Intra-laboratory validation of commercially available ELFA-based assay and PCR-based assay for detection of *Listeria monocytogenes* in food samples according to ISO 16140-2

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Summary

In this study, commercially available VIDAS system based on the enzyme linked fluorescent assay (ELFA; BioMérieux, Lyon, France) and the BAX system based on polymerase chain reaction (PCR; Hygiena, Camarillo, California, USA) were compared to the reference culture-based method (EN ISO 11290-1) to detect *Listeria monocytogenes* in food samples. The process was carried out in accordance with criteria for methods comparison established in EN ISO 16140-2 for validation of alternative microbiological methods. As a result of validation studies with food samples, sensitivity rates for samples in all food categories were determined as 97.2 % for the ELFA method and 97.9 % for the PCR method. Level of detection of the ELFA method per 25 g was determined to be 1.1–4.3 CFU and of the PCR method was determined to be 2.7–8.0 CFU. The method comparison study is a part of the validation process that takes place in analysis laboratories. Although the VIDAS System and BAX System have been used in food analysis laboratories for many years, this study is the first intralaboratory method validation study conducted in accordance with the latest version of ISO 16140-2 against a reference method.

Keywords

Listeria monocytogenes; rapid microbiological method; intra-laboratory validation; enzyme-linked flouorescent immunoassay; polymerase chain reaction

Food-borne pathogen *Listeria monocytogenes* is the causative agent of listeriosis that causes meningitis, encephalitis, septicemia, abortions and neonatal deaths in humans. Immune suppressed patients, AIDS and cancer patients, pregnant women, newborns and the elderly are in the risk group. The mortality rate in this group is 20–30 % [1, 2]. The fifth most frequently reported zoonotic disease in the European Union (EU) in 2021 was listeriosis, with a total of 2 183 cases reported [3]. This represents a 14 % increase in the EU notification rate from the previous year. The disease primarily affects individuals aged 64 years or older [3]. Listeriosis progresses in two different clinical forms, non-invasive and invasive. Although non-invasive forms that cause gastroenteritis are generally underestimated in many countries, invasive forms that cause bacteremia, neonatal infections or localized infections in various organ systems are an important public health concern [2].

L. monocytogenes is largely resistant to environmental factors that affect microbial growth, such as low pH, low temperature or high salt concentration. Therefore, it may overcome protection and safety barriers in food production processes and creates a potential risk to human health. The main sources of contamination are dairy products (raw and pasteurized milk, cheese, cream, butter), meat and meat products (delicatessen meat products, minced meat, pork, poultry), seafood (smoked fish, crustaceans, shellfish, molluscs), ready-to-eat foods, fruits, vegetables, fruit juices and vegetable juices [4].

Accurate and reliable determination of *L. monocytogenes* in food analysis laboratories is of utmost importance for food safety. Selective isolation of microorganisms and biochemical characterization in culture media, which are accepted as standard microbiological methods, are not commonly preferred in food analysis today when

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compared to new rapid technologies developed for detection of pathogenic microorganisms [5]. Although conventional cultural methods are economical, they are time-consuming as they require steps of pre-enrichment, selective enrichment and inoculation on selective agars. Therefore, a period of over a week is required for the identification and confirmation of the pathogenic microorganism present in a food sample, making rapid results impossible [6, 7].

To reduce the number of food-borne outbreaks, it is crucial to detect food-borne pathogenic microorganisms as quickly as possible in their initial growth stages. Quick results also prevent economic losses, as they provide early, risk-free marketing of food [8–11]. Some of the rapid methods are culture-based similar to the reference methods but do not require additional identification since they contain chromogenic or fluorogenic substrates. Some immunoenzymatic and molecular methods are automated and these systems have been used widely in recent years both in analytical laboratories and by quality control in food production [12]. Automated systems may provide an advantage over cultural methods in terms of giving fast results, having higher sensitivity, less labour requirements and the ability to analyse a large number of samples simultaneously. For this reason, they are particularly suitable for monitoring critical control points in the production process, controlling rapidly perishable food products, performing quality control analysis of imported and exported foods as well as managing potential food safety crises [12]. However, it is necessary to validate these automated systems before they can be used in routine analysis, especially through an accreditation process.

In many countries, culture-based method ISO 11290-1 for detection of *L. monocytogenes* in food, feed and environmental samples is used. It requires more than six days in case of positive results [13, 14]. Commercially available ELFA-based VIDAS or mini VIDAS system (BioMérieux, Lyon, France) and the PCR-based BAX system (Hygiena, Camarillo, California, USA), with dedicated kits of chemicals, reduce the detection time of *L. monocytogenes* to one or two days.

VIDAS LMO2 (BioMérieux) is a kit of chemicals to be used with VIDAS or mini VIDAS instruments. It provides rapid pathogen analysis based on enzyme-linked fluorescent immunoassay (ELFA) specific for *L. monocytogenes* surface antigens. All chemicals used in the test are supplied as ready-to-use materials and all the steps in the process are carried out automatically. Solid Phase Receptacle (SPR) pipettes (BioMérieux) used as solid phase are coated with *L. monocytogenes* antibodies, and they also act as a pipetting device. The enriched sample is transferred to a reagent strip. If the sample contains the target organism, its antigen binds to *L. monocytogenes* antibodies found inside SPR. In the next step, alkaline phosphatase-bound antibodies bind to the antigen-antibody complex in SPR. In the detection step, 4-methylumbelliferyl phosphate is added to the medium as a substrate and the conjugated enzyme reacts with the substrate to form 4-methylumbelliferone, which is fluorescent. Fluorescence is measured by the instrument and each sample is evaluated as positive or negative by the instrument.

BAX System is an automated system that detects the target microorganism using real-time polymerase chain reaction (PCR) with Scorpion primer-probe technology. For this system, all the reagents (primers, polymerase, nucleotides and a positive control) required for the *L. monocytogenes* PCR assay are combined in a small tablet in a microtube. This eliminates potential pipetting errors caused by the operator and minimizes the risk of cross-contamination. This system combines PCR with fluorescence detection in the instrument with no additional results interpretation required.

In this paper, intra-laboratory validation studies of VIDAS LMO2 and BAX *L. monocytogenes* 24E for identification of *L. monocytogenes* causing serious health problems was performed against ISO 16140-2 [15]. The ELFA-based method was performed using mini VIDAS System (BioMérieux) and the PCR method was performed using BAX System (Hygiena). For this validation process, the ISO 11290-1 is considered as the reference method [13, 14]. As validation parameters, three different studies were carried out: sensitivity study, relative level of detection study and an inclusivity/exclusivity study, so that the reference method and alternative methods could be compared.

MATERIALS AND METHODS

Preparation of bacterial strains

Lyophilized *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 19114 and *L. monocytogenes* ATCC 19116 cultures were used as reference strains for the experiments. For exclusivity study, *L. innocua* ATCC 51742, *Bacillus cereus* ATCC 10876 and *Escherichia coli* ATCC 8739 were also used. Reference strains were obtained commercially (Microbiologics, St. Cloud, Minnesota, USA). Lyophilized cultures were resus-

pended in 10 ml tryptic soy broth (TSB; Merck Millipore, Burlington, Massachusetts, USA) supplemented with 6 $g \cdot l^{-1}$ yeast extract (Merck Millipore) and were allowed to grow statically for 24 h at 37 °C (for *B. cereus* at 30 °C). After incubation, suspensions were streaked on tryptic soy agar (TSA, Merck Millipore) supplemented with 6 $g \cdot l^{-1}$ yeast extract and the plates were incubated for 24 h at 37 °C (for *B. cereus* at 30 °C). Overnight cultures were suspended in 1 $g \cdot l^{-1}$ peptone (Sigma Aldrich, St. Louis. Missouri, USA) and turbidity of the microbial suspensions was adjusted to 0.8 McFarland using a densitometer (Grant Instruments, Cambridge, United Kingdom). These cultures were diluted to desired concentration and suitable dilutions were used for validation experiments. To determine bacterial counts, appropriate dilutions were inoculated to TSA supplemented with 6 $g \cdot l^{-1}$ yeast extract using spreading method and were incubated at 37 °C for 24 h.

Selection of food samples

Several food groups were tested in order to observe a possible matrix effect. For this purpose, five main product categories were used in method comparison studies: meat and meat products, dairy products, seafood, fruit and vegetable products, and ready-to-eat foods.

Protocol of EN ISO 11290-1

Isolation procedure of *L. monocytogenes* was conducted according to the reference method ISO 11290-1 [13, 14]. This method involves using selective enrichment broth with subsequent solid agar medium to grow and differentiate target organisms from the natural flora residing in the food sample. The stages of the method were:

The samples (25 g) were weighed into a stomacher homogenization bag, which was followed by the addition of 225 ml of Half Fraser Broth (Oxoid, Basingstoke, United Kingdom).

Mixtures were homogenized by stomaching and were incubated for the first enrichment stage at 30 °C for 25 ± 1 h.

After incubation, 0.1 ml of the suspension was transferred into 10 ml of Fraser Broth (Oxoid) for the second enrichment stage and incubated at 37 °C for 24 ± 2 h.

At the end of the enrichment stages, growing organisms were streaked on Oxford, PALCAM and ALOA media (all Oxoid).

After 24 h incubation (48 h if necessary), characteristic colonies were determined.

The presumptive colonies were confirmed using appropriate morphological, physiological and biochemical tests.

Protocol of VIDAS-LMO2

The detection stages of *L. monocytogenes* according to VIDAS-LMO2 were:

The samples (25 g) were weighed into a stomacher homogenization bag, which was followed by the addition of 225 ml of Half Fraser Broth.

Mixtures were homogenized by stomaching and were incubated for the first enrichment stage at 30 °C for 24–26 h.

After incubation, 0.1 ml of the suspension were transfered into 10 ml of Fraser Broth for the second enrichment stage and incubated at 30 °C for 24–26 h.

A 500 µl aliquot of the second enrichment was pipetted to the stripe. The stripe was placed into mini VIDAS device and the procedure was performed according to manufacturer's instructions.

When the assay was completed, results were analysed automatically by the instrument.

Test values less than the threshold value indicated that the sample either did not contain *L. monocytogenes* antigens or contained *L. monocytogenes* antigens at a concentration below the detection limit. Test values greater than or equal to the threshold value indicated that the sample contained *L. monocytogenes*.

Protocol of BAX *L. monocytogenes* 24E

The BAX *L. monocytogenes* 24E procedure was performed as follows:

An amount of 25 g of the food sample was transferred to 225 ml of Half Fraser Broth, homogenized using stomacher device (Interscience, Saint Nom la Brétèche, France) and incubated at 37 ± 1 °C for 22–24 h.

A volume of 150 µl of protease solution (Hygiena) was added to 12 ml of lysis buffer (Hygiena) and 200 µl of the mixture was transferred to a microtube.

A volume of 5 µl of the enriched sample heated at 37 °C for 30 min was transferred into the lysis mixture.

Microtubes were incubated in a heating block for 30 min at 55 ± 2 °C and then for 10 min at 95 ± 3 °C.

Once the lysis was completed, the tubes were placed in 2–8 °C.

After 5 min, 30 µl lysates were removed and transferred into kit microtubes containing reagent tablets.

The microtube strips in a rack were placed in the BAX instrument and the PCR process proceeded automatically.

After PCR was finished, the system automatically provided results in the form of red (+) symbol for positives and the green (–) symbol for negatives.

Tab. 1. Categories and types of food samples used in the sensitivity study.

Food category	Food type	Number of samples		
		Negative results	Positive results	Total
Meat and meat products	Minced meat, piece of lamb, meatball mix, chicken wing, drumstick, ground turkey, sausage, fermented sausage, ham, pastrami	29	34	63
Dairy products	Milk, cheese, cream, yoghurt, curd, butter, ice cream, dried milk, casein	29	31	60
Seafood	Fish fillet, calamary, mussel, shrimp	30	32	62
Fruit and vegetables	Dried, chilled and fresh minced vegetable, fruit juice	37	23	60
Ready-to-eat foods	Ready-cooked, salad, delicatessen, cream-cake, dessert	22	30	52

Results were confirmed according to ISO 11290-1 standard [13, 14].

Inclusivity and exclusivity

Inclusivity study demonstrates the detectability of all pure target strains with alternative methods. *Listeria* type strains were inoculated into one sample from each food category and studied with ELFA and PCR methods. The exclusivity study is designed to investigate non-target strains that are not expected to be detected by alternative methods but are likely to cause cross-reactions. For this study, *L. innocua* ATCC 51742, *B. cereus* ATCC 10876, and *E. coli* ATCC 8739 strains were inoculated into a sample from each food category and studied with alternative methods.

Sensitivity and relative trueness

According to the ISO 16140-2 [15], sensitivity (*SE*) is defined as the percentage of positive samples that gives the correct positive signal. Relative trueness (*RT*) is the degree of correspondence between responses obtained by the reference method and the alternative method on identical samples. For calculation of sensitivity of the alternative method and relative trueness, numbers of positive agreement (*PA*), negative agreement (*NA*), positive deviation (*PD*), and negative deviation (*ND*) were defined.

Positive agreement (*PA*) is the number of samples presenting the positive results for both reference and alternative method.

Negative agreement (*NA*) is the number of samples presenting the negative results for both reference and alternative method.

Positive deviation (*PD*) is the number of samples negative for the reference method and positive for the alternative one. When test result is proven negative, the positive deviation becomes false positive (*FP*). However, in cases where the result is confirmed as positive, the positive deviation disappears.

Negative deviation (*ND*) is the number of samples positive for the reference method and negative for the alternative one. When test result is confirmed as positive, the negative deviation becomes false negative.

N is the total number of samples.

$$N = NA + PA + PD + ND \quad (1)$$

Cohen's kappa (κ) statistical method was used to determine the degree of concordance of alternative methods with the reference method. According to method validation studies, the degree of agreement between the two methods should be high. For this, κ should be greater than 0.80 [16].

For sensitivity study, naturally and artificially contaminated samples were analysed simultaneously by the reference and alternative methods. All positive results obtained from alternative methods were confirmed after the isolation from Fraser Broth on ALOA and PALCAM agar as described in ISO 11290-1 [13, 14].

Sixty dairy products, 63 meat products, 62 seafoods, 60 fruit and vegetable products and 52 ready-to-eat food samples were analysed and used for statistical calculations. Two hundred and ninety-seven (297) samples were analysed including 147 positives and 150 negatives. Fourteen of the positive samples were naturally contaminated, while the rest were artificially contaminated. The categories and types of studied samples are summarized in Tab. 1.

Level of detection and relative level of detection

The level of detection (LOD_{50}) is defined as the lowest concentration of microorganism obtained by a particular measurement procedure, where the probability of detection is 50 %. However, when it comes to microbiological methods, determining certain inoculation levels is not possible for most of the time. Therefore, ISO 16140-2 [15] proposes a statistical approach called the relative level of detection (*RLOD*), which is

based on the ratio of LOD_{50} values of a reference method and an alternative method. In order to calculate *RLOD*, a comparative study was carried out evaluating the detection level of the alternative methods versus the reference method.

For the detection level study, one matrix from each tested food category was artificially contaminated in accordance with ISO 16140-2 requirements [15]. For this purpose, five food types, namely, cream cheese, Turkish sausage, raw frozen bar fish, onion powder and ready-cooked soup, at three inoculation levels were analysed by each method. All food types were screened for the absence of *L. monocytogenes* by the culture-based method. Cheese and onion powder were inoculated with *L. monocytogenes* ATCC 19114, Turkish sausage and ready-cooked soup were inoculated with *L. monocytogenes* ATCC 19116 and bar fish was inoculated with *L. monocytogenes* ATCC 19111.

For artificial contamination, cultures developed on TSA medium during 18–24 h were prepared for suspension and were adjusted to 0.5 McFarland. In order to mimic food processes, the suspensions were exposed to some stress conditions such as low temperature and low pH buffer for 6 h. After stress exposure, the suspensions were serially diluted to the desired level. For four food types, one master sample was left uninoculated, a second master sample was spiked at a low level (expected final concentration of 1–10 CFU·ml^{-1}) and a third master sample was spiked at a high level (expected final concentration of 10–100 CFU·ml^{-1}). For each level, 25 g food were weighed into a stomacher homogenization bag and spiked with 0.25 ml culture suspension. The actual LOD_{50} value was determined by using the plate count method after spiking the samples. All sets were then analysed according to method protocols.

RESULTS AND DISCUSSION

In this study, the culture-based method ISO 11290-1 [14], rapid ELFA (VIDAS System), and PCR (BAX System) methods were compared at food analysis for presence of *L. monocytogenes*. In this comparison, ISO 11290-1 method was the reference method. As a requirement of the ISO 17025 laboratory accreditation standard [17], ISO 16140-2 [15] validation procedure was applied to determine intra-laboratory suitability of the rapid methods. Inclusivity and exclusivity of the methods were analysed using 3 different positive (target) strains and 3 different negative (non-target) strains, respectively. All *L. monocytogenes* strains tested gave positive results while no cross-reaction occurred with non-target strains.

For sensitivity studies, various types of food samples from different categories were analysed in parallel. As a result of the parallel studies conducted, the sensitivity value of the alternative method and the reference method and the relative trueness value of the alternative method were calculated. The sampling was designed to be approximately 50 % negative and 50 % positive to allow statistical calculations. Some of the food samples were naturally contaminated with *L. monocytogenes*. However, since enough naturally contaminated samples were not available for validation studies, reference cultures of *L. monocytogenes* were spiked in negative samples.

A sensitivity study was carried out to determine the difference in sensitivity between the results of the reference method and the results of the alternative method (Tab. 2, Tab. 3). As a result, 2 positive deviations (for meat and dairy products), 4 negative deviations (for dairy products, seafood and vegetables), and 3 false positive results (for meat and dairy products) were determined for the ELFA method. Regarding the PCR method, 3 positive deviations (for meat products, dairy products

Tab. 2. Sensitivity study results for the ISO and ELFA methods for detection of *L. monocytogenes* in food samples.

Food Category	Number of samples				*FP*	*N*	SE_{alt} [%]	SE_{ref} [%]	*RT* [%]
	PA	*NA*	*PD*	*ND*					
Meat and meat products	31	29	1	0	2	63	100.0	96.9	95.2
Dairy products	29	27	1	2	1	60	93.8	96.9	93.3
Seafood	31	29	1	1	1	62	96.9	100.0	96.8
Fruit and vegetables	23	36	0	1	0	60	95.8	100.0	98.3
Ready-to-eat foods	22	30	0	0	0	52	100.0	100.0	100.0
All categories	136	151	3	4	4	297	97.2	97.9	96.6

PA – positive agreement, *NA* – negative agreement, *PD* – positive deviation, *ND* – negative deviation, *FP* – false positive ratio for the alternative method, *N* – total number of samples, SE_{alt} – sensitivity for the alternative method, SE_{ref} – sensitivity for the reference method, *RT* – relative trueness.

Tab. 3. Sensitivity study results for the ISO and PCR methods for detection of L. monocytogenes in food samples.

Food Category	Number of samples				*FP*	*N*	SE_{alt} [%]	SE_{ref} [%]	*RT* [%]
	PA	*NA*	*PD*	*ND*					
Meat and meat products	32	28	1	1	1	63	97.1	97.1	95.2
Dairy products	30	28	1	1	0	60	96.9	96.9	96.7
Seafood	31	29	1	0	1	62	100.0	96.9	96.8
Fruit and vegetables	21	36	0	1	2	60	95.5	100.0	95.0
Ready-to-eat foods	21	30	0	0	1	52	100.0	100.0	98.1
All categories	135	151	3	3	5	297	97.9	97.9	96.3

PA – positive agreement, *NA* – negative agreement, *PD* – positive deviation, *ND* – negative deviation, *FP* – false positive ratio for the alternative method, *N* – total number of samples, SE_{alt} – sensitivity for the alternative method, SE_{ref} – sensitivity for the reference method, *RT* – relative trueness.

and seafood), 3 negative deviations (meat, dairy products and vegetables) and 5 false positives (for meat products, seafood, vegetables and ready-to-eat foods) were found. Sensitivity rates for samples in all food categories were 97.2 % and 97.9 % for ELFA method and PCR method, respectively.

Positive and negative deviation results were used to determine that the alternative methods gave results compatible with the reference method. Using deviation data, the difference ($ND - PD$) and the sum ($ND + PD$) were calculated. Since the same amounts of samples (25 g of food samples) were analysed by the three methods compared and the pre-enrichment steps were the same, final evaluation was made considering paired studies in the ISO 16140-2 standard [15]. Acceptance criteria for paired studies and results calculated for each alternative method are shown in Tab. 4. It was determined that the calculated ($ND + PD$) and ($ND - PD$) values were below the acceptability limit for each category and categories combined. As a result of the sensitivity study carried out, Cohen's kappa index showed a perfect agreement for both alternative methods ($\kappa = 0.96$).

LOD_{50} of the ELFA method per 25 g of samples was determined to be 1.1 CFU for meat products, 4.3 CFU for dairy products, 2.3 CFU for seafood, 3.4 CFU for fruit and vegetable products and 2.9 CFU for ready-to-eat food. LOD_{50} value of the PCR method was found to be 2.7 CFU for meat products, 5.5 CFU for dairy products, 8.0 CFU for seafood, 4.4 CFU for fruit and vegetable products, and 3.1 CFU for ready-to-eat food. The *RLOD* values were calculated according to the ISO 16140-2 standard [15]. *RLOD* data are presented in Tab. 5 and Tab. 6.

Values of combined *RLOD* were determined to be 0.871 (z test of 0.714) and 1.120 (z test of 0.621) for the PCR and ELFA methods, respectively. Since acceptance criterion for the *RLOD* value is 1.5 according to the ISO 16140-2 standard [15], the *RLOD* values obtained were below the acceptable limit. As a result, alternative and reference methods for the detection of *L. monocytogenes* demonstrated similar LOD_{50} values in the food matrices tested.

For the detection of *L. monocytogenes* in food industry, commercially available chromogenic selective media and immunological and nucleic acid-based rapid methods are currently used. DuPont Lateral Flow System (Qualicon Diagnostics, Camarillo, California, USA), Listeria-Tek ELISA (Organon Teknika, Jersey City, New Jersey, USA), Singlepath L'MONO (Merck Millipore), Solus (Solus Scientific, Mansfield,

Tab. 4. Acceptability limit parameters for a paired study and the difference between negative deviations and positive deviations.

Food Category	Number of samples					
	$ND + PD$		Acceptance limit	$ND - PD$		Acceptance limit
	ELFA method	PCR method		ELFA method	PCR method	
Meat and meat products	1	2	≤ 6	–1	0	≤ 3
Dairy products	3	2	≤ 6	1	0	≤ 3
Seafood	2	1	≤ 6	1	–1	≤ 3
Fruit and vegetables	1	1	≤ 6	1	1	≤ 3
Ready-to-eat foods	0	0	≤ 6	0	0	≤ 3
All food categories	6	6	≤ 14	2	0	≤ 5

ND – negative deviation, *PD* – positive deviation.

Tab. 5. Relative level of detection values for ELFA method.

Name	*RLOD*	$RLOD_{low}$	$RLOD_{upp}$	*b*	SD_b	*z*-test	*p*
Meat and meat products	0.872	0.383	1.987	–0.137	0.412	0.332	1.260
Dairy products	1.147	0.503	2.613	0.137	0.412	0.332	0.740
Seafood	1.382	0.622	3.074	0.324	0.400	0.810	0.418
Fruit and vegetables	1.207	0.531	2.745	0.189	0.411	0.459	0.646
Ready-to-eat foods	1.000	0.389	2.572	0.000	0.472	0.000	1.000
Combined	1.120	0.777	1.617	0.114	0.183	0.621	0.535

RLOD – relative level of detection, $RLOD_{low}$ – the lower limit of the 95% confidence interval for *RLOD*, $RLOD_{upp}$ – the upper limit of the 95% confidence interval for *RLOD*, *b* – natural logarithm of the *RLOD*, SD_b – standard deviation of *b*, *z*-test – absolute value of the statistic of the *z*-test with the null hypothesis H_0 ($b = 0$), *p* – *p*-value of the *z*-test.

Tab. 6. Relative level of detection values for PCR method.

Name	*RLOD*	$RLOD_{low}$	$RLOD_{upp}$	*b*	SD_b	*z*-test	*p*
Meat and meat products	0.761	0.237	2.441	–0.273	0.583	0.469	1.361
Dairy products	0.719	0.289	1.788	–0.329	0.455	0.724	1.531
Seafood	1.067	0.484	2.352	0.064	0.395	0.163	0.871
Fruit and vegetables	0.751	0.331	1.702	–0.286	0.409	0.700	1.516
Ready-to-eat foods	1.000	0.376	2.662	0.000	0.490	0.000	1.000
Combined	0.871	0.592	1.282	–0.138	0.193	0.714	1.525

RLOD – relative level of detection, $RLOD_{low}$ – the lower limit of the 95% confidence interval for *RLOD*, $RLOD_{upp}$ – the upper limit of the 95% confidence interval for *RLOD*, *b* – natural logarithm of the *RLOD*, SD_b – standard deviation of *b*, *z*-test – absolute value of the statistic of the *z*-test with the null hypothesis H_0 ($b = 0$), *p* – *p*-value of the *z*-test.

United Kingdom), Transia Plate (BioControl Systems, Bellevue, Washington, USA) and VIDAS LMX (BioMérieux) are the most widely used immunological methodologies for *L. monocytogenes* detection in food and environmental samples. While the analysis time is reduced to 24–48 h in these immunology-based methods, it is observed that when 25 g of samples were to be analyzed the detection limit varies between 0.2 CFU to 9 CFU [18]. In our study, the *LOD* value obtained in different matrices with the ELFA method- which gave results in 48 h was obtained between 1.1 CFU and 4.3 CFU per 25 g of sample. Molecular methods, especially the PCR-based ones, are rapid methods that are frequently preferred in the field of food microbiology, especially in pathogen analysis. BACGene (Eurofins GeneScan, Baden-Wurttemberg, Germany), BAX System (DuPont Nutrition and Health, New York, New York, USA), Gene-Up (BioMérieux) and GeneQuence (Neogen, Lansing, Michigan, USA) are molecular-based kits commercially used in *Listeria* analysis. With these rapid test kits, *L. monocytogenes* is determined in a very short time (approximately 20–24 h), and the detection limit varies between 0.3 CFU and 10^4 CFU for 25 g portions [18]. With the PCR kit used in our study, which gave results in 24 h, the *LOD* was found to be 2.7–8.0 CFU per 25 g of sample.

CONCLUSION

Although culture-based methods are inexpensive and can be used for every food matrix, they left their place to faster methods due to being time-consuming and labour-intensive. Reaching the result quickly and accurately in food microbiology analyses is important not only for ensuring food safety but also for supplying perishable products to the market. For this reason, rapid microbiological methods are given priority in both analysis laboratories and quality control laboratories of food manufacturers. In this study, two rapid methods widely used in analysis laboratories were validated for the detection of *L. monocytogenes* in various food matrices. In the light of the results obtained from the validation parameters, the two alternative methods can be used instead of the classical cultural method. However, there may be some restrictions regarding them. For example, in some cases, dead cells might cause a positive result. In extremely dark pigmented foods such as cocoa, black tea or coffee, additional treatment may be required to facilitate correct fluorescence reading. The presence of PCR inhibitory substances in some food matrices, such as spices, may cause problems in the analysis. In this respect, it is crucial for food analysis laboratories to validate the rapid microbiological methods they use in

various food matrices and thus eliminate the problems that may arise with possible false results.

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