Detection of listeria in food and environmental samples

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An EN ISO 16140 validation was carried out for the use of Bio-Rad's RAPID'L.Mono as the basis of a chromogenic method for the simultaneous detection of Listeria monocytogenes and Listeria spp. in food and the environment.

There are six species of listeria and they are ubiquitous, regular, non-sporing, Gram positive bacteria of which only Listeria monocytogenes is a human pathogen. The 25% mortality rate associated with listeriosis makes it a serious public health problem.

Outbreaks have most often been related to dairy products, meat products, vegetables or fishery products and such a constant threat has prompted the search for rapid microbiological methods for food screening for contamination by Listeria monocytogenes.

The presence of all listeria species is also interesting since they can be indicator micro-organisms.

Controlling Listeria spp. in the environment and in food is the most effective means of reducing the likelihood of product contamination by the pathogenic Listeria monocytogenes.

For this purpose, the RAPID'L.mono method underwent an AFNOR validation procedure according to the EN ISO 16140(1) requirements for the simultaneous detection of both Listeria monocytogenes and Listeria spp. parameters. The other five species are Listeria innocua, L. ivanovii, L. seeligeri, L. welshimeri and L. grayi.

Couples (matrix, strains)	Reference method	RAPID'L.mono method
Rillettes/L. monocytogenes 1/2c	0.7	0.7
L. welshimeri 1/2c	0.7	0.7
Raw milk/ L. monocytogenes 1/2b	0.4	0.4
Smoked salmon/L. monocytogenes 1/2a	0.7	0.7
Red cabbage/L. monocytogenes 4b	0.6	0.9
Process water/L. monocytogenes 1/2c	0.3	0.3
L. innocua 1/2c	0.6	0.6

Table 1. Relative detection levels (CFU/25g) of the reference and the RAPID'L.mono methods according to the Spearman-Karber test.

Materials and methods

All experiments were performed by the Institut Pasteur de Lille according to technical specifications and the ISO validation standard regarding inclusivity, exclusivity, relative detection limit, relative accuracy, sensitivity and specificity and inter-laboratory studies.

The reference method used for the detection of both Listeria monocytogenes and the other listeria species was the EN ISO 11290-1/A1:2004 standard.

Briefly, the main steps of the alternative RAPID'L.mono method are as follows:

- Enrichment in a half Fraser broth for 24 hr ± 2 hr at 30°C ± 1°C.
- The streaking of 0.1ml of the enrichment broth on a single RAPID'L.mono plate.
- The RAPID'L.mono plate is incubated at 37°C ± 1°C for 24 hr ± 2 hr for the detection of Listeria monocytogenes.
- Incubated at 37°C ± 1°C for 48 hr ± 2 hr for the detection of other listeria species.

The principle of the RAPID'L.mono agar combines the chromogenic detection of the phosphatidylinositol-specific phospholipase C and xylose fermentation.

This medium provides differentiation between Listeria monocytogenes, which produces blue colonies, and Listeria ivanovii, whose colonies are blue surrounded by a yellow halo. The colonies of other listeria species appear white or yellow.

The ability of this method to detect a wide range of Listeria monocytogenes belonging to different serotypes and all other Listeria species was assessed using 50 and 30 strains, respectively. Exclusivity capability was investigated with 43 non-targeted strains corresponding to 23 different species.

The relative detection level was defined by analysing, using both the reference and the RAPID'L.mono methods, five matrix/strain couples at four inoculation rates (rate 1: 0 CFU/g or /ml; rate 2: rate necessary to obtain 0-50% positives; rate 3: rate necessary to obtain 50-75% positives; rate 4: rate

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Table 2. Definition of food matrices and distribution of results for the detection of Listeria monocytogenes in different food categories.

Categories		Positive	Negative	Total
Meat products	Raw and seasoned meats, ready to cook and cooked meats, prepared dishes	45	38	83
Dairy products	Raw milk, cow, goat or sheep milk cheeses, desserts, powdered milk	39	62	101
Seafood	Fish filets and shellfish, smoked fish, prepared fish dishes	54	48	102
Vegetables	Fresh or packaged vegetables, frozen vegetables, seasoned vegetables	40	37	77
Environmental samples	Various waters surface samples, residues	71	49	120
Total		**249**	**234**	**483**

Categories		Positive	Negative	Total
Meat products	Raw and seasoned meats, ready to cook and cooked meats, prepared dishes	42	38	80
Dairy products	Raw milk, cow, goat or sheep milk cheeses, desserts, powdered milk	31	62	93
Seafood	Fish filets and shellfish, smoked fish, prepared fish dishes	32	48	80
Vegetables	Fresh or packaged vegetables, frozen vegetables, seasoned vegetables	36	37	73
Environmental samples	Various waters surface samples, residues	45	49	94
Total		**186**	**234**	**420**

Table 3. Definition of food matrices and distribution of results for the detection of listeria other than monocytogenes in different food categories.

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necessary to obtain 100% positives) and six replicates/inoculation rate.

The relative accuracy, sensitivity and specificity were evaluated for the RAPID'L.mono method in comparison to the reference method by testing 483 and 420 food and environmental samples, respectively, for Listeria monocytogenes and other listeria species detection.

Finally, to conclude the AFNOR validation process, an inter-laboratory study involving 15 different European laboratories was managed by the Institut Pasteur de Lille.

Some 24 samples at three contamination levels (Level 0: 0cfu/25g or /25ml; Level 1: slightly above the relative detection level; Level 2: 10-fold the relative detection limit) were analysed by both the reference and alternative methods in each laboratory.

In pure culture testing, 50/50 strains of Listeria monocytogenes produced typical blue colonies within 22 hours. The three strains of Listeria ivanovii tested showed blue colonies with a yellow halo.

All other listeria strains produced white or yellow colonies (13 L. innocua; 4 L. seeligeri; 6 L. welshimeri and 4 L. grayi).

Non-listeria strains were either inhibited or did not produce typical colonies.

The relative detection levels for both Listeria monocytogenes and other Listeria species are between 0.2 and 1.2 cells per 25g for the reference method and between 0.2 and 1.6 cells per 25g for the RAPID'L.mono method (Table 1).

Some 483 samples were tested for Listeria monocytogenes detection and 249 positive results were found with at least one method (Table 2).

Among these positive results, 19% were obtained with artificially contaminated samples using stressed strains according to the EN ISO 16140 standard and the AFNOR validation agency requirements.

The EN ISO 11290-1/A1 reference method found 244 positive results while the RAPID'L.mono method gave 243 positive results.

Some 420 samples were tested for the detection of listeria other than monocytogenes, and 186 positive results were found with at least one method (Table 3). 47% of positive results were obtained with artificially contaminated samples. 181 positive results were found with the reference method, while 179 positive results were found with the RAPID'L.mono method.

The relative accuracy, relative specificity and relative sensitivity of the RAPID'L.mono method were evaluated after an incubation time of 22 hours for Listeria monocytogenes detection and 48 hours for other listeria species detection (Table 4).

The sensitivities of both methods, calculated by taking into account all of the positive results obtained, were re-evaluated using the formula (PA + PD)/(PA + PD + ND) where PA, PD and ND are respectively Positive Agreement, Positive Deviation and Negative Deviation (Table 5).

The RAPID'L.mono method provided similar results to the reference method for both Listeria monocytogenes and other listeria species detection. Pasteurised milk contaminated with a wild strain of L. monocytogenes was used to carry out the inter-laboratory study. L1 and L2 contamination levels were 2.3 CFU/25ml and 26.3 CFU/25ml, respectively.

All the laboratories obtained comparable results with the reference and RAPID'L.mono methods. The variability of the alternative method (accordance and concordance) is identical to that of the reference method (Table 6).

Conclusion

The RAPID'L.mono validation study for the detection of L. monocytogenes and other listeria species was conducted according to the EN ISO 16140 standard.

The inclusivity/exclusivity study demonstrated the good specificity of the alternative method for both parameters.

Regarding the relative detection limit and relative accuracy, specificity and sensitivity, the performances of the RAPID'L.mono method were found to be comparable to those of the EN ISO 11290-1/A1 reference method. Lastly, the inter-laboratory study showed the good precision of the alternative method.

In conclusion, the RAPID'L.mono method has been demonstrated to be an efficient, convenient and cost saving rapid method for the detection of L. monocytogenes and all listeria species in food and environmental samples. ■

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Level	Accordance		Concordance	
	Reference method (%)	RAPID'L.mono method (%)	Reference method (%)	RAPID'L.mono method (%)
Level 0	100	100	100	100
Level 1	87	87	85	85
Level 2	100	100	100	100

Table 6. Accordance and concordance of the reference and RAPID'L.mono methods.

Table 4. Relative sensitivity, relative specificity and relative accuracy of RAPID'L.mono compared to EN ISO 11290-1/A1.

	Relative accuracy	Relative specificity	Relative sensitivity
Response to L. monocytogenes (22 hrs) (%)	98.1	98.3	98.0
Response to listeria other than monocytogenes (48 hrs) (%)	97.1	97.9	96.1

Table 5. Sensitivities of the reference and the RAPID'L.mono methods.

	Relative method	RAPID'L method
Response to L. monocytogenes (22 hrs) (%)	98.4	98.0
Response to listeria other than monocytogenes (48 hrs) (%)	97.3	96.2