods Manual
3. AS/NZS 1766.2.15:1998 Examination for specific organisms – ***Listeria monocytogenes*** in dairy products.
4. 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 A Microplate Technique to Determine Hemolytic Activity for Routine Typing of ***Listeria*** Strains. **24**:99 – 103.
5. Mira-Gutierrez J. and C.Perz De Lara and M.A. Rodriguez-Igesias. 1990. Identification of species of the genus ***Listeria*** by fermentation of carbohydrates and enzymatic patterns. Acta Microbiologica Hungarica **37**:123 – 129.
6. Wilkinson B.J. and D.Jones. 1977. A Numerical Taxanomic Survey of ***Listeria*** and Related Bacteria. J.Gen. Microbiol. 98: 399 – 421.
7. Lapage S.P, S.Bascombe, W.R. Willcox and M.A.Curtis. 1973 Identification of Bacteria by Computer: General Apects and Perspectives J.Gen. Microbiol. **77**: 273 -290

Colour chart

Microgen™ Listeria ID MID-67

Read microwell test strips at 24 hours

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