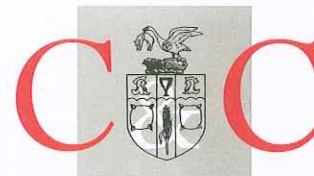


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Campden & Chorleywood Food
Research Association Group

**CAMPDEN MICROBIOLOGICAL METHOD ASSESSMENT SCHEME
(CMMAS)**

**ASSESSMENT OF
MICROGEN LISTERIA ID TEST KIT**

FINAL REPORT

PROJECT: MB/REP/88759

BY: Dr. Christopher L. Baylis

23rd August 2006

An independent assessment done by Campden & Chorleywood Food Research Association

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INTRODUCTION

Listeria species are widely distributed in nature and can be found in many foods, food ingredients, animal feed stuffs and environmental reservoirs. There are currently six species of *Listeria* (*L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. welshimeri* and *L. grayi*) with *L. monocytogenes* considered as the principal human pathogen of this genus although *L. ivanovii* and *L. seeligeri* have occasionally been associated with human disease. Although *L. monocytogenes* is the species most commonly associated with human disease, the conditions required for the growth of all of the species are often very similar. As *L. monocytogenes* may also be present in a sample containing other *Listeria* species, it is important not only to detect the presence of any *Listeria* species in a food sample but also to subsequently confirm which species are present.

Increased awareness of the presence of *Listeria* species in the human food chain, concerns over contamination by *L. monocytogenes* and regulatory requirements to test for this pathogen has led to greater testing for these bacteria and the need to rapidly and accurately identify the species present, especially if *L. monocytogenes* is among those isolated. Detection of *Listeria* species in foods has commonly been performed by classical selective culture methods followed by confirmation using traditional biochemical identification. Traditionally, identification has been achieved by performing a number of individual phenotypic tests which allow differentiation and identification of the different *Listeria* spp. The biochemical profile obtained with these tests is then used to identify the isolate. Today there are proprietary tests which incorporate a number of biochemical tests into a miniaturised multi-well format to identify *Listeria* spp. The results from these tests can be easily interpreted manually and the identity obtained with the aid of a computerised database which compares the results to the biochemical profiles derived from typical strains of each species.

Microgen *Listeria* ID is a rapid microwell based biochemical identification system capable of identifying individual *Listeria* species. The manufacturer claims this can be achieved from single colonies on selective agar plates and results are available after 18-24 hours. Microgen *Listeria* ID uses classical biochemical substrates in 11 of the 12 wells and an in-well micro-haemolysis test in well 12 (Table 1) to differentiate *Listeria innocua* from *Listeria monocytogenes*. For such a test to gain wide acceptance in laboratories the method must be reliable, easy-to-use and yield results that are comparable to the gold standard classical biochemical test methods. This assessment appraises data derived from a number of laboratories, including CCFRA, to give an independent verdict on the performance of Microgen *Listeria* ID method to identify isolates of *Listeria* spp.

PERFORMING THE TEST

Preliminary tests

Prior to the inoculation of the test strip, the following tests are recommended: Gram stain, oxidase, catalase, motility or alternatively use of a latex agglutination test e.g. Microscreen *Listeria* Latex Test (Microgen F48).

1. 18-24 hour test

Pick a single colony from an 18-24 hour culture on selective or non-selective media and emulsify in 2.5ml of *Listeria* suspending medium. Mix thoroughly to prepare a homogeneous suspension.

2. Inoculation

1. Remove a test strip from its foil pouch and place in the holding tray provided.
2. Warm the haemolysis reagent to room temperature. (For details of reagent preparation, guidance is provided in the Appendix of the instructions).
3. Remove lid from the test strip.
4. Using a sterile pasteur pipette place 4 drops (approximately 100 µl) of the bacterial suspension in each well.
5. Add 1 drop of haemolysis reagent to well 12.
6. Replace the lid.
7. Place 1 drop of the inoculum onto an appropriate non-selective medium for a purity check. Incubate at 35°C – 37°C.

3. Incubation

Incubate inoculated strips at 35-37°C for 18-24 hours in an incubator.

4. Reading of the test strips

8. Remove the strip(s) from the incubator.
9. Remove the lid.
10. Record all test results onto the report form. For assistance in interpreting the results refer to the colour chart and / or the table of reactions provided by the manufacturer (see Table 2).

5. Interpretation of test results

1. Comparison with reaction tables.
Compare the test results obtained with the predicted results in the Data Table or the dedicated software programme supplied by the manufacturer.
2. Profile register / computer aided identification package
A four digit code is used for the Microgen Listeria ID, each group of 3 reactions producing a single digit of the four-digit code. Using the results obtained, the indices of the positive reactions are circled. The sum of these indices in each group of three reactions forms the code number. The Profile Register or Microgen Computer Aided Identification Package may then be consulted for the identification choices.

TABLE 1
SUBSTRATES USED IN THE MICROGEN LISTERIA ID TEST

SUBSTRATE	CRITERIA
AESCULIN HYDROLYSIS ARABITOL FERMENTATION ALPHA-D-GLUCOSIDE FERMENTATION TREHALOSE FERMENTATION	Provide differentiation of <i>Listeria</i> spp. From other non- <i>Listeria</i> sp. All <i>Listeria</i> spp. (+) except for <i>L. grayi</i> which are Glucoside (-).
XYLOSE FERMENTATION	Provide differentiation between <i>L. grayi</i> , <i>L. monocytogenes</i> and <i>L. innocua</i> (-) and other species (+).
RHAMNOSE FERMENTATION	Provide differentiation between <i>L. monocytogenes</i> and <i>L. welshimeri</i> (+) and other species (-).
ALPHA-D-MANNOSIDE FERMENTATION	Provide differentiation between <i>L. ivanovii</i> and <i>L. seeligeri</i> (-) and other species (+).
D-TAGATOSE FERMENTATION	Provide differentiation between <i>L. welshimeri</i> (+) and other species (-).
D-RIBOSE FERMENTATION	Provide differentiation between <i>L. grayi</i> and <i>L. ivanovii</i> (+) and other species (-).
MANNITOL FERMENTATION	Provide differentiation between <i>L. grayi</i> (+) and other species (-).
GLUCOSE-1-PHOSPHATE FERMENTATION	Provide differentiation between <i>L. ivanovii</i> (+) and other species (-)
HAEMOLYSIS	Provide differentiation between <i>L. monocytogenes</i> , <i>L. ivanovii</i> and <i>L. seeligeri</i> (haemolytic, (+) and other <i>Listeria</i> spp. (non-haemolytic, (-).

TABLE 2
REACTIONS AND INTERPRETATION OF THE MICROGEN LISTERIA ID TEST

Well No.	Designation	Reaction Principle	Reaction		Comments
			Negative	Positive	
1	Esculin	Hydrolysis	Yellow	Black	
2	Mannitol	Sugar Fermentation	Purple	Yellow	Bromocresol Purple indicator changes colour when the sugar has been utilised producing acid.
3	Xylose		Purple	Yellow	
4	Arabitol		Purple	Yellow	
5	Ribose		Purple	Yellow	
6	Rhamnose		Purple	Yellow	
7	Trehalose		Purple	Yellow	
8	Tagatose		Purple	Yellow	
9	Glucose-1-Phosphate		Purple	Yellow	
10	Methyl-D-Glucose		Purple	Yellow	
11	Methyl-D-Mannose		Purple	Yellow	
12	Haemolysis	Haemolysis of sheep red blood cells	Red cell deposit	Brown	If the <i>Listeria</i> spp. under test is haemolytic then the red blood cells lyse to produce a brown colour throughout the well. If the <i>Listeria</i> spp. under test is non-haemolytic then the intact red blood cells will settle to the bottom of the well resulting in a discrete red deposit.

TEST METHOD

Test:	Identification of the Genus <i>Listeria</i> to the species level.
Sample types:	Pure isolates of <i>Listeria</i> species.
Product constituents:	The Microgen Listeria ID kit consists of: 20 test strips each sealed in a foil Pouch; 20 <i>Listeria</i> suspending media; 1 bottle of stabilised sheep red blood cells; 1 holding tray; 20 report forms.
Additional media/ reagents required:	Non-selective agar plates, reagents, media and consumables associated with the following: Gram stain, oxidase test, catalase test, motility test. Or Latex agglutination test and associated consumables.
Equipment required:	Inoculating loops; incubator (35-37°C); sterile pipettes; Computer to run the software, computer aided identification software (Product code: MID 60). Suitable storage for kit (e.g. 2-8°C refrigerator). Suitable waste disposal facilities (e.g. autoclave).
Kit shelf-life:	If kept in unopened foil pouches at 2-8°C, the kit is stable until the specified expiry date (1 year from date of manufacture).
Total Test Time:	18-24 hours.
Through-put: samples/person/day	Approximately 50.
Controls:	Controls are not specified but the recommended strains are listed in the Quality Control section of the product insert.
Training:	The strips are intended to be used by qualified and trained laboratory staff, using good microbiological techniques and aseptic procedures. However, full training can be provided by the manufacturer as required.

Technical Support: Provided by Microgen Bioproducts Ltd. in the UK.

Servicing/calibration: As the kit requires no specialist equipment, no servicing/calibration is required over and above that needed for standard laboratory equipment.

Cost (2005): List Price : 20 Tests - £110.00

Note Some of the additional items listed above are not specific to operating the Microgen Listeria ID test but are required to assist with the storage of kits and disposal of used tests. Some items are also required to perform the supplementary tests recommended by the manufacturer. It is therefore assumed that some of the items will already be in place in most microbiology laboratories and it would not be necessary to obtain these in order to perform this test.

Some of the data is taken from data provided by Microgen Bioproducts in the CMMAS questionnaire (see Appendix).

MANUFACTURER'S QUALITY CONTROL

A full quality control procedure is applied to each production lot of the Microgen Listeria ID. Each lot is tested against the following organisms.

<i>L. monocytogenes</i>	MBCC 305
<i>L. grayi</i>	MBCC 307 (ATCC 25400)
<i>L. innocua</i>	MBCC 306
<i>L. seeligeri</i>	MBCC 310
<i>L. welshimeri</i>	MBCC 308
<i>L. ivanovii</i>	MBCC 325 (NCTC 12701)

MANUFACTURER'S DETAILS

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PRACTICAL DATA

The Microgen Listeria ID kit has been evaluated extensively by the manufacturer in its own microbiology facility and tested by a number of laboratories. Some of this work is reproduced in this report to indicate the validity of the test in end user laboratories.

Manufacturer supplied information

To confirm the specificity of the Microgen Listeria ID the manufacturer has tested a range of *Listeria* spp. from its own culture collection (MBCC) against other commonly used micro-well *Listeria* biochemical identification systems i.e. API Listeria (bioMérieux) and Microbact 12L (Oxoid Ltd). The results from this comparison are presented in Table 3.

TABLE 3
SUMMARY OF MANUFACTURERS RESULTS COMPARING MICROBACT LISTERIA ID
TEST AGAINST TWO OTHER COMMERCIALY AVAILABLE IDENTIFICATION
SYSTEMS USING VARIOUS *LISTERIA* SPP.

Organism	Microgen Listeria ID	API Listeria	Microbact 12L
<i>L. monocytogenes</i>	59	59	59
<i>L. innocua</i>	22	22	22
<i>L. seeligeri</i>	9	9	9
<i>L. welshimeri</i>	7	7	7
<i>L. ivanovii</i>	4	4	4
<i>L. grayi</i>	4	4	4
Total	105	105	105

The results from the comparison study demonstrated to the Manufacturer that the Microbact Listeria ID kit gave equivalent performance to the other commercially available micro-well identification systems. No discrepant identification results between the test kits was reported in this study.

A chromogenic medium named Agar Listeria according to Ottaviani and Agosti (ALOA) has recently been incorporated into a number of international standard methods for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp. This medium has improved diagnostic properties for the recognition of *Listeria* spp and allows colonies of *L. monocytogenes* to be more easily differentiated from other *Listeria* spp. This medium has been adopted in two International Organization for Standardization (ISO) methods and also the US Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) method.

In response to the wide adoption of ALOA medium Microgen performed a small study to confirm that colonies of presumptive *Listeria monocytogenes* and other *Listeria* spp. recovered directly from this medium could be subjected to the Microgen Listeria ID test and to establish whether components of the medium interfered with the reactions of the test or had any adverse influence on the final result. The results of this study are presented in Table 4.

TABLE 4
RESULTS OF IDENTIFICATIONS USING THE MICROGEN LISTERIA ID TESTS OF
DIFFERENT *LISTERIA* SPP. RECOVERED FROM THE CHROMOGENIC ALOA MEDIUM

Organism	Microgen Listeria ID	ALOA	Expected Result
<i>L. monocytogenes</i>	23	23	23
<i>L. innocua</i>	14	14	14
<i>L. seeligeri</i>	3	3	3
<i>L. welshimeri</i>	3	3	3
<i>L. ivanovii</i>	3	3	3
<i>L. grayi</i>	3	3	3
Total	49	49	49

These results demonstrated that colonies taken directly from ALOA plates can be confirmed using the Microgen Listeria ID system and that there was no apparent interference by components of this medium.

AOAC-RI Approval

The manufacturer submitted this product to be evaluated by the AOAC-Research Institute for approval in 2004 and this was granted in September 2004. As part of this validation 91 strains of *Listeria* spp. were tested by the Microgen Listeria ID test (see Table 5).

TABLE 5
SUMMARY OF RESULTS OF INCLUSIVITY STUDY:

Organism	Microgen Listeria ID Code	Microgen Listeria ID Results	Confirmed Status (API Listeria)	Probability
<i>Listeria monocytogenes</i>	4547	55	55	All 1/1
<i>Listeria innocua</i>	4546	17	17	All 1/1
<i>Listeria seeligeri</i>	5445	8	8	All 1/1
<i>Listeria welshimeri</i>	5466	1	1	1/8
<i>Listeria welshimeri</i>	5466	1	1	1/8
<i>Listeria welshimeri</i>	5566	1	1	1/1
<i>Listeria welshimeri</i>	5566	1	1	1/1
<i>Listeria welshimeri</i>	5566	1	1	1/1
<i>Listeria ivanovii</i>	5455	3	3	All 1/3
<i>Listeria grayi</i>	6642	3	3	All 1/2
<i>Other</i>	NA	0	0	N/A
Total		91	91	

Conclusions of Inclusivity Study:

- All 91 *Listeria* species were correctly identified by Microgen Listeria ID in accordance with their confirmed status.
- Eight isolates did not give 1/1 probabilities (1/2 (3), 1/3 (3) or 1/8 (2) probabilities), however, the next possible species being suggested in each case was always greater than 1/1000.
- There was therefore no difficulty in confirming the true status of these isolates using the Microgen Listeria ID product.

Conclusions of AOAC-RI Study

The AOAC-RI study was based on the ability of this product to perform in an equivalent manner to the FDA-BAM method. The results achieved confirmed that the product produced very reproducible results with the various individual *Listeria* spp. The kit was also very robust and could be used to identify the individual *Listeria* spp. from colonies isolated on a wide range of selective and chromogenic agar plates. Furthermore, the software analysis has been specially adapted to warn the user if a non-*Listeria* spp. isolate has been introduced into the test. On screen a warning message as follows appears in red:

“Isolate may not be *Listeria* sp. – ESC and/or ARL and/or TRE are negative – Check Gram Stain, Motility, Oxidase, Catalase”.

As part of the AOAC-RI study the product was evaluated by Campden and Chorleywood Food Research Association (CCFRA). The following conclusions were made by CCFRA during this evaluation:

“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 microwell strip, whereas the FDA-BAM method took several days to obtain an identification result. Also, the Microgen Identification System Software was simple to use.”

Data supplied from external end-user trials

In addition to assessing data provided by the manufacturer, expert laboratories including CCFRA and independent studies, emphasis is also placed on data provided by end-users. The following section provides additional evidence of the performance of the Microgen Listeria-ID test compared with other commercial kits or traditional tests and the use of food isolates and type cultures.

(1) Contract Laboratory #1

Various routine food sample isolates were identified from colonies grown on selective agar plates (Oxford) using the Microgen Listeria ID and Microbact 12L systems:

TABLE 6
COMPARISON OF RESULTS OBTAINED BY A CONTRACT LABORATORY USING THE
MICROGEN LISTERIA ID TEST AND MICROBACT 12L (OXOID LTD)TEST

<i>Listeria</i> spp.	Microgen Listeria ID	Microbact 12L
<i>L. monocytogenes</i>	37	41
<i>L. innocua</i>	36	32
<i>L. grayi</i>	4	4
<i>L. welshimeri</i>	1	1
<i>L. ivanovii</i>	1	1
<i>L. seeligeri</i>	4	4
Total	83	83

All of the samples produced the same octal identification code, however, four cultures identified as *L. innocua* by the Microgen ID Listeria method were identified as *L. monocytogenes* by the Microbact 12L Listeria method (Table 6). These isolates were subsequently confirmed as non-haemolytic strains by the laboratory and are therefore considered as mis-identifications by the Oxoid Microbact system.

(2) Contract Laboratory #2

Various routine food isolates were identified from colonies grown on selective agar (Oxford) using the Microgen Listeria ID and Microbact 12L (Oxoid Ltd) systems:

TABLE 7
COMPARISON OF RESULTS OBTAINED BY A SECOND CONTRACT LABORATORY
USING THE MICROGEN LISTERIA ID TEST AND MICROBACT 12L (OXOID LTD) TEST

<i>Listeria sp.</i>	Microgen Listeria ID	Microbact 12L
<i>L. monocytogenes</i>	5	5
<i>L. innocua</i>	9	9
<i>L. grayi</i>	4	4
<i>L. welshimeri</i>	3	3
<i>L. ivanovii</i>	0	0
<i>L. seeligeri</i>	0	0
Total	21	21

The laboratory reported that both products produced expected results and that both products gave equivalent results with the strains tested.

(3) Contract Laboratory #3

Various routine food isolates were identified from colonies grown on selective agar (Oxford) using the Microgen Listeria ID and Microbact 12L systems:

TABLE 8
COMPARISON OF RESULTS OBTAINED BY A THIRD CONTRACT LABORATORY
USING THE MICROGEN LISTERIA ID TEST AND MICROBACT 12L (OXOID LTD) TEST

<i>Listeria</i> spp.	Microgen Listeria ID	Microbact 12L	Comments
<i>L. monocytogenes</i>	11*/**	11*	2 Poor ID
<i>L. innocua</i>	4	4	
<i>L. grayi</i>	0	1***	12L ID as <i>L. grayi</i>
<i>L. welshimeri</i>	1	1	
<i>Non-Listeria</i>	1***	0	MID 67 warns it's a non- Listeria
Total	17	17	

From the results achieved by Contract laboratory # 3 the laboratory demonstrated that the two kits gave broadly equivalent results with the same isolates (Table 8).

The results obtained from the isolates yielding discrepant results (Table 8) are presented in more detail in Table 9. One isolate (coded sample 2399029) was identified as a non-*Listeria* species by Microgen Listeria ID but as *L. grayi* by Microbact 12L. This result demonstrates the non-*Listeria* spp. isolate feature of the Microgen Software Program in this validation study. However, it is not known if further tests were performed on this isolate to establish the true identify of this organism. Consequently, this result cannot be categorically confirmed as a mis-identification by the Microbact 12L test.

For another isolate (Lab Sample 2466459**) both systems identified the isolate poorly as *L. monocytogenes*, however, the probability in Microgen Listeria ID at 1: 1,146 was much closer to the expected code than the Microbact 12L result with the very low probability of 1: 1,145,200. The results from a third isolate (Lab Sample 2397477*) may have been produced due to a mixed culture being introduced into the identification systems.

TABLE 9
RESULTS FOR THREE ISOLATES PREVIOUSLY GIVING DISCREPANT RESULTS

Lab No.	Microbact 12L	Software ID	Comment	Microgen ID	Software ID	Comment
2397477*	6567	L. mono poor	MAN & TAG Against	6567	L. mono poor	MAN & TAG Against
2466459**	4767	L. mono poor	RIB & TAG Against	4567	L. mono OK	TAG Against
2399029***	6261	L. grayi poor	ARL, TAG & HAEM against	6261	ARL Negative Check Pre-tests	Probable non-Listeria

Whilst the results from this laboratory do show some equivalence between the Microgen Listeria ID tests and Microbact 12L, some discrepant results were obtained which cannot be fully explained. The manufacturer has reported that further training of staff in this laboratory in the use of the Microgen Listeria ID system was undertaken following the trial as they considered the results obtained were not as consistent as would be expected for this product.

(4) Contract Food and Veterinary Laboratory

Various food isolates of *Listeria* spp. were identified using the Microgen Listeria ID and the classical set of standard biochemical tests (Haemolysis, CAMP test, Xylose & Rhamnose fermentations) on colonies grown on both selective agar and the non-selective medium Tryptone Soya Yeast Extract (TSYE) agar (Table 10) and the selective chromogenic medium ALOA (AES Laboratoire) (Table 11). The set of biochemical tests used following growth on TSAYE are generally regarded as the gold standard tests for the identification of *Listeria* spp.

TABLE 10
AGREEMENT BETWEEN IDENTIFICATION RESULTS BY MICROGEN LISTERIA ID
TEST AND CONVENTIONAL BIOCHEMICAL TESTS OF SINGLE COLONIES OF
***LISTERIA* GROWN ON TSAYE (24H)**

<i>Listeria species</i>	No. tested	No. correctly identified		Agreement (%)
		Microgen <i>Listeria</i> ID	Conventional method	
<i>L. monocytogenes</i>	49	49	49	100
<i>L. innocua</i>	25	25	25	100
<i>L. welshimeri</i>	5	5	5	100
<i>L. seeligeri</i>	5	5	5	100
<i>L. ivanovii</i>	16	16	16	100
<i>L. grayi</i>	0	NA	NA	NA
TOTAL	100	100	100	100

All of the isolates tested by the Microgen *Listeria* ID after 24h growth on TSAYE were correctly identified and there was 100% agreement with the results obtained using the “gold standard” biochemical tests (Table 10).

TABLE 11
AGREEMENT BETWEEN IDENTIFICATION RESULTS BY MICROGEN LISTERIA ID
TEST AND CONVENTIONAL BIOCHEMICAL TESTS OF SINGLE COLONIES OF
***LISTERIA* GROWN ON CHROMOGENIC ALOA MEDIUM (24H)**

<i>Listeria species</i>	No. tested	No. correctly identified		Agreement (%)
		Microgen <i>Listeria</i> ID	Conventional method	
<i>L. monocytogenes</i>	25	25	25	100
<i>L. innocua</i>	13	13	13	100
<i>L. welshimeri</i>	3	3	3	100
<i>L. seeligeri</i>	2	2	2	100
<i>L. ivanovii</i>	7	7	7	100
<i>L. grayi</i>	0	NA	NA	NA
TOTAL	50	50	50	100

The results obtained from colonies grown for 24h on ALOA medium gave correct identifications and there was 100% agreement with the results from the traditional method.

The results show that the Microgen Listeria ID system delivered equivalent results to the gold standard biochemical tests and that the product can be used on colonies taken from both non-selective and selective chromogenic media.

(5) NHS Food & Environmental Testing Laboratory

A Food and Environmental testing unit of a NHS Microbiology Department was commissioned to perform a small validation study to confirm the routine performance of the Microgen Listeria ID (MID 67) product. The laboratory routinely used API Listeria (bioMérieux) so the results achieved using API Listeria were compared to the Microgen Listeria ID product. Initially six strains covering each of the *Listeria* spp. were supplied by Microgen to test by the laboratory as a pre-qualification that they were achieving the confirmed results for these particular isolates (Table 12). Once the laboratory had confirmed their results for the six supplied strains they then proceeded to test their own *Listeria* spp. isolates. Therefore, the test panel comprised the following:

TABLE 12
SIX STRAINS OF GOOD PROVENANCE SUPPLIED BY MICROGEN
BIOPRODUCTS FOR TESTING BY THE LABORATORY

<i>Listeria</i> Species	Serotype	MBCC No	ATCC No	NCTC No	Type Strain
<i>L. monocytogenes</i>	1/2A	107	35152	7973	Proposed
<i>L. innocua</i>	6A	95	33090	11288	Yes
<i>L. seeligeri</i>	1/2B	110	35967	11856	Yes
<i>L. welshimeri</i>	6B	114	35897	11857	Yes
<i>L. ivanovii</i>	N/A	96	19119	11846	Yes
<i>L. grayi</i>	N/A	307	25400		

TABLE 13
RESULTS OBTAINED BY THE LABORATORY FOR SIX STRAINS
OF *LISTERIA* SUPPLIED BY MICROGEN BIOPRODUCTS FOR
TESTING BY THE LABORATORY

MBCC no.	Sample No. (identifier)	Organism	Microgen Listeria ID Identification	API Listeria Identification
107	MBCC 107	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
95	MBCC 95	<i>L. innocua</i>	<i>L. innocua</i>	<i>L. innocua</i>
110	MBCC 110	<i>L. seeligeri</i>	<i>L. seeligeri</i>	<i>L. seeligeri</i>
114	MBCC 114	<i>L. welshimeri</i>	<i>L. welshimeri</i>	<i>L. welshimeri</i>
96	MBCC 96	<i>L. ivanovii</i>	<i>L. ivanovii</i>	<i>L. ivanovii</i>
307	MBCC 307	<i>L. grayi</i>	<i>L. grayi</i>	<i>L. grayi</i>

The laboratory achieved the correct results using the strains supplied by Microgen Bioproducts (Table 13) and the laboratory proceeded to compare results for a number of suspect *Listeria* spp. isolated from food samples (Table 14). A summary of the results obtained for the food isolates is presented in Table 15.

TABLE 14
RESULTS OBTAINED WITH SUSPECT *LISTERIA* SPP ISOLATED FROM FOOD SAMPLES

Test no.	Sample No. (identifier)	Organism	Source (Food Type)	Microgen Listeria ID Identification	API Listeria Identification
1	139255	<i>L. monocytogenes</i>	Sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
2	136893	<i>L. monocytogenes</i>	Salad	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
3	136843	<i>L. monocytogenes</i>	Salad	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
4	134785	<i>L. monocytogenes</i>	Salad	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
5	141199	<i>L. monocytogenes</i>	Sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
6	126707	<i>L. monocytogenes</i>	Meat Pate	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
7	119398	<i>L. monocytogenes</i>	Cooked Rice	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
8	124866	<i>L. monocytogenes</i>	Cooked Ham	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
9	124811	<i>L. monocytogenes</i>	Cooked Fish	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
10	115990	<i>L. monocytogenes</i>	Mayonnaise	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
11	114540	<i>L. monocytogenes</i>	Swab	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
12	111285	<i>L. monocytogenes</i>	Cooked Beef	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
13	136685	<i>L. monocytogenes</i>	Sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
14	139056	<i>L. innocua</i>	Smoked Fish	<i>L. innocua</i>	<i>L. innocua</i>
15	129867	<i>L. innocua</i>	Swab	<i>L. innocua</i>	<i>L. innocua</i>
16	136233	<i>L. innocua</i>	Sandwich	<i>L. innocua</i>	<i>L. innocua</i>
17	124339	<i>L. innocua</i>	Fish Pate	<i>L. innocua</i>	<i>L. innocua</i>
18	113515	<i>L. innocua</i>	Butter	<i>L. innocua</i>	<i>L. innocua</i>
19	114856	<i>L. innocua</i>	Butter	<i>L. innocua</i>	<i>L. innocua</i>
20	139257	<i>L. welshimeri</i>	Fresh Bream	<i>L. welshimeri</i>	<i>L. welshimeri</i>
21	136073	<i>L. welshimeri</i>	Salad	<i>L. welshimeri</i>	<i>L. welshimeri</i>
22	134869	<i>L. welshimeri</i>	Cooked Ham	<i>L. welshimeri</i>	<i>L. welshimeri</i>
23	136464	<i>L. welshimeri</i>	Salad	<i>L. welshimeri</i>	<i>L. welshimeri</i>
24	141613	<i>L. monocytogenes</i>	Egg Fried Rice	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
25	136298	<i>L. seeligeri</i>	Soft Cheese	<i>L. seeligeri</i>	<i>L. seeligeri</i>

TABLE 15
SUMMARY OF RESULTS OBTAINED FOR *LISTERIA* ISOLATED FROM FOOD SAMPLES

<i>Listeria sp.</i>	Microgen Listeria ID	API Listeria
<i>L. monocytogenes</i>	15	15
<i>L. innocua</i>	7	7
<i>L. grayi</i>	1	1
<i>L. welshimeri</i>	5	5
<i>L. ivanovii</i>	1	1
<i>L. seeligeri</i>	2	2
Total	31	31

It is not known if the laboratory tested isolates directly from selective media but it is suspected that they may have been previously grown on a non-selective medium prior to the test. The results obtained by this laboratory showed 100% agreement between the Microgen Listeria test and the API Listeria test (Table 15).

(6) NHS Food & Environmental Testing Laboratory

A Food and Environmental testing unit of a NHS Microbiology Department was commissioned to perform a small validation study to confirm the routine performance of the Microgen Listeria ID (MID 67) product. The laboratory routinely used API Listeria test (bioMérieux) so the results achieved using API Listeria were compared to the Microgen Listeria ID product. Initially 6 strains covering all *Listeria* spp. were supplied by Microgen to test by the laboratory as a pre-qualification that they were achieving the confirmed results for these particular isolates (see Table 12). Once the laboratory had confirmed their results for the 6 supplied strains they then proceeded to test their own *Listeria* spp. isolates. All of the 6 strains were correctly identified by the laboratory (data not presented) and a number of food isolates were subjected to identification the two test kits (Table 16).

TABLE 16
RESULTS OBTAINED WITH SUSPECT *LISTERIA* SPP ISOLATED FROM FOOD SAMPLES

Test No.	Sample No.	Source (food type)	Microgen Listeria ID	API Listeria ID
1	56452	Cheese sandwich	<i>L. innocua</i>	<i>L. innocua</i>
2	49460	Chicken sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
3	50751	Caesar Salad	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
4	49460	Chicken sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
5	50608	Egg sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
6	55416	Gorgonzola	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
7	51272	Caesar salad	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
8	54027	Chicken pasta salad	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
9	49459	Cheese sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
10	49458	Tuna sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
11	56837	Cheese sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
12	56838	Tuna sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
13	56839	Egg sandwich	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
14	57519	Chicken fajita	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
15	57521	Cheese	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
16	57639	Smoked mussels	<i>L. welshimeri</i>	<i>L. welshimeri</i>
17	54027	Chicken pasta salad	<i>L. innocua</i>	<i>L. innocua</i>
18	54027	Chicken pasta salad	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>
19	54132	Cheddar cheese	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>

As with a previous laboratory it was not known if the laboratory had tested isolates directly from selective media but it is suspected that they may have been previously grown on a non-selective medium prior to the test. The results obtained by this laboratory showed 100% agreement between the Microgen Listeria test and the API Listeria test (Table 17).

TABLE 17
SUMMARY OF RESULTS OBTAINED FOR *LISTERIA* ISOLATED FROM FOOD SAMPLES

<i>Listeria</i> sp.	Microgen Listeria ID	API Listeria
<i>L. monocytogenes</i>	17	17
<i>L. innocua</i>	3	3
<i>L. grayi</i>	1	1
<i>L. welshimeri</i>	2	2
<i>L. ivanovii</i>	1	1
<i>L. seeligeri</i>	1	1
Total	25	25

(7) **NHS Food & Environmental Testing Laboratory**

A Food and Environmental testing unit of a NHS Microbiology Department was commissioned to perform a small validation study to confirm the routine performance of the Microgen Listeria ID (MID 67) product. The laboratory routinely used API Listeria (bioMérieux) so the results achieved using API Listeria were compared to the Microgen Listeria ID product. Initially, 11 strains covering all *Listeria* spp. were supplied by Microgen to be tested by the laboratory as a pre-qualification that they were achieving the confirmed results for these particular isolates. Once the laboratory had confirmed their results for the 11 supplied strains they then proceeded to test their own *Listeria* spp. isolates. Therefore, the test panel comprised the following:

TABLE 18
ELEVEN STRAINS OF *LISTERIA* SUPPLIED BY MICROGEN
BIOPRODUCTS FOR TESTING BY THE LABORATORY

Listeria Species	Serotype	MBCC No	ATCC No	NCTC No	Type Strain
<i>L. monocytogenes</i>	1/2A	107	35152	7973	Proposed
<i>L. innocua</i>	6A	95	33090	11288	Yes
<i>L. seeligeri</i>	1/2B	110	35967	11856	Yes
<i>L. welshimeri</i>	6B	114	35897	11857	Yes
<i>L. ivanovii</i>	N/A	96	19119	11846	Yes
<i>L. grayi</i>	N/A	307	25400	N/A	No
<i>L. monocytogenes</i>	N/A	305	N/A	N/A	No
<i>L. innocua</i>	N/A	306	N/A	N/A	No
<i>L. welshimeri</i>	N/A	308	N/A	N/A	No
<i>L. seeligeri</i>	N/A	310	N/A	N/A	No
<i>L. ivanovii</i>	N/A	325	12701	N/A	N/A

TABLE 19
RESULTS OBTAINED BY THE LABORATORY FOR ELEVEN STRAINS OF *LISTERIA*
SUPPLIED BY MICROGEN BIOPRODUCTS FOR TESTING BY THE LABORATORY

Test No.	Sample No. (identifier)	Organism	NCTC No.	ATCC No.	Type Strain	Microgen Listeria ID Identification	API Listeria Identification
107	MBCC 107	<i>L. mono</i>	7973	35152	proposed	<i>L. mono</i> (4547)	<i>L. mono</i> (6550)
95	MBCC 95	<i>L. innocua</i>	11288	33090	Yes	<i>L. innocua</i> (4546)	<i>L. innocua</i> (7510)
110	MBCC 110	<i>L. seeligeri</i>	11856	35967	Yes	<i>L. seeligeri</i> (5445)	<i>L. seeligeri</i> (3310)
114	MBCC 114	<i>L. welshimeri</i>	11857	35897	Yes	<i>L. welshimeri</i> (4466)	<i>L. welshimeri</i> (7311)
96	MBCC 96	<i>L. ivanovii</i>	11846	19119	Yes	<i>L. ivanovii</i> (4445)	<i>L. ivanovii</i> (3350)
307	MBCC 307	<i>L. grayi</i>	N/A	25400	No	<i>L. grayi</i> (6642)	<i>L. grayi</i> (7120)
1	MBCC 305	<i>L. mono</i>	N/A	N/A	No	<i>L. mono</i> (4547)	<i>L. mono</i> (6510)
2	MBCC 306	<i>L. innocua</i>	N/A	N/A	No	<i>L. innocua</i> (4546)	<i>L. innocua</i> (7510)
3	MBCC 308	<i>L. welshimeri</i>	N/A	N/A	No	<i>L. welshimeri</i> (4566)	<i>L. welshimeri</i> (7511)
4	MBCC 310	<i>L. seeligeri</i>	N/A	N/A	No	<i>L. seeligeri</i> (5445)	<i>L. seeligeri</i> (3310)
5	MBCC 325	<i>L. ivanovii</i>	12701	N/A	Yes	<i>L. ivanovii</i> (4455)	<i>L. ivanovii</i> (3250)

L. mono denotes *Listeria monocytogenes*

The laboratory achieved the correct results using the strains supplied by Microgen Bioproducts (Table 19) and the laboratory proceeded to compare results for a number of suspect *Listeria* spp. isolated from food samples (Table 20). A summary of the results obtained for the food isolates is presented in Table 21.

TABLE 20
RESULTS OBTAINED WITH SUSPECT *LISTERIA* SPP ISOLATED FROM FOOD SAMPLES

MBC C No.	Sample No.	Organism	Source (food type)	Microgen Listeria ID	API Listeria ID
6	6524	<i>L. innocua</i>	Pork tongue	<i>L. innocua</i> (4546)	<i>L. innocua</i> (7510)
7	6483	<i>L. innocua</i>	Ox tongue	<i>L. innocua</i> (4546)	<i>L. innocua</i> (7510)
8	7642	<i>L. seeligeri</i>	Cheese sandwich	<i>L. seeligeri</i> (5445)	<i>L. seeligeri</i> (3310)
9	7719	<i>L. seeligeri</i>	Ox tongue	<i>L. seeligeri</i> (5445)	<i>L. seeligeri</i> (3310)
10	7746	<i>L. seeligeri</i>	Ox tongue	<i>L. seeligeri</i> (5445)	<i>L. seeligeri</i> (3310)
11	7911	<i>L. monocytogenes</i>	Ox tongue	<i>L. monocytogenes</i> (4547)	<i>L. monocytogenes</i> (6510)
12	7888	<i>L. monocytogenes</i>	Turkey sandwich	<i>L. monocytogenes</i> (4547)	<i>L. monocytogenes</i> (6510)
13	6104	<i>L. monocytogenes</i>	Cooked pork	<i>L. monocytogenes</i> (4547)	<i>L. monocytogenes</i> (6510)
14	5753	<i>L. monocytogenes</i>	Cooked turkey	<i>L. monocytogenes</i> (4547)	<i>L. monocytogenes</i> (6510)
15	5756	<i>L. innocua</i>	Cooked chicken	<i>L. innocua</i> (4546)	<i>L. innocua</i> (7510)
16	5185	<i>L. monocytogenes</i>	Cooked chicken	<i>L. monocytogenes</i> (4547)	<i>L. monocytogenes</i> (6510)
17	5189	<i>L. monocytogenes</i>	Chicken sandwich	<i>L. monocytogenes</i> (4547)	<i>L. monocytogenes</i> (6510)
18	7716	<i>L. monocytogenes</i>	Ox tongue	<i>L. monocytogenes</i> (4545)	<i>L. monocytogenes</i> (2510)
19	7718	<i>L. monocytogenes</i>	Ox tongue	<i>L. monocytogenes</i> (4547)	<i>L. monocytogenes</i> (6510)

As with two previous laboratories it was not known if this laboratory had tested isolates directly from selective media but it is suspected that they may have been previously grown on a non-selective medium prior to the test. The results obtained by this laboratory showed 100% agreement between the Microgen Listeria test and the API Listeria test (Table 21).

TABLE 21
SUMMARY OF RESULTS OBTAINED FOR *LISTERIA* ISOLATED FROM FOOD SAMPLES

<i>Listeria sp.</i>	Microgen Listeria ID	API Listeria
<i>L. monocytogenes</i>	10	10
<i>L. innocua</i>	5	5
<i>L. grayi</i>	1	1
<i>L. welshimeri</i>	2	2
<i>L. ivanovii</i>	2	2
<i>L. seeligeri</i>	5	5
Total	25	25

CONCLUSIONS

This report details results obtained in food testing laboratories for the identification of members of the Genus *Listeria*. The Microgen Listeria ID system performed well against three other recognised systems, classical standard biochemical tests, API Listeria (bioMérieux) and Microbact 12L (Oxoid Ltd.).

The manufacturer has also demonstrated that the test can be performed directly on colonies grown on chromogenic media, such as ALOA medium, as well as other selective agars e.g. Oxford agar. Furthermore, the manufacturer recommends that only one colony is taken directly from a selective or non-selective agar plate used per test and that the test should be read between 18-24 hours. However, although there appears to be no adverse effect on this test from selective agents used in many selective *Listeria* media, as with other tests that also enable isolates to be taken directly off selective primary isolation plates, care must be taken to ensure that the isolate taken is pure and that sufficient growth is available to ensure correct performance of the test. Failure to observe this could potentially result in incorrect results. If in doubt we would recommend that colonies from primary isolation plates are streaked for purity or that a purity plate is streaked at the same time as performing the test. This way the test could be repeated if the test results indicate a mixed culture.

The overall results of the end-user trial are summarised in Table 22.

TABLE 22
SUMMARY OF RESULTS OBTAINED DURING THE END-USER RING TRIAL

<i>Listeria sp.</i>	No. correctly Identified	
	Microgen Listeria ID	Comparison method*
<i>L. monocytogenes</i>	168	172
<i>L. innocua</i>	102	98
<i>L. ivanovii</i>	28	28
<i>L. welshimeri</i>	22	22
<i>L. seeligeri</i>	19	19
<i>L. grayi</i>	11	12
<i>Non-Listeria</i>	1	0
Total	351	351

The “comparison systems” used varied and included the API Listeria test (81 isolates) which was used in 3 laboratories, Microbact 12L (121 isolates) which was used in 3

laboratories and conventional biochemical tests (150 isolates) which were used in 1 laboratory. Furthermore, most of the tests were performed on colonies grown on standard selective Oxford agar, however, in some cases the new chromogenic agar ALOA was used. The use of different agars containing selective agents and dyes did not appear to affect the test, although the user would be advised to check first with the manufacturer or conduct trials if a different medium was to be used in future.

All of the studies comparing Microgen Listeria ID and either API Listeria (bioMérieux) or conventional biochemical tests gave equivalent results. In the case of Microbact 12L (Oxoid Ltd) there were some minor differences in the interpretation of the results but not in the reactions produced in the test. Four discrepant identifications were noted in validation study (1). These discrepancies did not demonstrate differences in the substrate reactions as the isolates produced the same codes in Microgen Listeria ID as Microbact 12L.

It was understood from the Manufacturer that the updated software program for use with the Microgen Listeria ID test was reported to be extremely useful by end-users especially the feature whereby it also informs the user if they have inadvertently introduced a non-Listeria into the test. This was demonstrated during the trials when a non-Listeria isolate was inadvertently tested by the laboratory performing study (3).

The Microbact software delivered an identification of *L. grayi* although the isolate was negative for Arabitol which indicated that it was a non-Listeria species isolate. However, it is not known if this isolate was subjected to further identification tests to confirm this.

Results from this study highlight the need for users to receive proper training and in some instances to be more careful and proficient in the use and interpretation of this and similar kinds of identification tests. Unlike genotypic tests, phenotypic tests (i.e. biochemical tests) can be more subjective to interpret and test results can be inadvertently misinterpreted. Furthermore, the results from biochemical tests rely on strains giving typical profiles with the range of test substrates in the panel. If an atypical strain is encountered i.e. one with a mutation that renders it unable to ferment a particular carbohydrate, or which has lost or acquired the ability to lyse blood cells i.e. non-haemolytic *L. monocytogenes* or haemolytic *L. innocua*, the results may be incorrect. During this study the discrepancies were probably due to errors in the interpretation as the Microgen Listeria ID kit was only compared against another phenotypic test.

The manufacturer also supplied data generated in their own laboratory and a comprehensive AOAC-RI report which further supports the performance of the test. These studies demonstrate the robustness, stability, reproducibility, specificity and equivalence in performance of this test compared to the international FDA/BAM method.

Overall, the Microgen Listeria ID test gave equivalent results to the other commercially available micro-well biochemical identification products for the identification of species belonging to the Genus *Listeria*. The product is robust and the manufacturer claims that it can be used on colonies grown on a wide range of selective and chromogenic agars. However, if colonies are being taken from primary isolation plates the user must ensure that growth is derived from a single well isolated colony, otherwise the test could give misleading or incorrect results if the isolate is mixed. This is also true of other similar kits and if possible the laboratory would be advised to perform identification on colonies that have been purified on a non-selective medium first as recommended in many of the reference methods for the identification of *Listeria* spp.

Appendix: CMMAS Questionnaire information supplied by Microgen Bioproducts

CAMPDEN MICROBIOLOGICAL METHODS ASSESSMENT SCHEME (CMMAS)

Method Validation Scheme Questionnaire to Manufacturers.

All information will be held in confidence.

SECTION 1

TEST INFORMATION

1.1. Company Information

Name and Address: Microgen Bioproducts Limited,
1 Admiralty Way,
Camberley, Surrey GU15 3DT

Contact Name: Dr. Stuart Clark
Technical Director

Telephone: 01276 600081
FAX: 01276 600151
E. mail: sales@microgenbioproducts.com
Website: www.microgenbioproducts.com

1.2. Name of your Product

Microgen Listeria ID (Product Code MID-67).

1.3. Microbiological target (e.g. organism / toxin) to be analysed.
(presumptive or confirmed result if applicable).

Confirmed identification of individual *Listeria* species.

1.4. Basis of the technique with brief details of how your product works.

Is there a requirement for the user to establish a microbiological calibration curve prior to application? No.

Please supply a method instruction sheet when returning this questionnaire.

Confirmed identification of all *Listeria* species using their abilities/inabilities to ferment certain substrates in conjunction with an in well haemolysis test to differentiate *L. monocytogenes* from *L. innocua*.

1.5. Product Components (i.e. what is included in the product purchased?).

Individual pouches (1 test/pouch) x 20.

Suspending media x 20.

Holding tray x 1.

Stabilised sheep red blood cells x 1 bottle (5ml).

Report cards x 20.

Product Insert (Instructions for use including colour chart).

1.6. What additional equipment/reagents are required for the complete test, i.e. sample to results?

None – All are provided.

1.6.1. Incubators (numbers, temperatures).

Incubator (not CO₂) set at 35 - 37°C.

- 1.6.2. Waterbaths (numbers, temperatures).
N/A.
- 1.6.3 Refrigerators (temperature required).
2 - 8°C for storage of kit.
- 1.6.4 Centrifuges (RCF required, volumes, refrigerated).
N/A.
- 1.6.5. Other Microbiological Equipment (autoclave, steamer, etc.).
N/A.
- 1.6.6. Any other equipment.
Inoculation Loops.
- 1.6.7 Pipettes (standard, automatic).
Yes.
- 1.6.8. Specialist instruments (manipulators, readers, washers, etc.).
N/A.
- 1.6.9. Diluents (types/volumes).
N/A.
- 1.6.10. Media (types/volumes of broth & agar).
N/A.

1.6.11. Any other chemicals/reagents (including specific cleaning chemicals & disinfectants).

N/A.

1.7. Reagent pack size.

1.7.1. What pack sizes are available?

N/A.

1.7.2. How many tests can be performed from one reagent test pack of each size?

N/A.

1.8. What is the shelf-life of your product?

12 months from date of manufacture.

1.8.1. as supplied (specify storage conditions).

2 - 8°C.

1.8.2. after reconstitution (detail individual components & storage conditions).

N/A.

1.9. Sample types (meat, milk, water, swabs, etc.) that your product is aimed at.

Isolates from any source.

1.10. What is the total elapsed time from setting up sample to obtaining:

1.10.1 a negative result?

< 24 hours.

- 1.10.2 a positive result (please state whether presumptive or confirmed)?
< 24 hours – confirmed.
- 1.10.3 a confirmed positive result?
- 1.11 If your product forms part of the test, specify elapsed time.
- 1.12 What is the test "hands on" time for your product i.e. actual technical time needed to prepare and perform the test.
- 1.12.1 for the minimum number of samples (specify)?
1 test takes < 2 minutes.
- 1.12.2 for the optimal number of samples (specify)?
N/A. Conformations done as required.
- 1.13. Once the test result from your product has been obtained, what if any, work is required to confirm the result?
- 1.13.1. Is any further confirmatory testing required.
No
- 1.13.2. Please specify the type of confirmatory test required.
- 1.13.3. Details of any confirmatory tests that you recommend and indicate time required.
- 1.14. What is the test throughput of your product? (No. samples/person/8h day).
N/A.

1.15. What positive/negative controls are required when using your product (specify type & frequency)?

1.16. Are the controls specified in (15) provided with your product, if not what do you recommend?

<i>L. monocytogenes</i>	(ATCC 35152, NCTC 7973)
<i>L. innocua</i>	(ATCC 33090, NCTC 11288)
<i>L. grayi</i>	(ATCC 19120, NCTC 10815)

QUALITY

1.17. What Quality Control measures are taken by the manufacturer? If applicable please return an example of QC data for your product?

Quality reports available on separate sheets.

1.18. What quality control information do you routinely supply to users?

Supplied on request.

SAFETY

1.19. What hazardous materials are incorporated in or are required to be used with your product or its components? Indicate which are supplied with your product.

See package insert supplied.

1.20. Is a hazard data sheet available for your product or components? Please return with completed questionnaire.

Yes.

1.21. Please give any waste disposal details? (Autoclave, sharps, chemicals, etc.).

Used strips should be autoclaved.

TRAINING & SERVICING

- 1.22. What training do you recommend users have before using your product routinely?. (Detail costs if applicable).

Minimal training is required and is always offered to users free of charge.

- 1.23. Does your product contain a software programme? If so, please supply information concerning updating of computer programmes.

A windows based dedicated identification software package is recommended to users and is available from the manufacturer (Product Code MID 60).

1.24.

- 1.24.1. What external calibration/servicing do you recommend for equipment you supply (please supply a copy of typical service and/or calibration contract(s) and prices).

N/A.

- 1.24.2. State frequency and cost of a single visit.

N/A.

- 1.24.3. State frequency and cost of a single calibration visit.

N/A.

- 1.25. What user calibration is required to ensure that the equipment you supply works optimally?

N/A.

ECONOMICS

1.26.

1.26.1. What is the cost per single sample (including controls) based on your current list price? (Please supply a copy of your current price list).

£ 5.50.

1.26.2. What is the cost per sample for 100 samples (including controls)?

£ 330.00 per 100 samples.

1.27. What is the cost of any special instrumentation that you supply? (please give details and list prices).

N/A.

2.2.2. Artificial inoculation

- Number of different microorganisms/toxins etc. studied (include numbers of target and non-target types used).
- Number of different sample types used (please list categories).
- Sensitivity of your product and total test procedure indicating minimum detection level and percentage of false negatives.

2.2.3. Uninoculated samples

- Number of different sample types used (please list categories).
- Sensitivity of your product and total test procedure indicating minimum detection level and percentage of false negatives.
- Selectivity of your product and total procedure indicating any specific inaccuracies e.g. false positives of false negatives.

2.3. Confirmation

- Number of different microorganisms studied (include numbers of target and non-target types used).
- Sensitivity of your product indicating minimum detection level and percentage of false negatives.
- Selectivity of your product indicating any specific inaccuracies e.g. false positives.