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Short Communication

Enzyme-linked imunoassays for the detection of *Listeria* sp. and *Salmonella* sp. in sausage: A comparison with conventional methods

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Abstract

This study was carried out comparing the conventional methods (ISO 11290-1 and BAM method, 2008) and system mini-Vidas® (Biomerieux), for detection of *Listeria* sp. and *Salmonella* sp. in cooled sausage. The immunoenzymatic method has shown to be effective for the detection of target pathogens, it has presented itself as an excellent screening method.

Key words: cooled sausage, *Salmonella* sp., *Listeria* sp., system mini-Vidas®.

An appropriate and efficient method for detecting microorganisms in food has been constantly searched for by researchers (Giombelli, 2000). The choice of methodology to be adopted in conducting the microbiological analysis of any product should be guided by the following parameters: the desired accuracy, cost, time of analysis, acceptance of the method by official agencies and the scientific community, simplicity of operation, training, and the analyst's qualifications, availability of reagents, culture media and other supplies (Franco and Landgraf, 2008).

The detection of *Salmonella* sp. and *Listeria* sp. in food is often carried out by the traditional method designate classical method. Although they show some variations in the selection of culture media and in the form of sample preparation, they basically involve four steps that can be applied to any type food: pre-enrichment, selective enrichment, isolation on solid selective media, and complete identification of the colonies through biochemical and serological testing (Boer and Beumer, 1999; Giombelli and Lopes da Silva, 2002; Reis *et al.*, 2002; Silva *et al.*, 2004).

In recent years, several methods have been proposed for the rapid detection and among them there are the immunological techniques. The enzyme immunoassays (EIA) may contribute to accelerate and simplify the detection of various types of pathogens in foods (1) and are most commonly used because they present several advantages: simplicity, rapidity, sensitivity, specificity and convenience as a screening method (Franco and Landgraf, 2008).

The objective of this study was to compare results obtained with the conventional culture methods for detection of *Listeria* sp. (ISO standard 11290-1) (ISO, 1996) and *Salmonella* sp. (BAM method) (Hitchins, 2003) in a cold sausages, compared to the mini-Vidas® system, as well as identify the prevalence of colds sausage contaminated with *Listeria monocytogenes* and species of the genus *Salmonella*, and perform the evaluation of the analysis time waived for identification of *Salmonella* sp. and *Listeria* sp. to conventional methods (BAM and ISO) and rapid methods (mini-Vidas *Listeria* and mini-Vidas *Salmonella*) (Biomerieux).

We collected 51 samples of cold sausage purchased at different retailers in the state of Paraná, and produced in the southern region of Brazil. It was acquired by the sample, the minimum amount of 250 g in order to maintain the representative recommended by the RDC (Board Resolution) of 12 02/01/2001 (RDC, 2001).

In all evaluations characterized the presence and absence of pathogens in 25 g of sample tested. For the detection of *Listeria* sp. employed the ISO 11290-1 method (conventional method), and the system mini-Vidas® (Biomerieux). For the *Salmonella* sp. using the conventional method we used conventional methodology described in Bacteriological Analytical Manual (BAM) *online*

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(Hitchins, 2003), created by the Food and Drug Administration (FDA), Compendium of Methods for the Microbiological Examination of Foods (1992) and rapid method, using the system mini-Vidas® (Biomerieux).

The system mini-Vidas® is a method of qualitative automated enzyme immunoassay analysis that uses the technique ELFA (Enzyme Linked Flourescent Assay) consisting of a mixture of monoclonal capture antibodies with high specificity for antigen detection. All the test steps are performed automatically using disposable plastic galleries, consisting of compartments containing the reactants needed for the test. Therefore, the reading and interpretation of results are also automatic.

The conventional culture method for *Listeria* sp. consisted of: 01) Primary Enrichment: 25 g of sample were added 225 mL of Half Fraser broth supplemented with SR 0166E (Oxoid), this was incubated at 30 °C for 24 h. 02) Secondary Enrichment: 1 mL of primary enrichment was added to 9 mL of Fraser broth supplemented with SR 0155E (Oxoid) and incubated at 37 °C for 48 h. 03) Selective Plating: it was the rifling in duplicate plating on selective media: Palcam agar supplemented with SR 0150E (Oxoid), Oxford agar selective for *Listeria* sp. supplemented with SR 0140E (Oxoid). The confirmation occurred after by rifling in tubes Triptycase soy agar supplemented with 0.6% yeast extract (TSA-YE) (Difco) and incubated at 30 °C / 24 to 48 h. It was evaluated the motility characteristic umbrella tube using sulfide indol motility with addition of 0.05% chloride trifeniltetrazolium (Sigma®) (SIM changed), and incubated at 25 °C for up to 7 days and daily observation. The confirmation biochemical occurred in parallel with API *Listeria* system® (Biomerieux), according to the manufacturer's instructions.

Mini-Vidas® system *Listeria* sp. was developed from the 48 h of enrichment Fraser broth and subsequent inactivation cell occurred by transferring 2 mL sterile tube, heated in a water bath at 95 °C - 100 °C for 15 min, the tube was cooled immediately in a cold water bath. It was transferred 0.5 mL of the suspension to the well-inactivated sample LIS mini-Vidas® (Development of the methodology as recommended by the manufacturer). In this case we proceeded to the selective plating, biochemical identification, adopting the same methodology of the conventional procedure.

The conventional culture method for *Salmonella* sp. consisted of: 01) Pre-non-selective enrichment broth: 25 g of sample were added to 225 mL of pre-enrichment broth (ADPT) and incubated at 35 ± 0.2 °C for 24 h. 02) Selective enrichment broth: 1.0 mL pre-enrichment broth was transferred to 10.0 mL of Selenite Cystine Broth (SC) and 0.1 mL to 10.0 mL of Rappaport-Vassiliadis Broth Soy Peptone (RVS) which were incubated at 42 ± 0.2 °C for 24 h. 03) Selective Differential Plating: the selective enrichment broth was streaked on SS agar and HE agar incubated at 35 ± 0.2 °C for 24 h. The biochemical and serological confirmation occurred after plating tube LIA agar and TSI agar, incubated at 35 ± 0.2 °C for 24 h and from these serologic testing performed in somatic polyvalent glass sheet. In addition the API 20E® system (Biomerieux) has been developed according to the manufacturer's instructions.

The mini-Vidas® system *Salmonella* sp. was performed from the secondary enrichment broth, where 1 mL of each broth was transferred to the broth M, incubated at 42 ± 0.2 °C for 24 h. The cell inactivation occurred by transferring 2 mL of the secondary enrichment for sterile tube, heated in a water bath at 95 °C - 100 °C for 15 min, the tube was cooled immediately in a cold water bath. It was transferred 0.5 mL of the suspension to the well-inactivated sample SLM mini-Vidas® (Development of the methodology as recommended by the manufacturer). In this case we proceeded to the selective plating, biochemical identification and serology, adopting the same methodology of the conventional procedure.

In statistical analysis we calculated the estimates of sensitivity, specificity, prevalence, false positive error rate and false negative error rate according to the model proposed by Hanrahan and Madupu (1994). For comparative analysis between the methods of pathogens mini-Vidas® and the conventional method the latter was considered as standard for pathogen identification.

Considering the two methods of analysis, the prevalence of *Listeria* sp. was 52.9%, and of these, 13.7% corresponded of *L. monocytogenes*, whereas *L. gray* was reported in 19.7% and *L. innocua* and *L. welshimeri* were identified, 13.7% and 5.9% of cases respectively. The presence of *Salmonella* was recorded in 3.9% of samples.

In the study of *Listeria* sp. 28 samples were positive for methods ISO 11290-1 and also the mini-Vidas® system, however, there was only one positive sample by the method mini-Vidas® (false positive), and one sample by the ISO method (false negative) (Table 1). Observing the comparative evaluation between the methodologies, a sen-

Table 1 - Positive results observed by mini-Vidas® method and conventional method for *Salmonella* sp. and *Listeria* sp. in cooled sausage

Diagnosis	*Salmonella* sp.		*Listeria* sp.	
	BAM standard	Mini-Vidas®	ISO standard	Mini-Vidas®
Prevalence	06	11	28	28
Exclusive insulation	0	05	01	01

sitivity of 96.7% and a specificity of 94.7% (Table 2) was found.

During the *Salmonella* sp. study 11 positive samples were verified by the system mini-Vidas® and only 06 for the methodology BAM, so 05 samples were positive only if the method mini-Vidas®, considered these false positive (Table 1). There was the presence of false positives attributed by the detection of other bacteria, these belonging to the family *Enterobacteriaceae*. The method mini-Vidas® compared to the BAM method for identification of *Salmonella* sp. produced a sensitivity of 100% and a specificity of 88.89% (Table 2).

Comparing the response time of methods for identification of *Salmonella* sp., it was observed that the negative result is identified for both method (mini-Vidas® and Bam) in 72 h. However, in case of positive result mini-Vidas® method requires 24 h to more than the BAM method, this is, dispensation additional 24 h to the culture broth of the "M", used in the confirmatory method of mini-Vidas® (Table 3).

However for the study of *Listeria* sp., it was observed that the method mini-Vidas®, presented a negative result in 48 h, whereas the conventional method needs 120 h. Nevertheless, the positive result would occur in 264 h by the method mini-Vidas® and 288 h by the ISO methodology. Considering that the international law determines absence of the genus *Listeria*, the rapid method mini-Vidas® provides an advantage over the conventional method ISO reducing the 72 h analysis (Table 4).

The mini-Vidas® method when compared to the ISO method, demonstrated that it is able to identify very low concentrations of viable microorganisms in the sample. It is noteworthy that a positive sample for *Listeria* sp. is not always pathogenic, causing part of this group only *Listeria monocytogenes* which corresponds to an incidence of only 13.7% of 53.9% of the total.

Lepper *et al.* (2002) developed a similar study conducted by comparing the system with the mini-Vidas® *Salmonella*, BAM classical culture in 6 types of products: milk chocolate, milk powder, egg powder, soy flour, black pepper and "raw land". The researcher noted that the two methods were concordant for 1266 of 1440 samples.

Blackburn *et al.* (2008) promoted the detection of *Salmonella* sp. using the mini-Vidas® system was compared with a conventional method for the detection of *Salmonella* sp. in 141 samples and naturally contaminated

Table 2 - Comparison of results between the mini-Vidas® method and conventional method for *Salmonella* sp. and *Listeria* sp. in cooled sausage.

Diagnosis	Salmonella sp. (BAM standard X mini-Vidas®)	Listeria sp. (ISO standard X mini-Vidas®)
True positive	6	31
True negative	40	18
False positive	5	1
False negative	0	1
Sensibility	100%	96.80%
Specifciity	88.89%	94.70%
Prevalence	11.76%	62.74%

Table 3 - Description of the analysis time for identification of *Salmonella* sp.

		Activity	Time worked	Total time
BAM	Result negative	Pre-enrichment broth non selective	24 h	72 h (3 days)
		Seletive enrichment broth	24 h	
		Selective and differential plating	24 h	
	Result positive	Pre-enrichment broth non selective	24 h	96 h (4 days)
		Seletive enrichment broth	24 h	
		Selective and differential plating	24 h	
		Culture TSI and LIA + serology	24 h	
Mini-Vidas® *Salmonella*	Result negative	Pre-enrichment broth non selective	24 h	72 h (3 days)
		Seletive enrichment broth	24 h	
		Seletive enrichment broth "M" + cell inactivation + insertion of the antigen in the cap in mini-Vidas® machine	24 h	
	Result positive	Pre-enrichment broth non selective	24 h	120 h (5 days)
		Seletive enrichment broth	24 h	
		Seletive enrichment broth "M" + cell inactivation + insertion of the antigen in the cap in mini-Vidas® machine	24 h	
		Selective and differential plating	24 h	
		Culture TSI and LIA + serology	24 h	

Table 4 - Description of the analysis time for identification of *Listeria* sp.

		Activity	Time worked	Total time
ISO	Result negative	Primary enrichment	24 h	120 h (5 days)
		Secondary enrichment	48 h	
		Selective plating	48 h	
	Result positive	Primary enrichment	24 h	288 h (12 days)
		Secondary enrichment	48 h	
		Selective plating	48 h	
		Culture of Indol and evaluation for the motility	168 h	
Mini-Vidas® *Listeria*	Result negative	Primary enrichment	24 h	48 h (2 days)

foods. As a result there was an overall agreement of 92.9% between the methods.

In the present study it was found that the mini-Vidas® methodology was effective for the detection of target pathogens, and they presented themselves as excellent screening methods, so the present work contributes to the introduction of a faster method (especially when researching the pathogen *Listeria* sp.) with the reliability expected by analysts, developing this forms a new reality facing the accuracy of results, favoring the satisfaction of the producer to send to the market a safe product, providing advantages in marketing directly to consumers by ensuring more reliable products to be consumed.

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