

## A method and medium for the electrical detection of *Listeria* spp. from food

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Received 6 March 1994; revision received 6 June 1994; accepted 21 June 1994

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### Abstract

The development of a liquid medium for the detection of *Listeria* spp. by capacitance monitoring of food samples previously enriched in UVM 1 broth is described. Rapid growth of *Listeria monocytogenes* was shown to occur in liquid media with selectivity based on antibiotics found in Oxford agar. The final capacitance medium contained higher concentrations of the Oxford selective agents than Oxford agar and did not require the esculin/ferric ammonium citrate reaction to be observed. The medium relied upon the ability of *Listeria* spp. to induce a greater than 30% change in capacitance within 30 h. When run in parallel with the *Listeria* spp. test samples of a large food company, the method gave far fewer false-positive results than Fraser broth.

**Keywords:** *Listeria*; Electrical; Detection; Enrichment; Medium

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### 1. Introduction

The rapid detection of *Listeria* spp. in food samples is complicated due to a need for enrichment and the large number of false-positive results which derive from differential enrichment media (Warburton et al., 1991a,b) such as Fraser broth (Fraser and Sperber, 1988). The traditional methods need skilled interpretation of reactions, and time delays are caused by requiring colony formation of

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*Listeria* spp. Colonies may take two or more days to develop aerobically on selective plates such as Oxford agar (Curtis et al., 1989). Oxford agar has been reported to be marginally the best isolation medium for *Listeria monocytogenes* (Walker et al., 1990; Lund et al., 1991; Warburton et al., 1991a,b), though it may inhibit some *L. monocytogenes* lithium chloride sensitive strains (Anon., 1993).

Novel methods, for example using ELISA (e.g. *Listeria*-Tek, Organon Teknika; or MiniVidas, BioMerieux) or gene probe technology (e.g. Accuprobe, Gen-probe) are extremely rapid but expensive. The major source of the running costs with these methods is the need to examine large numbers of false-positive results from primary screening.

Previous work published on the electrical detection of *Listeria* spp. has mainly relied upon conversion of standard media (Phillips and Griffiths, 1989; Bolton, 1990). Conversion of solid aerobic media for use in liquid culture changes the growth environment significantly and as *Listeria* spp. are facultative anaerobes (Farber and Peterkin, 1991), this change may be beneficial to these bacteria. Hancock et al. (1993) reported the development of a new medium containing moxalactam and proflavine hydrochloride as selective agents, with impedance monitoring in order to identify enrichment samples inoculated with combinations of various *Listeria* spp. and other bacteria in pure and mixed cultures.

The Vitec-Bactometer (BioMerieux) produces graphs of percentage change of impedance, conductance or capacitance versus time in a medium and also gives the time to detection. This detection time is a measure of the time taken for the rate of change in the measured parameter to reach a predetermined value and is inversely proportional to the log of viable bacteria inoculated into the medium (Richards et al., 1978): this theory is used when quality control laboratories apply electrical detection to bacterial count estimation. An estimate of the growth rate can also be calculated from the difference in detection times for two different dilutions. Detection of the growth of a pure culture of bacteria by the Bactometer generally occurs when the cell concentration reaches  $10^6 - 10^7$  CFU/ml (Firstenberg-Eden and Eden, 1984). Data can also be analyzed to give measurements of the maximum change from the initial value.

Electrical media design for specific microorganism detection from food is difficult due to the complex nature of metabolism by mixed culture. Microbially induced capacitance changes in media are less well understood than those of conductance, which makes prediction of the net result of metabolism untenable (Owens, 1985). The ideal isolation medium for *Listeria* spp. would not allow other genera to grow or metabolize; this ideal is generally realized to be practically unobtainable. Using a "typical curve" identification is also not practical because the presence of other bacteria interferes with capacitance change. The pure culture approach by Phillips and Griffiths (1989) successfully identified a direction for further research, but problems caused by food samples were not addressed. Bolton (1990) applied conductance monitoring to *L. monocytogenes* detection but also did not approach the problems of mixed culture. Hancock et al. (1993) suggested that observation of a Bactometer detection time within 36 h was sufficient to identify positive cheese samples in their medium.

## 2. Materials and methods

### 2.1. Media preparation

Preparation of the Bactometer base medium was as follows; 20 g/l tryptone, 20 g/l yeast extract, 17.5 g/l lithium chloride (all Oxoid), 4 g/l D-glucose (BDH) and 1 g/l esculin (Sigma) were dissolved and sterilized at 121°C for 15 min. Additional constituents were added by making stock solutions and filter sterilizing the required volume into the Bactometer base medium. Finally, the pH was aseptically adjusted to  $7.2 \pm 0.1$ . Other media were prepared in accordance with the manufacturers instructions.

### 2.2. Methods

(i) *Pure culture*. Three lots of Bactometer base medium were prepared and 10 ml/l of a 5% (w/v) ferric ammonium citrate (Sigma) solution added to each. *Listeria* selective supplements [Oxford formulation] (Oxoid) which contain 200 mg cycloheximide, 5 mg colistin sulphate, 1.25 mg acriflavin, 0.5 mg cefotetan and 2.5 mg fosfomycin per vial were reconstituted according to manufacturers instructions and added to the three media at one, one and a half and double the concentration recommended for Oxford agar (2, 3 and 4 vials/l).

Two test cultures were used; *L. monocytogenes* 3a (NCTC 5105) and an esculin positive *Enterococcus faecalis* (University of Surrey Collection Number 363), which grew well on Oxford agar.

The test organisms were inoculated into tryptone soy broth plus 20 g/l yeast extract (TSBYE, Oxoid) and incubated at 30°C for 24 h. One ml volumes of the three media were added to Bactometer module wells and 0.1 ml of a dilution series of the test organisms in maximum recovery diluent (MRD, Oxoid) added, with each test being done in triplicate. The Bactometer (Model 120SC, BioMerieux) was set to measure capacitance for a test time of 24 h at 30°C.

(ii) *Inoculated food*. Twenty g samples of food were aseptically added to 180 ml of *Listeria* selective broth base plus *Listeria* selective supplements [UVM 1 formulation] (UVM 1, Oxoid) in quadruplicate and homogenized in a stomacher for 2 min. The four foods used were live natural yoghurt, white sliced bread, salted butter and pasteurized milk. Following dilution in MRD, cultures of the two organisms previously grown in TSBYE (24 h at 30°C) were inoculated into each UVM 1 food enrichment in the following estimated final concentrations: (a) 15 CFU/ml *L. monocytogenes*, (b) 17 CFU/ml *E. faecalis*, (c)  $1.7 \times 10^5$  CFU/ml *E. faecalis* and 15 CFU/ml *L. monocytogenes*, (d) no inoculation.

The levels of organisms in the inoculum were determined using standard plate count techniques on nutrient agar (Oxoid) for 48 h at 30°C.

UVM 1 was incubated for 24 h at 30°C and then 0.05 ml transferred into individual wells in the Bactometer modules, into which 2 ml of medium had previously been added. Each test was duplicated at this stage. The medium used consisted of the Bactometer base medium with four Oxford selective supplement

vials/l and 20 ml/l of 5% (w/v) ferric ammonium citrate solution. The Bactometer was set to measure capacitance for 24 h at 30°C. Confirmation of growth was performed by re-isolating the bacteria on Oxford agar (48 h, 30°C) from the Bactometer module wells by the streak plate method.

(iii) *Field trial.* A trial on food and environmental samples being tested for *Listeria* at a large food manufacturers quality control laboratory was performed. Media used were as above but with 15 ml/l of 5% (w/v) ferric ammonium citrate solution (medium A) and medium A with 20 mg/l nalidixic acid (Sigma) (medium B). Nalidixic acid was filter sterilized into the medium from a stock solution containing 0.5 g/l. The *Listeria* detection method used at the company was based upon a modified version of the USDA method. The method used UVM 1 (24 h, 30°C), Fraser broth (24–48 h, 30°C), Oxford agar (48 h, 30°C) (all Oxoid) with confirmation of *Listeria* spp. by ELISA (Organon Teknika) from Fraser broth and confirmation of *L. monocytogenes* by the Accuprobe gene probe method (Gen-Probe, San Deigo, California) from typical colonies on Oxford agar.

Two ml/well of each of Bactometer media A and B were inoculated with 0.05 ml of the UVM 1 enrichments at the same time as the Fraser broth. A Vitec-Bactometer (Model M64, BioMerieux) was set to monitor capacitance for 48 h at 30°C. No confirmations were made on results from the Bactometer. Comparison of the results with the laboratory test confirmations were used to define the positive reaction in the Bactometer. The 64 samples tested included factory environmental hygiene samples (38), product samples such as meats, cream cakes, cheeses and milks (20), UVM 1 positive controls inoculated with *L. monocytogenes* (2) and sterile UVM 1 negative controls (4).

### 3. Results

#### 3.1. (i) *Pure culture*

Negative controls gave no detection times or positive change in capacitance (Table 1). All *L. monocytogenes* inoculated wells were esculin-positive (black). Generation time estimation (Firstenberg-Eden and Eden, 1984) was performed by averaging the difference in detection times of consecutive dilutions and dividing the product by  $\log_2$  of the dilution factor (approximately 3.322).

#### 3.2. (ii) *Inoculated food*

All the *E. faecalis* inoculated foods gave small negative curves (the largest being –7.9% change from initial capacitance value; see Table 2). Only *L. monocytogenes* inoculated tests were esculin-positive. *L. monocytogenes* inoculated tests all gave a >38% change in capacitance, with rapid rates of increase from detection to peak values. Typical *Listeria* spp. colonies were seen only on Oxford agar plates inoculated from food samples a and c of the Bactometer wells.

Table 1  
Effects of the variation of Oxford antibiotic supplement concentration on the growth and signal production of *L. monocytogenes* and *E. faecalis*

Supplement concentration vials/l	Inoculated organism	Average detection times of dilutions (h)				Average % capacitance change	Estimated generation time (min)
		10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>		
2	<i>L. monocytogenes</i>	–	–	10.4	13.7	85	58
	<i>E. faecalis</i>	–	–	2.5	ND	53	–
3	<i>L. monocytogenes</i>	6.6	8.2	11.6	14.0	131	45
	<i>E. faecalis</i>	2.0	ND	ND	ND	13	–
4	<i>L. monocytogenes</i>	5.8	8.8	11.9	14.3	118	51
	<i>E. faecalis</i>	ND	ND	ND	ND	0	–

ND, not detected; –, not done.

### 3.3. (iii) Field trial

The four confirmed *Listeria* spp. laboratory results were complete sub-sets of the positive Bactometer results except those for medium B esculin hydrolysis (Table 3). One Oxford agar plate gave typical-looking colonies that were not

Table 2  
The effect of different levels of *L. monocytogenes* and *E. faecalis* either singly or in combination in UVM 1 broth on the subsequent change of capacitance in the Bactometer

Food added to UVM 1	Inoculum added to UVM 1	Average detection time (h)	Average maximum increase in capacitance (%)	Average rate of increase from detection to peak (%/h)
Yoghurt	a	3.4	68.0	10.9
	b	ND	0	–
	c	2.3	39.5	10.2
	d	ND	0	–
Bread	a	0.4	70.5	10.7
	b	ND	0	–
	c	0.6	63.7	11.2
	d	ND	0	–
Butter	a	2.4	67.0	10.8
	b	ND	0	–
	c	1.1	70.6	10.2
	d	ND	0	–
Milk	a	2.5	66.2	10.2
	b	ND	0	–
	c	1.9	69.0	13.5
	d	ND	0	–

a, 15 cfu/ml of *L. monocytogenes*; b, 17 cfu/ml of *E. faecalis*; c, 1.7 × 10<sup>5</sup> cfu/ml of *E. faecalis* and 15 cfu/ml of *L. monocytogenes*; d, none; ND, not detected.

Table 3

Correlations of the 64 laboratory tests with three results given by the two Bactometer media are displayed

Bactometer positive results	Laboratory positive results			
	Fraser	Oxford	<i>Listeria</i> spp. (ELISA)	<i>L. monocytogenes</i> (gene probe)
	17	5	4	3
Correlation of both test results being positive				
<i>Medium A</i>				
Detection	42	15	5	3
Esculin	18	9	4	3
> 30%, < 30 h	10	6	4	3
<i>Medium B</i>				
Detection	44	15	5	3
Esculin	11	7	3	2
> 30%, < 30 h	6	5	4	3

Detection: positive if a 'detection time' was given during the 48-h test.

Esculin: positive if a darkening of the medium was seen after 24 or 48 h.

> 30%, < 30 h: positive if greater than 30% change in capacitance was reached within 30 h.

confirmed as *Listeria* spp. Within 48 h, blackening faded in some Bactometer wells leaving them indistinguishable from negative controls. The greater than 30% increase in capacitance in less than the 30 h cut-off axes on the Bactometer graphs had two and zero results (for the medium A and B, respectively), just outside the limits that were not included as being positive. The laboratory confirmed *Listeria* spp. results gave an average time at which 30% increase in capacitance was reached in the Bactometer of 22.75 h for medium A and 24.25 h for medium B. Two of the four *Listeria* spp. positive samples were media control tests and the other two were environmental samples. One of the two was confirmed as *L. monocytogenes*. The other two positive samples in Bactometer medium B that were not reported by the laboratory as containing *Listeria* spp. both gave large capacitance change curves, with an average time to reach 30% increase in capacitance of 19 h. One of the two positives was a product sample that was Fraser broth negative.

## 4. Discussion

### 4.1. (i) Pure culture

Preliminary work, in agreement with Phillips and Griffiths (1989), suggested that *Listeria* spp. were good candidates for the application of electrical detection as growth caused large, typically shaped changes in capacitance over time. The Oxford agar selective agents were added to a medium broth base that fulfilled the minimal nutrient requirements for *L. monocytogenes* (Premaratne et al., 1991).

Increasing antibiotic concentration resulted in a significant inhibition of *E. faecalis* without unacceptably slowing the growth of *L. monocytogenes* beyond the rate reported by Petran and Zottola (1989) for the Scott A strain in tryptic soy broth at 30°C. The addition of glucose to the medium may reduce the antimicrobial effects of the Oxford agar antibiotic supplements (Sousa and Prista, 1988) and increase *Listeria* spp. yield (Premaratne et al., 1991).

*E. faecalis* induction of capacitance change was proportional to the inoculum size (see Table 1) and the inoculation medium effected the shape of the pure culture capacitance curve (results not included). This led to the halving of the inoculum to 0.05 ml and doubling the Bactometer medium volume to give a 1:40 dilution ratio for subsequent UVM 1 food enrichments. The esculin reaction at this stage was thought to be necessary as a secondary observation of a positive result. Due to problems encountered during preliminary work with *L. monocytogenes* inoculated foods that gave weak esculin reactions, the concentration of ferric ammonium citrate was increased.

#### 4.2. (ii) Inoculated food

The foods selected had properties that may have been expected to cause problems either with regard to the growth of *L. monocytogenes* or to the electrical properties of the Bactometer medium (high fat, mineral, carbohydrate and acid content). The results showed that little or no effects due to foods were seen on the percentage or rate of change of capacitance. The *L. monocytogenes* inoculated tests gave large capacitance responses. The pH of yoghurt should inhibit the growth of *L. monocytogenes* (Farber and Peterkin, 1991) but the presence of yoghurt in the UVM 1 broth did not greatly increase the time to detection of *L. monocytogenes*. All detection times were under 4 h, suggesting very good growth of the small inoculum of *L. monocytogenes* in the UVM 1 enrichment broth. *E. faecalis* did not show a detection time or a positive change of capacitance. In mixed cultures, a 10<sup>4</sup> times greater inoculum of *E. faecalis* than *L. monocytogenes* did not inhibit the *L. monocytogenes* detection or change the subsequent rate of increase of capacitance, but reduced the total percentage change observed with the yoghurt sample.

#### 4.3. (iii) Field trial

Preliminary experiments with environmental samples (sponge swabs of drains and other dirty surfaces) in the Bactometer showed that some samples were esculin positive with small positive response curves. Subsequent isolation on Oxford and nutrient agar demonstrated the presence of Gram-positive, oxidase-negative, catalase-positive, spore-forming rod-shaped bacteria. The field trial therefore used two media, one of which contained 20 mg/l of nalidixic acid, which was reported by Phillips and Griffiths (1989) to reduce the growth of *Bacillus* spp., but still support the growth of various *Listeria* strains in liquid culture.

The food laboratory and Bactometer media test results were in agreement (Table 3). The esculin results were recorded by laboratory staff who did not know the identity of the Bactometer wells. Visual analysis of the resulting curves from confirmed positive and negative results showed a distinctive difference. Whilst many tests showed an increase in capacitance, very few gave substantial (> 30%) changes. Those that did included all the samples confirmed as containing *Listeria* spp. by the laboratory. The addition of nalidixic acid to medium B reduced the number of false-positive results (using the > 30% change criterion) from six to two and reduced the number of near positive results. Taking the laboratory confirmation results as correct, if the Bactometer > 30% change in < 30 h results had been used instead of the laboratory secondary enrichment procedure, a large saving of labour, time and ELISA tests would have resulted, reducing the number of false-positives from 13/17 to 2/6.

The benefits of using a test that needs no qualitative human decision of the outcome should outweigh the comparative uncertainty of the result, as long as proper calibration of the system is made. The stringency (% change/time) of the test may be adjusted to suit the criterion of the laboratory, primary enrichment method or sample type, as long as the test out performs the alternative methods available. Further work is needed to investigate the effects of different foods (especially cheese) on the medium and to correlate the detection time to actual numbers of *Listeria* spp.

## Acknowledgements

The authors would like to thank the laboratory staff of the University of Surrey and Grand Metropolitan Foods Europe for their assistance and especially to acknowledge the help and support of Alec Kyriakides. This work was made possible by donations of materials from Grand Metropolitan Foods Europe and a Bactometer 120SC by BioMerieux, Grafton Way, Basingstoke to the University of Surrey.

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