Evaluation of mini-VIDAS rapid test for detection of *Listeria monocytogenes* from production lines of fresh to cold-smoked fish

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Abstract

This study was conducted to evaluate the efficacy of the mini-VIDAS *Listeria monocytogenes* (LMO) system (BioMérieux Vitek, Inc., Missouri, USA) for detection of *L. monocytogenes* in environmental and fish samples from three Portuguese cold-smoking plants and from their fresh fish suppliers. Mini-VIDAS-LMO is a fully automated system that uses fluorescent ELFA (Enzyme Linked Fluorescent Assay) technology for detection of *Listeria monocytogenes* antigens in food. It can be a rapid screening method alternative to time consuming classical isolation and identification. Two hundred and ninety five samples were tested in mini-VIDAS-LMO and in parallel by the ISO 11290-1 traditional protocol. The mini-VIDAS-LMO detected 8 of the 11 confirmed positive samples and presented 11 false positive results. The specificity of the mini-VIDAS-LMO found in this experiment was 0.96 and the sensitivity 0.73.

Keywords: Cold-smoked fish; *Listeria monocytogenes*; Rapid immunological-based methods

1. Introduction

Listeria monocytogenes is a ubiquitous psychrotrophic bacterium responsible for foodborne infections worldwide. Although *L. monocytogenes* was ocasionally found in foods in the past, it was only within the last few years that it has become established as a foodborne pathogen for specific segments of the population, such as foetuses and immunocompromised people (Brackett, 1988). The pathogen has been consistently isolated from production lines of fresh to cold-smoked fish (Farber, 1991; Dillon et al., 1992; Gibson, 1992; Ben Embarek, 1994; Fuchs and Nicolaides, 1994; Jemmi and Keusch, 1994; Duarte et al., 1995; Eklund et al., 1995; Rørvik et al., 1995; Vaz-Velho et al., 1998). To our knowledge, the first suspected outbreak of listeriosis caused by cold-smoked fish however was reported only recently (Ericsson et al., 1997).

For a food company operating a positive release system, traditional detection methods for *L. monocytogenes* in foods lead to lengthy delays before the product is known to be ''safe''. Developments of sensitive and rapid immunological and genetic-based methods suitable for routine monitoring of food products have been achieved (Dever et al., 1993; Beumer, 1997), but the balance between accuracy, reduction in time required and the corresponding

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cost, does not always make implementation of these methods commercially worthwhile.

Mini-VIDAS *L. monocytogenes* (LMO) (BioMérieux Vitek, Inc., Missouri, USA) is a fully automated instrumental system that uses fluorescent ELFA (Enzyme Linked Fluorescent Assay) technology for detection of listerial antigens in food. It can be a rapid screening method alternative to time-consuming classical isolation and identification.

This study was conducted to evaluate the efficacy of the mini-VIDAS-LMO system for detection of *L. monocytogenes* in environmental and fish samples along the salmon, salmon–trout, and swordfish cold-smoking processing chains of three Portuguese factories and their fresh fish suppliers.

2. Materials and methods

A total of 295 environmental and fish samples were collected and analysed for *L. monocytogenes*, respectively at sites before the production head and along the process line of three Portuguese cold-smoking fish plants. All the smoking plants use fresh salmon, imported from Norway, salmon–trout from two Portuguese trout farms and swordfish from different suppliers. The fresh salmon from the importer and the salmon–trout, water, containers, ice, and polystyrene boxes of the trout farms were also analysed.

The fresh salmon and salmon–trout samples and the environmental samples from the trout farms were transported to the laboratory inside cold portable insulated boxes, refrigerated overnight and analysed the following day.

Due to the distance between the fish smoking plants and the laboratory (600 km), all the environmental and fish samples along the processing chain of each factory were maintained in refrigerated conditions and were analysed up to one week after collection. All the fresh fish and environmental samples from the fresh fish suppliers were analysed 4–6 h after being collected.

2.1. Sampling procedure

Ten centimetres squared of the fresh fish skin and surfaces were swabbed (5 swabs per sample) and the swabs were placed in 25 ml of 0.1% (w/v) peptone water [1 g/l of Tryptone (LabM) + 5 g/l of NaCl (Merck)]. Water samples were collected in a sterile 500 ml bottle for later filtration. Twenty five grams of processed fish samples were collected in sterile plastic bags. The samples were transported to the laboratory inside cold portable insulated boxes.

Peptone water instead of Fraser broth was chosen as a pre-enrichment broth because it has been concluded that it will improve the recovery of *L. monocytogenes* in swabbed samples (Vaz-Velho et al., 1999).

The ISO 11290-1 analytical protocol was followed together with the mini-VIDAS-LMO (BioMérieux) protocol.

2.2. Principle of the VIDAS-LMO procedure

The VIDAS-LMO assay is an enzyme-linked fluorescent imunnoassay (ELFA) performed in the automated VIDAS instrument. This instrument can analyse 12 samples simultaneously after the first assay where 3 reagent strips are used respectively as standard, positive and negative controls. A pipette tip-like disposable device, the solid phase receptacle (SPR), serves as the solid phase as well as the pipette for the assay. The SPR is coated with anti-*L. monocytogenes* antibodies. Reagents for the assay are in the sealed reagent strips. An aliquot of the enrichment sample is placed in the reagent strip and the sample is cycled in and out of the SPR for a specific length of time. *L. monocytogenes* antigens present in the sample will bind to the anti-*L. monocytogenes* antibodies coating the interior of the SPR. Antibodies conjugated with alkaline phosphatase are cycled in and out of the SPR and will bind to any *L. monocytogenes* antigen bound to the SPR wall. A fluorescent substrate, 4-methyl-umbelliferyl phosphate is introduced in the SPR. Enzyme remaining on the SPR wall will then catalyze the conversion of the substrate to the fluorescent product, 4-methyl-umbelliferone. The intensity of fluorescence is measured at 450 nm by the optical scanner in the VIDAS and is expressed in RFV (relative value of fluorescence). When the assay is completed, the results are analysed automatically by the computer, a test value is generated together and a report is printed for each sample. The test value (TV) =

sample RFV/standard FV. The result is negative if $TV < 0.05$. The result is positive if $TV \geq 0.05$. A positive result must be confirmed following standard plating procedures using the remaining enrichment broth stored at 2–8°C.

2.3. Isolation procedure

The water and ice thawed samples were filtered (0.45 μm, 47 mm diameter membrane filters, Gelman Science) and the filters were placed in 20 ml of primary enrichment broth, Fraser base (Merck) with half concentration of selective agents (Merck). The fresh fish and environmental samples swabs in 25 ml of peptone water were transferred to 225 ml of primary enrichment broth and homogenised. The 25 g of processed fish were placed in 225 ml of primary enrichment broth and homogenised in the Stomacher (Seward 400) for 2 min. All the samples were incubated at 30°C for 24 h.

For the ISO method, 0.1 ml of these primary enrichments were transferred to 10 ml of secondary enrichment Fraser broth (35°C, 24–48 h). All the enrichments, whether showing growth or not, were subcultured by streaking onto Oxford (Merck) and PALCAM (Merck) selective agars (both incubated at 30°C, 48 h). Typical colonies (5 per plate) were streaked on Tryptone Soy Yeast Extract agar (Tryptone Soy Broth (Lab M) + 6 g/l yeast extract (Lab M) + 12 g/l agar (Lab M)) and incubated at 37°C for 24 h.

For the mini-VIDAS-LMO and for the processed fish samples, 1 ml of the primary enrichment cultures (30°C for 24 h) were transferred to 10 ml of Fraser broth and, after 24 h of incubation at 30°C, 0.5 ml of each bacterial suspension was transferred into the sample well of the mini-VIDAS-LMO reagent strip. For the environmental and fresh fish samples, 0.5 ml of the primary enrichment Fraser broth (48 h, 30°C) were directly transferred to the mini-VIDAS-LMO wells. The results were automatically obtained after 70 min.

The sample was considered positive or negative if it was confirmed, respectively, positive or negative by the reference conventional cultural method (ISO 11290-1). The rate of correct positives, named sensitivity (%), was defined as the number of correct positives over this last plus the number of false negatives; the rate of correct negatives, named specificity (%), was defined as the number of correct negatives over this last plus the number of false positives.

2.4. Confirmation and identification procedures

All the isolates were confirmed to the genus level by Gram, catalase and oxidase tests, and tumbling motility (Tryptone Soy Broth, 25°C, 24 h), and to the species level by API *Listeria* (BioMérieux) and by the CAMP test with *Staphylococcus aureus* ATCC 25923 and *Rhodococcus equi* NCTC 1691 on sheep blood agar plates (BioMérieux).

3. Results and discussion

In this study 234 fish and 61 environmental samples were tested in mini-VIDAS-LMO and confirmed by the ISO 11290-1 traditional protocol.

L. monocytogenes was recovered from 11 samples namely, fresh swordfish (1), fresh salmon–trout (1), vacuum packed cold-smoked salmon–trout (1), fresh salmon (3), vacuum packed cold-smoked salmon (3), salmon after filleting (1) and the water from the lake where the salmon–trout is farmed (1).

The mini-VIDAS-LMO detected 8 of the 11 confirmed positive samples and presented 11 false-positive results (Table 1).

The specificity of mini-VIDAS-LMO found in this experiment was 0.96. Despite the AFNOR validation (Nr BIO-12/3-03/96) of the VIDAS-LMO that concluded that no reactions with other species of *Listeria* nor other bacterial groups tested had occurred, in the present study 11 false positive results were found. All of these false positive results were found in the fresh salmon–trout samples. Seven of these false positives were found to contain only *Listeria innocua* and no *Listeria* spp. were recovered from the other four false positive samples. However, the four false positive samples, where no *Listeria* spp. were confirmed to be present, presented a high level of contamination of esculin-positive competitive microflora not identified in this study.

The sensitivity of mini-VIDAS-LMO found in this experiment was 0.73. The mini-VIDAS-LMO, compared to the ISO protocol, gave three false negative

Table 1
Evaluation of mini-VIDAS-LMO rapid method for detection of *Listeria monocytogenes* from production lines of fresh to cold-smoked fish

	mini-VIDAS LMO	ISO 11290-1
Correct positives	8	11
Correct negatives	273	284
False positives	11	0
False negatives	3	0
Total	295	295

results. The three false negatives were found in fresh salmon (1) and smoked salmon (2) samples.

In one of the false-negative smoked salmon samples only one colony growing on Oxford and Palcam agars was found. Mini-VIDAS-LMO failure to detect *L. monocytogenes* from this sample was almost certainly due to the low numbers of the target organism. Further typing of the above strain and of the other strain recovered from the same kind of product, showed they both belonged to serovar 4b.

The use of a liquid medium as a second enrichment, especially when the target organisms are in low numbers, may lead to inhibition by the competitive flora. As was reported by Beumer (1997a), after comparing different protocols for the detection of *Listeria* spp., the best performance was achieved by the protocol which included a solid medium as a second stage in the enrichment procedure.

The other mini-VIDAS-LMO false negative result was a sample of fresh salmon collected at the head of the production line of one of the cold-smoking plants found to be contaminated with *L. monocytogenes* by traditional isolation/confirmation procedures. This salmon was from a different source/importer. Further typing of the strain showed that it belonged to a serotype 1/2a and a phage type never found in all the Portuguese environmental and fish samples analysed.

In the AFNOR validation of the VIDAS LMO method, 4 samples of smoked fish artificially contaminated with different concentrations of *Listeria monocytogenes* 4b strains, respectively, 22, 43, 44 and 86 CFU/25 g, although recognized by the AFNOR VO8-055 reference detection protocol were not detected by VIDAS-LMO kit. However, the VIDAS-LMO kit gave positive results when tested with the same products but inoculated with lower concentrations (9 and 18 CFU/25 g) of the same strains. They also noticed the random nature of VIDAS LMO detection of *Listeria monocytogenes* serovars 1/2a and 4a.

In respect of the intrinsic sensitivity, the AFNOR validation (Nr BIO-12/3-03/96) of VIDAS-LMO pointed to an optimum detection level between 4×10^5 and 10^6 cells/ml. In order to ascertain the minimum level of detection in a food sample this method was compared to the traditional AFNOR V 08-055, and in 82 analysed samples, inoculated with 4 strains of *L. monocytogenes* in 5 different concentrations, 71 were positives in the VIDAS-LMO method and 66 by the traditional method. Five samples theoretically contaminated were not detected by any of the methods.

In the current study, only qualitative analyses were performed and the ISO 11291-1 protocol was used as a reference method and it was assumed to have 100% sensitivity and specificity. However, it is known that no method can fully detect *L. monocytogenes* if present in naturally contaminated samples. The selective plating media used in traditional cultural procedures are not designed to differentiate *Listeria* spp. and the selection of five suspect colonies at random from such media could lead to the detection only of other *Listeria* spp. than *L. monocytogenes* even though the latter was present on the plate (Beumer et al., 1997b).

As far as we know, only Beumer et al. (1997a) compared the VIDAS-LMO and other rapid methods for the detection of *L. monocytogenes* from foodstuffs, but, unfortunately, it was only for naturally contaminated meat products.

Comparative studies with VIDAS-LMO for detection of *L. monocytogenes* from cold-smoked fish products have not previously been reported in a scientific journal.

However, due to the low levels of *L. monocyto-*

genes and the high levels of competitive microflora found in Portuguese cold-smoked products, which eventually interfere with the sensitivity/specificity of the method, it is doubtful if mini-VIDAS-LMO can be considered a suitable method for screening these type of products for the presence of *L. monocytogenes*.

Acknowledgements

The authors gratefully acknowledge EC FAIR Project CT95-1207 ''Spoilage and Safety of Cold-Smoked Fish'' for financial support.

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