

MICROGEN® LISTERIA ID – Optimizing Listeria Identification

It is now well established that the genus *Listeria* consists of the following 6 species: *L.monocytogenes*, *L.innocua*, *L.seeligeri*, *L.welshimeri*, *L.ivanovii* and *L.grayi*. *L.innocua* was originally classified as *L.monocytogenes* until it was determined that that this species in fact contained 2 distinct groups based on their ability to produce a haemolytic reaction on sheep blood agar (1). Subsequently *L.monocytogenes* were defined as the haemolytic virulent strains whilst the new species *L.innocua* were the non haemolytic avirulent strains. The name *innocua* was derived from the Latin word for harmless. *L.welshimeri* and *L.seeligeri* are well established as non haemolytic avirulent species (2), whilst *L.ivanovii* was formerly classified as *L.monocytogenes* serovar 5 until it was reclassified in 1984 (3).

In the 1980's, the incidence of cases of listeriosis increased significantly. This increase was accompanied by a number of foodborne outbreaks caused by *L.monocytogenes*. *Listeria*

spp. are ubiquitous in the environment, *L.monocytogenes* being the major human pathogen whilst *L.ivanovii* is rarely documented as causing human illness (4). It is therefore important that *Listeria spp.* are correctly identified.

Currently all standard identification methods rely on the fermentation of sugars and haemolytic reactions. Microgen Bioproducts have developed a simple yet comprehensive biochemical

identification system for the identification of all species of Listeria. The creation of this system began with the development of a well defined product specification. The key elements of this specification are shown in Table 1. and represent the key elements raised by customers after considerable consultation. To ensure that design elements 1 – 4 were achieved, a computer based analysis was performed on a comprehensive database derived from a number of extensive taxonomic studies (5). The computer based

MICROGEN® Listeria ID DESIGN BRIEF

- ◆ Perform the identification directly from selective media
- ◆ Confirm the isolate as *Listeria spp.*
- ◆ Clear differentiation of all 6 species of Listeria
- ◆ Substrates based on conventional methods
- ◆ Eliminate the need for separate haemolysis or CAMP test
- ◆ Substrates conform to international standards
- ◆ No assembly required
- ◆ Easy to set up and inoculate.
- ◆ Clear interpretation of substrate reactions
- ◆ Results available within 18 – 24 hours from a single colony
- ◆ Supported by a comprehensive computer based identification package.

Table 1. Microgen® Listeria ID Design Brief

analysis was performed using a number of well established algorithms (6,7) which have been incorporated into a program (MIOS) developed *de novo* by Microgen (Figure 1.) This analysis

but modified to provide long term stability of the reagent. The final test panels were evaluated using 105 strains of *Listeria spp* obtained from a wide range of food sources. The identification of these

isolates was performed using the Microgen® Listeria ID and compared to the identification achieved using 2 alternative identification systems.

All final identifications using the Microgen® Listeria ID were confirmed using the Microgen® Identification System Software.

The results of this investigation indicated that all 105 isolates examined were correctly identified by the Microgen® Listeria ID as a stand alone system ie. without the need for additional tests (Table 3).

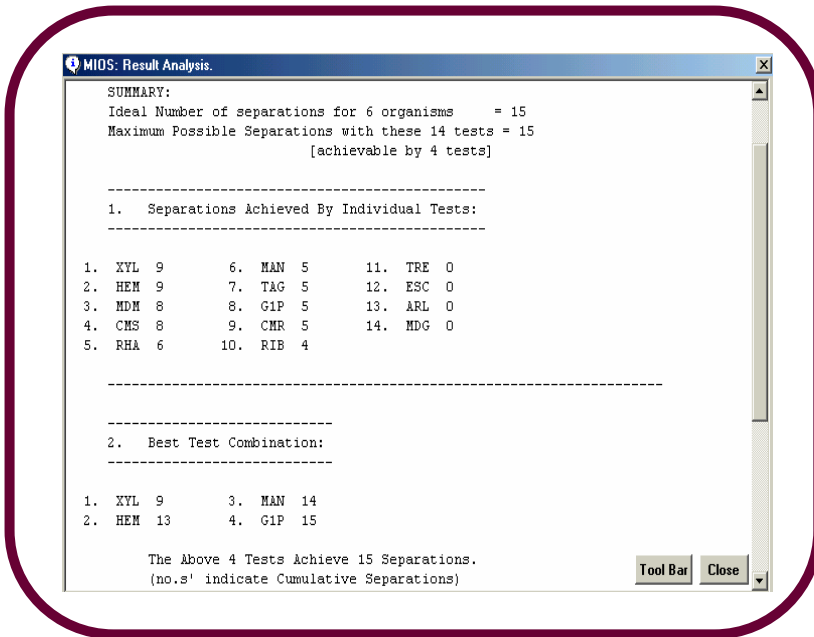


Figure 1. MIOS Database Development Program Developed by Microgen

resulted in the selection of the tests shown in Table 2. The final selection of tests was specifically made to ensure that substrates were suitable for both the confirmation of *Listeria spp.* (Esculin, Arabitol and Trehalose) whilst others contribute to the differentiation of the 6 individual *Listeria spp.* .

All substrates were formulated by the Microgen Bioproducts R & D team to provide identical performance to established conventional substrate formulations (8,9) in a microwell format. The microwell haemolysin test which is critical to the differentiation of *L.innocua* and *L.monocytogenes* was based on the methods of Dominguez Rodriguez (10)

Esculin Trehalose Arabitol	Confirmation of <i>Listeria spp.</i> , >95% positive
Xylose	Differentiates <i>L.monocytogenes</i> and <i>L.innocua</i> from other species
Rhamnose	Differentiates <i>L.monocytogenes</i> and <i>L.innocua</i> from other species
Methyl-D-Glucoside	Differentiates <i>L.grayi</i> from other species
Mannitol	Differentiates <i>L.grayi</i> from other species
Ribose	Differentiates <i>L.grayi</i> and <i>L.ivanovii</i> from other species
Methyl-D-Mannoside	Differentiates <i>L.ivanovii</i> and <i>L.seeligeri</i> from other species
Glucose-1-Phosphate	Differentiates <i>L.ivanovii</i> from other species
Tagatose	Differentiates <i>L.welshimeri</i> from other species
Haemolysis	Differentiates <i>L.monocytogenes</i> from <i>L.innocua</i>

Table 2. Substrate Validation for Listeria Identification

Organism	API
<i>L.monocytogenes</i>	59
<i>L.innocua</i>	22
<i>L.seeligeri</i>	9
<i>L.welshimeri</i>	7
<i>L.ivanovii</i>	4
<i>L.grayii</i>	4
Total	105

Strains of *Listeria spp* examined (above)

REFERENCE ID	MICROGEN ID	
	Positive	Negative
Positive	59 (A)	0 (B)
Negative	0 (C)	46 (D)

Specificity = 100% $[A/(A + B) \times 100]$

Sensitivity = 100% $[D/(C + D) \times 100]$

False Positive = 0% $[(C/(A + B)) \times 100]$

False Negative = 0% $[(B/(C + D)) \times 100]$

The Microgen Listeria ID is deemed to be equivalent to the Reference Methods (B=0, NO FALSE NEGATIVE RESULTS WITH THE ALTERNATIVE SYSTEM)

Table 3. Results of Microgen® Listeria Validation Study

References:

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2. Rocourt J and P.A. Grimont (1983) *Listeria welshimeri* sp. nov. and *L.seeligeri* sp. nov. Int. J. Syst. Bacteriol. **33**: 866 – 869.
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10. Dominguez Rodriguez L, J.A. Vazquez Boland, J.F. Fernandez Garayzabal, and G.Suarez Fernandez (1986) Microplate Technique to Determine Hemolytic Activity for Routine Typing of *Listeria* Strains. J.Clin.Microbiol **24**: 99 - 103

ORDERING INFORMATION:

MID-64 Microgen® GN-ID A
MID-65 Microgen® GN-ID B
MID-61 Microgen® GN-ID Reagent Pack

MID-67 Microgen® Listeria-ID

MID-60 Microgen® ID System Software

MICROGEN™ ID BACTERIAL IDENTIFICATION LAUNCH PACK

The new Microgen® ID Bacterial Identification Systems for Gram Negative Bacilli and *Listeria spp.* are now available. To assist existing and new customers in familiarising themselves with these products we have made available a comprehensive support package. This package contains:

- ◆ Technical Bulletins
- ◆ Material Safety Data Sheets
- ◆ Draft SOP's to assist in documentation
- ◆ Data Tables
- ◆ Quick Reference Charts
- ◆ Colour Charts
- ◆ Draft Validation Study Protocols
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TECHNICAL NOTE

Non Haemolytic *Listeria monocytogenes* – occasionally the isolation of non haemolytic *Listeria monocytogenes* is reported. The identification of such strains requires the use of molecular methods as biochemical based methods will produce an identification of *L.innocua*. The question asked in such situations is “what is the significance?”. A number of extensive studies have been published on the epidemiology of listeriosis and the role of non haemolytic strains of *L.monocytogenes* (1). All of these reports confirm that non haemolytic strains of *L.monocytogenes* are avirulent and therefore irrelevant in the context of human and animal pathogenicity.

1. Camilli A., H. Goldfine and D.A. Portnoy (1991) *Listeria monocytogenes* mutants lacking phosphatidylinositol – specific phospholipase C are avirulent. J. Exp. Med. 173: 751 – 754

FOR MORE INFORMATION ON THE MICROGEN® ID SYSTEMS FOR GRAM NEGATIVE BACILLI OR LISTERIA, PLEASE CONTACT YOUR LOCAL MICROGEN DISTRIBUTOR OR COMPLETE THE INFORMATION REQUEST FORM ENCLOSED AND RETURN IT TO MICROGEN BIOPRODUCTS LTD, UK.

MICROGEN BIOPRODUCTS LTD
1 Admiralty Way
Camberley
Surrey
United Kingdom GU15 3DT

Phone: +44 1276 600081
Fax: +44 1276 600151
E-mail: sales@microgenbioproducts.com
Web: www.microgenbioproducts.com

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