

# Performance of a new chromogenic plating medium for the isolation of *Listeria monocytogenes* from marine environments

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## ABSTRACT

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**Aims:** This study investigated the performance of a new chromogenic plating medium for the detection of *Listeria monocytogenes* from naturally contaminated samples obtained from marine environments in Morocco in comparison with the conventional plating media PALCAM and Oxford.

**Methods:** A total of 479 marine samples (sea water, sediment and mussels) were collected from 16 littoral sites in the region of Agadir (western centre of Morocco). They were examined for the presence of *L. monocytogenes* using a slight modification of the standardized French method (AFNOR V 08-055) for the detection of *L. monocytogenes* from food and three different isolation media: PALCAM, Oxford and a new chromogenic plating medium.

**Results and Significance of the Study:** The Oxford and the new chromogenic plating media were found relatively more efficient than the PALCAM medium for the isolation of *L. monocytogenes* (chi-square test,  $P < 0.05$ ) from marine samples. However, the new chromogenic plating medium was significantly more selective for *L. monocytogenes* ( $P < 0.005$ ) than the two other isolation media as 87.5% of the suspect colonies on this medium were indeed confirmed through identification of the isolates vs 12.7% for Oxford and only 3.8% for the PALCAM medium.

**Keywords:** Chromogenic medium, isolation, *Listeria monocytogenes*, marine environments, performance, selective plating media.

## INTRODUCTION

Since 1981 when *Listeria monocytogenes* has been recognized as an agent of food-borne infection (Rocourt *et al.* 2000), this pathogen has been associated with a numerous outbreaks of food-borne listeriosis; foods involved include vegetables, dairy products, meat products and seafoods (Farber and Peterkin 1991). The presence of this pathogen in foods is a major concern to the food industry and public health regulators (Cassiday *et al.* 1989). Detection and enumeration of *L. monocytogenes* from environments such as food, which can be heavily contaminated with other

organisms are often difficult (Golden *et al.* 1988). One of the major challenges of the food sanitarians is to identify and apply reliable and rapid techniques for the recovery of this pathogen from foods (Foret and Dorey 1997).

In general, the current conventional procedures for the detection of *L. monocytogenes* from foods suffer from a certain lack of performance particularly in foods where this pathogen is expected to occur in low numbers and/or in debilitated condition. Moreover, the presence of non-listeriae bacteria or even the very competitive *L. innocua* may interfere with the recovery of *L. monocytogenes*.

The plating media PALCAM and Oxford have been developed for the selective isolation of listeriae on the basis of the hydrolysis of esculin, which contribute to the differentiation of the listeriae from the other bacteria (Mc

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Lauchlin 1987). Although these media have been found efficient for the isolation of listeriae from food products with injured listeriae cells and/or rich in competitive microflora (Ben Embarek 1994), they are not able to allow distinctive visualization of *L. monocytogenes* colonies. Therefore, their use as plating media does not permit a rapid detection of this pathogen from foods (Scotter *et al.* 2001). The need for isolation media capable of clearly differentiating *L. monocytogenes* colonies from the other listeriae colonies and particularly from those of *L. innocua* has been expressed by Poyski *et al.* (1993). Thus, different chromogenic media such as ALOA chromogenic medium have been developed to respond to this need (Carles *et al.* 1997; Vlaemynck *et al.* 2000; Smith *et al.* 2001).

The present work aims to assess the performance of a new chromogenic plating medium CHROMagar™ *Listeria* (CHROMagar Microbiology, Paris, France) in comparison with the conventional plating media PALCAM and Oxford for the selective isolation of *L. monocytogenes*. This comparative study was based on the tentative recovery of this pathogen from samples obtained from naturally contaminated marine products using the three considered isolation media.

## MATERIALS AND METHODS

### Sampling procedure

Within the public health monitoring programme of the Moroccan southern Atlantic coast between the towns of Essaouira and Tan-Tan, a total of 479 marine samples were collected during a 2-year period (2001–02) from 16 sites located on the shores of the Atlantic coast of the region of Agadir (western centre of Morocco). These included 161 samples of sea water, 160 samples of marine sediment and 158 samples of mussels (*Mytilus galloprovincialis*).

All the samples were collected at low tide. Samples of sea water were taken at a depth of 20 cm under the sea surface and those of marine sediment at the surface of sea ground. These two types of samples were collected in separate sterile flasks while those of mussels were introduced into plastic sterile bags. All the samples were transported at the laboratory in ice boxes and then stored at 6–8°C before being analysed within 16 h of sampling.

### Detection of *L. monocytogenes*

The standardized French method (AFNOR NF V 08-055, 1993) for the detection of *L. monocytogenes* in food was used with a slight modification. Portions of 25 ml of sea water or 25 g of marine sediment were introduced aseptically into sterile 500-ml flasks containing 225 ml of half strength Fraser broth (E. Merck, Darmstadt, Germany) and incubated

for 24 h at 30°C. However, mussels (from three to 10 according to their size) were scrubbed, rinsed with tap water and then flamed with ethylic alcohol before being opened aseptically. The flesh with the intervalvar liquor (25 g) were added to 225 ml of half strength Fraser broth (E. Merck) and homogenized for 40–60 s at 1400 g using a Waring blender and then incubated at 30°C for 24 h. A 0.1 ml subsample of each of the primary enriched cultures was added to 10 ml of full strength Fraser broth (E. Merck) and kept for 24 h at 37°C. A loopfull of the secondary enrichment culture was streaked onto each of the following isolation plating media: PALCAM (E. Merck), Oxford (E. Merck) and CHROMagar™ *Listeria* (CHROMagar Microbiology). The latter medium is composed of a base and a supplement. The base contains the followings ingredients: 23 g l<sup>-1</sup> peptones and meat extract; 5 g l<sup>-1</sup> sodium chloride; 15 g l<sup>-1</sup> agar and 17.5 g l<sup>-1</sup> special chromogenic mix. The inoculated plates were incubated for 24–48 h at 37°C. A number of one to three suspect colonies showing the typical morphology of listeriae on these three isolation media (blackish colonies on PALCAM, dark grey/green colonies with depressed black centre on Oxford, blue colonies with white halo on CHROMagar™ *Listeria*) were selected at random from the plates, checked for purity on Columbia agar and kept on slants of this medium for 24 h at 37°C before their final identification. This latter was based on reactions to confirm the genus affiliation: Gram stain, catalase test, motility at 25°C, urease activity, indole production, esculin hydrolysis and methyl red and Voges Proskauer tests. Then, the listeriae-confirmed isolates were speciated using the API-*Listeria* strips (Bio-Mérieux, Marcy l'Etoile, France) and the β-haemolysis test on sheep blood agar.

### Statistical analysis

The chi-square test was used to compare the results of recovery rate of *L. monocytogenes* from marine samples with respect to the type of the isolation plating media used.

## RESULTS AND DISCUSSION

### Occurrence of listeriae in the marine samples

In general, 31 (6.5%) and seven (1.5%) of the overall samples were found to be contaminated with *Listeria* spp. and *L. monocytogenes* respectively (Table 1). Proportions of 8.1, 6.5 and 3.1% of sea water, 6.9, 6.2 and 0.6% of sediments and 4.5, 3.4 and 0.6% of mussels contained organisms of *Listeria* spp., *L. innocua* and *L. monocytogenes* respectively. *L. ivanovii* was detected only once and was from a single sediment sample (Table 1). These results are somewhat surprising as bivalve molluscs like mussels are expected to contain more listeriae than sea water and

**Table 1** Prevalence of *Listeria* spp. in samples from marine environments

Marine sample (samples no.)	<i>Listeria</i> spp.			
	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	
Sea water (161)	13 (8.1)	5 (3.1)	11 (6.5)	None
Sediment (160)	11 (6.9)	1 (0.6)	10 (6.2)	1 (0.6)
Shellfish (158)	7 (4.5)	1 (0.6)	6 (3.4)	None
Total (479)	31 (6.5)	7 (1.5)	27 (5.6)	1 (0.2)

Values are presented as *n* (%).

sediment as these molluscs are able to filter large amounts of water and subsequently concentrate pathogens (Huss 1994). However, the relative higher incidence of listeriae in sea water samples compared with sediment samples shown in this work, has been reported also by Colburn *et al.* (1990) in a study on the incidence of *Listeria* species in a California coast estuarine environment. Although the real explanation for this phenomenon was not given, these authors have attributed it to certain factors such as differences in the levels of indigenous competing bacteria between the two types of samples. Otherwise, the presence of listeriae in sea water may indicate a relatively recent contamination as their levels tend to decrease rapidly in the marine environment due to the effects of dilution by the large volumes of sea water (Colburn *et al.* 1990). Furthermore, listeriae were reported to be less commonly isolated than enteric bacteria such as salmonellae or *Escherichia coli* in the marine environment (Monfort *et al.* 2000) because of different factors although they are known, in general, to be highly resistant in high salt concentrations (Shahamat *et al.* 1980).

### Comparative performances of the isolation media

The performance of the three plating media for the isolation of *L. monocytogenes* in terms of sample type (sea water, marine sediment or mussels) was not investigated because of the small number of samples positives for *L. monocytogenes*. Data regarding the relative performance of the three isolation media used in this study, for the recovery of different *Listeria* species from the overall examined samples are presented in Table 2. However, before discussing these results, it should be remembered, that because the PALCAM and Oxford plating media are based on the hydrolysis

of esculin which is a common characteristic to all the *Listeria* species, they cannot differentiate *L. monocytogenes* from the other *Listeria* species, whereas the CHROMagar™ *Listeria* medium is intended to differentiate the phospholipase C-producing listeriae which are *L. monocytogenes* and *L. ivanovii*. It is noticeable from the data showed in Table 2 that the Oxford and CHROMagar™ *Listeria* plating media were relatively more efficient than the PALCAM medium for the isolation of *L. monocytogenes* ( $P < 0.05$ ). In effect, all the seven samples found positives for this pathogen have been detected with both the Oxford and the CHROMagar™ *Listeria* medium, while only two samples were found positives with the PALCAM medium. As expected, the CHROMagar™ *Listeria* was very selective for *L. monocytogenes* as seven of eight samples presumptively *L. monocytogenes* positive on this plating medium were indeed confirmed later as positives for this pathogen (87.5%). The data from Table 2 indicate that the Oxford medium was more selective than the PALCAM medium for the isolation of *L. monocytogenes* ( $P < 0.05$ ) as seven of 30 presumptively *Listeria*-positive samples contained in fact this micro-organism (23.3%) compared with only two *L. monocytogenes*-positive samples of 22 *Listeria* presumptives (9.1%) when using the PALCAM medium. In addition, as expected, none of the presumptively *L. monocytogenes*-positive samples on CHROMagar™ *Listeria* medium contained *L. innocua* while this latter made up the major parts of presumptive *Listeria* samples on both PALCAM (20/22; 90.9%) and Oxford (26/30; 86.6%). As already mentioned by several authors (Petran and Swanson 1993; Mc Donald and Sutherland 1994; Carles *et al.* 1997), *L. innocua* (a nonpathogen species for humans) can mask the presence of *L. monocytogenes* on both Oxford and PALCAM agars because of differences in growth rates of *Listeria* spp. in selective broths. Thus, species identification from a definite number of colonies per plate can be biased (Scotter *et al.* 2001). The antagonistic effect of *L. innocua* over *L. monocytogenes* has been attributed by some authors (Yokohama *et al.* 1998; Kalmokoff *et al.* 1999) to the production of phages as well as bacteriocin-like substances by this organism. Recently developed chromogenic isolation media such as CHROMagar™ *Listeria* are then capable of improving greatly the visual differentiation of these two *Listeria* species.

**Table 2** Recovery rate of *Listeria monocytogenes* from marine samples according to type of the plating isolation medium used

Isolation medium	No. of samples	<i>Listeria</i> spp.	<i>Listeria</i>			<i>L.m./Listeria</i> [ratio (%)]
			<i>L.m.</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	
Oxford	479	30 (6.3)	7 (1.5)	22 (5.4)	None	7/30 (23.3)
PALCAM	479	22 (4.6)	2 (0.4)	20 (4.2)	None	2/22 (9.1)
CHROMagar	479	ND	7	None	1	ND

Values are presented as *n* (%) unless indicated.

*L.m.*, *Listeria monocytogenes*; ND, not determined.

Isolation medium	No. of suspect colonies	<i>Listeria</i> spp.	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. ivanovii</i>
Oxford	71*	49 (69)	9 (12.7)	40 (56.3)	None
PALCAM	53*	46 (86.8)	2 (3.8)	44 (83)	None
CHROMagar	8†	ND	7 (87.5)	None	1 (12.5)

Values are presented as *n* (%). ND, not determined.

\*Number of *Listeria* suspect colonies.

†Number of *L. monocytogenes* suspect colonies.

Figures in Table 3 are relative to the performance of each of the examined plating media with regard to the isolation of *L. monocytogenes* from the total suspicious colonies isolated from that medium. It is important to indicate that in samples found to be positives for *L. monocytogenes*, the number of colonies showing the phenotypic characteristics of *L. monocytogenes* on the CHROMagar™ *Listeria* medium was very small (mostly one colony per sample). This explains the low number of *L. monocytogenes* suspect colonies (eight colonies) observed on CHROMagar™ *Listeria* medium (Table 3). However, seven of them were identified as *L. monocytogenes* (87.5%) confirming the high selectivity of this medium to this micro-organism. Data from Table 3 show that although PALCAM was able to detect more listeriae among the *Listeria* suspect colonies (46/53; 86.8%) than did Oxford (49/71; 69%), colonies confirmed as *L. monocytogenes* were not as many on this medium (2/46; 3.8%) as on Oxford (9/49; 12.7%). This difference was found to be significant ( $P < 0.05$ ). Most of the *Listeria* suspect colonies on PALCAM (83%) belonged to *L. innocua* species vs 56.3% on Oxford. Thus, it is clear that *Listeria* suspect colonies on either PALCAM or Oxford should first be confirmed to the genus level and then submitted to appropriate tests to differentiate *L. monocytogenes* from the other *Listeria* species. This procedure is time and labour consuming and may take several days. Furthermore, the tests required to ascertain the identity of the isolate may make this procedure costly. Subsequently, the use of plating media able to direct visual differentiation of *L. monocytogenes* colonies from the other listeriae colonies, such as CHROMagar™ *Listeria*, should theoretically overcome these drawbacks. The results obtained in this present study indicate that the concordance was perfect between Oxford and CHROMagar™ *Listeria* for the recovery of *L. monocytogenes* from naturally contaminated marine samples as both plating media have been able to isolate this pathogen from the same number of positive samples. Nevertheless, CHROMagar™ *Listeria* had an advantage over Oxford as it can visually differentiate *L. monocytogenes* colonies from all other *Listeria* colonies and particularly those of *L. innocua* which are found, very often, predominant on the conventional plating media such

**Table 3** Performance of CHROMagar™ *Listeria* medium in comparison with two other plating media for the isolation of *Listeria monocytogenes* from the total suspect colonies isolated from each medium

as Oxford or PALCAM. Although CHROMagar™ *Listeria* cannot visually differentiate colonies of *L. monocytogenes* from those of *L. ivanovii*, this latter species is fortunately less frequently encountered than *L. innocua* in the environment (Rocourt and Seeliger 1985; Lovett and Twedt 1988). Therefore, the probability of a *L. monocytogenes* suspect colony on CHROMagar™ *Listeria* to belong indeed to this species is very high as shown in this present study. Because of its high level of selectivity, the CHROMagar™ *Listeria* plating medium, has also the advantage of exempting the microbiological laboratories from performing the haemolysis test, which is critical in the routine procedure for the differentiation of *L. monocytogenes* from *L. innocua*. This test has caused numerous problems to laboratories because of difficulties in reading and interpreting the results of the haemolysis reaction (Scotter *et al.* 2001).

In conclusion, this study has demonstrated that isolation plating media able to target specifically the detection of *L. monocytogenes* such as CHROMagar™ *Listeria* are highly recommendable as they allow a relatively rapid presumptive response and an economy on the analysis cost.

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