

## Microgen™ Listeria ID Gains AOAC Approval

### Microgen™ Listeria ID (MID 67) was granted AOAC Research Institute Performance Tested Methods (PTM) Status (Certificate No. 060402)

The Microgen Bioproducts Listeria Identification Kit, Microgen™ Listeria ID (MID 67) has been granted AOAC Research Institute Performance Tested Methods (PTM) Status by the internationally recognised AOAC International. The achievement of Performance Tested Methods Status is a complex task requiring extensive performance validation including confirming the ability of the kit to accurately identify *Listeria* spp. from a wide range of sources, the ability of the kit to discriminate clearly between *Listeria* spp. and non *Listeria* spp., shelf life validation and the general robustness of the kit under extremes of testing conditions including inoculum variation and incubation temperature. In addition, compliance with BAM Methods must be confirmed.

The AOAC International has examined the Microgen™ Listeria ID (MID 67) kit and has recognised that it is not only does the kit fulfill its intended purpose i.e. identify *Listeria* spp., but it is able to readily discriminate non *Listeria* spp. complies with the BAM Method and meets all of the criteria for robustness both in terms of usability and storage required of a kit to meet their stringent requirements for PTM Status.

*Listeria species* are a common source of contamination in a wide variety of foods and food ingredients. The genus comprises 6 different species, all of which have distinct phenotypical characteristics which may be used to identify or differentiate these species. The most commonly found species are *Listeria monocytogenes*, a pathogenic species and *Listeria innocua*, considered as non-pathogenic. Microgen Bioproducts Ltd. has developed a biochemical identification system which enables laboratories to rapidly confirm which *Listeria* species they have isolated directly from the selective isolation plate. The product needs only one colony and delivers its results in 18-24 hours. An important feature of this product is an inwell haemolysis test which is based on the ability of a *Listeria species* isolate to lyse red

blood cells. This is one of the key pathogenicity factors related to Phospholipase C and provides a clear discrimination between *Listeria monocytogenes* (haemolytic/ pathogenic) and *Listeria innocua* (non-haemolytic/ non-pathogenic).

### Summary of AOAC-RI Validated Claims

The Microgen™ Listeria ID kit has been AOAC validated to confirm that the product will identify all 6 members of the genus correctly and that the serotype of the isolate does not affect the result. Furthermore, studies were undertaken to confirm that the product would not mistakenly identify non-*Listeria* (closely related bacteria) as *Listeria* species if they were introduced into the product. The method was also directly compared to the FDA/BAM online method. The product

claims to be able to confirm *Listeria* species colonies from a range of selective and non-selective media. This was also confirmed during the extensive ruggedness studies.

## Description of the Method

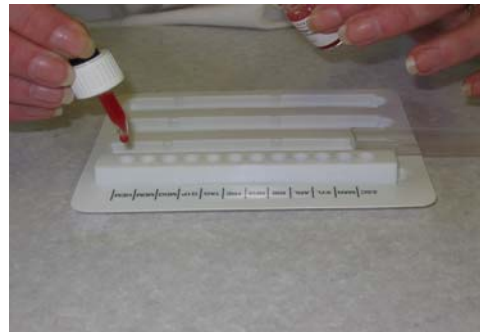
Microgen™ *Listeria* ID has been designed to enable users to generate rapid confirmation of the identity of any *Listeria* species which they isolate from food or food ingredient samples. The product is normally used on “*Listeria* like colonies” that have been isolated on a selective agar plate. One colony is then taken and suspended in the *Listeria* suspending broth (supplied in the kit). The bacterial suspension is then added to all 12 wells of the test strips comprising each identification panel. Finally the haemolysin reagent (containing stabilised red blood cells) is added to well twelve. The micro-well strip is then incubated for 18-24 hours in a non-fan assisted incubator at 35-37°C.

Next day the substrate reactions are read, the differentiation of positive and negative reactions being based on simple colour changes. Microwell 1 is Esculin Hydrolysis and should have turned black for all *Listeria* species isolates tested. The next 10 microwells are sugar fermentation reactions which will either remain purple (negative) or turn yellow (positive) The final test, haemolysis (microwell 12) will either show a button of red blood cells with clear inoculum (negative) or a cloudy inoculum with no red cell button visible (positive / haemolytic), see Figure 2 Microgen™ *Listeria* ID substrate reactions. The results are recorded and used to produce a four digit code which is input into the dedicated software programme. The programme analyses the four digit code and suggests the most probable *Listeria* species.

## Test Kit Features

The Microgen™ *Listeria* ID kit offers several advantages over conventional methods and other miniaturised biochemical test systems for *Listeria* species identification. The product can be used directly on bacterial colonies taken directly from selective agar plates; most other systems require the test to be performed on colonies from non-selective plates. Furthermore, only one colony is required per test so there is no possibility of multiple species being mixed in a single identification. All *Listeria*-like colonies can be

tested separately. In addition, the Microgen™ *Listeria* ID test strips provide a self-contained test system which delivers the complete result without recourse to additional confirmatory tests such as CAMP or a separate blood plate for haemolysis. To support the identification system, Microgen Bioproducts has produced a dedicated software program which interprets the results achieved in the test strip and delivers a most probable species result to confirm the identity of the isolate under test. This software package now incorporates a feature which ensures that the introduction of a non-*Listeria* species isolate into the test system will be identified by the software program. The program now prompts the operator to go back and re-confirm that the isolate under test is indeed a member of the genus *Listeria*, using standard pre-tests of gram stain (positive), Oxidase (negative), Catalase (positive) and motility at 25°C but non-motile at 37°C.



**Figure 1. Addition of Haemolysin Reagent to the MID *Listeria* ID System**

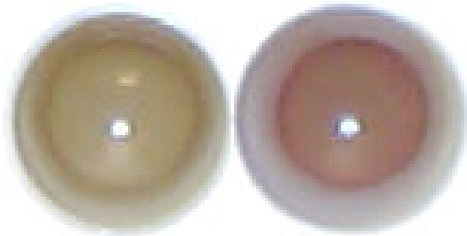
## Independent Validation Study

An external validation study was performed on behalf of the AOAC RI by Campden & Chorleywood Food Research Association. Comparing the Microgen *Listeria* ID with the online FDA/BAM method<sup>1</sup>, they found that the two methods delivered equivalent results and commented “The Microgen *Listeria*-ID Test was easy and quick to carry out compared to performing the identification procedures in the FDA-BAM method. In addition, the Microgen Test gave an identification result within 24 hours of inoculating the micro-well strip, whereas the FDA-BAM method took several days to obtain an identification result. Also, the Microgen Identification System Software was simple to use”.

## Internal Validation Studies

**Inclusivity:** A total of 91 confirmed *Listeria species* isolates were tested using the Microgen *Listeria* ID product and all were successfully confirmed by the kit.

**Exclusivity:** A total of 32 non-Listerias (closely related bacteria) were tested in the Microgen *Listeria* ID product and all were successfully rejected by the system as non-Listerias.



**Figure 2. Interpretation of Haemolysin Reactions, Positive (left), Negative (Right)**

**Method Comparison:** 10 *Listeria species* including 5 different *Listeria monocytogenes* serotypes strains and one strain each of the other five species (*innocua*, *seeligeri*, *welshimeri*, *ivanovii* and *grayi*) were tested in direct comparison to the online FDA/BAM method with complete agreement in the results achieved.

**Ruggedness:** A range of studies were undertaken to demonstrate the robustness of the identification system. These studies included tests to confirm that the kits were capable of withstanding the extremes of temperature during international shipping, the shelf-life of 1 year from the date of manufacture, and the ability of the product to be used on colonies isolated on a range of selective agar plates (including chromogenic agars).

## Conclusions

The food industry needs to be vigilant when it comes to controlling microbiological contamination throughout its production processes. One of the key bacterial pathogens which can find its way into a wide range of food products is *Listeria monocytogenes*. Whilst a number of the *Listeria* species are not considered as potential human pathogens *Listeria*

<i>Listeria Species</i>	ATCC No.	NCTC No.	Sero-Type	ID Code	Microgen ID Result	Probability	FDA BAM Result
<i>L. monocytogenes</i>	35152	7973	1/2A	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	N/A	10887	1/2B	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	N/A	N/A	1/2C	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	N/A	11994	4B	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	N/A	4883	4C	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. innocua</i>	33090	11288	6A	4546	<i>L. innocua</i>	1/1	<i>L. innocua</i>
<i>L. seeligeri</i>	35967	11856	1/2B	5445	<i>L. seeligeri</i>	1/1	<i>L. seeligeri</i>
<i>L. welshimeri</i>	35897	11857	6B	5566	<i>L. welshimeri</i>	1/1	<i>L. welshimeri</i>
<i>L. ivanovii</i>	19119	11846	N/A	5455	<i>L. ivanovii</i>	1/3	<i>L. ivanovii</i>
<i>L. grayi</i>	25400	N/A	N/A	6642	<i>L. grayi</i>	1/2	<i>L. grayi</i>

N/A = Not Applicable  
 ATCC No. = American Type Culture Collection, USA  
 NCTC = National Collection of Type Cultures, UK

**Figure 3. Summary of Method Comparison Results**

*monocytogenes* certainly is. It is important that food testing laboratories isolating *Listeria* species from food manufacturing environments can rapidly confirm which *Listeria* species they are dealing with. The Microgen Listeria ID kit delivers a complete, rapid, reliable and easy to use system for discriminating *Listeria monocytogenes* from other *Listeria* species isolates.

## NOW AVAILABLE

### AOAC-RI Validated Microgen Identification System Software Update

#### Features Include:

Updated databases

Automatically scans for non targeted species such as non-*Listeria* spp. or oxidase positive species with gram negative identification systems

## MICROGEN™ LISTERIA

### Rapid screening of Listeria like organisms prior to biochemical confirmation.

To confirm the presence of *Listeria* spp. a number of tests are normally used including esculin hydrolysis, oxidase, catalase and motility (differential at 25°C and 37°C). Microgen™ *Listeria* latex was developed as an alternative to this battery of tests.

Although individually these tests are inexpensive, they are time consuming to perform. The use of a simple latex agglutination test provides a real time 2 minute test for the confirmation to genus level of *Listeria* spp. prior to biochemical identification and species confirmation

FOR MORE INFORMATION ON THE MICROGEN IDENTIFICATION PRODUCTS AND THE MICROGEN IDENTIFICATION SYSTEM SOFTWARE OR ANY OF THE OTHER MICROGEN PRODUCT, PLEASE CONTACT YOUR LOCAL MICROGEN DISTRIBUTOR

**MICROGEN BIOPRODUCTS LTD**  
1 Admiralty Way  
Camberley  
Surrey  
United Kingdom GU15 3DT  
Phone: +44 1276 600081  
Fax: +44 1276 600181  
E-mail:  
[sales@microgenbioproducts.com](mailto:sales@microgenbioproducts.com)  
Web: [www.microgenbioproducts.com](http://www.microgenbioproducts.com)

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