DECIDE WITH CONFIDENCE

FOOD SAFETY

# VIDAS® *L. monocytogenes* Xpress (LMX)
## Ultra Performance Summary

Food safety professionals have a variety of test kits to choose from when looking to fill a need in their laboratory. One of the main criteria used in making their decision is an evaluation of the certifications the test kit has received. Validation studies provide a user with confidence in the performance of the test kit. This document summarizes the certifications granted to the bioMérieux VIDAS® *L. monocytogenes* Xpress (LMX) test method. The VIDAS LMX method was certified by the AOAC Research Institute *Performance Tested Method*℠ (PTM) program after the completion of a single laboratory study and an independent laboratory study. These studies included an evaluation of the following parameters: inclusivity, exclusivity, robustness, lot-to-lot/stability and method comparison. The VIDAS LMX method was also adopted as an AOAC Official Method of Analysis (OMA), First Action status after the completion of a multi-laboratory method comparison study. Additionally, the VIDAS LMX method has been certified NF VALIDATION (AFNOR) as an alternative method for the detection of *Listeria monocytogenes* through the completion of an independent single laboratory study and a multi-laboratory study. All of the validation studies demonstrated that the VIDAS LMX method was statistically equivalent to the corresponding reference method for the matrices tested with 95% confidence.

BIOMÉRIEUX INDUSTRY

### AOAC Performance Tested Method: PTM#091103

In September 2011, the VIDAS LMX Assay was granted *Performance Tested Method*℠ (PTM) status by the AOAC Research Institute for the detection of *Listeria monocytogenes* in a variety of foods. The VIDAS LMX method was validated according to harmonized PTM and *Official Methods of Analysis* guidelines (2002). Results of the validation study demonstrated the ability of the VIDAS LMX method to: 1) detect 60 different *L. monocytogenes* strains; 2) correctly show negative results for 31 non- *L. monocytogenes* strains; 3) support a 12-month shelf life and quality of the test kit; 4) perform appropriately after varied protocol parameters including enriched sample boiling time (4, 5, 6 min), sample temperature after boiling (10, 25, 50°C) and time reagents held at room temperature (after refrigeration) prior to performing VIDAS LMX method (0, 30, 60, 120 min); and 5) demonstrate no statistically significant differences when compared to a reference method for 7 claimed matrices using a 25g test portion size: deli ham, processed American cheese, vanilla ice cream, frozen spinach, cooked shrimp, smoked white fish, creamy peanut butter. Independent testing included method comparison studies for 3 matrices: deli ham, vanilla ice cream, cooked shrimp.

Through the AOAC Research Institute GovVal program, five matrices were evaluated for their performance compared to the USDA/FSIS MLG and the MFHPB-30 references methods: deli turkey, deli ham, liver pate, raw fermented sausage, turkey hot dogs. This study was validated according to a protocol written following the AOAC Microbiology Guidelines (2012) and approved by AOAC, Health Canada and Canadian Food Inspection Agency representatives.

In January 2013, the VIDAS LMX Assay matrix extension study was approved by the AOAC Research Institute when 4 matrices (deli ham, deli turkey, ground beef and queso fresco) were evaluated using a 125g test portion size. This study was validated according to a protocol written using the AOAC Microbiology Guidelines (2012).

### AOAC Official Method: Certificate No: 2013.11

In June 2013, the VIDAS LMX Assay was granted First Action AOAC *Official Methods of Analysis*℠ (OMA) status by the AOAC Research Institute for the detection of *L. monocytogenes* after the completion of the AOAC PTM evaluation and a collaboratively studied evaluation of raw queso fresco at both 25g and 125g. Fourteen different laboratories received twelve replicate test portions from each of the three contamination levels of matrix were analyzed by VIDAS LMX and the AOAC OMA 993.12 reference method. Statistical analysis was conducted according to the probability of detection model and showed no statistically significant difference in the number of positive test portions detected by the VIDAS LMX method and the OMA 993.12 method.

### AFNOR – NF Validation: Certificate No: BIO 12/27 – 02/10

In April 2010, the VIDAS LMX Assay was certified NF VALIDATION as an alternative analysis method for the detection of *L. monocytogenes* in food products for humans and environmental samples. This validation was obtained by comparison with the reference method described in the international standard ISO 11290-1 according to the standard EN ISO 16140 (2003). In the single laboratory study, the results showed a relative sensitivity level of 90.5% for the VIDAS LMX method for all food and environmental surface testing. In the interlaboratory study, twelve different laboratories evaluated eight replicate test portions from each of the three contamination levels. Each test portion was analyzed by the VIDAS LMX and the ISO 11290-1 reference method. All presumptive positive test portions were confirmed following plating on selective agar and identification. The results showed a relative sensitivity level of 97.9% for the VIDAS LMX method.

PERFORMANCE DOCUMENT VIDAS® *L. monocytogenes* Xpress (LMX)

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# VIDAS® *L. monocytogenes* Xpress (LMX)

Catalog Number - REF 30 123

## Contents of the VIDAS LMX Kit

- 60 LMX Strips
- 60 LMX SPR®s
- Standard LMX
- LMX Positive Control
- Negative Control
- 1 MLE Card (Master Lot Entry)
- 1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib

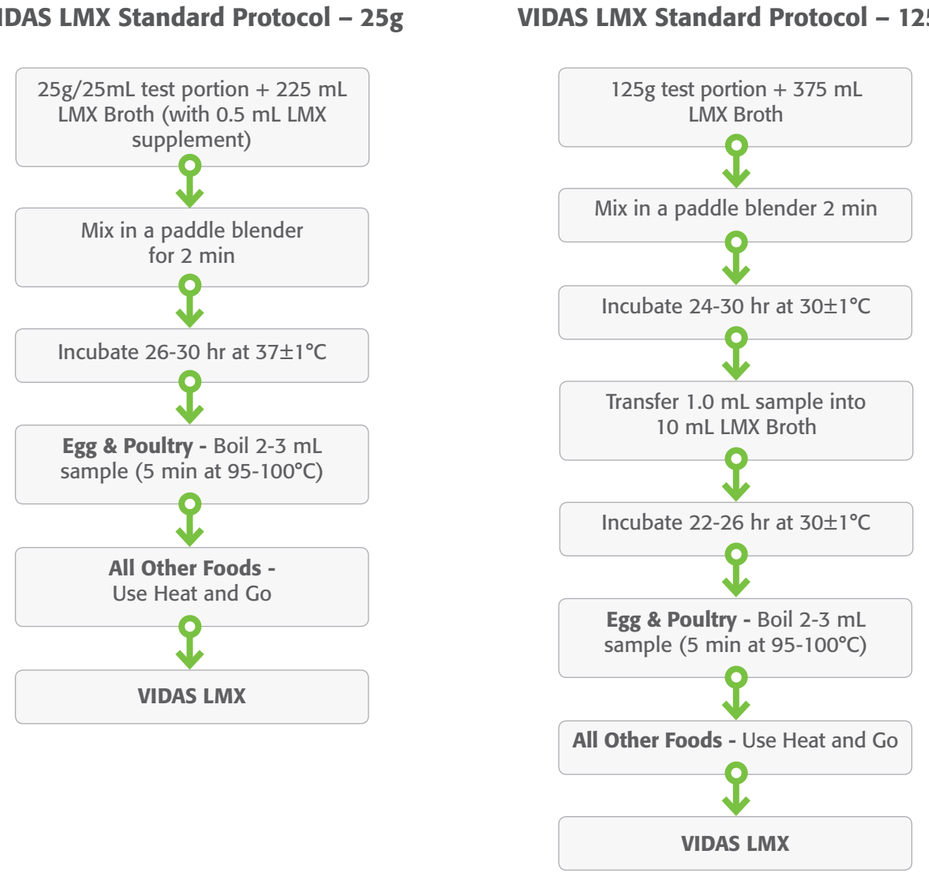
## Principle of the Assay

VIDAS® *Listeria monocytogenes* Xpress (LMX) method is for use on the automated VIDAS instrument for the detection of *Listeria monocytogenes* antigens using the ELFA method (Enzyme Linked Fluorescent Assay). The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device. The interior of the SPR is coated with proteins specific for *Listeria monocytogenes* receptors. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. All assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. An aliquot of enrichment broth is dispensed into the reagent strip. The *Listeria monocytogenes* receptors present will bind to the interior of the SPR. Unbound components are eliminated during the washing steps. The proteins conjugated to the alkaline phosphatase are cycled in and out of the SPR and will bind to any *Listeria monocytogenes* receptors, which are bound to the SPR wall. A final wash step removes unbound conjugate. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of the substrate into a fluorescent product (4-Methyl-umbellliferone) the fluorescence of which is measured at 450 nm. At the end of the assay, results are automatically analyzed by the instrument, which calculates a test value for each sample. This value is then compared to internal references (thresholds) and each result is interpreted as positive or negative.





**Figure 1.** Flow diagrams showing the VIDAS LMX standard protocols




## *Performance Tested Method*℠ (PTM)

The AOAC *Performance Tested Methods*℠ (PTM) program was formed in 1992 and is a method certification program for proprietary methods. Methods certified as *Performance Tested*℠ were found to perform according to the manufacturer's documented claims and are used throughout the global marketplace and within the regulatory arena. The PTM program offers certification as an endpoint for method evaluation or as an entry to method validation for programs requiring increased confidence and method reproducibility information. Validation study protocols are written according to AOAC Microbiology Guidelines (2002 or 2012) and include the following technical requirements: inclusivity/exclusivity, method developer method comparison, independent laboratory method comparison, robustness, product consistency, product stability and instrument variation (where applicable) studies. More information can be found at www.aoac.org.

### *Official Methods of Analysis℠* (OMA)

*The Official Methods of Analysis℠* (OMA) program is offered by AOAC INTERNATIONAL and evaluates chemistry, microbiology, and molecular biology methods. It also evaluates traditional bench-top methods, instrumental methods, and proprietary, commercial, and/or alternative methods. Validation study protocols are written according to AOAC Microbiology Guidelines (2002 or 2012) and include the following technical requirements: inclusivity/exclusivity, method developer method comparison and collaborative method comparison studies. More information can be found at www.aoac.org.

### AFNOR Validation Study

The NF VALIDATION mark has been widely recognized in France since the 1990s and is now well-established in Europe and internationally. It is a completely separate European certification system, operating alongside the technical validation systems of NordVal (inter-governmental validation system of 5 Nordic countries) and AOAC (North American technical validation system). Validation study protocols are written according to EN ISO 16140 and include the following technical requirements: inclusivity/exclusivity, single laboratory methods comparison and an inter-laboratory studies. More information can be found at http://www.afnor-validation.com/afnor-validation-food-industry/food-industry.html.

### GovVal Study

The GovVal program was based on the PTM program operated by the AOAC Research Institute. Candidate test kit methods were evaluated using a validation protocol approved by AOAC, the Canadian Food Inspection Agency and Health Canada, using blind coded, randomized samples. Sample preparation and independent testing of many test kit methods, as well as the MFHPB-30 reference method, was done by an AOAC RI approved independent laboratory. The GovVal program was designed to evaluate previously AOAC-approved methods for the specific needs of regulatory agencies to enforce their standards for regulatory testing, in this case *Listeria monocytogenes* in ready-to-eat meats and *Listeria* species on stainless steel surfaces as compared to the Health Canada reference method.

**Table A. Validation Study Technical Requirements**

| Study Type | AOAC PTM | AOAC OMA | AFNOR |
|---|---|---|---|
| Method Developer | ● | ● | |
| Independent | ● | | ● |
| Collaborative | | ● | ● |





## AOAC PTM Validation Study

PTM Certification#: 091103

PTM Certified: September 2011

PTM Matrix Extension Certified: January 2013

Guideline document – Original Study: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Qualitative and Quantitative Food Microbiological Official Methods of Analysis (2002)

Guideline document – Matrix Extension and GovVal: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (2012)

Reference methods: USDA/FSIS Microbiological Laboratory Guidebook (8.07), US-FDA Bacteriological Analytical Manual Chapter 10 (2003), AOAC Official Method of Analysis 993.12 and Microbiology Food Health Protection Branch (MFHPB-30, Feb 2011)

**Method Comparison (Tables B and C):** The original method comparison study was performed at both the method developer (internal) and independent laboratories. Matrices (6 internal and 3 independent) were inoculated with *L. monocytogenes.* For each matrix, twenty replicates at one inoculation level (0.2-2 cfu/25g) and 5 uninoculated replicates were tested by both the VIDAS LMX and appropriate reference method. Primary enrichments for each method were confirmed using the traditional confirmation methods and ALOA chromogenic agar.

The data collected in the GovVal study was incorporated into the VIDAS LMX PTM study. There were 5 matrices evaluated using twenty replicates at two inoculation levels (0.2-2 cfu/test portion and 2-5 cfu/test portion) and 5 uninoculated replicates that were tested by the VIDAS LMX and the reference methods, USDA/FSIS MLG and MFHPB-30. Primary enrichments for each method were confirmed using the traditional confirmation methods and ALOA chromogenic agar followed by identification.

A matrix extension study was performed on four matrices at a larger test portion size, 125g. Each matrix was evaluated using twenty replicates at two inoculation levels (0.2-2 cfu/test portion and 2-5 cfu/test portion) and 5 uninoculated replicates that were tested by the VIDAS LMX and the appropriate reference method. Primary enrichments for each method were confirmed using the traditional confirmation methods and ALOA chromogenic agar followed by identification.

In the method comparison studies there were no significant differences between the VIDAS LMX method and the reference methods using unpaired Chi-square or the POD test at 5% level for the majority of the matrices evaluated. The only matrix that showed a significant difference, with the VIDAS LMX method resulting in a higher number of positive test portions, was turkey hot dogs (25g).

**Inclusivity/Exclusivity (Tables D and E):** The inclusivity studies demonstrated that the VIDAS LMX method could detect all 50 *L. monocytogenes* strains. For the exclusivity testing, all 30 non-*L. monocytogenes* strains tested negative by the VIDAS LMX method.

**Lot-to-lot/Stability:** Stability and lot-to-lot variation of the VIDAS LMX method was evaluated over 12 months at 2-8°C using *L. monocytogenes* and non-*L. monocytogenes* strains on three different test kit lots. There was no loss of stability over the 12 months or any difference between the three different test kits supporting the 12-month shelf life and the quality of the product.

**Ruggedness:** Minor variations to the protocol parameters, including enriched sample boiling time (4, 5, 6 min), sample temperature after boiling (10, 25, 50°C) and time reagents held at room temperature (after refrigeration) prior to performing VIDAS LMX method (0, 30, 60, 120 minutes). There were no differences seen in number of positive results after with each of the variations in the protocol.

# Official Methods of Analysis (OMA) Validation Study

Certificate No: 2013.11

Certification date: June 2013

Guideline document: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (2012)

Reference Method: AOAC 993.12 Listeria monocytogenes in Milk and Dairy Products

### Inclusivity/Exclusivity & Method Developer Method Comparison

See the AOAC PTM study sections for evaluation results of these studies.

**Collaborative Study: Queso fresco - 25g (Table F):** Queso fresco (soft Mexican cheese, 25g) was analyzed by thirteen laboratories. The matrix was artificially contaminated with *L. monocytogenes* at two levels: a high level 3.81 CFU/25g (95% confidence interval of 3.06, 5.48) and a low level of 0.55 CFU/25g (95% confidence interval of 0.43, 0.70). A set of uncontaminated control test portions were also included at 0 CFU/25g. Twelve replicate test portions from each of the three contamination levels of matrix were analyzed. Two sets of test portions (72 total) were sent to each laboratory for analysis by VIDAS LMX and the AOAC 993.12 reference method due to different sample enrichments for each method. All test portions were confirmed by streaking the non-heated enrichment broth to ALOA and Oxford agar followed by identification.

Statistical analysis was conducted according to the probability of detection (POD). Results obtained by the different confirmation protocols showed no significant differences between the options evaluated.

**Collaborative Study: Queso fresco - 125g (Table F):** Queso fresco (soft Mexican cheese, 125g) was analyzed by twelve laboratories. The matrix was artificially contaminated with *Listeria monocytogenes* at two levels: a high level 5.41 CFU/125g (95% confidence interval of 3.53, 8.30) and a low level of 0.59 CFU/125g (95% confidence interval of 0.46, 0.74). A set of uncontaminated control test portions were also included at 0 CFU/25g. Twelve replicate test portions from each of the three contamination levels of matrix were analyzed. Two sets of test portions (72 total) were sent to each laboratory for analysis by VIDAS LMX and the AOAC 993.12 reference method due to different sample enrichments for each method. All test portions were confirmed by streaking the non-heated enrichment broth to ALOA and Oxford agar followed by identification.

Statistical analysis was conducted according to the probability of detection (POD). Results obtained by the different confirmation protocols showed no significant differences between the options evaluated.





## Table B. AOAC PTM Method Developer Method Comparison Study Details

| Matrix | Inocluating Organism | Test portion size(s) | Reference Method Comparison |
|---|---|---|---|
| Liver paté[b] | *L. monocytogenes* 1/2a, ATCC 49594[d] | 25g | USDA/FSIS MLG 8.07 & MFHPB-30 |
| Raw fermented sausage[b] | *L. monocytogenes* 3b, CWD 1591[d] | 25g | USDA/FSIS MLG 8.07 & MFHPB-30 |
| Turkey hot dogs[b] | *L. monocytogenes* 1/2b, ATCC BAA-751 plus 10x *L. innocua* 4ab[d] | 25g | USDA/FSIS MLG 8.07 & MFHPB-30 |
| Sliced deli turkey[b] | *L. monocytogenes* 3c, FSL-J1_049[d] | 25g | USDA/FSIS MLG 8.07 & MFHPB-30 |
| Sliced deli turkey[a] | *L. monocytogenes* 4b, ATCC 51776[d] | 125g | USDA/FSIS MLG 8.07 |
| Sliced deli ham[c] | *L. monocytogenes* 4b, ATCC 19115[d] | 25g | USDA/FSIS MLG 8.07 & MFHPB-30 |
| Sliced deli ham[a] | *L. monocytogenes* ATCC 7644[d] | 125g | USDA/FSIS MLG 8.07 |
| Ground beef[a] | *L. monocytogenes* 4b, ATCC 13932 | 125g | USDA/FSIS MLG 8.07 |
| Queso fresco[a] | *L. monocytogenes 4a*, ATCC 19114 | 125g | AOAC OMA 993.12 |
| Vanilla ice cream | *L. monocytogenes* 1 | 25g | AOAC OMA 993.12 |
| Processed cheese | *L. monocytogenes* 1 | 25g | AOAC OMA 993.12 |
| Frozen spinach | *L. monocytogenes* 4, Scott A | 25g | FDA BAM Ch. 10 (2003) |
| Peanut butter | *L. monocytogenes* 4a | 25g | FDA BAM Ch. 10 (2003) |
| Cooked shrimp | *L. monocytogenes* 3b | 25g | FDA BAM Ch. 10 (2003) |
| Smoked white fish | *L. monocytogenes* 1/2b | 25g | FDA BAM Ch. 10 (2003) |

[a]Matrix validated in PTM matrix extension study
[b]Matrix validated as part of GovVal program
[c]Matrix validated in both PTM and GovVal
[d]Heat-stressed at 55°C for 10 minutes in a water bath

## Table C. AOAC PTM Method Comparison Study Results

| | INTERNAL LABORATORY DATA | Test portion size | Enrichment broth | Level | VIDAS LMX | | Reference | $X^2$ | Sensitivity, % | Specificity, % | False pos, % | False neg, % | dPOD CI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Presumptive | Confirmed | | | | | | | P vs C[a] | C vs R[b] |
| Meat and Poultry | Deli ham[a] | 25g | LMX | Low | 10 | 10 | 13 | 0.89 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | -0.15 [-0.41, 0.15] |
| | | | | High | 16 | 16 | 18 | 0.76 | 100 | 100 | 0 | 0 | 0 [-0.25, 0.25] | -0.10 [-0.33, 0.13] |
| | Deli ham | 125g | LPT | Low | 16 | 16 | 15 | 0.07 | 100 | 100 | 0 | 0 | 0 [-0.25, 0.25] | 0.05 [-0.21, 0.29] |
| | Deli turkey[a] | 25g | LMX | Low | 15 | 15 | 15 | 0.00 | 100 | 100 | 0 | 0 | 0 [-0.26, 0.26] | 0 [-0.26, 0.26] |
| | | | | High | 15 | 15 | 17 | 0.61 | 100 | 100 | 0 | 0 | 0 [-0.26, 0.26] | -0.1 [-0.34, 0.15] |
| | Deli turkey | 125g | LPT | Low | 16 | 16 | 15 | 0.07 | 100 | 100 | 0 | 0 | 0 [-0.25, 0.25] | 0.05 [-0.21, 0.29] |
| | Ground beef | 125g | LPT | Low | 7 | 7 | 7 | 0.00 | 100 | 100 | 0 | 0 | 0 [-0.24, 0.24] | 0 [-0.24, 0.24] |
| | Liver paté[a] | 25g | LMX | Low | 11 | 11 | 10 | 0.10 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | 0.05 [-0.24, 0.33] |
| | | | | High | 15 | 15 | 12 | 1.00 | 100 | 100 | 0 | 0 | 0 [-0.26, 0.26] | 0.15 [-0.13, 0.40] |
| | Raw fermented sausage[a] | 25g | LMX | Low | 9 | 9 | 10 | 0.10 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | -0.05 [-0.33, 0.24] |
| | | | | High | 11 | 12 | 10 | 0.10 | 100 | 100 | 0 | 0 | -0.05 [-0.33, 0.24] | 0.05 [-0.24, 0.33] |
| | Turkey hot dogs[a] | 25g | LMX | Low | 10 | 10 | 8 | 0.39 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | 0.10 [-0.19, 0.37] |
| | | | | High | 20 | 20 | 13 | 8.27 | 100 | 100 | 0 | 0 | 0 [-0.16, 0.16] | 0.35 [0.12, 0.57] |
| Dairy | Processed American cheese | 25g | LMX | Low | 5 | 5 | 5 | 0.00 | 100 | 100 | 0 | 0 | 0 [-0.26, 0.26] | 0 [-0.26, 0.26] |
| | Vanilla ice cream | 25g | LMX | Low | 10 | 10 | 13 | 0.78 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | -0.15 [-0.41, 0.15] |
| | Queso fresco | 125g | LPT | Low | 11 | 11 | 11 | 0.00 | 100 | 100 | 0 | 0 | 0 [-0.26, 0.26] | 0 [-0.26, 0.26] |
| Vegetable | Frozen spinach | 25g | LMX | Low | 4 | 4 | 5 | 0.21 | 100 | 100 | 0 | 0 | 0 [-0.25, 0.25] | -0.05 [-0.30, 0.21] |
| Seafood | Cooked shrimp | 25g | LMX | Low | 14 | 14 | 13 | 0.01 | 100 | 100 | 0 | 0 | 0 [-0.27, 0.27] | 0.05 [-0.23, 0.32] |
| | Smoked white fish | 25g | LMX | Low | 8 | 8 | 8 | 0.00 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | 0 [-0.28, 0.28] |
| Misc. | Creamy peanut butter | 25g | LMX | Low | 8 | 8 | 10 | 0.39 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | -0.10 [-0.37, 0.19] |
| | INDEPENDENT LABORATORY DATA | Test portion size | Enrichment broth | Level | VIDAS LMX | | Reference | $X^2$ | Sensitivity, % | Specificity, % | False pos, % | False neg, % | dPOD CI | |
| | | | | | Presumptive | Confirmed | | | | | | | P vs C[a] | C vs R[b] |
| | Deli ham | 25g | LMX | Low | 16 | 16 | 14 | 0.52 | 100 | 100 | 0 | 0 | 0 [-0.25, 0.25] | 0.10 [-0.17, 0.35] |
| | Vanilla ice cream | 25g | LMX | Low | 8 | 8 | 12 | 1.56 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | -0.20 [-0.46, 0.10] |
| | Cooked shrimp | 25g | LMX | Low | 7 | 7 | 6 | 0.11 | 100 | 100 | 0 | 0 | 0 [-0.28, 0.28] | 0.05 [-0.23, 0.32] |

[a]Two levels evaluated in the GovVal study

[a] VIDAS LMX presumptive vs confirmed
[b] VIDAS LMX vs reference method

$$\text{Mantel Haenszel Chi sq } (X^2) = \frac{(n-1)(ad-bc)^2}{(a+b)(a+c)(b+d)(c+d)}$$

N = total number of samples, a = candidate +, b = candidate -, c = reference +, d = reference -

**Acceptability Criteria**
$X^2 \leq 3.84$ indicates no significant difference (at the 0.05 level) between the two methods.

**Sensitivity** = VIDAS presumptive + (that confirmed +)/VIDAS confirmed +
**Specificity** = VIDAS presumptive – (that confirmed -)/VIDAS confirmed -
**False positive** = 100-sensitivity
**False negative** = 100-specificity

**POD** = x/N, where x is the number of positive test portions and N is the total number of test portions
**dPOD** = the difference between any two POD values

**Acceptability Criteria**
Confidence interval of a dPOD contains zero indicates no significant difference (at the 0.05 level) between the two methods



PERFORMANCE DOCUMENT VIDAS® *L. monocytogenes* Xpress (LMX)

## Table D. Complete Inclusivity List

| | Strain | Origin | | Strain | Origin |
|---|---|---|---|---|---|
| 1 | *Listeria monocytogenes* 1/2a | ATCC 35152 | 31 | *Listeria monocytogenes* 1/2c | Ground beef |
| 2 | *Listeria monocytogenes* 1/2a | Smoked salmon | 32 | *Listeria monocytogenes* 1/2c | Beef in sauce |
| 3 | *Listeria monocytogenes* 1/2a | Pizza | 33 | *Listeria monocytogenes* 1/2 | Smoked salmon |
| 4 | *Listeria monocytogenes* 1/2a | Raw milk cheese | 34 | *Listeria monocytogenes* 1/2 | Chicken |
| 5 | *Listeria monocytogenes* 1/2a | Raw milk cheese | 35 | *Listeria monocytogenes* 3b | SLCC 2540 |
| 6 | *Listeria monocytogenes* 1/2a | Rillettes (pate) | 36 | *Listeria monocytogenes* 3c | SLCC 2479 |
| 7 | *Listeria monocytogenes* 1/2a | Raw milk cheese | 37 | *Listeria monocytogenes* 4a | ATCC 19114 |
| 8 | *Listeria monocytogenes* 1/2a | Smoked salmon | 38 | *Listeria monocytogenes* 4b | Salad |
| 9 | *Listeria monocytogenes* 1/2a | Raw milk cheese | 39 | *Listeria monocytogenes* 4b | Raw milk cheese |
| 10 | *Listeria monocytogenes* 1/2a | Chicken breast | 40 | *Listeria monocytogenes* 4b | ATCC 19115 |
| 11 | *Listeria monocytogenes* 1/2a | Ground beef | 41 | *Listeria monocytogenes* 4d | ATCC 19117 |
| 12 | *Listeria monocytogenes* 1/2a | Sausage | 42 | *Listeria monocytogenes* 4e | ATCC 19118 |
| 13 | *Listeria monocytogenes* 1/2a | Rabbit terrine | 43 | *Listeria monocytogenes* 4e | Reblochon (raw milk cheese) |
| 14 | *Listeria monocytogenes* 1/2a | Fried potatoes | 44 | *Listeria monocytogenes* 4e | Raw milk cheese |
| 15 | *Listeria monocytogenes* 1/2a | Fish | 45 | *Listeria monocytogenes* 7 | SLCC 2482 |
| 16 | *Listeria monocytogenes* 1/2a | Feed (soya) | 46 | *Listeria monocytogenes* | Sausage |
| 17 | *Listeria monocytogenes* 1/2a | Fried potatoes | 47 | *Listeria monocytogenes* | Salmon |
| 18 | *Listeria monocytogenes* 1/2b | Raw milk cheese | 48 | *Listeria monocytogenes* | Spinach |
| 19 | *Listeria monocytogenes* 1/2b | Pork tongue | 49 | *Listeria monocytogenes* | Cheese (Neufchatel) |
| 20 | *Listeria monocytogenes* 1/2b | Cream of chicken liver | 50 | *Listeria monocytogenes* | Mozzarella |
| 21 | *Listeria monocytogenes* 1/2b | Raw milk cheese | 51 | *Listeria monocytogenes* | Fish |
| 22 | *Listeria monocytogenes* 1/2b | SLCC 2755 | 52 | *Listeria monocytogenes* | Vegetable |
| 23 | *Listeria monocytogenes* 1/2b | Pork ears | 53 | *Listeria monocytogenes* | Ham |
| 24 | *Listeria monocytogenes* 1/2c | Sausage from MontbŽliard | 54 | *Listeria monocytogenes* | Ground beef |
| 25 | *Listeria monocytogenes* 1/2c | Ground beef | 55 | *Listeria monocytogenes* | Cheese (coulommier) |
| 26 | *Listeria monocytogenes* 1/2c | Beef | 56 | *Listeria monocytogenes* | Environment |
| 27 | *Listeria monocytogenes* 1/2c | Ground beef | 57 | *Listeria monocytogenes* | Environment |
| 28 | *Listeria monocytogenes* 1/2c | Pork belly | 58 | *Listeria monocytogenes* | Environmental sponges |
| 29 | *Listeria monocytogenes* 1/2c | Raw milk cheese | 59 | *Listeria monocytogenes* | French fries |
| 30 | *Listeria monocytogenes* 1/2c | Environmental sponge | 60 | *Listeria monocytogenes* | Beef |

## Table E. Complete Exclusivity List

| | Strain | Origin |
|---|---|---|
| 1 | *Bacillus cereus* | Beets |
| 2 | *Bacillus mycoides* | Environmental sample |
| 3 | *Bacillus stearothermophilus* | Dairy |
| 4 | *Bacillus sphaericus* | Meat |
| 5 | *Streptococcus bovis* | Institut Pasteur Collection |
| 6 | *Enterococcus durans* | Meat |
| 7 | *Streptococcus equinus* | Institut Pasteur Collection |
| 8 | *Enterococcus faecalis* | ATCC 19433 |
| 9 | *Enterococcus faecium* | Institut Pasteur Collection CIP 5433 |
| 10 | *Streptococcus anginosus* | Institut Pasteur Collection |
| 11 | *Lactobacillus casei* | Dairy |
| 12 | *Jonesia denitrificans* | Institut Pasteur Collection |
| 13 | *Staphylococcus intermedius* | Institut Pasteur Collection |
| 14 | *Staphylococcus aureus* | Yogurt |
| 15 | *Staphylococcus epidermidis* | Yogurt |
| 16 | *Micrococcus* | Environmental sample |
| 17 | *Listeria seeligeri* | French fries |
| 18 | *Listeria grayi* | ATCC 25 401 |
| 19 | *Listeria seeligeri* | Environmental sample |
| 20 | *Listeria welshimeri* | Salmon |
| 21 | *Listeria ivanovii* | Institut Pasteur Collection |
| 22 | *Listeria ivanovii* | Environmental sample |
| 23 | *Listeria ivanovii* | Institut Pasteur Collection |
| 24 | *Listeria innocua* | Raw milk cheese |
| 25 | *Listeria innocua 6b* | Ground beef |
| 26 | *Listeria ivanovii* | Institut Pasteur Collection |
| 27 | *Listeria seeligeri 1/2b* | Pork tongue |
| 28 | *Listeria welshimeri* | Sausage |
| 29 | *Listeria innocua* | Gorgonzola |
| 30 | *Listeria seeligeri* | Raw milk cheese |
| 31 | *Listeria ivanovii* | Ground beef |





## Table F. AOAC OMA Collaborative Study Data Summary

| Matrix | Level (CFU/test portion) | VIDAS LMX | | Reference (N=156) | $X^{2a}$ | Sensitivity | Specificity | False pos | False neg | dLPOD[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Presumptive (N=156) | Confirmed (N=156) | | | | | | | P vs C[a] | C vs R[b] |
| Queso fresco (25g) | 0.55 (0.43, 0.70) | 77 | 75 | 69 | 0.23 | 97 | 100 | 3 | 0 | 0.01 [-0.10, 0.13) | 0.04 [-0.08, 0.15] |
| | 3.81 (3.06, 5.48) | 156 | 156 | 153 | 1.50 | 100 | 100 | 0 | 0 | 0 [0.02, 0.02] | 0.02 [-0.01, 0.06] |
| **Matrix** | **Level (CFU/test portion)** | **VIDAS LMX** | | **Confirmed (N=156)** | **$X^{2a}$** | **Sensitivity** | **Specificity** | **False pos** | **False neg** | **dLPOD[b]** | |
| | | **Presumptive (N=156)** | **Confirmed (N=156)** | | | | | | | **P vs C[a]** | **C vs R[b]** |
| Queso fresco (125g) | 0.59 (0.46, 0.74) | 70 | 70 | 69 | 0.01 | 100 | 100 | 0 | 0 | 0 [-0.12, 0.12] | 0.10 [-0.10, 0.13] |
| | 5.41 (3.53, 8.30) | 144 | 144 | 144 | 0 | 100 | 100 | 0 | 0 | 0 [-0.03, 0.03] | 0 [-0.03, 0.03] |

[a] VIDAS LMX presumptive vs confirmed
[b] VIDAS LMX vs reference method

$$\text{Mantel Haenszel Chi sq } (X^2) = \frac{(n-1)(ad-bc)^2}{(a+b)(a+c)(b+d)(c+d)}$$

N = total number of samples, a = candidate +, b = candidate -, c = reference +, d = reference -

**Acceptability Criteria**
$X^2 \leq 3.84$ indicates no significant difference (at the 0.05 level) between the two methods.

**Sensitivity** = VIDAS presumptive + (that confirmed +)/VIDAS confirmed +
**Specificity** = VIDAS presumptive – (that confirmed -)/VIDAS confirmed -
**False positive** = 100-sensitivity
**False negative** = 100-specificity

**POD** = x/N, where x is the number of positive test portions and N is the total number of test portions
**dPOD** = the difference between any two POD values

**Acceptability Criteria**
Confidence interval of a dPOD contains zero indicates no significant difference (at the 0.05 level) between the two methods

# AFNOR NF Validation Study

Certificate No: BIO 12/27 – 02/10

Certification date: February 2010

Guideline document: ISO 16140, Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods (2003)

Reference Methods: ISO 11290-1, Microbiology of the food chain -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp.

### Independent Expert Laboratory Study

**Inclusivity/exclusivity (Tables D and E):** The inclusivity studies demonstrated that the VIDAS LMX method could detect 50/50 *L. monocytogenes* strains tested after enrichment in LMX broth. For the exclusivity testing, 30/30 non-*L. monocytogenes* strains tested negative by the VIDAS LMX method.

**Relative sensitivity (Tables G and H):** The purpose of these tests was to evaluate the performance of the VIDAS LMX method with respect to the ISO 11290-1 reference method, on test portions naturally and artificially contaminated with *L. monocytogenes,* for the categories falling within the scope. The study evaluated 384 test portions that were inoculated with a wide variety of *L. monocytogenes* strains and analyzed at a level to achieve approximately 50% positive test portions with the following breakdown: meat – 62, dairy – 125, vegetables – 71, seafood – 66, environmental – 60. The results demonstrated that there are no statistically significant differences between the alternative method (90.5%) and the reference method (90%) when analyzing sensitivity values.

**Relative level of detection (Table I):** The objective of these tests was to determine the level of contamination needed to obtain about 50% of positive results and 50% negative results. Various "food matrix-strain" pairs were studied in parallel with the reference method and the VIDAS LMX method, for the studied categories. The results demonstrated that there are no statistically significant differences between the alternative method (0.2-1.8 cfu/25g) and the reference method (0.2-1.3 cfu/25g).

**Inter-laboratory Study (Tables J and K):** Pasteurized milk (25mL) was analyzed by twelve laboratories in this inter-laboratory study. The matrix was artificially contaminated with *L. monocytogenes* at two levels: a high level of 22.2 CFU/25g (95% confidence interval of 14, 33) and a low level of 3.26 CFU/25g (95% confidence interval of 0.7, 9.5). A set of uncontaminated control test portions were also included for each matrix at 0 CFU/25g. Eight replicate test portions from each of the three contamination levels of matrix were analyzed. Two sets of test portions (48 total) were sent to each laboratory for analysis by VIDAS LMX and the ISO 11290-1 reference method due to different test portion enrichments for each method. All test portions were confirmed following plating on selective agar and identification. The results demonstrated that there are no statistically significant differences between the alternative method (97.9%) and the reference method (100%) when analyzing sensitivity values.



PERFORMANCE DOCUMENT VIDAS® *L. monocytogenes* Xpress (LMX)

## Table G. AFNOR Independent Expert Laboratory Study Summary

| | Matrix | Protocol Type | Enrichment broth | Test portion size | Number Positive | Number Negative | Total |
|---|---|---|---|---|---|---|---|
| Poultry | Raw | General | LMX | 25g | 9 | 11 | 20 |
| | Raw seasoned | General | LMX | 25g | 10 | 10 | 20 |
| | Delicatessen | General | LMX | 25g | 11 | 11 | 22 |
| | | | | **TOTALS** | **30** | **32** | **62** |
| Other meat | Raw milk & pasteurized milk chssese (cow) | General | LMX | 25g | 12 | 5 | 17 |
| | Pasteurized milk cheeses (goat, ewe) | General | LMX | 25g | 13 | 13 | 26 |
| | Raw milk cheese | General | LMX | 25g | 33 | 28 | 61 |
| | Desserts, milk powders | General | LMX | 25g | 9 | 12 | 21 |
| | | | | **TOTALS** | **67** | **58** | **125** |
| Environment | Fish fillets & shellfish | General | LMX | 25g | 13 | 3 | 16 |
| | Smoked fish | General | LMX | 25g | 10 | 24 | 34 |
| | Ready-made meal | General | LMX | 25g | 8 | 8 | 16 |
| | | | | **TOTALS** | 31 | 35 | 66 |
| | Frozen | General | LMX | 25g | 9 | 12 | 21 |
| | Raw/fresh | General | LMX | 25g | 13 | 13 | 26 |
| | Seasoned | General | LMX | 25g | 10 | 14 | 24 |
| | | | | **TOTALS** | 32 | 39 | 71 |
| | Various waters | General | LMX | 25g | 9 | 10 | 19 |
| | Surface samples | General | LMX | 25g | 11 | 9 | 20 |
| | Residues & scraps | General | LMX | 25g | 10 | 11 | 21 |
| | | | | **TOTALS** | 30 | 30 | 60 |
| | | | | **OVERALL TOTALS** | **190** | **192** | **384** |

## Table H. AFNOR Independent Expert Laboratory Study Data Summary – Relative Sensitivity

| | PA | NA | ND | PD | N | Relative Sensitivity, % | |
|---|---|---|---|---|---|---|---|
| | | | | | | VIDAS LMX | Reference |
| **All products** | 153 | 194 | 18 | 19 | 384 | 90.5 | 90 |
| **All foods (except raw milk cheese and environmental** | 105 | 134 | 10 | 12 | 261 | 91.1 | 91.1 |
| **Totals:** | **258** | **328** | **28** | **31** | **645** | | |
| | **PA** | **NA** | **ND** | **PD** | **N** | | |
| **Meat products** | 24 | 32 | 3 | 3 | 62 | | |
| **All Dairy** | 56 | 58 | 6 | 5 | 125 | | |
| **Other dairy products** | 32 | 30 | 2 | 0 | 64 | | |
| **Raw milk cheese** | 24 | 28 | 4 | 5 | 61 | | |
| **Fish** | 27 | 35 | 2 | 2 | 66 | | |
| **Vegetable** | 22 | 39 | 3 | 7 | 71 | | |
| **Environmental** | 24 | 32 | 4 | 2 | 62 | | |

PA – positive agreement (Candidate +/Reference +)
NA – negative agreement (Candidate -/Reference -)
ND – negative deviation (Candiate -/Reference +)
PD – positive deviation (Candidate +/Reference -)
N – total number of samples
Relative Sensitivity, (PA + PD)/(PA + PD + ND)

## Table I. AFNOR Independent Expert Laboratory Study Data Summary – Relative Detection Limit

| Matrix | Test portion size | Strain | Relative detection level (CFU/25g or 25mL) with confidence interval[a] $LOD50_{50}$ | |
|---|---|---|---|---|
| | | | Reference Method | VIDAS LMX Method |
| Rillette | 25g | *L. monocytogenes* 1/2b | 0.7 [0.4 - 1.3] | 1.1 [0.6 - 1.8] |
| Raw milk | 25g | *L. monocytogenes* 1/2b | 0.5 [0.3 - 0.9] | 0.4 [0.2 - 0.7] |
| Raw milk cheese | 25g | *L. monocytogenes* 1/2b | 0.7 [0.4 - 1.3] | 0.6 [0.4 - 1.0] |
| Worn red cabbage | 25g | *L. monocytogenes* 4b | 0.5 [0.3 - 0.8] | 0.6 [0.4 - 1.0] |
| Smoked salmon | 25g | *L. monocytogenes* 1/2a | 0.5 [0.3 - 0.9] | 0.4 [0.2 - 0.7] |
| Process water | 25g | *L. monocytogenes* 1/2c | 0.4 [0.2 - 0.7] | 0.3 [0.2 - 0.6] |

[a] $LOD_{50}$: estimated level of contamination enabling positive detection using the alternative method in 50% of cases.





## Table J. AFNOR Inter-laboratory Study – Testing Results

| Level | # positive/total | |
|---|---|---|
| | VIDAS LMX | Reference |
| Level 0 (0 CFU/25g) | 0/96 | 0/96 |
| Low level (3.26 CFU/25g) | 96/96 | 96/96 |
| High level (22.2 CFU/25g) | 91/96 | 95/96 |

## Table K. AFNOR Inter-laboratory Study – Relative Sensitivity

| Level | PA | NA | ND | PD | N | Relative Sensitivity, % | |
|---|---|---|---|---|---|---|---|
| | | | | | | VIDAS | Reference |
| Level 0 (0 CFU/25g) | - | 96 | - | - | 96 | 97.9 | 100 |
| Low level (3.26 CFU/25g) | 96 | - | - | 2 | 96 | | |
| High level (22.2 CFU/25g) | 92 | - | 4 | 1 | 96 | | |

PA – positive agreement (Candidate +/Reference +)
NA – negative agreement (Candidate -/Reference -)
ND – negative deviation (Candiate -/Reference +)
PD – positive deviation (Candidate +/Reference -)
N – total number of samples
Relative Sensitivity, (PA + PD)/(PA + PD + ND)

# Glossary of Terms

**Chi square –** Test for significant difference; results less than 3.84 indicates no significant difference between methods.

**Collaborative Study (AOAC) –** A validation study performed by multiple laboratories to estimate critical VIDAS LMX method performance parameters. See also inter-laboratory study.

**Confirmed result –** The qualitative response from the confirmatory phase of the VIDAS LMX method.

**False negative –** A VIDAS negative test result that was confirmed to be culturally positive from the corresponding VIDAS enrichment (AOAC studies).

**False positive –** A VIDAS positive test result that was not confirmed culturally from the corresponding VIDAS enrichment (AOAC studies).

**Fractional recovery –** Validation criterion that is satisfied when an unknown sample yields both positive and negative responses within a set of replicate analyses. The proportion of positive responses should fall within 25% and 75% and should ideally approximate 50% of the total number of replicates in the set.

**Inter-laboratory Study (AFNOR) –** A validation study performed by multiple laboratories to estimate critical VIDAS LMX method performance parameters. See also collaborative study.

**Limit of detection –** The VIDAS system is able to detect a single target cell in a specific test portion following enrichment.

**Negative agreement –** Results for both the VIDAS LMX method and the reference method are negative.

**Negative deviation –** Result for the VIDAS LMX method is negative and the result for the reference method is positive.

**Presumptive result –** The qualitative response from the presumptive phase of the VIDAS LMX method that includes a confirmatory phase.





**Probability of Detection (POD) –** The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent.

**Positive agreement –** Results for both the VIDAS LMX method and the reference method are positive.

**Positive deviation –** Result for the VIDAS LMX method is positive and the result for the reference method is negative.

**Relative sensitivity –** Ability of the alternative method to detect the analyte when it is detected by the reference method

**Sensitivity –** The number of VIDAS positive test results that were confirmed to be positive from the VIDAS enrichment divided by the total number of confirmed positive VIDAS enrichments.

**Specificity –** The number of VIDAS negative test results that were confirmed to be negative from the VIDAS enrichment divided by the total number of confirmed negative VIDAS enrichments.

**Test portion –** A specified quantity of the sample that is taken for analysis by the method.

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