Evaluation of the Microgen® Listeria ID for the Identification of Listeria species.

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Introduction

All current standard methods for the isolation and identification of ***L. monocytogenes*** employ Oxford and/ or Palcam Agar as the primary isolation medium (1,2,3). Using these media, ***L. monocytogenes*** and all other ***Listeria spp.*** grow as black colonies, 1 – 2 mm in diameter often with a black halo developing around the colony after extended incubation. These typical colonies offer no features to assist in the differentiation of ***L. monocytogenes*** from the other 5 species comprising this genus. Once isolated, the further differentiation of the members of this genus relies upon the performance of a range of key biochemical tests including mannitol, rhamnose and xylose fermentation, haemolysis and possibly the CAMP Test (4).

The Microgen® Listeria ID (Figure 1.) is a complete identification system for the differentiation of the 6 currently recognised species of Listeria. The identification system comprises aesculin hydrolysis, 10 carbohydrate fermentation tests plus a micro well haemolysin test for the differentiation of ***L. monocytogenes*** and ***L. innocua*** (Table 1.). The final analysis of the results is achieved using the Microgen® ID System Software Package (Figure 2.).

The Microgen® Listeria ID (Microgen Bioproducts, UK) was compared to the Microbact™ Listeria (Medvet Science, Australia) for the identification of ***L. monocytogenes***, with discrepant results being confirmed using conventional methods.

Figure 1. *Microgen® Listeria ID Kit*

Materials and Methods

The Microgen® Listeria ID was evaluated using the protocols described in AS/NZ 4659.3:1999: Guide to determining the equivalence of food microbiology test methods Part 3: Confirmation Tests.

Bacterial Strains

A total of 83 isolates from a range of food samples submitted to Leatherhead Food International, Surrey, UK for routine Listeria investigation were examined. All cultures were maintained in the –80°C culture collection until identified. Prior to investigation, all cultures were subcultured onto sheep blood agar plates and incubated for 18 – 24 hours at 37°C.

Substrate	Relevance to Listeria Identification
Aesculin Hydrolysis	Confirms ***Listeria spp.*** – All strains positive
Mannitol Fermentation	Differentiates ***L. grayi*** (+) from all other species (-)
Xylose Fermentation	Differentiates ***L. monocytogenes*** & ***L. innocua*** (-) from all other species (+)
Arabitol Fermentation	Confirms ***Listeria spp.*** – All strains positive
Ribose Fermentation	Differentiates ***L. grayi*** and ***L. Ivanovii*** (+) from all other species (-)
Rhamnose Fermentation	Differentiates ***L. monocytogenes*** & ***L. innocua*** (-) from all other species (+)
Trehalose Fermentation	Confirms ***Listeria spp.*** – All strains positive
Tagatose Fermentation	Differentiates ***L. welshimeri*** (+) from all other species (-)
Glucose-1-Phosphate Fermentation	Differentiates ***L. ivanovii*** (+) from all other species (-)
Methyl-D-Glucose Fermentation	Differentiates ***L. grayi*** (-) from all other species (+)
Methyl-D-Mannose Fermentation	Differentiates ***L. ivanovii*** & ***L.seeligeri*** (-) from all other species (+)
Haemolysis	Differentiates ***L. monocytogenes*** & ***L. ivanovii***, the pathogenic species(+) from all other species (-)

Table 1. *Substrates included in the Microgen® Listeria ID and their relevance to the differentiation of the various Listeria spp.*

Identification

Both test kits evaluated employ similar inoculation procedures. A single colony was selected and emulsified thoroughly in the specific suspending medium provided by the kit manufacturer. Approximately 100µl (3 drops from a pasteur pipette) of the inoculum was added to each well of the respective test systems and the test strips incubated at 37°C for 18 – 24 hours. Following incubation, the individual micro wells were read in accordance with the manufacturers instructions. All results were recorded on the work sheets provided and interpreted using the database systems provided for each system.

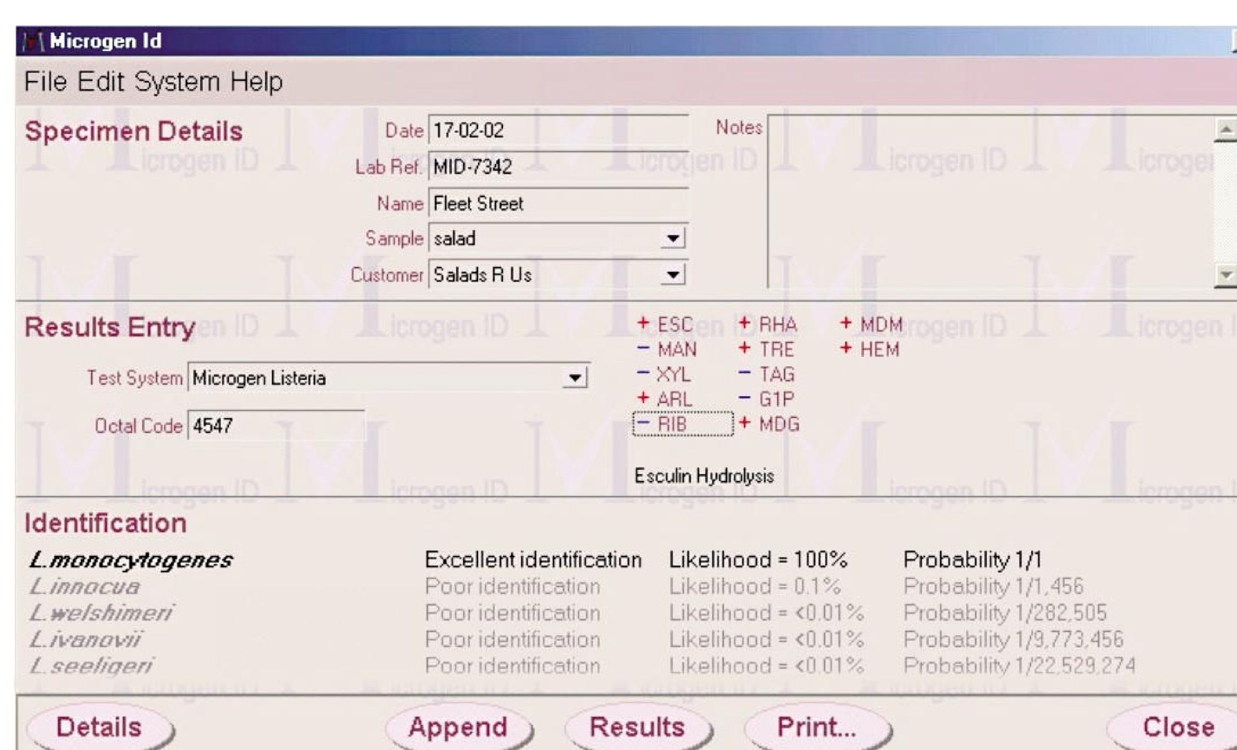

Figure 2. *Microgen® ID System Software Package Identification Screen.*

Results

A total of 83 isolates (Table 2.) were examined using both identification systems under evaluation. All 83 (100% specificity) isolates were correctly identified by the Microgen® Listeria ID and 79 (91% specificity) isolates were correctly identified using the Microbact™ Listeria (Table 3.). In all instances where discrepant results were obtained, the Microbact™ Listeria identified haemolysin negative isolates as ***L. monocytogenes***. In each of these instances, the Microgen® Listeria ID identified the isolates as ***L. innocua***. The final identification of each of these isolates was subsequently confirmed as ***L. innocua*** after repeating the haemolysis test on sheep blood agar plates. In each instance the isolate was confirmed as being non haemolytic. Further confirmation of this identification was made using an independent identification system.

Isolate	Number Tested
L. monocytogenes	37
L. innocua	36
L. ivanovii	1
L. seeligeri	4
L. welshimeri	1
L. grayi	4
Total	**83**

Table 2. *Isolates identified in the comparative trial*

	Microgen® Listeria ID	Microbact™ Listeria
Sensitivity:	100%	100%
Specificity:	100%	91%
False Positive:	0%	11%
False Negative:	0%	0%

Table 3. *Results of Microgen® Listeria ID Identifications*

Discussion

The accurate identification of ***L. monocytogenes*** is dependent upon the effective differentiation of this species from ***L. innocua***. These two species of Listeria are morphologically identical on conventional isolation media and indistinguishable based on carbohydrate fermentation tests. The key differentiating tests are the production of haemolysin and the development of a positive CAMP Test by ***L. monocytogenes***. Although both of the identification systems examined included a haemolysin reaction and despite the fact that in all cases the haemolysin reactions obtained from all isolates agreed, the final results obtained using the respective data analysis tools provided resulted in different identifications. In all instances the final identification of ***L. innocua*** was confirmed by the performance of independent identification tests.

The identification of haemolysin negative isolates as ***L. monocytogenes*** the Microbact™ Listeria was of major concern with the potential for both Public Health and economic ramifications.

The Microgen® Listeria ID was found to be easy to inoculate with the individual test reactions being clearly defined and easily interpreted. The haemolysin reaction supplied with the Microgen® Listeria ID was also found to be easier to interpret than that supplied with the Microbact™ Listeria. The major advantage of the Microgen® Listeria ID was however found to be in the composition of the data base accompanying the computerised identification system.

References

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4. Christie, R., Atkins, N.E. and Munch – Petersen, E. (1944) A note on lytic phenomenon shown by group B streptococci. Aust. J. Exp.Biol.Med.Sci., **22**: 197