



## Evaluation of the Detection Methods used for Investigation of *Listeria* and *Listeria monocytogenes*

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### Abstract

This study investigated the occurrence of *Listeria spp.* in samples of fresh cheese and ricotta comparing the results obtained using the conventional methodology (ISO 11290-1) and automated enzyme immunoassay systems (mini-VIDAS<sup>®</sup>LIS, mini-VIDAS<sup>®</sup>LMO2, mini-VIDAS<sup>®</sup>LDUO-LIS and mini-VIDAS<sup>®</sup>LDUO-LMO). The conventional method was used as gold standard for the methods comparisons. The sensitivities for the identification of *Listeria* by mini-VIDAS<sup>®</sup>LIS and mini-VIDAS<sup>®</sup>LDUO-LIS were 73.3% and 64.7%, respectively. For *L. monocytogenes* identification the sensitivities were 83.3% (mini-VIDAS<sup>®</sup>LMO2) and 62.5% (mini-VIDAS<sup>®</sup>LDUO-LMO). The specificity parameter obtained by the mini-VIDAS<sup>®</sup> systems ensured the detection of the target microorganism. As a conclusion, the mini-VIDAS<sup>®</sup>LIS reagent system can be used to quickly detect *L. monocytogenes* followed by the mini-VIDAS<sup>®</sup>LMO2 system.

**Keywords:** Food borne diseases; *Listeria*; Mini-VIDAS<sup>®</sup> system; Listeriosis

### Introduction

Despite the use of different techniques to ensure the quality and safety of food, foodborne diseases (FD) remain a public health problem. Various microorganisms can cause FD, including the *Listeria monocytogenes* bacteria, which is able to survive and proliferate in food kept at refrigeration temperature [1,2].

Among the six species currently recognized in the genus *Listeria*, only *L. monocytogenes* is typically implicated in human FD called Listeriosis. It has been estimated that 99% of all human listeriosis cases are caused by consumption of contaminated food products especially dairy products such as fresh cheese and ricotta. *Listeria monocytogenes* is responsible for cases of abortion, meningitis, and septicemia. The high mortality rate of about 20–30% in those developing listeriosis (pregnant women, elderly and immunocompromised persons) makes *L. monocytogenes* a serious human pathogen [3,4].

The detection of *Listeria spp.* traditionally involve culture methods based on selective enrichment and plating followed by the characterization of *Listeria spp.* based on colony morphology, sugar fermentation and haemolytic properties. This method is the gold standard; but it can take up to five days to obtain a result. Recently, alternative methods have been proposed for the detection of *Listeria spp.* in food. These methods include immunological techniques such as the mini-VIDAS<sup>®</sup> system (BioMerieux Vitek, Inc., Missouri, USA): a fully automated instrumental system that uses fluorescent ELFA (Enzyme Linked Fluorescent Assay) technology for detection of listerial antigens in food [5–8]. It can be a rapid screening method alternative to time consuming classical isolation and identification.

In this study, three different reagent systems of the mini-VIDAS<sup>®</sup> system were compared to the conventional method for detection of *Listeria sp.* in fresh cheese samples and ricotta in the state of Paraná. In addition, the prevalence of samples contaminated with *Listeria monocytogenes* was investigated [9].

A total of 106 samples of 26 different brands of fresh cheeses (77 samples) and ricotta (29 samples) were collected and analyzed with the conventional method for search *Listeria sp.* and the mini-VIDAS<sup>®</sup> system (Biomérieux) LIS (*Listeria sp.*), LMO2 (*Listeria monocytogenes*) and LDUO (simultaneous detection of *Listeria sp.*, *Listeria monocytogenes*) featuring presence or absence of the microorganism in 25 grams of sample [10].

The conventional method followed the ISO 11290-1: 1996 protocol that basically involves four steps: pre-enrichment, selective enrichment, isolation on solid selective media, and complete identification of the colonies through biochemical and serological testing [11]. Primary Enrichment was performed using Half Fraser broth. In the secondary enrichment was used Fraser broth supplemented with SR 0155E (Oxoid). Selective plating was performed in duplicate plating on selective medias: Palcam agar supplemented with SR 0150E (Oxoid), Oxford agar selective for *Listeria sp.* supplemented with SR 0140E (Oxoid), Oxoid Chromogenic *Listeria* Agar (OCLA) and Chromogenic *Listeria* Agar (ISO). Typical colonies were confirmed using tubes of Triptycase soy agar supplemented with 0.6% yeast extract (TSA-YE) (Difco). Evidence for catalase activity was investigated and motility was evaluated using sulfide indol motility with addition of 0.05% chloride trifeniltetrazolium (Sigma<sup>®</sup>) (SIM changed) that allow the detection of sulfide production, indole formation and motility. The biochemical confirmation occurred in parallel with API *Listeria* system<sup>®</sup> (Biomérieux), according to the manufacturer's instructions. This biochemical test was designed for the genus *Listeria* and includes 10 biochemical differentiation tests in a microtube format for species identification [7].

The mini-VIDAS<sup>®</sup> system was developed from the 48 h of enrichment Fraser broth and subsequent inactivation cell in a water bath at 95°C - 100°C for 15 min. Then, an aliquot is placed into a reagent strip which is coated with antibodies and contains all the ready-to-use reagents (wash solution, conjugate and substrate) required. All assay steps are performed automatically by the instrument. Finally, the fluorescence is measured by the optical scanner in the apparatus and analyzed automatically by the computer [12]. The mini-VIDAS<sup>®</sup> LIS was employed for the detection of *Listeria sp.* The mini-VIDAS<sup>®</sup> LMO2 was used for detection and confirmation of *Listeria monocytogenes* and the mini-VIDAS<sup>®</sup> LDUO was used for

the simultaneous determination of *Listeria* sp and *Listeria monocytogenes*. The immunological techniques described above consist of screening methods that require confirmation of positive results by the conventional method. In this case we proceeded to the selective plating and biochemical identification, adopting the same methodology of the conventional procedure.

The results obtained using the different methods employed for the determination of *Listeria* sp and *Listeria monocytogenes* were analyzed with a nonparametric method (Chi-square test) by the Excel 2007 program and the Statistical Package for the Social Sciences SPSS 17.0 (Windows and Mac). Percentage calculations, proportions and charts were used for descriptive statistics. To determine the concordance between the positive and negative results obtained by the conventional method (ISO 11290-1) and the different mini-VIDAS<sup>®</sup> systems, the overall percentage of agreement was calculated by the percentage of positive and negative diagnoses in compliance with all of the assessments.

In this work, the conventional method was used as reference to prove if samples were negative or positive for *Listeria* sp. Results identified as *Listeria* and as absence of *Listeria* by the conventional method were considered as true positives and true negatives results, respectively. The sensitivity and specificity of the methods were evaluated by the equation of Beumer et al. [10].

Of the 106 analyzed samples, 11 (10.37%) tested positive to *Listeria* by the conventional method (true positive results). Contamination with the *Listeria* genus was observed in 9 fresh cheese (11.7%) and 3 ricottas (10.3%) and the *Listeria monocytogenes* species were isolated from 5 of these samples (4.7%): 2 samples from cheese and three from ricotta. In the remaining 7 samples, the species *Listeria seeligeri* (3.8%), *Listeria ivanovii* (1.9%) and *Listeria innocua* (0.9%) were observed. Six (23%) of the 26 brands analyzed had contamination problems. These results showed that there was no significant difference between the contaminated ricotta and cheese samples ( $p= 0.846$ ). Therefore, the contamination percentage in the two products was similar regardless of the nature of the sample.

The results from the mini-VIDAS<sup>®</sup> systems (Biomerieux<sup>®</sup>) revealed 10 samples with positive results in at least one of the three evaluated reagent systems (LIS, LMO2 and LDUO) with the highest number of positive results presented by mini-VIDAS<sup>®</sup> LIS system (8 samples) and the mini-VIDAS<sup>®</sup> LDUO-LIS (6 samples). The mini-VIDAS<sup>®</sup> system was capable of identified 4 samples (3.8%) as *Listeria monocytogenes*, 3 (2.8%) as *Listeria seeligeri*, 2 (1.9%) as *Listeria ivanovii* and 1 (0.9%) as *Listeria innocua* (Table 1).

Sample	Brand / product	Conv	mini-VIDAS <sup>®</sup> system				API <i>Listeria</i>
			LIS	LMO2	LDUO		
					LIS	LMO	
1	L / cheese	X	X	X	X	X	<i>L. monocytogenes</i>
2	F / cheese	X	X				<i>L. seeligeri</i>
3	F / cheese	X					<i>L. seeligeri</i>
4	F / cheese	X	X				<i>L. seeligeri</i>
5	F / cheese	X	X		X		<i>L. seeligeri</i>
6	O / ricotta	X	X	X	X	X	<i>L. monocytogenes</i>
7	O / ricotta	X	X	X			<i>L. monocytogenes</i>
8	O / ricotta	X	X	X	X		<i>L. monocytogenes</i>
9	K / cheese	X					<i>L. monocytogenes</i>
10	D / cheese	X	X				<i>L. ivanovii</i>
11	D / cheese				X		<i>L. ivanovii</i>
12	U / cheese	X			X		<i>L. innocua</i>

**Table 1:** Positive results for *Listeria* sp. considering the brand, product type and different methods of detection Data: mini-VIDAS<sup>®</sup>LIS was employed for the determination of *Listeria* sp. mini-VIDAS<sup>®</sup>LMO2 system specific for the detection of *Listeria monocytogenes*. Mini-VIDAS<sup>®</sup>LDUO used for the simultaneous determination of *Listeria* sp (LDUO-LIS) and *Listeria monocytogenes* (LDUO-LMO). API *Listeria* was used for species identification. Conv: conventional method.

Comparing the results obtained in the conventional method with the mini-VIDAS<sup>®</sup> systems results, false negative results were observed using the mini-VIDAS<sup>®</sup> LIS (4 samples), mini-VIDAS<sup>®</sup>LMO2 (1 sample) system, mini-VIDAS<sup>®</sup> LDUO-LIS (6 samples) and mini-VIDAS<sup>®</sup>LDUO-LMO (3 samples) systems. Only one false positive result was founded using the mini-VIDAS<sup>®</sup>LDUO-LIS system. The

other entire samples tested negative by the conventional method presented negative results in the tested mini-VIDAS<sup>®</sup> systems. Therefore, for *Listeria* genus detection, a positive correlation between the three evaluated reagent systems (LIS, LMO2 and LDUO) was found for only four samples and for *L. monocytogenes* identification

there was a positive correlation between the three methods for only two samples.

Therefore, the methods capable of detecting *Listeria* (mini-VIDAS<sup>®</sup>LIS and mini-VIDAS<sup>®</sup>LDUO-LIS) showed 100.0% and 99.0% of specificity, respectively. The sensitivity was 73.3% for the mini-

VIDAS<sup>®</sup>LIS and 64.7% for the mini-VIDAS<sup>®</sup>LDUO-LIS. For the methods used to detect *Listeria monocytogenes* (mini-VIDAS<sup>®</sup>LMO2 and mini-VIDAS<sup>®</sup>LDUO-LMO) a specificity of 100.0% was observed. The sensitivity was 83.3% for the mini-VIDAS<sup>®</sup>LMO<sub>2</sub> and 62.5% for the mini-VIDAS<sup>®</sup>LDUO-LMO (Table 2).

	Method	Positive samples	Negative samples	False-negatives <sup>a</sup> samples	False-Positives <sup>b</sup> samples	Total
<i>Listeria sp</i>	Conventional	11	95	0	0	106
	mini-VIDAS <sup>®</sup> LIS	8	98	4	0	106
	mini-VIDAS <sup>®</sup> LDUO-LIS	5	101	6	1	106
<i>L. monocytogenes</i>	Conventional	5	101	0	0	106
	mini-VIDAS <sup>®</sup> LMO <sub>2</sub>	4	102	1	0	106
	mini-VIDAS <sup>®</sup> LDUO-LMO	2	104	3	0	106

**Table 2:** False positive and false negative values considering the results obtained by the different methods used to identify the genus *Listeria* and *Listeria monocytogenes* <sup>a</sup> = negative result in the mini-VIDAS<sup>®</sup> tested system and positive result in the conventional method <sup>b</sup> = positive result in the mini-VIDAS<sup>®</sup> tested system and negative result in the conventional method.

## Discussion and Conclusion

In this work, *Listeria spp.* in samples of fresh cheese and ricotta was investigated comparing the results obtained using the conventional methodology (ISO 11290-1) and automated enzyme immunoassay systems (mini-VIDAS<sup>®</sup>LIS, mini-VIDAS<sup>®</sup>LMO<sub>2</sub> and mini-VIDAS<sup>®</sup>LDUO). The obtained results showed that 10.37% of the samples were contaminated with genus *Listeria* and 4.7% of the analyzed products tested positive for the presence of *L. monocytogenes*, classifying them as unfit for human consumption. Furthermore, the presence of other *Listeria* species does not rule out the possibility that *Listeria monocytogenes* may be present even if it was not detected. In addition, the species *L. ivanovii* and *L. seeligeri* found in this study have been reported to cause disease in humans [13]. These results indicate that care must be taken in the production process in order to prevent contamination at critical points.

National and international data published over the last 10 years for the occurrence of *Listeria monocytogenes* in cheese and similar products demonstrated similar infection rates than the rates reported in the present work. The comparison of different works investigating the occurrence of *Listeria monocytogenes* is difficult due to the variety of the analytical methods used and the great diversity of cheese types analyzed in these works. The use of different culture media and sampling standards may result in different levels of detection. This difficulty is compounded in Brazilian studies by the fact that typically only Brazilian products (i.e., Minas fresh cheese and curd cheese) are analyzed. Accordingly, there is an absence of similar foreign products that can be used for comparison [5].

The choice of methodology for microbiological testing of food should be guided by the required accuracy, cost analysis, time, and acceptance of the method by official bodies and the scientific community. Additional factors that require consideration include the operational, training and qualification of the analyst, availability and quality of reagents, culture media and other supplies, and availability of lab space [7].

From the methods comparison performed in this work, the specificity parameter obtained by the mini-VIDAS<sup>®</sup> systems ensured the detection of the target microorganism. In the case of the sensibility parameter, it was demonstrated that although of practical interest, the immunoassays screening techniques were not able to detect all positive samples. In cases of mini-VIDAS<sup>®</sup>LMO<sub>2</sub> positive results, the identification of *Listeria monocytogenes* must be confirmed using the conventional method from the second enrichment. In addition, the mini-VIDAS<sup>®</sup>LIS followed by mini-VIDAS<sup>®</sup>LMO<sub>2</sub> system reduced the analysis time for the detection of *Listeria monocytogenes*. The analysis time for negative results would be 48 hours, which is significantly less than the minimum 7 days recommended for the conventional method.

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