NMKL - NordVal International
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NordVal International Certificate

Issued for:	**RAPID'*L.mono***
NordVal No:	022
First approval date:	20 November 2005
Renewal date:	02 May 2024
Valid until:	02 May 2026

RAPID'*L.mono*

Manufactured and supplied by:

Bio-Rad Laboratories,
Bio-Rad Laboratories,
3 Blvd Raymond Poincare,
92430 Marnes-la-Coquette, France

fulfils the requirements of the NordVal validation protocol 1. The reference method is EN ISO 11290-2:2017: Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp - Part 1: Detection method and Part 2: Enumeration method.

NordVal International has reviewed the method and the validation studies conducted by the expert laboratory ISHA, France, in accordance with EN ISO 16140-2:2016. NordVal has concluded that it has been satisfactorily demonstrated that the requirements of the NordVal validation protocol are fulfilled for RAPID'*L.mono,* there are no statistical differences in the performances of RAPID*'L.mono* and the reference method for the detection and the enumeration of *Listeria monocytogenes* and the detection of *Listeria spp.* in foods and environmental samples.

The production of the RAPID'*L.mono* is fulfilling the requirements given in ISO 9001.

Date: 02 May 2024

Yours sincerely,

Hrólfur Sigurðsson

Hrólfur Sigurðsson
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

The principle of the RAPID'*L.mono* medium relies on the chromogenic detection of the *Listeria monocytogenes* phosphatidylinositol-specific phospholipase C and on the inability of these species to metabolise xylose. After 24 ± 2 hours of incubation, *Listeria monocytogoenes* forms characteristics blue (pale blue, grey blue to dark blue) colonies without a yellow halo. Colonies formed by other species of *Listeria* are white, with or without a yellow halo. The particularity of *Listeria ivanovii* species, infrequently found in food matrices, should be noted: it presents blue-green colonies with a yellow halo (xylose positive character). This halo can appear after 24 to 48 hours of incubation. The selective mixture in the medium allows the inhibition of most interfering flora (Gram- positive and Gram-negative bacteria, yeast and mould). Thus RAPID'*L.mono* detects *Listeria monocytogenes* in 24 hours and other *Listeria* species in 24 and 48 hours.

FIELD OF APPLICATION

The method is applicable for the detection and enumeration of *Listeria monocytogenes* and for the detection of other *Listeria* species in a broad range of food and environmental samples.

HISTORY

Original studies for this certificate were conducted in 2001 (inclusivity/exclusivity) and in 2005 (relative trueness and Inter Laboratory Study (ILS)) by Institut Pasteur de Lille, France, according to the NordVal Protocol and validated according to ISO 16140. Selectivity results are from this study. A renewal study was carried out in 2017/2018 according to ISO 16140-2:2016 to test for relative trueness and accuracy profile for enumeration of *Listeria monocytogenes*.

METHOD COMPARISON STUDY

Relative trueness study

The relative trueness is illustrated by the use of a Bland-Altman plot, i.e. the difference (bias) between paired samples analysed with the reference method and the alternative method respectively, plotted against the mean values obtained by the reference method. In the plot, Upper and Lower limits are included as the bias ± 2 times the standard deviation of the bias. The Bland-Altman Plot in Figure 1 illustrates the difference obtained in the enumeration of total *Listeria monocytogenes* in foods by the alternative and the reference method, respectively. A total of 105 samples of meat products, dairy products, seafood products, vegetal products, composite foods (ready to eat, ready to reheat) and environmental samples were analysed.

Conclusions of the relative trueness study

The results of the Bland-Altman Plot (Figure 1) provide a visual observation on the amount of bias and extreme results. It is expected that no more than one in 20 (5%) data values will lie outside the upper and lower limits. In this study 9 out of 105 results (8.6%) were outside the limits. However, the relative trueness is considered as satisfactory.

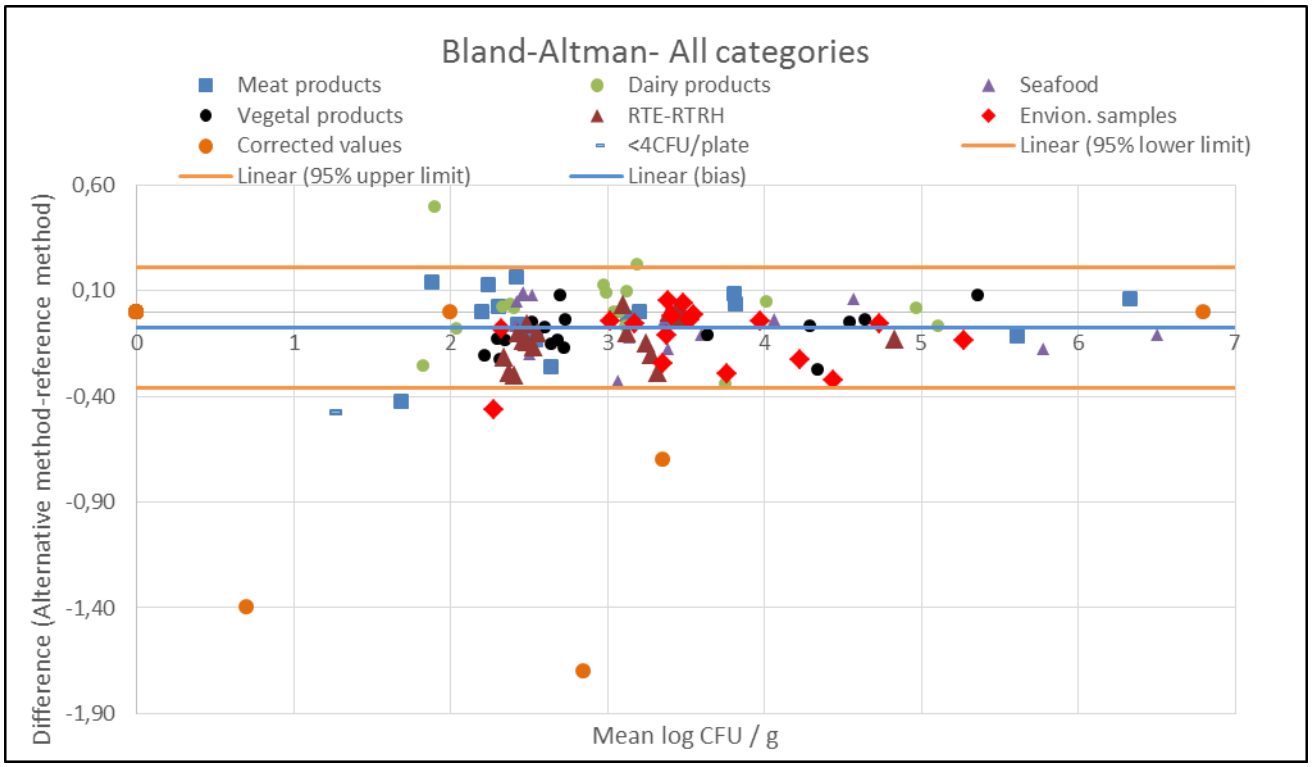

Figure 1. Bland-Altman difference plot for all categories: Comparison of reference method versus Alternative method

Accuracy profile study

The accuracy profile study is a comparative study between the results obtained by the reference and the results of the alternative method. This study is conducted using artificially contaminated samples. One type per category is tested for this.

Food matrices

For each of the 5 food categories and environmental samples, one type of food/sample was selected and two batches of each type were inoculated. Of the 6 samples, there were 2 at a low level (2.0 cfu/g), 2 at a medium level (2.5 cfu/g) and 2 at a high level (3.5 cfu/g) of contamination. For each of the 6 samples per category and two batches of each, 5 replicate test portions were tested. Non–inoculated samples (5) of each product type were also tested. Each sample was bulk inoculated and separate replicate test portions examined.

The observed profiles are within the Acceptance Limit, AL of ± 0.5 log cfu/g.

All the accuracy profiles, shown in figure 2, fulfil the performance criteria and the alternative method is accepted as being equivalent to the reference method.

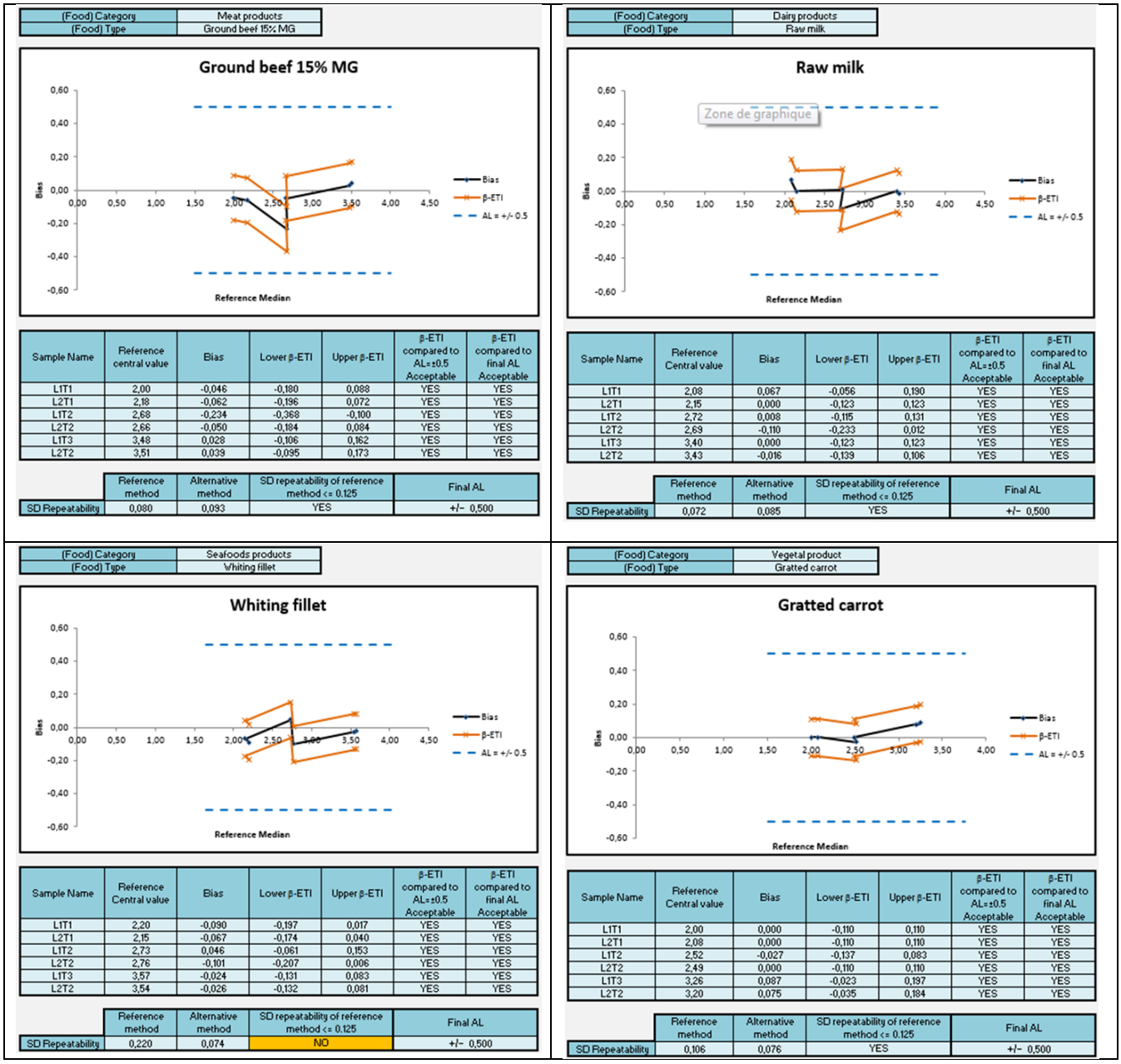

Figure 2. Accuracy profiles (β = 80%, AL = 0.5)

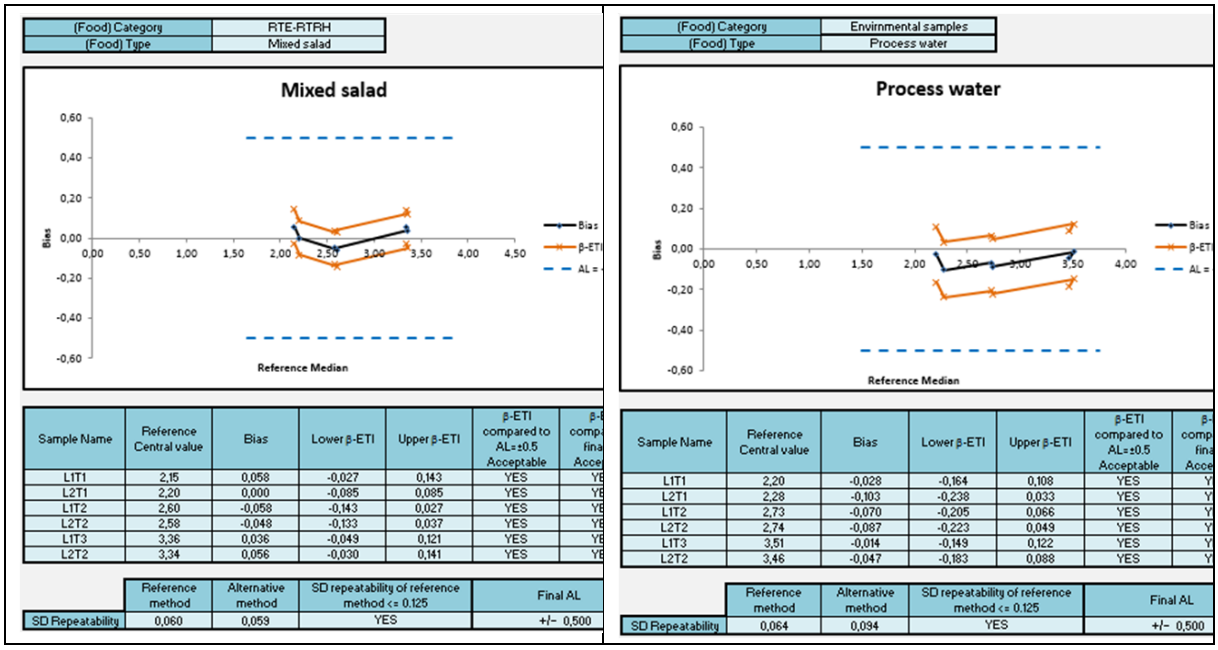

Figure 2 continued. Accuracy profiles (β = 80%, AL = 0.5)

***Detection of Listeria* other than *monocytogenes* after 48 hours of incubation**

In 2006, 420 product samples were analysed: 99 were naturally contaminated, 87 artificially contaminated and 234 non-contaminated. All the samples were analysed once by the two methods. The following results were obtained:

Matrices	*PA	NA	ND	PD	Sum	**Relative trueness RT (%)**	**Sensitivity alternative method SE_{alt} (%)**	**Sensitivity reference method SE_{ref} (%)**	**FPR (%)**
Meat products	41	38	1	0	80	98.8	97.6	100	0.0
Fish products	28	62	1	2	93	96.8	96.8	93.5	3.2
Dairy products	29	48	1	2	80	96.3	96.9	93.8	4.2
Vegetable products	33	37	3	0	73	95.9	91.7	100	0.0
Environment samples	43	49	1	1	94	97.9	97.8	97.8	2.0
Total	174	234	7	5	420	97.1	96.2	97.3	2.1

* PA = number of obtained results that are positive with both the alternative and the reference method

NA = number of obtained results that are negative with both the alternative and the reference method.

ND = number of obtained results that are negative with the alternative method and positive with the reference method (possible false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method (possible false positive)

FPR, false positive ratio;

For the response to *Listeria* other than *monocytogenes*, the results demonstrate that the agar plates must be incubated for 24 hours, and for 24 additional hours in the case of absence of colonies or of weak growth. After 48 hours of incubation, the relative accuracy obtained is 97.1%, the relative sensitivity is 96.1% and the relative specificity is 97.9%. Twelve discordant results were obtained: 5 supplemental positive results and 7 false negative results.

The positive samples by the alternative method being confirmed positive samples, the

sensitivities and specificities were recalculated with respect to all of the positive results and are:

- 96.2% sensitivity for the alternative method, - 97.3% sensitivity for the reference method.

The sensitivity is satisfactory (≥ 95%) for all matrices.

Selectivity; Inclusivity and exclusivity

Listeria monocytogenes
Inclusivity: 183 strains of *Listeria monocytogenes* were detected out of the 184 tested.
Exclusivity: 51 strains of *Listeria* spp. other than *L. monocytogenes* and 43 strains of other genera were tested. There was no typical reaction of *Listeria monocytogenes* for these strains.

Listeria* other than *monocytogenes

Inclusivity: 50 strains of *Listeria monocytogenes* were detected out of the 50 tested.

30 strains of *Listeria* other than *monocytogenes* were detected out of the 30 tested

Exclusivity: The study conducted in 1998 and 1999 of 47 strains not belonging to the geneus *Listeria monocotygtes* did not detect presence of any cross-reaction, even with strains listed in the bibliography as having PLPLC activity: *Bacillus cereus, Clostridium perfringens* and *Staphylococcus aureus.*

For the accuracy study performed in 2006, some strains were identified because they produced colonies that looked similar to *Listeria* other than *monocytogenes*; these were *Bacillus, Enterococcus faecium*, *Oekskovia xanthineolytica, Gardnerella vaginalis* and *Lactobacillus*. However, these few strains have a different appearance from *Listeria* in the GRAM test.

INTERLABORATORY STUDY

Eleven laboratories participated in the interlaboratory study from 2005. A strain of *Listeria monocytogenes* 1/2b (L37) isolated from a diary product was incubates. The matrix was pasteurized milk. Eight samples were prepared, two with each at four levels: zero, lower (100 CFU/ml), middle (1000 CFU/ml) and higher (10000 CFU/ml). The results are given in the table below.

Levels	No. of labs	Mean (log cfu/g)	S_R (log cfu/g)	Bias (log cfu/g)	Lower limit (log cfu/g)	Upper limit (log cfu/g)	± AL (log cfu/g)
1.98	11	1.92	0.18	-0.06	-0.30	0.19	0.5
3.01	11	2.97	0.08	-0.05	-0.16	0.07	0.5
4.02	11	4.02	0.07	0	-0.09	0.09	0.5

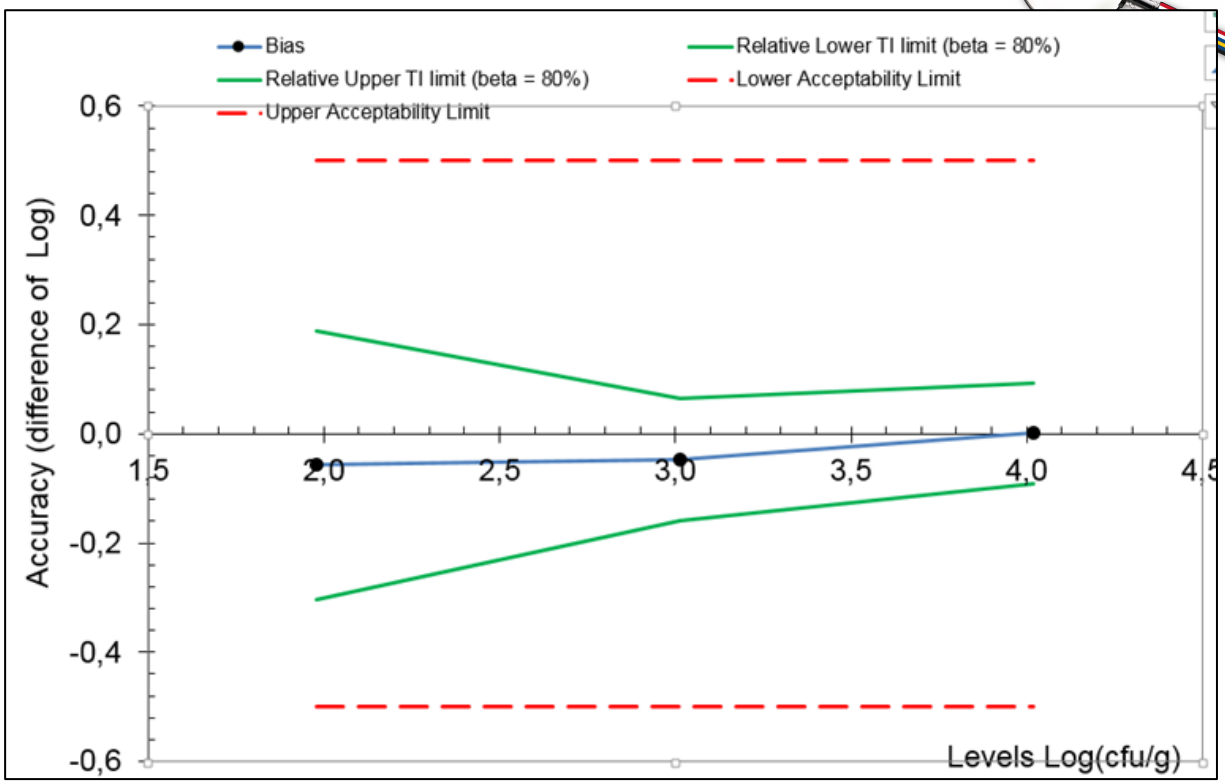

Figure 3. Graphical representation of the accuracy profile of the interlaboratory study for *Listeria*

CONCLUSION

The studies have shown that there are no statistical differences in the performances of RAPID*'L.mono* and the reference method for the detection and the enumeration of *Listeria monocytogenes* and the detection of *Listeria spp.* in foods and environmental samples.