Evaluation of the Rosco system for the identification of *Listeria* species

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Summary. The Rosco system was used to identify previously confirmed isolates of the seven currently recognised species of *Listeria*. These included reference cultures and recent isolates from clinical material, food products and environmental sources. The system identified all correctly. Results were obtained after 4 h if heavy inocula, as suggested by the manufacturers, were used. The method may be used to aid identification of isolates of *Listeria* from clinical and non-clinical specimens and would be of particular value in laboratories examining small numbers of isolates relatively infrequently. Essential tests not included in the system are β -haemolysis on sheep-blood agar and the CAMP test.

Introduction

Human listeriosis, with few exceptions, is caused by *Listeria monocytogenes*, a gram-positive aerobic and facultatively anaerobic bacillus. Microbiological and epidemiological evidence from sporadic^{1,2} and epidemic³ cases of listeriosis has implicated various foodstuffs as the source of the organism. Contaminated food products are now regarded as the primary means of transmission of *L. monocytogenes*.³ These findings have led to extensive investigations into the prevalence of *L. monocytogenes* and other species of *Listeria* in food. Products which may contain these organisms include dairy and meat products, chicken, seafood, vegetables⁴ and pre-cooked, chilled meals.⁵

Listeria spp. are widely distributed in the environment and, with improvements in isolation techniques, clinical laboratories are faced with the task of examining food samples as suspected sources of infection when clinical cases have been diagnosed. Speed is crucial in the investigation of any presumed food-borne infection; therefore, a simple, robust method that will speciate *Listeria* rapidly and accurately is needed.

The identification of presumptive *Listeria* spp. relies heavily on the results of carbohydrate fermentation tests. Traditionally these tests may require up to 7 days to obtain results.⁶ Other, more rapid, methods do not identify *L. monocytogenes* accurately⁷ or are more suitable for large numbers of isolates.⁸ We have evaluated the ability of the Rosco system (Rosco Diagnostica, Taastrup, Denmark) for determining carbohydrate fermentation reactions of fastidious organisms to identify isolates of *Listeria* to species level. This system detects pre-formed enzymes and,

therefore, does not depend on growth of the test organism. Furthermore, sterile inoculation conditions are not required. However, as with all other commercially available systems for the identification of *Listeria* spp., separate determination of β -haemolysis and the result of the CAMP test are also needed for definitive identification.⁶

Materials and methods

Strains

One hundred and fifty-five isolates of *Listeria* spp. were examined; 71 were supplied by others and 84 were from our own laboratory (tables I and II). Cultures were maintained on blood agar or tryptose phosphate agar slopes at 4°C. All were also identified and speciated by the procedures described by McLauchlin.⁶

Identification

Cultures were incubated for 18–24 h in air at 37°C on 5% horse-blood agar and the presence of β -haemolysis was noted. A heavy suspension of growth—corresponding to MacFarland's standard 8: number of cells $c. 2 \times 10^9$ /ml—was made in sterile saline and 0.25-ml samples were dispensed into six 100 $\times$ 10 mm tubes. Trehalose, salicin, mannitol, rhamnose, xylose and α -methyl mannosidase tablets (Rosco) were then added and each tube was briefly agitated on a vortex mixer.

After incubation at 37°C, tests for the fermentation of trehalose, salicin, mannitol, rhamnose and xylose were considered to be positive if a yellow-orange (4 h) or yellow (24 h) colour was observed. Tests were

Table I. Isolates of *L. monocytogenes*

Reference culture and identification numbers	Serotype	Source of culture	Origin (where known)
NCTC 4883	4c	National Collection of Type Cultures, Central PHL, Colindale, London	Fowl myocardium
NCTC 5105	3a	National Collection of Type Cultures, Central PHL, Colindale, London	Man
NCTC 5214	4a	National Collection of Type Cultures, Central PHL, Colindale, London	Sheep brain
NCTC 9862	2	National Collection of Type Cultures, Central PHL, Colindale, London	Neonatal meningitis
NCTC 9863	4b	National Collection of Type Cultures, Central PHL, Colindale, London	Rabbit
NCTC 10357	1a	National Collection of Type Cultures, Central PHL, Colindale, London	Sheep
NCTC 10890	7	National Collection of Type Cultures, Central PHL, Colindale, London	Human faeces
CLIP 12501	3a	Institut Pasteur, Paris, France	
CLIP 12502	3b	Institut Pasteur, Paris, France	
CLIP 12503	3c	Institut Pasteur, Paris, France	
LH1-4, 6, 7, 10, 12, 14, 15, 18, 19, 26, 32, 34	1	Public Health Laboratory, Hull	
LH5, 8, 9, 11, 13, 16, 17, 20-25, 27-31, 33		Public Health Laboratory, Hull	
YL1, 2, 4-8	4	Yorkshire Water, York	Sewage
YL3	1	Yorkshire Water, York	Sewage
TW5, 17	1	University of Leeds	Cook-chill meal
TW23, 39	4	University of Leeds	Pre-cooked chicken
LC1, 2, 4, 5, 7-12	4	University of Leeds	Human blood (placenta)
LC3, 6	1	University of Leeds	Human blood
EC2, 3, 4A, 8, 12, 40G, A1B, 420, 438, 81C, 82	4	University of Leeds	EC2-12 pre-cooked chicken
EC15A, 16A, 17A, 18A, 19A, 20H, 29, 31-33, 40A, 43G, 59, 62, 65, 67, 70, 71, 81A, 87, 93, 109, 110	1	University of Leeds	EC15-19 Raw chicken EC20-110 pre-cooked chicken
DM32, 35, 40, 47, 49, 60, 75, 90, 100	1	University of Leeds	Soft cheese
NY2, 3, 4, 6, 16	1	University of Leeds	Soft cheese
NY5, 14	4	University of Leeds	Soft cheese

deemed to be negative if, at 4 h, a pink colour or, at 24 h, an orange or pink colour was observed. A positive test for α -methyl mannosidase was the development of a yellow colour after 4 h or 24 h. The tube remained colourless if the result was negative.

Results and discussion

With heavy inocula, all tests could be read after incubation for 4 h. The manufacturers suggest that lighter inocula—McFarland's standard 2: number of cells $c. 6 \times 10^8/\text{ml}$ —may be used. We found that when this low density of inoculum was used the results were

difficult to interpret after incubation for 4 h and tests for α -mannosidase activity and rhamnose fermentation gave false-negative results. With both inocula, results of all tests were unequivocal after incubation for 24 h. The results obtained with all isolates were in complete accordance with those obtained by conventional tests (table III). It should be noted that *L. monocytogenes* and *L. innocua* yielded identical results in the system and that these organisms should be distinguished by the presence or absence of haemolysis on sheep-blood agar and the results of the CAMP test with *Staphylococcus aureus*.

McGowan *et al.*⁷ recommended the API STREP

Table II. Isolates of *Listeria* spp. other than *L. monocytogenes*

Reference strains and numbers	Species	Source	Origin of isolates
CLIP7842	<i>L. ivanovii</i>	Institut Pasteur	Sheep
ATCC19119	<i>L. ivanovii</i>	University of Würzburg, Germany	
NCTC11007	<i>L. ivanovii</i>	National Collection of Type Cultures	
KC1714	<i>L. ivanovii</i>	US Department of Agriculture, Cincinnati, OH	
CI94	<i>L. innocua</i>	US Department of Agriculture, Cincinnati, OH	
NCTC11288	<i>L. innocua</i>	University of Würzburg	Bovine brain
NCTC11289	<i>L. innocua</i>	University of Würzburg	Human faeces
CLIP8011	<i>L. innocua</i>	Institut Pasteur	Cook-chill meals
TW4, 8, 11	<i>L. innocua</i>	University of Leeds	
EC15B, 17C, 18E, 190, 101	<i>L. innocua</i>	University of Leeds	
CLIP100100	<i>L. seeligeri</i>	Institut Pasteur	Pre-cooked chicken
LA15	<i>L. seeligeri</i>	US Department of Agriculture	
ATCC35967	<i>L. seeligeri</i>	University of Würzburg	
TW21	<i>L. seeligeri</i>	University of Leeds	
EC1, 4C, 16C, 23, 40E, 41A, 42A, 43A	<i>L. seeligeri</i>	University of Leeds	
2434KA	<i>L. welshimeri</i>	US Department of Agriculture	Corn
SLCC5334	<i>L. welshimeri</i>	University of Würzburg	
CLIP8149	<i>L. welshimeri</i>	Institut Pasteur	
NCTC10812	<i>L. murrayi</i>	National Collection of Type Cultures	
ATCC2540	<i>L. murrayi</i>	University of Würzburg	
SLCC2080	<i>L. grayi</i>	University of Würzburg	Corn
NCTC10815	<i>L. grayi</i>	National Collection of Type Cultures	

Table III. Biochemical reactions of *Listeria* spp. in the Rosco system

Species tested (n)	Result obtained (expected)† in tests with					
	trehalose	salicin	mannitol	rhamnose	xylose	α -methyl mannosidase*
<i>L. monocytogenes</i> (120)	+	+	-	+	-	+
<i>L. ivanovii</i> (4)	+	+	-	-	+	-
<i>L. innocua</i> ‡ (12)	+	+	-	+ (V, 72.2%)	-	+
<i>L. seeligeri</i> (12)	+	+	-	-	+	-
<i>L. welshimeri</i> (3)	+	+	-	V, 33% (V, 66%)	+	+
<i>L. murrayi</i> (2)	+	+	+	+ (V, 66%)	-	+
<i>L. grayi</i> (2)	+	+	+	-	-	+

+, $\geq 90\%$ of strains gave positive results; -, $\leq 10\%$ of strains gave positive results; V, variable, percentage of positive results.

* Substrate is *p*-nitrophenyl- α -D-mannopyranoside.

† Data from references 9, 10, 12.

‡ *L. monocytogenes* and *L. innocua* gave identical results. These species are distinguished by β -haemolysis on blood agar and a positive CAMP test with *S. aureus* for *L. monocytogenes*.

system (API, La Balme des Grottes, France) for the rapid identification of isolates of *Listeria* from human clinical material. This system is intended for the identification of streptococci and does not include tests for xylose and rhamnose fermentation or α -methyl-D-mannosidase activity; therefore, it is not possible to identify *L. monocytogenes* conclusively with the API STREP system. These authors also suggested that the fermentation of ribose was a useful character for differentiating *L. ivanovii* from *L. monocytogenes*, but c. 10% of *L. monocytogenes* isolates also ferment this carbohydrate.⁹ Thus, identification of *Listeria* spp. on the basis of ribose fermentation is inconclusive.⁸ Rocourt and Catimel reported that the API 50CH system was successful for the speciation of *Listeria*.⁹ This system is relatively expensive, and employs a large number of substrates, many of which are not relevant to the identification of *Listeria* spp. Furthermore, it does not lend itself to the examination of large numbers of isolates. The Mast ID system (Mast Laboratories, Bootle, Merseyside) employs a multipoint inoculation method and can be used to determine carbohydrate fermentation, aesculin hydrolysis and acetoin production.⁸ Although this system permits the examination of large numbers of strains, it requires incubation for 18–24 h and the preparation of individual agar plates for each test. If the maximum 21 isolates per plate are examined, then the cost per isolate is the lowest of all the commercial *Listeria* speciation systems which are at present available and

compares very favourably with a conventional "in house" method.

Recently, Robinson *et al.* have described another commercially available kit for the identification of *Listeria* spp.¹¹ This system identified 98.1% of 137 isolates of *Listeria* correctly. The technique employs 15 biochemical tests, several of which are not pertinent to the identification of *Listeria* spp., e.g., urease production, and it does not include a test for α -mannosidase activity. This system is relatively expensive.

The Rosco system was relatively inexpensive and could be read after incubation for 4 h if heavy inocula were employed. The system also included a test for aesculin hydrolysis. This test would be useful when neither enrichment broth nor plating medium contain a ferric ammonium citrate-aesculin indicator system. This technique is only slightly more expensive than the Mast ID system.

In conclusion, we recommend the Rosco system as a rapid, inexpensive method for the identification of *Listeria* spp. from human, food and environmental sources, particularly in laboratories that speciate *Listeria* infrequently and when a long shelf life (carbohydrates 2 years, α -mannosidase 4 years) of the test system is important.

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