A new capacitance medium for presumptive detection of *Listeria* spp. from cheese samples

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Abstract

The paper presents a new medium and a method for the rapid presumptive detection of *Listeria* spp. in cheese samples by electrical capacitance changes. The Bactometer based capacitance broth (LED medium) required a 30% change in signal within 30 h to identify presumptive positive results. Of 32 cheese samples tested, 10 were found to contain *Listeria* spp. using a Fraser broth screening method and 12 using the LED medium and method. The LED method also gave 16 fewer (total = 1) false presumptive positive results. Results show the LED method to be superior to Fraser broth in regard to both the number of false presumptive positive and confirmed false negative results detected. The method appears to be suitable as a reliable rapid screen for the presence of *Listeria* spp. in cheese.

Keywords: Bactometer; Electrical detection; *Listeria*; Portuguese cheese; Presumptive media

1. Introduction

Cheeses, especially soft varieties, are closely associated with food-borne listeriosis outbreaks both in Europe and North America [1–3]. Pasteurization effectively eliminates the bacterium and most cheeses possess a pH that inhibits *Listeria monocytogenes* growth. Due to infection during manufacturing, micro-environments in and around the product, poor handling and distribution or a combination of these, higher pH cheeses may become high inoculum sources of the bacterium. Cheese is also associated with other bacterial genera capable of giving false positive results in *Listeria* media, most notably *Bacillus* spp. and *Enterococcus* spp. [4].

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Rapid detection or enumeration of *L. monocytogenes* in food samples is difficult as it grows quite slowly on selective media, is usually present in quite low numbers and damaged cells may be inhibited by selective agents [2,5].

Much has been published on the application of new immunological and genetic technologies, for instance polymerase chain reaction [6,7], gene-probe [8,9], and ELISA [10] for detecting *Listeria* spp. or *L. monocytogenes*. These tests are successful in reducing the detection or confirmation time but are generally expensive in reagents, and most require an enrichment to be performed.

The balance between relevance, accuracy, time and cost of microbiological screening has to be attained within a food company's quality programme, with time and cost usually given greatest priority. For this reason improvements in the standard F.D.A. and U.S.D.A.-F.S.I.S. methods [11], which are slow, are required.

Fraser broth [12] is widely used as an alternative to UVM 2 in the U.S.D.A.-F.S.I.S. method; it is the first stage at which negative results may be rejected and it is known to be a good *Listeria* spp. cultural medium. Fraser broth has problems with large numbers of false presumptive positive results [12–14]. Any possibly false positive cultures have to be allowed at least 2 days on agar before they are rejected as negative, or, if alternative rapid confirmation techniques are used on the broth cultures, expensive test kits are wasted.

Initial work on the application of the Bactometer to *Listeria* spp. isolation from artificially inoculated food samples [15] resulted in the use of Oxford agar antibiotic supplements in a liquid medium. This medium allowed the detection of *Listeria* spp. by the observation of greater than 30% change in capacitance within 30 h when inoculated from UVM 1 primary enrichment broths. The objective of the current work was to test that the medium and method was able to identify naturally contaminated cheese samples.

2. Materials and methods

2.1. Primary enrichment

Samples of four varieties of cheese (very soft, Queijo Fresco; soft, Camembert and Queijo Serra das Estrelas; and semi-soft, Queijo Flamengo) were bought in a range of retail outlets and refrigerated before use. 25 g samples were taken aseptically and homogenized in 225 ml of UVM 1 (Oxoid) in a stomacher (Seward 400) for 2 min. These primary selective enrichment broths were incubated at 30°C for 24 h before secondary differential enrichment.

2.2. Secondary enrichment

Listeria Electrical Detection medium (LED) was made from a base of 20 g l^{-1} of tryptone (Oxoid), 20 g l^{-1} of yeast extract (Oxoid), 17.5 g l^{-1} lithium chloride (Oxoid), 4 g l^{-1} glucose (BDH) and 1 g l^{-1} esculin (Sigma). After sterilization of

the base 4 vials l^{-1} of Oxford Selective Supplements (Oxoid) and 20 mg l^{-1} of a nalidixic acid (Sigma) filter sterilized stock solution were added. The pH was then aseptically adjusted to 7.2 ± 0.1.

A Vitec-Bactometer (Model M64, BioMèrieux) was used to monitor capacitance during the test. 0.05 ml of each primary selective enrichment were added to 2 ml of the LED medium that had been prewarmed to 30°C in the Bactometer module wells; all tests were replicated at this stage. The Vitec-Bactometer was set to monitor capacitance for a test time of 40 h at 30°C. Positive results were taken as those that gave greater than 30% change in capacitance within 30 h. All wells were streaked onto Oxford agar (Oxoid) after the test and incubated (30°C, 48 h).

At the same time 0.1 ml of primary selective enrichment medium was transferred to 10 ml of Fraser broth [12] and incubated (30°C, 48 h). Fraser results (blackening) were noted after 24 h and 48 h. All enrichment samples of were streaked onto Oxford agar (Oxoid) and incubated (30°C, 48 h).

2.3. Confirmation

Confirmation was performed to the genus level by first producing a pure sub-culture of Oxford typical colonies onto tryptone soy agar (TSA, 37°C, 24 h) (Oxoid), noting: the appearance by Henrys' illumination, catalase and oxidase reaction from the TSA plate and tumbling motility from tryptone soy broth ((Lab M), 22°C, 24 h) inoculated from the TSA plate. Further confirmation by filter-sterilised sugar fermentation tests of D-raffinose (0.5% (w/v)) and sorbitol, D-arabitol, xylitol and trehalose (all 0.75% (w/v)) (all from Sigma) in Purple broth (Difco) were used to add certainty of identification for up to five typical colonies per Oxford agar plate (where available). Purple broths were incubated at 30°C for 5–7 days. API *Listeria* (BioMérieux) biochemical tests were performed on all isolates that gave typical looking colonies on Oxford agar but atypical results for any of the following tests and on some typical isolates.

Finally, all confirmed *Listeria* spp. isolates were inoculated back into UVM 1, incubated (30°C, 24 h) and transferred to LED medium (0.05 ml UVM 1 into 2 ml LED) in the Bactometer to ensure that each produced >30% change in capacitance within 30 h at 30°C.

3. Results

The complete confirmation procedure of the U.S.D.A.-F.S.I.S. *L. monocytogenes* method was not followed due to the problems of obtaining sheep and horse blood for Camp and Columbia blood agar locally; the final reports are only of *Listeria* spp. except where API *Listeria* tests were performed. The additional sugar fermentation tests were used to reduce the possibility of error in the *Listeria* spp. identification. All isolates identified as *Listeria* spp. were oxidase negative, catalase positive, Gram positive rods, exhibiting tumbling motility, Henry

illumination positive, sorbitol and D-raffinose negative and D-arabitol, xylitol and trehalose positive in Purple broth fermentations. The sugar fermentation results all agreed with the API *Listeria* results and seemed an accurate but slow method of confirmation of *Listeria* spp.

The two positive results confirmed from the LED medium method but not the Fraser broth method were re-streaked from Fraser broth onto Oxford agar after 48 h, but still did not give typical colonies.

All *Listeria* sp. isolates when re-inoculated into the Bactometer in pure culture from UVM 1 gave >30% change in capacitance within 30 h.

4. Discussion

In preliminary experiments using local cheeses known to contain *Listeria* spp. (results not shown) difficulties with interpretation of capacitance curves due to variable initial values of capacitance led to problems in confirmation of the presence of the bacteria. Ferric ammonium citrate was removed from the previously reported medium to make LED medium as the observation of the esculin reaction had been found to be unnecessary, subsequent experiments showed much improved results (Table 1). It was therefore concluded that in this case ferric ammonium citrate was detrimental to the efficiency of the medium.

The LED medium tests were performed in duplicate in order to balance the volumes of UVM I transferred and to indicate the internal variation of the Bactometer. These duplicates gave two confirmed positive results that spanned the 30% change in 30 h cut-off. This intra-sample variation may have been due to competitive inhibition of *Listeria* spp. growth in the medium, very low initial numbers and poor distribution of bacteria in the primary enrichment or poor technique. The particle size and the adhesive nature of some cheeses make accurate transfer of small volumes difficult. Discounting the two tests spanning 30%/30 h the average intra-sample variation of time to reach 30% change in capacitance was small (0.5 h) and showed a large range (2–29 h).

The LED method gave a greater number of confirmed *Listeria* spp. positive results (12/32) than the Fraser broth method (10/32). For two samples confirmed as *Listeria* spp. positive by the LED method *Listeria* spp. colonies were not isolated on Oxford agar plates from positive Fraser broths; it was noted that the corresponding isolates from LED medium gave comparatively weak catalase reactions. The LED method also gave far fewer false presumptive positive results (1/32) than the Fraser broth method (19/32). The 65.5% false presumptive positive rate for Fraser broth is within the range reported for other products by other authors [12–14,16]. The number of confirmed positive samples from each cheese type was inversely proportional to the hardness of the cheese; however the number of false presumptive positive results from Fraser broth was not. This may indicate that other esculin hydrolysing organisms capable of growing in Fraser broth are not as sensitive as *Listeria* spp. to the preservative effects of harder cheeses.

Table 1
Presumptive and confirmed results of 32 cheese samples tested for *Listeria* spp.

Cheese sample[a]	Times to 30% change (h)	Bactometer presumptive	Confirmed *Listeria* spp.[b]	Fraser broth presumptive	Confirmed *Listeria* spp.[b]
Fr 1	23/34	+/−	+	+	+
2	4/4	+/+	+	+	+
3	23/23	+/+	+	+	+
4	20/34	+/−	−[f]	+	−
5	14/14	+/+	+[e]	+	+
6	17/17	+/+	+[d]	+	+
7	2/2	+/+	+	+	−[f]
8	2/2	+/+	+	+	+
Ca 1	−/−	−/−	−	+	−
2	28/36	+/−	+	+	+
3	−/−	−/−	−	+	−
4	40/−	−/−	−	−	−
5	26/29	+/+	+	+	−
6	11/10	+/+	+[e]	+	+
7	−/−	−/−	−	+	−
8	−/−	−/−	−	+	−[f]
Se 1	17/17	+/+	+[e]	+	+
2	−/−	−/−	−	+	−
3	−/−	−/−	−	+	−
4	16/17	+/+	+[e]	+	+
5	−/−	−/−	−	+	−
6	−/−	−/−	−	+	−
7	−/−	−/−	−	−	−
8	−/−	−/−	−	−	−
Fl 1	−/−	−/−	−	+	−
2	−/−	−/−	−	+	−
3	−/−	−/−	−	+	−
4	−/−	−/−	−	+	−
5	−/−	−/−	−	+	−
6	−/−	−/−	−	+	−
7	−/−	−/−	−	+	−
8	−/−	−/−	−	+	−

[a] Cheese Types: **Fr**; Queijo Fresco, **Ca**; Camembert, **Se**; Queijo Serra das Estrelas, **Fl**; Queijo Flamengo.
[b] API *Listeria* Biochemical Results: [e]: *L. monocytogenes*, [d]: *L. innocua*, [e]: *L. seeligeri*, [f]: Not a *Listeria* spp.

The results suggest that by using the LED method the number of presumptive positive samples requiring confirmation at the end of secondary enrichment could be substantially reduced. This would lead to saving of time and reagents in the confirmation phase of the analysis. The reduction in false presumptive positives is achieved without increasing the number of false negative results. Furthermore the low numbers of false positive results (3.1%) suggest that the Bactometer method may be employed as a rapid screen as part of a company's internal G.M.P. programme.

Acknowledgments

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