DECIDE WITH CONFIDENCE

FOOD SAFETY

VIDAS® UP *Listeria* (LPT)
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® UP *Listeria* (LPT) test method. The VIDAS LPT method was adopted as an AOAC Official Method of Analysis (OMA), First Action status after the completion of a single laboratory method comparison study, an inclusivity/exclusivity study and a multi-laboratory method comparison study. Additionally, the VIDAS LPT method has been certified NF VALIDATION (AFNOR) as an alternative method for the detection of *Listeria* through the completion of an independent single laboratory study and a multi-laboratory study. All of the validation studies demonstrated that the VIDAS LPT method was statistically equivalent to the corresponding reference method for the matrices tested with 95% confidence.

BIOMÉRIEUX INDUSTRY

AOAC Official Method: Certificate No: 2013.10

In June 2013 the VIDAS® UP *Listeria* (VIDAS LPT) Assay was granted First Action AOAC *Official Methods of Analysis℠* (OMA) status by the AOAC Research Institute for the detection of *Listeria* after the completion of the single laboratory method comparison study of nineteen different matrices (15 foods and 4 environmental surfaces) and a collaboratively studied evaluation of queso fresco (soft Mexican cheese, 25g and 125g). Fourteen different laboratories evaluated each test portion size. Twelve replicate test portions from each of the three contamination levels of matrix were analyzed by VIDAS LPT and the AOAC 993.12 reference method. Statistical analysis was conducted according to the probability of detection model. The data showed that there were three matrices with a statistically significant difference in the number of positive test portions detected by the VIDAS LPT method and the AOAC 993.12 method at the 0.05 level (hot dogs, mixed salad and stainless steel) where the VIDAS LPT method resulted in a greater number of test portions with recovery of the target organism.

AFNOR – NF Validation: Certificate No: BIO 12/33 – 05/12

In May 2012, the VIDAS® UP *Listeria* (VIDAS LPT) Assay was certified NF VALIDATION as an alternative analysis method for the detection of *Listeria* 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 ISO 16140 (2003). In the single laboratory study, the results showed a relative sensitivity level of 91.2% for the VIDAS LPT method for all food and environmental surface testing. In the interlaboratory study, sixteen different laboratories evaluated eight replicate test portions from each of the three contamination levels. Each test portion was analyzed by the VIDAS LPT 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 93.8% for the VIDAS LPT method.

PERFORMANCE DOCUMENT VIDAS® UP *Listeria* (LPT)

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VIDAS® *UP Listeria* (LPT)

Catalog Number - REF 30 126

Contents of the VIDAS LPT Kit

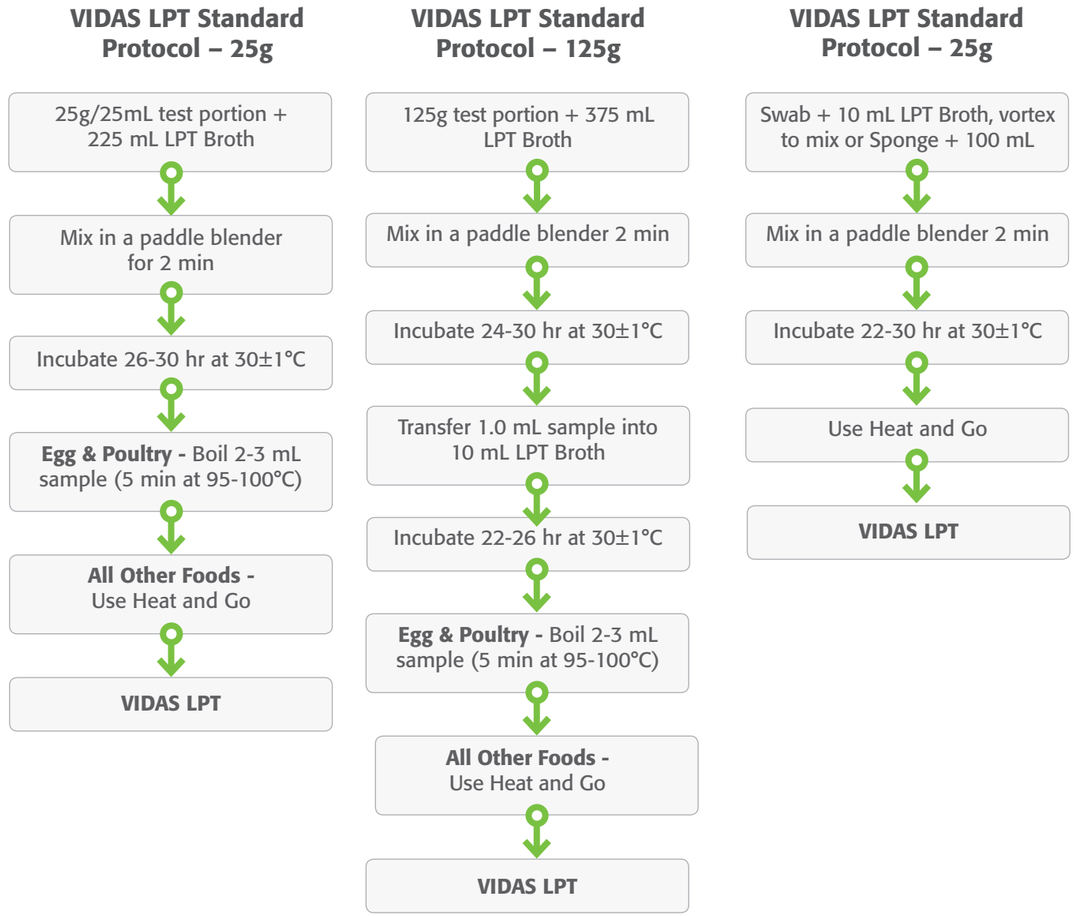
- 60 LPT Strips
- 60 LPT SPR®s
- Standard LPT
- LPT Positive Control
- Negative Control
- 1 MLE Card (Master Lot Entry)
- 1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib

Principle of the Assay

The VIDAS® LPT test is a recombinant phage protein based technology designed for use with the automated enzyme-linked fluorescent assay VIDAS® or mini-VIDAS® instruments for next day detection of *Listeria* in a variety of foods and select environmental samples. The novel method utilizes a single primary enrichment in LPT broth. The Solid Phase Receptacle serves as the solid phase as well as the pipetting device for the assay. The SPR is coated with proprietary detection proteins specific for *Listeria.* Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips. The instrument performs all of the assay steps automatically. The user places the sample into the reagent strip. Then the sample is cycled in and out of the SPR for a specific length of time. *Listeria* receptor targets present in the sample will bind to the specific capture proteins, which are coated on the interior of the SPR. Unbound sample components are washed away. Specific proteins conjugated to alkaline phosphatase are cycled in and out of the SPR and will bind to any *Listeria* receptor targets, which are bound to the capture proteins on the SPR wall. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The bound enzyme conjugate catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm.

PERFORMANCE DOCUMENT VIDAS® UP *Listeria* (LPT)

Figure 1. Flow diagrams showing the VIDAS LPT standard protocols



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 (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 inter-laboratory studies. More information can be found at http://www.afnor-validation.com/afnor-validation-food-industry/food-industry.html.

Table A. Validation Study Technical Requirements

Study Type	AOAC OMA	AFNOR
Method Developer	●	
Independent		●
Collaborative	●	●

Official Methods of Analysis (OMA) Validation Study

Certificate No: 2013.10

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

Method Comparison (Tables B and C): The method comparison study was performed at a contract laboratory commissioned by the Method Developer. Nineteen matrices were inoculated with *Listeria* species. For each matrix, twenty replicates at one inoculation level (0.2-2 cfu/25g) and 5 uninoculated replicates were tested by both the VIDAS LPT and 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 LPT method and the reference methods using unpaired Chi-square or the POD test at 5% level for the majority of the matrices evaluated. The following matrices showed a significant difference, with the VIDAS LPT method resulting in a higher number of positive test portions: beef hot dogs (25g), bagged mixed salad (25g) and stainless steel (sponge).

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

Collaborative Study: Queso fresco - 25g (Table F) Queso fresco (soft Mexican cheese, 25g) was analyzed by thirteen laboratories. The matrix was artificially contaminated with *Listeria innocua* (ATCC 33090) at two levels: a high level 5.38 CFU/25g (95% confidence interval of 3.60, 8.36) and a low level of 0.63 CFU/25g (95% confidence interval of 0.49, 0.79). 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 LPT 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* (ATCC 19115) 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 LPT 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), Sampling device	Reference Method Comparison
Deli ham	*L. seligeri* (IMVS 1150)[a]	25g	USDA/FSIS 8.07
Deli ham	*L. monocytogenes* (ATCC 7644)[a]	125g	USDA/FSIS 8.07
Pepperoni	*L. innocua* (ATCC 33090)[a]	25g	USDA/FSIS 8.07
Beef hot dogs	*L. monocytogenes* (4b) SM Scott A[a]	25g	USDA/FSIS 8.07
Ground beef	*L. monocytogenes* (ATCC 13932)	125g	USDA/FSIS 8.07
Chicken nuggets	*L. welshimeri* (ATCC 35897)[a]	25g	USDA/FSIS 8.07
Chicken liver paté	*L. monocytogenes* (SM 20V)	25g	USDA/FSIS 8.07
Deli turkey	*L. monocytogenes* (ATCC 51776)	125g	USDA/FSIS 8.07
Vanilla ice cream	*L. ivanovii* (ATCC 13124)	25g	AOAC 993.12
Queso fresco	*L. monocytogenes* (ATCC 19114)	25g, 125g	AOAC 993.12
Cooked shrimp	*L. grayii* (ATCC 700545)	25g	FDA-BAM Ch. 10 (Apr 2011)
Smoked salmon	*L. monocytogenes* (ATCC 19115)	25g	FDA-BAM Ch. 10 (Apr 2011)
Cantaloupe	*L. welshimeri* (ATCC 43549)	25g	FDA-BAM Ch. 10 (Apr 2011)
Bagged mixed salad	*L. monocytogenes* (ATCC 1971)	25g	FDA-BAM Ch. 10 (Apr 2011)
Peanut butter	*L. seligeri* (ATCC 35697)	25g	FDA-BAM Ch. 10 (Apr 2011)
Black pepper	*L. innocua* (ATCC 33090)	25g	FDA-BAM Ch. 10 (Apr 2011)
Plastic	*L. welshimeri* (ATCC 35897)[b]	swab	FDA-BAM Ch. 10 (Apr 2011)
Concrete[b]	*L. monocytogenes* (NCTC 11994)	swab	FDA-BAM Ch. 10 (Apr 2011)
Stainless steel[b]	*L. monocytogenes* (SM stock culture)	sponge	FDA-BAM Ch. 10 (Apr 2011)
Ceramic[b]	*L. innocua* (ATCC 33090)	sponge	FDA-BAM Ch. 10 (Apr 2011)

[a]Heat stressed culture used for inoculation - 18 to 24 hour culture (10 mL) heated at 50°C for 10 min
[b]Co-inoculated with competitor organism, Enterococcus faecalis (SM 244), target:competitor at 1:10
[c]Lyophilzed culture
IMVS - , ATCC - American Type Culture Collection, SM - Silliker Microtech, NCTC - National Collection of Type Cultures

Table C. AOAC PTM Method Comparison Study Results

INTERNAL LABORATORY DATA	Test portion size	VIDAS LPT		Reference	X^2	Sensitivity, %	Specificity, %	False pos, %	False neg, %	dPOD CI		dPOD CI
		Presumptive	Confirmed							P vs C[a]	C vs R[b]	
Deli ham	25g	9	9	6	0.94	0.94	100	0	0	0	0.15	-0.28, 0.28
Deli ham	125g	16	16	15	0.14	0.14	100	0	0	0	0.05	-0.25, 0.25
Deli turkey	125g	11	11	12	0.10	0.10	100	0	0	0	0.05	-0.28, 0.28
Ground beef	125g	7	7	7	0.00	0.00	100	0	0	0	0	-0.27, 0.27
Pepperoni	25g	15	15	10	2.60	2.60	100	0	0	0	0.25	-0.26, 0.26
Beef hot dogs	25g	6	6	1	4.22	4.22	100	0	0	0	0.25	-0.27, 0.27
Chicken nuggets	25g	5	5	5	0.00	0.00	100	0	0	0	0	-0.26, 0.26
Chicken liver paté	25g	11	11	8	0.88	0.88	100	0	0	0	0.15	-0.28, 0.28
Bagged mixed salad	25g	20	20	15	5.57	5.57	100	0	0	0	0.25	-0.16, 0.16
Cantaloupe	whole	12	12	10	0.40	0.40	100	0	0	0	0.10	-0.28, 0.28
Cooked shrimp	25g	16	16	13	1.10	1.10	100	0	0	0	0.15	-0.25, 0.25
Smoked salmon	25g	14	14	15	0.12	0.12	100	0	0	0	0.05	-0.27, 0.27
Queso fresco	25g	9	9	11	0.39	0.39	100	0	0	0	0.10	-0.28, 0.28
Queso fresco	125g	11	11	11	0.00	0.00	100	0	0	0	0	-0.28, 0.28
Vanilla ice cream	25g	18	18	15	1.52	1.52	100	0	0	0	0.15	-0.21, 0.21
Peanut butter	25g	15	15	16	0.14	0.14	100	0	0	0	0.05	-0.26, 0.26
Black pepper	25g	8	8	10	0.40	0.40	100	0	0	0	0.10	-0.28, 0.28
Plastic	swab	18	18	15	1.50	1.50	100	0	0	0	0.15	-0.21, 0.21
Stainless steel	sponge	11	10	1	11.61	11.61	100	10	0.45	0.45	0.45	-0.24, 0.33
Ceramic	sponge	6	6	3	1.26	1.26	100	0	0	0	0.15	-0.27, 0.27
Concrete	swab	14	14	15	0.12	0.12	100	0	0	0	0.05	-0.27, 0.27

[a] VIDAS presumptive vs confirmed
[b] VIDAS 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

Table D. Complete Inclusivity List

	Strain	Origin		Strain	Origin
1	*Listeria grayi*	Smoked salmon	26	*Listeria monocytogenes* 1/2a	Collection (ATCC 19120)
2	*Listeria grayi*	Ground beef	27	*Listeria monocytogenes* 1/2b	Frozen french fries
3	*Listeria grayi*	Sausage	28	*Listeria monocytogenes* 1/2b	Collection (CIP 103213)
4	*Listeria innocua*	Rosted apple	29	*Listeria monocytogenes* 1/2c	Cow liver
5	*Listeria innocua*	Shelfish	30	*Listeria monocytogenes* 1/2c	Epoisses (raw milk cheese)
6	*Listeria innocua*	Environmental sample	31	*Listeria monocytogenes* 1/2c	Spinach
7	*Listeria innocua*	Maroilles (Raw milk cheese)	32	*Listeria monocytogenes* 1/2c	Boulette d'Avesnes (cheese)
8	*Listeria innocua*	Cheese	33	*Listeria monocytogenes* 1/2c	Ground beef
9	*Listeria grayi*	Ground beef	34	*Listeria monocytogenes* 1/2a	Sausage from Toulouse
10	*Listeria grayi*	Porc breast	35	*Listeria monocytogenes* 1/2b	Chicken
11	*Listeria grayi*	Munster (raw milk cheese)	36	*Listeria monocytogenes* 1/2b	Gorgonzola (cheese)
12	*Listeria innocua*	Sausage of Montbéliard	37	*Listeria monocytogenes* 1/2c	Smoked halibut
13	*Listeria innocua*	Environmental sample	38	*Listeria monocytogenes* 1/2c	Roquefort (raw milk cheese)
14	*Listeria innocua*	Herring filet	39	*Listeria monocytogenes* 1/2c	Ground beef
15	*Listeria innocua*	Collection (SLCC 2540)	40	*Listeria monocytogenes* 1/2c	Environmental sample
16	*Listeria innocua*	Collection (SLCC 2479)	41	*Listeria monocytogenes* 1/2c	Collection
17	*Listeria grayi*	Collection (ATCC 19114)	42	*Listeria monocytogenes* 1/2a	Cheese
18	*Listeria grayi*	Salad	43	*Listeria monocytogenes* 1/2b	Porc tongue
19	*Listeria grayi*	Environmental sample	44	*Listeria monocytogenes* 1/2b	Frozen french fries
20	*Listeria innocua*	Collection (ATCC 19117)	45	*Listeria monocytogenes* 1/2c	Raw milk cheese
21	*Listeria innocua*	Collection (ATCC 19118)	46	*Listeria monocytogenes* 1/2c	Ground beef
22	*Listeria innocua*	Reblochon (Raw milk cheese)	47	*Listeria monocytogenes* 1/2c	Collection (ATCC 35897)
23	*Listeria innocua*	Spinach	48	*Listeria monocytogenes* 1/2c	Pâté
24	*Listeria innocua*	Fish fillet	49	*Listeria monocytogenes* 1/2c	Ham
25	*Listeria grayi*	Cooked mixed vegetables	50	*Listeria monocytogenes* 1/2a	Salmon

Table E. Complete Exclusivity List

	Strain	Origin		Strain	Origin
1	*Bacillus cereus*	Beets	16	*Klebsiella pneumoniae*	Celery
2	*Bacillus cereus*	Custard	17	*Lactobacillus casei*	Dairy product
3	*Bacillus cereus*	Environmental sample	18	*Lactobacillus plantarum*	Collection
4	*Bacillus coagulans*	Collection	19	*Micrococcus spp.*	Environmental sample
5	*Bacillus sphaericus*	Ground beef	20	*Pseudomonas putida*	Fish
6	*Bacillus stearothermophilus*	Dairy product	21	*Pseudomonas putida*	Mushrooms
7	*Brochotrix thermosphacta*	Ground beef	22	*Rhodococcus equi*	Meat product
8	*Candida albicans*	Collection	23	*Rhodotorula rubra*	Pastries
9	*Corynebacterium flavescens*	Collection (ATCC 10340)	24	*Saccharomyces cerevisiae*	Coffee extract
10	*Enterobacter cloacae*	Egg products	25	*Serratia marcescens*	Raw milk
11	*Enterococcus faecalis*	Collection (ATCC 19433)	26	*Staphylococcus aureus*	Yaourt
12	*Enterococcus faecalis*	Tarama	27	*Staphylococcus epidermidis*	Collection (ATCC 12228)
13	*Enterococcus faecium*	Collection (CIP 5433)	28	*Staphylococcus hyicus*	Meat product
14	*Enterococcus faecium*	Collection	29	*Streptococcus bovis*	Collection
15	*Jonesia denitrificans*	Collection	30	*Streptococcus bovis*	Collection (CIP 5623)

Table F. AOAC OMA Collaborative Study Data Summary

Matrix	Level (CFU/test portion)	N	VIDAS LPT		Reference	X²[a]	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 *Listeria innocua* ATCC 33090	0	156	1	0	0	–	99.4	100	0.6	0	0.01 (-0.02,0.04)	0.00 (-0.02,0.02)
	0.63 (0.49,0.79)	156	80	78	76	0.01	98.7	100	1.3	0	0.01 (-0.10,0.13)	0.01 (-0.10,0.13)
	5.48 (3.60,8.36)	156	156	156	156	–	100	100	0	0	0.00 (-0.02,0.02)	0.00 (-0.02,0.02)
	0	156	1	0	0	–	99.4	100	0.6	0	0.01 (-0.02,0.04)	0.00 (-0.02,0.02)
	0.63 (0.49,0.79)	156	80	78	76	0.01	98.7	100	1.3	0	0.01 (-0.10,0.13)	0.01 (-0.10,0.13)
	5.48 (3.60,8.36)	156	156	156	156	–	100	100	0	0	0.00 (-0.02,0.02)	0.00 (-0.02,0.02)
Queso fresco - 125g *Listeria monocytogenes* ATCC 19115	0	144	0	0	0	–	100	100	0	0	0.00 (-0.03,0.03)	0.00 (-0.03,0.03)
	0.59 (0.46,0.74)	144	70	70	69	0.00	100	100	0	0	0.00 (-0.12,0.12)	0.01 (-0.13,0.13)
	5.41 (3.53,8.30)	144	144	144	144	–	100	100	0	0	0.00 (-0.03,0.03)	0.00 (-0.03,0.03)
	0	144	0	0	0	–	100	100	0	0	0.00 (-0.03,0.03)	0.00 (-0.03,0.03)
	0.59 (0.46,0.74)	144	70	70	69	0.00	100	100	0	0	0.00 (-0.12,0.12)	0.01 (-0.13,0.13)
	5.41 (3.53,8.30)	144	144	144	144	–	100	100	0	0	0.00 (-0.03,0.03)	0.00 (-0.03,0.03)

[a] VIDAS presumptive vs confirmed
[b] VIDAS 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 12/33 – 05/12

Certification date: May 2012

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

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 LPT method could detect 50/50 *Listeria* strains tested after enrichment in LPT broth. For the exclusivity testing, 30/30 non-Listeria strains tested negative by the VIDAS LPT method.

Relative sensitivity (Tables G and H): The purpose of these tests was to evaluate the performance of the VIDAS LPT method with respect to the ISO 11290-1 reference method, on test portions naturally and artificially contaminated with *Listeria,* for the categories falling within the scope. The study evaluated 345 test portions that were inoculated with a wide variety of *Listeria* species and analyzed at a level to achieve approximately 50% positive test portions with the following breakdown: meat – 63, dairy – 60, vegetables – 62, seafood – 66, environmental – 94. The results demonstrated that there are no statistically significant differences between the alternative method (91.2%) and the reference method (92.4%) 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® LPT method, for the studied categories. The results demonstrated that there are no statistically significant differences between the alternative method (0.3-1.1 cfu/25g or mL) and the reference method (0.3-1.5 cfu/25g or mL).

Inter-laboratory Study (Tables J and K): Cottage cheese (25g) was analyzed by seventeen laboratories in this inter-laboratory study. The matrix was artificially contaminated with *Listeria monocytogenes* at two levels: a high level of 30.8 CFU/25g (95% confidence interval of 29.6, 32.0) and a low level of 3.5 CFU/25g (95% confidence interval of 3.0, 4.1). 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 LPT 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 (93.8%) and the reference method (88.7%).

Table G. AFNOR Independent Expert Laboratory Study Summary

	Matrix	Protocol Type	Enrichment broth	Test portion size	Number Positive	Number Negative	Total
Meat	Raw	LPT broth	LPT broth	25g	15	9	24
	Seasoned, prepared for cooking	LPT broth	LPT broth	25g	9	9	18
	Cold cuts, ground meats	LPT broth	LPT broth	25g	9	12	21
				TOTALS	**33**	**30**	**63**
Dairy	Raw milk & raw milk cheese	LPT broth	LPT broth	25g	13	10	23
	Cheese (goat, sheep)	LPT broth	LPT broth	25g	6	10	16
	Yogurts, milk powder	LPT broth	LPT broth	25g	11	10	21
				TOTALS	**30**	**30**	**60**
Vegetables	Frozen	LPT broth	LPT broth	25g	11	5	16
	Fresh or ready-to-eat	LPT broth	LPT broth	25g	10	8	18
	Cooked or seasoned	LPT broth	LPT broth	25g	10	18	28
				TOTALS	31	31	62
Seafood	Fresh fillets & crustaceans	LPT broth	LPT broth	25g	14	6	20
	Smoked fish	LPT broth	LPT broth	25g	7	16	23
	Cooked fish	LPT broth	LPT broth	25g	10	13	23
				TOTALS	31	35	66
Environmental	Process water - general protocol	LPT broth	LPT broth	25mL	10	11	21
	Residue - general protocol	LPT broth	LPT broth	swab or sponge	11	9	20
	Surface sample - specific protocol	LPT broth	LPT broth	swab or sponge	24	29	53
				TOTALS	45	49	94
				OVERALL TOTALS	**170**	**175**	**345**

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

	PA	NA	ND	PD	N	Relative Sensitivity, %	
						VIDAS LPT	Reference
General Protocol	122	146	14	10	292	90.4	93.2
Specific Protocol	20	29	1	3	53	95.8	87.5
Totals:	**142**	**175**	**15**	**13**	**345**	91.2	92.4
	PA	**NA**	**ND**	**PD**	**N**		
Meat products	24	30	5	4	63		
Dairy	25	30	2	3	60		
Vegetable	29	31	2	0	62		
Seafood	25	35	4	2	66		
Environmental (total)	39	49	2	4	94		
General protocol	19	20	1	1	41		
Specific protocol	20	29	1	3	53		

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 LPT Method
Rillettes	25g	*L. monocytogenes*	0.5 (0.3-0.9)	0.5 (0.3-1.1)
Raw milk	25mL	*L. invanovii*	0.6 (0.4-0.9)	0.5 (0.3-1.0)
Red cabbage	25g	*L. welshimeri*	0.7 (0.3-1.5)	0.5 (0.2-1.0)
Smoked salmon	25g	*L. monocytogenes* 1/2a	0.7 (0.4-1.2)	0.4 (0.2-0.8)
Surface samples	sponge	*L. innocua*	0.5 (0.3-1.0)	0.6 (0.4-1.1)

[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 LPT	Reference
Level 0: 0 CFU/25g	0/112	0/112
Low level: 3.5 CFU/25g CI = 3.0 - 4.1	72/112	64/112
High level: 30.8 CFU/25g CI = 29.6 - 32	110/112	108/112

Table K. AFNOR Inter-laboratory Study – Relative Sensitivity

Level	PA	NA	ND	PD	N	Relative Sensitivity, %	
						VIDAS LPT	Reference
Level 0: 0 CFU/25g	0/112	0/112	-	-	96	93.8	88.7
Low level: 3.5 CFU/25g CI = 3.0 - 4.1	72/112	64/112	-	2	96		
High level: 30.8 CFU/25g CI = 29.6 - 32	110/112	108/112	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 LPT method performance parameters. See also inter-laboratory study.

Confirmed result – The qualitative response from the confirmatory phase of the VIDAS LPT 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 LPT 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 LPT method and the reference method are negative.

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

Presumptive result – The qualitative response from the presumptive phase of the VIDAS LPT 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 LPT method and the reference method are positive.

Positive deviation – Result for the VIDAS LPT 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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