

NordVal International Certificate

Issued for:	Microbiologique Listeria Test Kit
NordVal No:	064
First approval date:	21 October 2025
Valid until:	11 November 2027

Manufactured and supplied by:

Microbiologique, inc.
8315 Lake City Way NE
Seattle, WA 98115

NordVal International has reviewed the method validation documentation. The validation was conducted by Molecular Epidemiology Inc., DBA IEH Laboratories & Consulting Group according to ISO 16140-2. The reference method was ISO 11290-1 (May 2017): Microbiology of the food chain-Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. – Part 1: detection method.

NordVal International concludes that it has been satisfactorily demonstrated that the data and interpretations comply with the EN ISO 16140-2:2016 requirements and demonstrate comparable performance of the alternative method Listeria IEH Test Kit (Microbiologique, WA USA), to the ISO reference method for the detection of *Listeria* spp. and *Listeria monocytogenes* in raw milk and dairy products, raw and ready to eat meat products, eggs and egg products, fish and seafood, multicomponent foods, and environmental samples.

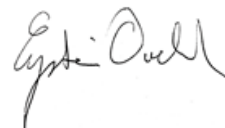
The production of the kits fulfills the requirements outlined in ISO 9001.

Date: 11. November 2025

Yours sincerely,

Hrólfur Sigurðsson

Hrólfur Sigurðsson
Chair of NordVal International



Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

Detection of *Listeria* spp. and *Listeria monocytogenes* is performed with the Real-time PCR **IEH Listeria RT-PCR kit** (Product Code: PM-28). The kit contains Resuspension buffer for cell lysis, Real-time PCR buffer, and pre-dispensed lyophilized Taq bead. The Listeria RT-PCR buffer (Microbiologique, Product Code: B-1237) employs three (3) fluorophores: ROX for *Listeria monocytogenes*, HEX for *Listeria* spp., and Cy5 for the internal amplification control (IAC). This multiplex fluorescence detection system enables screening and confirmation of *Listeria* spp. and *Listeria monocytogenes* in a single reaction.

Table 1. Fluorophores and their corresponding targets of IEH LM-PCR kit.

FLUOROPHORE	TARGET			
		<i>L. monocytogenes</i>	<i>Listeria</i> spp. other than <i>L. monocytogenes</i>	Non- <i>Listeria</i> strains
ROX	<i>L. monocytogenes</i>	Positive	Negative	Negative
HEX	<i>Listeria</i> spp.	Positive	Positive	Negative
CY5	Internal Amplification Control	Positive	Positive	Positive

• Enrichment protocols

- For food: 25 g food +225 mL M1GP the IEH proprietary enrichment medium (1:10 dilution) followed by incubation for 21 ± 1 h at 36 ± 1°C.
- For process water: 100 mL water + 100 mL double concentrated M1GP medium (M1GP 2×) followed by incubation for 21 ± 1 h at 36 ± 1°C.
- For Sponge and/or swab: 1 sponge or 1 swab + 90 mL M1GP. Incubation for 21 ± 1 h at 36 ± 1°C.

• Lysis step

- Dispense 5 µL of Resuspension buffer to a micro-centrifuge tube or 8 strip tubes.
- Transfer 45 µL of enrichment to the tubes and mix well by pipetting.
- Incubate the tubes at 35 ± 2°C for 20 min.
- Transfer 2 µL of resuspended sample to the Listeria RT-PCR buffer tubes.
- After all samples have been transferred, place the lysis buffer tubes in the thermal cycler and run the INC37 program (37°C for 10 min and 99°C for 5 min).
- Amplification and real-time PCR.
- Mix the sample in the lysis buffer by pipetting up and down several times, then transfer 25 µL from the PCR lysis tube to the corresponding Taq bead tube.
- Place the Taq bead tubes in the real-time machine and initiate the PCR program (95°C for 10m, 45 cycles of 95°C for 20s, 60°C for 30s, and 72°C for 30s).

FIELD OF APPLICATION

The Listeria IEH RT-PCR kit Microbiologique (Product Code: PM-28) is designed for horizontal testing of food matrices and environmental samples. Accordingly, the comparison studies will be performed with 6 test categories summarized in **Table 2**.

METHOD COMPARISON STUDY

Selectivity study

One hundred *Listeria* strains were tested by IEH Listeria RT-PCR for inclusivity study. All strains for the inclusivity test were obtained either from ATCC (American Type Culture Collection) or from a private collection maintained by Molecular Epidemiology, Inc. (MEI, <https://molecularepi.com/>). Fifty strains of *L. monocytogenes* were used for the inclusivity test. Additionally, fifty *Listeria* species strains, which included 18 strains of *L. innocua*, 15 strains of *L. welshimeri*, 10 strains of *L. seeligeri*, 2 strains of *L. grayi*, and 5 strains of *L. ivanovii*, were tested as well. All the inclusivity strains tested were detected by the alternative method accordingly.

For exclusivity there were tested 30 unrelated strains representing 17 genera of bacteria commonly associated with foods and environmental samples. All the exclusivity strains were showing negative results with the alternative method.

Table 2. *Listeria* strains used for artificial inoculation

Category	Type	<i>Listeria</i> spp.	<i>Listeria monocytogenes</i>
1. Raw milk and dairy products	A	<i>L. ivanovii</i> ATCC 700402	<i>L. monocytogenes</i> ATCC 51773
	B	<i>L. innocua</i> ATCC 33090	<i>L. monocytogenes</i> ATCC 35152
	C	<i>L. ivanovii</i> ATCC 19119	<i>L. monocytogenes</i> ATCC 19116
2. Raw and ready-to-eat meat products	A	<i>L. ivanovii</i> ATCC 49953	<i>L. monocytogenes</i> ATCC 7644
	B	<i>L. grayi</i> ATCC 25402	<i>L. monocytogenes</i> ATCC 15313
	C	<i>L. ivanovii</i> ATCC 700402	<i>L. monocytogenes</i> ATCC 19114
3. Eggs and egg products	A	<i>L. seeligeri</i> ATCC 35967	<i>L. monocytogenes</i> ATCC 19113
	B	<i>L. ivanovii</i> ATCC BAA-139	<i>L. monocytogenes</i> ATCC 19115
	C	<i>L. innocua</i> ATCC 33090	<i>L. monocytogenes</i> ATCC 19116
4. Fish & seafood	A	<i>L. innocua</i> ATCC 51742	<i>L. monocytogenes</i> ATCC 19115
	B	<i>L. ivanovii</i> ATCC 49953	<i>L. monocytogenes</i> ATCC 35152
	C	<i>L. grayi</i> ATCC 19120	<i>L. monocytogenes</i> ATCC 7644
5. Multicomponent food	A	<i>L. innocua</i> ATCC 33091	<i>L. monocytogenes</i> ATCC 15313
	B	<i>L. ivanovii</i> ATCC 700402	<i>L. monocytogenes</i> ATCC 19114
	C	<i>L. grayi</i> ATCC 700545	<i>L. monocytogenes</i> ATCC 51773
6. Environmental samples	A	<i>L. ivanovii</i> ATCC 700402	<i>L. monocytogenes</i> ATCC 35152
	B	<i>L. ivanovii</i> ATCC 49954	<i>L. monocytogenes</i> ATCC 19117
	C	<i>L. welshimeri</i> ATCC 43550	<i>L. monocytogenes</i> ATCC 19115

Sensitivity study

The sensitivity parameters calculated for each category and type are shown in **Table 3**.

Table 3. Calculation of the relative accuracy (RT), the relative sensitivity (SE), and the false-positive ratio (FPR) per category.

Category	PA	NA	PD	ND	PPND	PPNA	N	SE _{alt} %	SE _{ref} %	RT %	FPR %
1. Raw milk and dairy products	38	22	0	0	0	0	60	100.0	100.0	100.0	0.0
2. Raw and ready-to-eat meat products	38	24	0	0	0	0	62	100.0	100.0	100.0	0.0
3. Eggs and egg products	37	23	0	0	0	0	60	100.0	100.0	100.0	0.0
4. Fish & seafood	37	21	0	1	0	1	60	97.4	100.0	96.7	4.8
5. Multicomponent food	42	18	0	0	0	0	60	100.0	100.0	100.0	0.0
6. Environmental samples	36	23	0	0	0	1	60	100.0	100.0	98.3	3.3
Total	228	131	0	1	0	2	362	99.6	100.0	99.2	1.5

Acceptability Limit of the alternative method

The acceptability limit (AL) was based on the difference of deviations. For the five food matrices, the AL is 5.0 for unpaired studies, which has to be met by the alternative method in **Table 4**. The results for process water samples were calculated separately, for which a threshold of 3.0 applies to each preparation method.

One negative deviation and one positive deviation were observed in Type C of Category 4. The presence of *L. monocytogenes* was confirmed by the reference method (ISO 11290-1). The absence of *Listeria* spp. was confirmed as well.

The observed values for ((ND+PPND)-PD) meet the acceptability limit (AL) for each individual category and for all the combined categories (observed values are less than AL) in **Table 4**.

Table 4. Acceptability limit of the sensitivity for paired and unpaired study.

Category		Type	(ND+PPND)-PD	Acceptability limit (AL)
1	Raw milk and dairy products	a	Raw milk	0
		b	Soft cheese (e.g. Brie Munster)	0
		c	Butter	0
		Total		0
2	Raw and ready-to-eat meat products	a	Fresh meats (e.g. meat cuts, Carpaccio)	0
		b	Minced meat, meat preparations	0
		c	Fermented or dried meat	0
		Total		0
3	Eggs and egg products	a	Eggs (unprocessed, raw)	0

Category		Type	(ND+PPND)-PD	Acceptability limit (AL)
		b	Egg products (e.g. heat processed yolk, egg white whole egg liquids)	0
		c	Dry (egg powder)	0
		Total		0
4	Fish & seafood	a	Raw fish (unprocessed)	0
		b	Cooked fish products	0
		c	Smoked or cured fish	1
		Total		1
5	Multicomponent food	a	Hot meals	0
		b	Cooked chilled foods, boiled rice or pasta	0
		c	Bagged raw vegetable salads with dressing	0
		Total		0
6	Environmental samples	a	Process water	0
		b	Dust	0
		c	Wipes	0
		Total		0
All categories			1	6.0

The discordant result after enrichment broth and lysate is presented in **Table 5**.

Table 5. Discordant result after enrichment broth and lysate.

Category	PD	ND+PPND	(ND+PPND)-PD	AL
1 Raw milk and dairy products	0	0	0	5.0
2 Raw and ready-to-eat meat products	0	0	0	
3 Eggs and egg products	0	0	0	
4 Fish & seafood	0	1	1	
5 Multicomponent food	0	0	0	
6 Environmental samples	0	0	0	3.0
Total	0	1	1	6.0

Calculation of the Relative Level of Detection

The LOD is defined as the LOD₅₀, for which 50% of the tests give a positive result. Based on the data of the alternative method LOD₅₀ detection limit and lower and upper confidence limit will be calculated using the excel spreadsheet: PODLOD_ver10a.xls The results are summarized in **Table 6**.

Table 6. Level of detection at 50% (CFU / sample portion) for Category 1 to 5 according to AOAC, Wilrich and Wilrich (2009). The environmental sample (category 6) has been calculated separately since the sample volume is different from other categories.

Category #	Artificial contamination	Reference method	Alternative method
1. Raw milk and dairy products	<i>L. monocytogenes</i> ATCC 51773	0.696 [0.402 / 1.205]	0.779 [0.443 / 1.369]
	<i>Listeria innocua</i> ATCC 33090	0.735 [0.418 / 1.293]	0.657 [0.379 / 1.138]
2. Raw and ready-to-eat meat products	<i>L. monocytogenes</i> ATCC 7644	0.623 [0.365 / 1.065]	0.623 [0.365 / 1.065]
	<i>L. ivanovii</i> ATCC 49953	0.623 [0.365 / 1.065]	0.623 [0.365 / 1.065]
3. Eggs and egg products	<i>L. monocytogenes</i> ATCC 19113	0.779 [0.443 / 1.369]	0.727 [0.414 / 1.278]
	<i>Listeria seeligeri</i> ATCC 35967	0.751 [0.426 / 1.324]	0.751 [0.426 / 1.324]
4. Fish & seafood	<i>L. monocytogenes</i> ATCC 19115	0.689 [0.398 / 1.191]	0.588 [0.343 / 1.006]
	<i>Listeria innocua</i> ATCC 51742	0.623 [0.365 / 1.065]	0.623 [0.365 / 1.065]
5. Multicomponent food	<i>L. monocytogenes</i> ATCC 7644	0.696 [0.402 / 1.205]	0.696 [0.402 / 1.205]
	<i>Listeria ivanovii</i> ATCC 700402	1.045 [0.557 / 1.961]	1.045 [0.557 / 1.961]
6. Environmental samples	<i>L. monocytogenes</i> ATCC 35152	0.779 [0.443 / 1.369]	0.623 [0.365 / 1.065]
	<i>Listeria welshimeri</i> ATCC 43550	0.735 [0.418 / 1.293]	0.657 [0.379 / 1.138]
Combined (Category 1 to 5)	<i>L. monocytogenes</i>	0.695 [0.543 / 0.888]	0.679 [0.531 / 0.868]
	<i>Listeria</i> spp. other than <i>L. monocytogenes</i>	0.736 [0.572 / 0.946]	0.720 [0.561 / 0.924]

Level of detection at 50% (CFU / sample portion) for Category 1 to 5 according to AOAC, Wilrich and Wilrich (2009). The environmental sample (category 6) has been calculated separately since the sample volume is different from other categories.

The LOD 50% varies from 0.62 to 1.04 CFU/sample for the reference method and from 0.59 to 1.04 CFU/sample for the alternative method.

The relative level of detection (RLOD) is defined as follows:

$$\text{RLOD} = \frac{\text{LOD}_{\text{alt}}}{\text{LOD}_{\text{ref}}}$$

For calculation according to annex D, ISO16140-1 (2016) the excel method from file: RLOD_MCS_clause_5-1-4-2_V3_2015-08-15.xlsm was used. The results are summarized in **Table 7**, respectively.

Table 7. Summary of RLOD calculations per category 1 to 5. The environmental sample (category 6) was calculated separately since the sample volume differed from other categories.

Category	Strain	RLOD	RLODL	RLODU	b= ln(RLOD)	sd(b)	z-Test statistic	p- value
1. Raw milk and dairy products	<i>L. monocytogenes</i> ATCC 51773	1.119	0.509	2.464	0.113	0.394	0.286	0.775
	<i>Listeria innocua</i> ATCC 33090	0.893	0.406	1.966	-0.113	0.394	0.286	1.225
2. Raw and ready-to-eat meat products	<i>L. monocytogenes</i> ATCC 7644	1.000	0.471	2.123	0.000	0.376	0.000	1.000
	<i>L. ivanovii</i> ATCC 49953	1.000	0.471	2.123	0.000	0.376	0.000	1.000
3. Eggs and egg products	<i>L. monocytogenes</i> ATCC 19113	1.000	0.455	2.196	0.000	0.393	0.000	1.000
	<i>Listeria seeligeri</i> ATCC 35967	1.000	0.453	2.209	0.000	0.396	0.000	1.000
4. Fish & seafood	<i>L. monocytogenes</i> ATCC 19115	0.895	0.416	1.929	-0.110	0.384	0.288	1.227
	<i>Listeria innocua</i> ATCC 51742	1.000	0.464	2.154	0.000	0.384	0.000	1.000
5. Multi-component food	<i>L. monocytogenes</i> ATCC 7644	1.000	0.463	2.160	0.000	0.385	0.000	1.000
	<i>Listeria ivanovii</i> ATCC 700402	1.000	0.431	2.318	0.000	0.420	0.000	1.000
6. Environmental samples	<i>L. monocytogenes</i> ATCC 35152	0.800	0.367	1.743	-0.223	0.389	0.573	1.433
	<i>Listeria welshimeri</i> ATCC 43550	0.893	0.406	1.965	-0.113	0.394	0.286	1.225
Combined (1 to 5)	<i>L. monocytogenes</i>	0.999	0.706	1.414	-0.001	0.174	0.006	1.005
	<i>Listeria</i> spp.	0.978	0.685	1.396	-0.023	0.178	0.127	1.101

The RLOD met the AL fixed at 2.5 for unpaired studies for all the tested matrix and strain pairs.

INTERLABORATORY STUDY

The study was carried out in pasteurized milk with 10 collaborators and 5 participating laboratories. The samples were artificially contaminated with *Listeria innocua* and *Listeria monocytogenes* at 3 inoculation levels.

Contamination levels:

Level 0: Uninoculated

Level 1: Low (0.23 – 0.26 CFU / 25 mL)

Level 2: High (25 CFU / 25 mL)

Each collaborator received 30 samples and analysed them with the alternative and the reference method. The samples were shipped at 4°C to the participants, who reported the temperature upon arrival and processed the samples immediately.

The results from the laboratories that participated in the ILS are shown for the reference method and alternative method in **Table 8** to **Table 11**, respectively.

Table 8. Number of positive results at different levels – *L. monocytogenes* with reference method.

REFERENCE METHOD						
	L0		L1		L2	
Collaborators	N	Pos	N	Pos	N	Pos
1	8	0	8	3	8	8
2	8	0	8	1	8	8
3	8	0	8	2	8	8
4	8	0	8	1	8	8
5	8	0	8	2	8	8
6	8	0	8	1	8	8
7	8	0	8	3	8	8
8	8	0	8	3	8	8
9	8	0	8	2	8	8
10	8	0	8	1	8	8
Total	80	0	80	19	80	80

Table 9. Number of positive results before and after confirmation at different contamination levels – *L. monocytogenes* with alternative method.

ALTERNATIVE METHOD	LO			L1			L2		
	N	before conf.	after conf.	N	before conf.	after conf.	N	before conf.	after conf.
1	8	0	0	8	2	2	8	8	8
2	8	0	0	8	1	1	8	8	8
3	8	0	0	8	1	1	8	8	8
4	8	0	0	8	1	1	8	8	8
5	8	0	0	8	0	0	8	8	8
6	8	0	0	8	3	3	8	8	8
7	8	0	0	8	0	0	8	8	8
8	8	0	0	8	3	3	8	8	8
9	8	0	0	8	2	2	8	8	8
10	8	0	0	8	1	1	8	8	8
Total	80	0	0	80	14	14	80	80	80

Table 10. Number of positive results at different levels – *Listeria* spp. With reference method.

REFERENCE METHOD	L0		L1		L2	
	N	Pos	N	Pos	N	Pos
Collaborators						
1	8	0	8	0	8	8
2	8	0	8	5	8	8
3	8	0	8	6	8	8
4	8	0	8	4	8	8
5	8	0	8	5	8	8
6	8	0	8	3	8	8
7	8	0	8	3	8	8
8	8	0	8	4	8	8
9	8	0	8	4	8	8
10	8	0	8	3	8	8
Total	80	0	80	37	80	80

Table 11. Number of positive results before and after confirmation at different contamination levels – *Listeria* spp. With alternative method.

ALTERNATIVE METHOD	Number of positive results before and after confirmation at different contamination levels – <i>L. spp.</i>								
	LO			L1			L2		
Collaborators	N	before conf.	after conf.	N	before conf.	after conf.	N	before conf.	after conf.
1	8	1	0	8	2	2	8	8	8
2	8	2	0	8	5	5	8	8	8
3	8	1	0	8	4	4	8	8	8
4	8	1	0	8	4	4	8	8	8
5	8	1	0	8	2	2	8	8	8
6	8	0	0	8	5	5	8	8	8
7	8	1	0	8	5	5	8	8	8
8	8	0	0	8	4	4	8	8	8
9	8	1	0	8	5	5	8	8	8
10	8	0	0	8	3	3	8	8	8
Total	80	8	0	80	39	39	80	80	80

SPECIFICITY

Listeria monocytogenes

Specificity for the reference method (SP_{ref}) = 100%

Specificity for the alternative method (SP_{alt}) = 100%

Listeria spp.

Specificity for the reference method (SP_{ref}) = 100%

Specificity for the alternative method (SP_{alt}) = 100%

SENSITIVITY

Listeria monocytogenes (L1)

Alternative method	Reference method		Total
	R+	R-	
A+	14	0	14
A-	5	61	66
Total	19	61	80

Sensitivity for the alternative method (SE_{alt}) = 73.7%

Sensitivity for the reference method (SP_{ref}) = 100%

Relative trueness = 93.8%

False positive ratio for alternative method = 0%

Listeria spp.

Alternative method	Reference method		Total
	R+	R-	
A+	37	2	39
A-	0	41	41
Total	37	43	80

Sensitivity for the alternative method (SE_{alt}) = 100%

Sensitivity for the reference method (SP_{ref}) = 94.9%

Relative trueness = 97.5%

False positive ratio for alternative method = 0%

CONCLUSION

The completed studies demonstrate that the alternative method fulfills the requirements of the NordVal International Protocol No. 1 / ISO 16140-2 and provides equivalent results as the reference method.