P-013 A New System for the Rapid Isolation, Detection and Identification of *Listeria* Species from Food Samples

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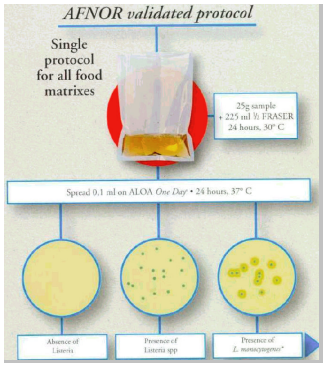
Abstract

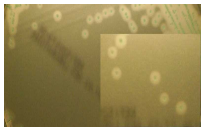
Listeria monocytogenes is considered an important food borne pathogen. By combining two existing commercial products, a chromogenic agar ALOA (AES Laboratoire) and a biochemical identification system Microgen Listeria ID (Microgen Bioproducts), we have been able to deliver full biochemical identification of all *Listeria* species isolated from food samples in three days. The food sample is processed then incubated for 24 hours in half Fraser broth. The broth sample is used to inoculate an ALOA chromogenic agar plate, after 24 hours the colonies can be interpreted to give a presumptive identification due to the colour of the colonies on the ALOA plate. *Listeria* species produce blue/green colonies with *Listeria monocytogenes* and *Listeria ivanovii* producing a halo (clear zone) around their colonies. Any other organisms that may grow in this system produce white colonies so they are easily differentiated from true *Listeria* species colonies. Individual *Listeria* species colonies are then used directly from the chromogenic agar to undergo full biochemical identification using the Microgen Listeria ID system. There is no need to transfer the cells onto non-selective agar or to set up a blood agar plate as the Microgen Listeria ID system contains a micro-haemolysis well.

ALOA has been fully validated under the AFNOR regulatory framework in France and is also cited in the BAM method. In the AFNOR studies ALOA plates delivered more colonies per plate at 24 hours than PALCAM or OXFORD at 48 hours and *Listeria monocytogenes* colonies were recovered from samples artificially contaminated with between 1-10 CFU/25g. In both the ALOA and the alternative method (Oxford) 100% of positive samples produced colonies of *Listeria monocytogenes* from a range of food samples. The ALOA / Microgen Listeria ID combined system has recently been approved in France by AFNOR.

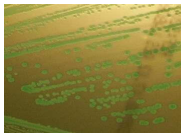
This system offers the food-testing laboratory a rapid culture based method for the isolation, early presumptive identification (day 2) and full biochemical speciation of contaminating *Listeria* species in three days without the need to invest in instrumentation.

Chromogenic Culture Method





Photograph 1a: L. monocytogenes on ALOA characteristic blue/green colonies with clear halo



Photograph 1b: L. innocua on ALOA characteristic green colonies

Summary of AFNOR Validation Study

Dairy Products:

ALOA Method	ISO Method		Total
	+	-	
+	34	3	37
-	0	53	53
Total	34	56	90

Concordance: 96.66% **Artificially contaminated: 19/90**

Meat Products:

ALOA Method	ISO Method		Total
	+	-	
+	33	0	33
-	0	30	30
Total	33	30	63

Concordance: 100% **Artificially contaminated: 2/63**

Fish Products:

ALOA Method	ISO Method		Total
	+	-	
+	43	0	43
-	0	38	38
Total	43	38	81

Concordance: 100% **Artificially contaminated: 20/81**

Vegetable Products:

ALOA Method	ISO Method		Total
	+	-	
+	30	0	30
-	0	60	60
Total	30	60	90

Concordance: 100% **Artificially contaminated: 27/90**

All Products:

ALOA Method	ISO Method		Total
	+	-	
+	140	3	143
-	0	181	181
Total	140	184	324

Concordance: 99.07% **Artificially contaminated: 68/324**

Performance Evaluation 1:Contract Food and Veterinary Laboratory

A contract Food and Veterinary Laboratory in France tested various food isolates of *Listeria* sp. using the Microgen Listeria ID and the classical set of standard biochemical tests (Haemolysis, CAMP, Xylose & Rhamnose)* on colonies grown on both selective agar TSYE and the chromogenic agar ALOA (AES Laboratoire):

TSYE Results:

Listeria species	*Standard Tests**	*Microgen Listeria ID*	*Concordance*
L monocytogenes	49	49	=100%
L. innocua	25	25	=100%
L. welshimeri	5	5	=100%
L. seeligeri	5	5	=100%
L. ivanovii	16	16	=100%
L grayi	0	0	
Total	100	100	= 100%

ALOA Results:

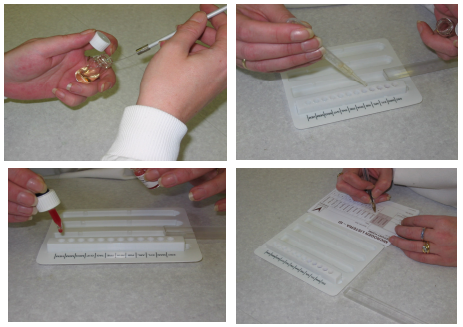
Listeria species	*Standard Tests**	*Microgen*	*Listeria*	*ID Concordance*
L monocytogenes	25	25		=100%
L. innocua	13	13		=100%
L. welshimeri	3	3		=100%
L. seeligeri	2	2		=100%
L. ivanovii	7	7		=100%
L grayi	0	0		
Total	50	50		= 100%

Performance Evaluation 2: Manufacturer Supplied Data

The Microgen Listeria ID system was compared to another miniaturised biochemical identification system for the differentiation of Listeria sp., the API Listeria ID system.

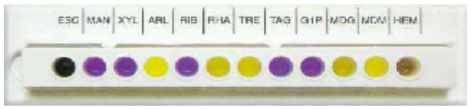
Organism	Microgen Listeria ID	API Listeria
L. monocytogenes	59	59
L. innocua	22	22
L seeligeri	9	9
L. welshimeri	7	7
L. ivanovii	4	4
L. grayi	4	4
Total	105	105

Conclusion: The performance of the Microgen Listeria ID system matches that of the API Listeria system



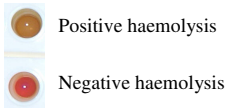
Photographs 3a-3d: Set-up and read Microgen Listeria ID

3a. Emulsify 1 colony in Listeria suspending broth
3b. Inoculate wells 1-12 with 100ul of suspending broth
3c. Inoculate 100ul of red blood cell solution into well 12
3d. Record results after 18-24 hours on report cards provided



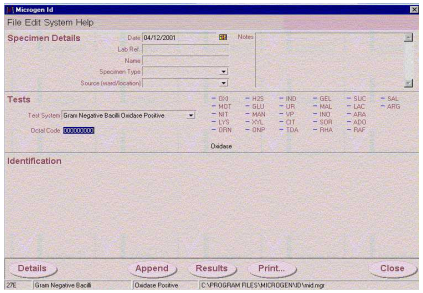
Photograph 4: A typical result from the Microgen Listeria ID strip. Aesculin positive black, positive results yellow and negative results purple for the 10 sugars, heamolysis positive brown liquid.

Photograph 5: Haemolysis Positive brown liquid, Negative clear liquid carpet of red cells



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

Table 2: The Microgen Listeria ID Substrates and how they are used to differentiate individual Listeria species one from another.



Photograph 6: Microgen ID Software Programme

Once the results have been noted they can be interpreted by reference to the product insert, see Table 3, or input into theMicrogen ID programme (Product Code: MID 60).

The software programme will deliver a probability result to determine which Listeria species has been isolated

	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.innocua	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.ivanovi	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

Table 3: Listeria database

This database reflects the probability that a Listeria species will produce a positive result in each substrate well. Please note that Listeria species are normally expected to be positive for Aesculin, Arabitol and Trehalose. These substrates are treated as four sets of three reactions weighted (4,2,1) giving a four digit code which is interpreted by the MID-60 software, see above Photograph 5.

CONCLUSIONS

- **ALOA differentiates L. monocytogenes/ivanovii from other Listeria species after 24 hour (presumptive result)**
- **Microgen Listeria ID then delivers full identification in a further 18-24 hours (Confirmation)**
- **A single colony grown on ALOA is used to inoculate the Microgen Listeria ID**
- **The Microgen Listeria ID system uses classical substrates**
- **ALOA has been written into the new draft ISO method & is AFNOR approved**
- **The system matches the current ISO method performance**
- **Microgen Listeria ID is currently undergoing AOAC RI**
- **The micro-haemolysis well avoids the need for additional tests**
- **Microgen Listeria ID can also be performed on colonies isolated on a range of selective media**

Acknowledgements

The authors thank Mrs Jessica Sprigens (Microgen Bioproducts) for her assistance in producing this poster. The authors also thank Ms Sarah Seymour (Microgen Bioproducts) for performing the Manufacturers validation study of Microgen Listeria ID.

The authors would also wish to thank the Laboratoire de Touraine, Tours, France for performing the validation study of Microgen Listeria ID tested direct from ALOA plates in comparison with classical biochemical testing.