CHROMagar™ Listeria method for detecting *Listeria monocytogenes*

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Summary: ***CHROMagar™ Listeria*** *method allows detection of* Listeria monocytogenes *after a single enrichmen step in Fraser 1/2 broth, isolation on this chromogenic medium and detection of blue colonies with white halo Compared to the ISO 11290-1 standard method,* ***CHROMagar™ Listeria*** *method is simpler and faster since* L. innocua *does not appear as false positive as with Palcam and Oxford media. Typical colonies are confirmed as* Listeria monocytogenes *and most negative samples are detected in 2 days.*

Introduction

Listeria monocytogenes (*L. m*) is a foodborne pathogen isolated from a large variety of foods. While traditional methods take 5 to 8 days for the detection of *L. m*, chromogenic media allow detection in 4 days, and detection of most negative in 2 days. The aim of this study was to assess the **CHROMagar™ Listeria** method's performances versus the conventional method ISO 11290-1 for the detection of *L. m* in food products and environmental samples.

Materials and methods

CHROMagar™ Listeria method allowing detection of *Listeria monocytogenes* has been compared to the traditional ISO 11290-1 method. Both methods start by an enrichment step in Fraser 1/2 broth 24h at 30°C but **CHROMagar™ Listeria** method skips the second enrichment step with direct isolation from Fraser 1/2 on the agar medium, using 100 µl instead of 10 µl. *Listeria monocytogenes* are detected on **CHROMagar™ Listeria** as blue and regular colonies surrounded by a white halo. See figure 1.

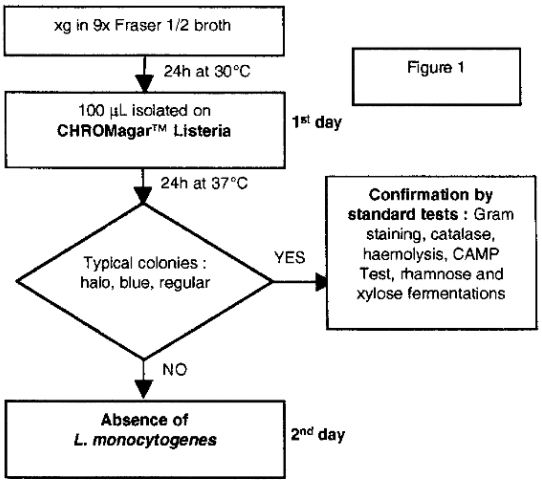

Figure 1

Results

Specificity

84 pure strains including 50 strains of *L. monocytogenes* from collections or from food matrices were regenerated in TSB broth for 24h and 100 µl were isolated on **CHROMagar™ Listeria**. After 24h incubation at 37°C, 100 % of the *L. m* strains showed the typical aspect of blue colonies with white halo and 100 % of the non *L. monocytogenes*, non-*L.ivanovii* strains showed a non-typical aspect (white colonies, yellow colonies, blue colonies without halo, inhibited).

Detection limit

The minimum level of detection with **CHROMagar™ Listeria** method was studied. Four different food matrices were chosen with their total aerobic mesophilic (30°C) flora measured : local chopped pork meat 1.4 10^5/g, raw milk 3.5 10^4/g, packaged salad 1.1 10^7/g and smoked salmon 76/g. These matrices were inoculated at level of 0 cfu/25g, 1-10 cfu/25g, 2-20 cfu/25g, 5-50 cfu/25g, 10-100 cfu/25g, with four different *L. m* strains : serotype 1/2a from raw milk, serotype 4e from parsley, serotype 1/2b from smoked salmon, serotype 4e from chopped pork meat. Detection of *L. m* was done in double with **CHROMagar™ Listeria** method and ISO 11290-1 method. There was a 100 % agreement between both metods with the four matrices. All non-contaminated samples were negative and all contaminated samples were positive.

Accuracy

470 food and environmental samples were analysed in parallel with both methods for detection of *L. m*. Food samples were chosen from four categories : milk products (raw and pasteurised cheeses, raw milk from cow and goat), meat products (raw and cooked pork and poultry meat), seafood (smoked salmon and trout, shellfish, fish and shellfishj pates, fresh fish), vegetables and environment samples (vegetables, milk industry and fish industry samoles). Most samples, studied over a period of 6 months, were naturally contaminated by *L. m* (and 36.7 % samples were artificially contaminated by 20 to 50 colony forming units stressed for 5 min at 50°C added into 25 g sample).

ISO 11290-1 method / CHROMagar Listeria	Positive	Negative	Total
Positive	112	**6**	118
Negative	**2**	350	352
Total	114	356	470

Table 1 : Results on accuracy

462 samples were concordant by both methods including 112 positive samples. Agreement of the two methods was 98.3 %. Six samples were detected only with **CHROMagar™ Listeria** method and there were 2 false negative samples by **CHROMagar™ Listeria** method, thus a detection yield of 103.5 % versus the ISO 11290-1 method.

Collaborative study

12 laboratories were provided with 8 raw milk samples (total flora of 2.1 10^3/g) being contaminated by 0, 6, 32 and 64 bacteria/25g. Sample temperature upon arrival was kept between 4.2°C and 5.6°C. There was 100 % agreement between all 12 laboratory results : all and only *L. m* contaminated samples were found positive with **CHROMagar™ Listeria** method.

Conclusion

➡ **CHROMagar™ Listeria** has excellent sensitivity and specificity close to 100% while Palcam and Oxford yield many false positive black colonies.

➡ **CHROMagar™ Listeria** method has an excellent detection yield, seemingly higher than the ISO method.

➡ **CHROMagar™ Listeria** method has an excellent low limit detection level of *L.monocytogenes* seemingly under 4 cfu/25g.

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