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Evaluation of API 20 STREP system for identifying *Listeria* species

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SUMMARY The API 20 STREP system was used to identify 146 known strains from seven species of the genus *Listeria*, including both pathogenic and environmental strains. The gallery was easy to use and tests, with the exception of leucine arylamidase (LAP) and starch fermentation (AMD), were simple to interpret. Identification to genus level was satisfactory but differentiation between species was poor. Using the API 20 STREP the haemolytic species *L monocytogenes, seeligeri,* and *ivanovii* could easily be differentiated from the non-haemolytic species *L welshimeri, innocua, grayii* and *murrayi*. Of the haemolytic species, *L monocytogenes* could not be distinguished from *L seeligeri* but *L ivanovii* could be separated from the two other haemolytic species because it fermented ribose. Non-haemolytic *L welshimeri* could not be differentiated from non-haemolytic *L innocua,* but mannitol and ribose fermenting non-haemolytic *L grayi* and *L murrayi* were easily differentiated from the other two non-haemolytic species.

The API 20 STREP identified *Listeria* in four hours and therefore might be used for rapid identification of strains causing infection in man. It would, however, not be useful to identify environmental isolates when speciation is important.

The incidence of human listeriosis is increasing in the United Kingdom.[1] Methods for isolation of *Listeria* have improved[2] and there is greater understanding of the pathogenesis of the infection and its association with the presence of *Listeria* in food. The use of rapid rather than traditional methods for identification of *Listeria* species, however, may result in misidentification.[2-4] The API 50 CH and API-ZYM systems have already been used to identify *Listeria*[4] but neither of these are widely used in the United Kingdom. The current API 20 STREP system was introduced before the advances in *Listeria* taxonomy were made in the early 1980s.[2] We therefore examined the ability of API 20 STREP to identify *Listeria* species.

Material and methods

One hundred and forty six *Listeria* species were used; 119 strains of *Listeria monocytogenes,* eight strains of *Listeria seeligeri,* six strains of *Listeria ivanovii,* four strains of *Listeria welshimeri,* seven strains of *Listeria innocua* and one strain each of *Listeria grayi* and *murrayi*. They were stored on blood agar or Dorset egg slopes at 4°C for up to 18 months before being tested. The original isolates were obtained from our own laboratory, Professor R Postlethwaite, department of bacteriology, University of Aberdeen, Dr R Fenton, School of Agriculture, Aberdeen, The National Collection of Type Cultures and Dr J McLauchlin, Central Public Health Laboratory, Colindale, London, Professor H P R Seeliger, University of Würzburg, West Germany, and Dr J Rocourt, Pasteur Institute, Paris, France.

The organisms were taken from stored cultures on to blood agar plates and incubated at 37°C for 18–24 hours. Individual colonies were then streaked on to blood agar plates and incubated anaerobically at 37°C for 18–24 hours. From the resultant growth a heavy suspension was made in 2 ml sterile distilled water and used to inoculate the first half of the API 20 STREP strip (tests acetoin production (VP) to arginine dihydrolase (ADH)). About 0·5 ml of the same suspension was added to the ampoule of API 20 STREP medium provided and then inoculated into the second half of the strip (tests ribose fermentation (RIB) to glycogen fermentation (GLYG), and on to sheep and horse blood agars to test for haemolysis. Mineral oil was used to overlay cupules arginine dihydrolase (ADH) to glycogen fermentation (GLYG). After four

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hours' incubation at 37°C reagents ZYM A and ZYM B were added to cupules pyrrolidonylarylamidase (PYRA) to LAP; ninhydrin was added to the hyppurate hydrolysis (HIP) cupule and VP and VP 2 reagents were added to the VP cupule (reagents supplied by API). After 10 minutes the results obtained in the tests were then interpreted according to the manufacturer's guide sheet. Haemolysis was assessed on both horse and sheep blood after overnight culture.

Results

Aesculin (ESC), ADH, and carbohydrate reactions were read at four and 24 hours, and were for the most part easily scored. The profile numbers given by each species are shown in table 1. After four hours' incubation 56% of strains gave positive LAP tests, but this test was difficult to interpret. In contrast, the VP, HIP, ESC and trebalose fermentation (TRE) tests were easier to read at four hours and the reactions universally positive. AMD fermentation was often difficult to interpret and therefore scored as +/− for calculating profile numbers (table 2). API profiles read at four hours showed that all *L monocytogenes* strains gave good, very good, or excellent identification (table 1). At 24 hours' incubation ESC, TRE, and AMD were all positive and a variable number of strains fermented lactose, raffinose, and ribose. Eighty per cent of the 146 strains fermented lactose and one strain fermented raffinose. All of the *L ivanovii* strains and both *L murrayi* and *L grayi* strains fermented ribose;

Table 1 *Four hour API 20 STREP number profiles*

Profile	*Strain*	*Per cent giving profile*	*APILAB software* ID*
7000010	*L monocytogenes*	13·3 (16)	Good
	L seeligeri		
7000010/1	*L monocytogenes*	16·6 (20)	
	L seeligeri	25·0 (2)	
	L welshimeri	25·0 (1)	
	L ivanovii	33·3 (2)	
7000011	*L monocytogenes*	7·5 (9)	Very good
	L innocua	14·3 (1)	
7040010	*L monocytogenes*	13·3 (16)	Very good
	L seeligeri	12·0 (1)	
	L ivanovii	33·3 (2)	
	L innocua	14·3 (1)	
7040010/1	*L monocytogenes*	40·0 (48)	
	L seeligeri	25·0 (2)	
	L welshimeri	50·0 (2)	
	L ivanovii	33·3 (2)	
	L innocua	71·4 (5)	
7040011	*L monocytogenes*	8·3 (19)	Excellent
	L seeligeri	25·0 (2)	
	L welshimeri	25·0 (1)	
7040110/1	*L grayi/murrayi*	100·0 (2)	Very doubtful

*APILAB software will only identify *L monocytogenes*.

Table 2 *API 20 STREP profiles of Listeria species read after four and 24 hours' incubation*

Species	*Four hour*		*24 hour*	
	Profile	*Per cent strains*	*Profile*	*Per cent strains*
L monocytogenes	7000010	13·3 (16)	7000015	9·2 (11)
	7000010/1*	16·6 (20)	7000415	30·0 (36)
	7000011	7·5 (9)	7040015	14·2 (17)
	7040010	13·8 (16)	7040415	45·0 (54)
	7040010/1	40·0 (48)	7040455	0·8 (1)
	7040011	8·3 (10)		
L seeligeri	7000010	12·5 (1)	7000415	25·0 (2)
	7000010/1	25·0 (2)	7002415	12·5 (1)
	7040010	12·5 (1)	7040015	25·0 (2)
	7040010/1	25·0 (2)	7040415	37·5 (3)
	7040011	25·0 (2)		
L welshimeri	7000010/1	25·0 (1)	7000411	25·0 (1)
	7040010/1	50·0 (2)	7040411	75·0 (3)
	7040011	25·0 (1)		
L ivanovii	7000010/1	33·0 (2)	7002015	16·6 (1)
	7040010	33·0 (2)	7002415	16·6 (1)
	7040010/1	33·0 (2)	7042015	16·6 (1)
			7042415	33·0 (2)
			7042455	16·6 (1)
L innocua	7000011	14·3 (1)	7000411	14·3 (1)
	7040010	14·3 (1)	7040411	87·1 (6)
	7040110/1	71·4 (5)		
L gray/murrayi	7040110/1	100·0 (2)	7042511	100·0 (2)

*AMD test equivocal therefore scored 0/1.

Table 3 *24 hour API 20 STREP number profile*

Profile	*Strain*	*Per cent giving profile*		*APILAB software* ID*
7000015	*L monocytogenes*	9·2	(11)	Excellent
7000415	*L monocytogenes*	30·0	(36)	Very good
	L seeligeri	25·0	(2)	
7000411	*L welshimeri*	25·0	(1)	Very good
	L innocua	14·3	(1)	
7002015	*L ivanovii*	16·6	(1)	Good
7002415	*L ivanovii*	16·6	(1)	Good
	L seeligeri	12·0	(1)	
7040015	*L monocytogenes*	14·2	(17)	Excellent
	L seeligeri	25·0	(2)	
7040411	*L welshimeri*	75·0	(3)	Excellent
	L innocua	87·7	(6)	
7040415	*L monocytogenes*	45·0	(54)	Excellent
	L seeligeri	38·0	(3)	
7040455	*L monocytogenes*	0·8	(1)	Very doubtful
7042015	*L ivanovii*	16·6	(1)	Very good
7042415	*L ivanovii*	33·3	(2)	Good
7042455	*L ivanovii*	16·6	(1)	Very doubtful
7042511	*L grayi/murrayi*	100·0	(2)	Very doubtful

*APILAB software will only identify *L monocytogenes*.

none of the other species except one strain of *L seeligeri* was reactive in this test. All strains of *L monocytogenes, seeligeri,* and *ivanovii* tested were haemolytic on horse and sheep blood agar, while none of the *L innocua, welshimeri, grayi* and *murrayi* strains produced detectable haemolysins. API profiles after 24 hours gave good, very good, or excellent identification for all but one strain of *L monocytogenes* (the raffinose fermenter), but, as the APILAB software does not recognise other *Listeria* species, *L monocytogenes* can be misidentified (table 3).

Discussion

Rocourt and Catmel reported that 82% of their 70 *Listeria* strains were LAP positive compared with only 56% in this study and 97% on the API data base.[4] These differences may be related to difficulty in interpreting the test. Lactose fermentation was reported with 90% of strains using the API 50 CH[4] and 95–100% using conventional methods.[5,6] Eighty per cent of our strains were lactose fermenters, which is in keeping with those observations. Similarly, only 0·8% of the *L monocytogenes* we tested fermented raffinose; previously raffinose fermentation has been reported to be rare.[7] Ribose was fermented by all five strains of *L ivanovii* while no *L monocytogenes* and only one strain of *L seeligeri* fermented this carbohydrate. Ribose fermentation therefore deserves further investigation as a differential test for speciation of *Listeria*.

Haemolysis around *Listeria* colonies is useful in differentiating between species because all the *L monocytogenes, L ivanovii,* and *L seeligeri* strains we tested were haemolytic on both sheep and horse agar while *L welshimeri, L innocua, L grayi* and *L murrayi* were not. This is in keeping with a considerable volume of reported work.[4] Of the haemolytic species, *L seeligeri* cannot be differentiated from *L monocytogenes* by the use of the API 20 STREP, but *L ivanovii* can as it ferments ribose. The non-haemolytic species *L innocua* and *L welshimeri* cannot be differentiated by API 20 STREP but the non-haemolytic, mannitol and ribose fermenting *L grayi* and *L murrayi* are easily differentiated from other *Listeria* spp. The API 20 STREP is thus adequate to identify *Listeria* to the genus level and this can be achieved in four hours.

The APILAB software package is out of date with regard to *Listeria* taxonomy, however, and the strip is not especially useful for speciation of *Listeria*. This is important when identifying environmental isolates such as those found in food stuffs, where *L monocytogenes* is not the only *Listeria* isolated. Most, if not all, *Listeria* causing disease in man in the United Kingdom, however, are likely to be *L monocytogenes*, and the API 20 STREP may be of greatest use in laboratories which do not stock the range of conventional biochemical tests required for provisional identification.[2] Referral to a central reference laboratory for serotyping, speciation, and, if necessary, phage typing, is recommended.

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