DECIDE WITH CONFIDENCE

FOOD SAFETY

VIDAS® *Campylobacter* (CAM)
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® *Campylobacter* (CAM) test method. The VIDAS CAM 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. Additionally, the VIDAS CAM method has been certified NF VALIDATION (AFNOR) as an alternative method for the detection of through the completion of an independent single laboratory study and a multi-laboratory study. All of the validation studies demonstrated that the VIDAS CAM method was statistically equivalent to the corresponding reference method for the matrices tested with 95% confidence.

BIOMÉRIEUX INDUSTRY

AOAC Performance Tested Method: PTM#051201

In May 2011, the VIDAS CAM Assay was granted Performance *Tested Method*℠ (PTM) status by the AOAC Research Institute for the detection of *Campylobacter* in selected foods. The VIDAS CAM method was validated according to AOAC Microbiology Guidelines (2002). Results of the validation study demonstrated the ability of the VIDAS CAM method to: 1) detect 50 different *Campylobacter* strains; 2) correctly show negative results for 30 non-*Campylobacter* 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 CAM method (0, 30, 60, 120 min); and 5) demonstrate no statistically significant differences when compared to a reference method for the following matrices: chicken carcass rinsate (30 mL), turkey carcass (sponge), fresh raw pork (25g), raw chicken breast (25g) processed chicken nuggets (25g). Independent testing included method comparison studies for chicken carcass rinsate (30 mL).

AFNOR – NF Validation: Certificate No: BIO 12/29 – 05/10

In May 2010, the VIDAS CAM Assay was certified NF VALIDATION as an alternative analysis method for the detection of *Campylobacter* in poultry products, other meat products and environmental samples. This validation was obtained by comparison with the reference method described in the international standard ISO 10271-1 according to the standard EN ISO 16140 (2003). In the single laboratory study, the results showed a relative sensitivity level of 98% (confirmed results using CampyFood Agar, CFA) and 94.8 (confirmed results using modified Charcoal Cefoperazone Deoxycholate, mCCDA) for the VIDAS CAM method for all food and environmental testing. In the interlaboratory study, test portions were evaluated by eleven different laboratories. Eight replicate test portions from each of the three contamination levels of matrix were analyzed by VIDAS CAM and the ISO 10271-1 reference method. All presumptive positive test portions were confirmed following direct plating on both CFA and mCCDA agar. The results showed a relative sensitivity level of 92.2% for the VIDAS CAM method.

PERFORMANCE DOCUMENT VIDAS® *Campylobacter* (CAM)

Table of Contents

VIDAS® *Campylobacter* (CAM)

Catalog Number - REF 30 111

Contents of the VIDAS CAM Kit

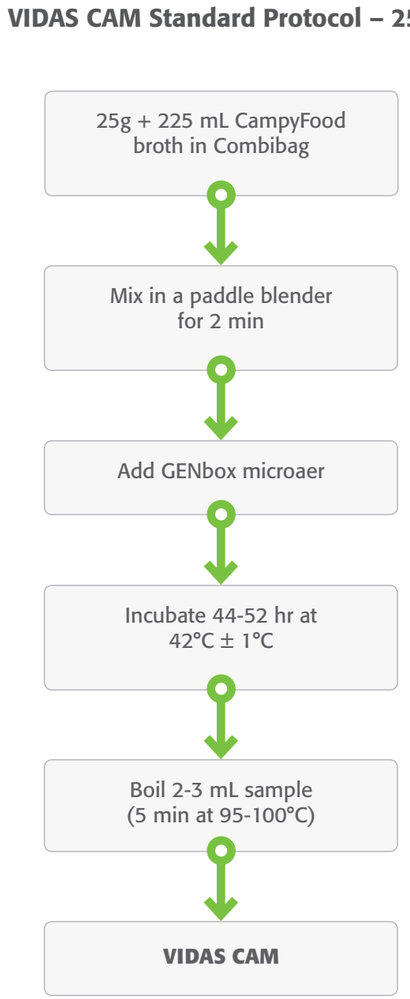
- 60 CAM Strips
- 60 CAM SPR®s
- Standard CAM
- CAM Positive Control
- Negative Control
- 1 MLE Card (Master Lot Entry)
- 1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib

Principle of the Assay

The VIDAS *Campylobacter* assay is an enzyme-linked fluorescent immunoassay (ELFA) for the detection of *Campylobacter* antigen using the automated VIDAS instrument. The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device. The interior of the SPR is coated with anti-*Campylobacter* antibodies. The other reagents for the assay are ready-to-use and predispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. Part of the enrichment broth is dispensed into the reagent strip. The antigens present will bind to the anti-*Campylobacter* antibodies coating the interior of the SPR. Unbound sample components are washed away. Antibodies conjugated with alkaline phosphatase are cycled in and out of the SPR and will bind to any *Campylobacter* antigens which are bound to the antibodies on the SPR wall. Unbound conjugate is eliminated during the washing steps. During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methylumbelliferone) the fluorescence of which is measured at 450 nm. At the end of the assay, the results are analyzed automatically by the instrument. A test value, which is compared to stored standards (thresholds) and an interpretation (positive, negative) are generated for each sample.

PERFORMANCE DOCUMENT VIDAS® *Campylobacter* (CAM)

Figure 1. Flow diagrams showing the VIDAS CAM standard protocol



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.

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.

Table A. Validation Study Technical Requirements

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

AOAC PTM Validation Study

PTM Certification#: 051201

PTM Certified: May 2011

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

Reference methods: USDA/FSIS Microbiological Laboratory Guidebook (41.00, Jan. 2010) and ISO 10272-1 Standard, Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method

Method Comparison (Tables B and C): The method comparison study was performed at both the method developer (internal) and independent laboratories. For each matrix, twenty replicates at one inoculation level (0.2-2 cfu/25g) and 5 uninoculated replicates were tested by both the VIDAS CAM and appropriate reference method. Primary enrichments for each method were confirmed using reference method agar (mCCDA for food products or Campy-Cefex for carcass rinse and turkey sponge) and CampyFood Agar (CFA).

In the method comparison studies there were no significant differences between the VIDAS CAM method and the reference methods using unpaired Chi-square or the POD test at 5% level for any of the matrices evaluated.

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

Lot-to-lot/Stability: Stability and lot-to-lot variation of the VIDAS CAM method was evaluated over 12 months at 2-8°C using *Campylobacter* and non-*Campylobacter* 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 CAM method (0, 30, 60, 120 min). There were no differences seen in the number of positive results with each of the variations in the protocol.

Table B. AOAC PTM Method Developer Method Comparison Study Details

Matrix	Test portion size(s)	Inocluating Organism	Reference Method Comparison
Chicken carcass rinse	30 mL	*C. coli* ATCC 33559	USDA/FSIS 41.00
Turkey carcass	sponge	*C. jejuni* ATCC 29428	USDA/FSIS 41.00
Fresh raw pork	25g	*C. lari* ATCC 43675	ISO 10272-1
Raw chicken breast	25g	*C. jejuni* ATCC 33291	ISO 10272-1
Frozen chicken nuggets	25g	*C. coli BAA* 1061	ISO 10272-1

Table C. AOAC PTM Independent Expert Laboratory Study – Internal Data

INTERNAL LABORATORY DATA	Test portion size	Enrichment broth	VIDAS CAM		Reference	X^2	Sensitivity, %	Specificity, %	False pos, %	False neg, %	dPOD CI	
			Presumptive	Confirmed							P vs C[a]	C vs R[b]
Chicken carcass rinse	30 mL	CFB	13	13	13	0.00	100	100	0	0	0 [-0.28, 0.28]	0 [-0.28, 0.28]
Turkey carcass	sponge	CFB	16	16	14	0.52	100	100	0	0	0 [-0.25, 0.25]	0.1 [-0.17, 0.35]
Fresh raw pork	25g	CFB	16	16	15	0.14	100	100	0	0	0 [-0.25, 0.25]	0.05 [-0.21, 0.30]
Raw chicken breast	25g	CFB	18	18	13	3.49	100	100	0	0	0 [-0.21, 0.21]	0.25 [-0.01, 0.48]
Processed chicken nuggets	25g	CFB	3	3	5	0.61	100	100	0	0	0 [-0.23, 0.23]	-0.1 [-0.34, 0.15]
INDEPENDENT LABORATORY DATA	**Test portion size**	**Enrichment broth**	**VIDAS CAM**		**Reference**	**X^2**	**Sensitivity, %**	**Specificity, %**	**False pos, %**	**False neg, %**	**dPOD CI**	
			Presumptive	**Confirmed**							**P vs C[a]**	**C vs R[b]**
Chicken carcass rinse	30 mL	CFB	14	14	12	0.43	100	100	0	0	0 [-0.27, 0.27]	0.1 [-0.18, 0.36]

[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

	Organisms	*Source*		*Organisms*	*Source*
1	*Campylobacter jejuni*	Turkey	26	*Campylobacter jejuni*	Poultry
2	*Campylobacter coli*	Turkey	27	*Campylobacter jejuni*	Poultry
3	*Campylobacter coli*	Turkey	28	*Campylobacter jejuni*	Poultry
4	*Campylobacter jejuni*	Turkey	29	*Campylobacter jejuni*	Poultry
5	*Campylobacter coli*	Turkey	30	*Campylobacter jejuni*	Poultry
6	*Campylobacter jejuni*	Turkey	31	*Campylobacter jejuni*	Poultry
7	*Campylobacter coli*	Turkey	32	*C. jejuni s.jejuni*	Poultry carcass
8	*Campylobacter jejuni*	Turkey	33	*C. jejuni s.jejuni*	Chicken filet
9	*Campylobacter coli*	Turkey	34	*C. jejuni s.jejuni*	Chicken neck peal
10	*Campylobacter coli*	Turkey	35	*C. jejuni s.jejuni*	Chicken filet
11	*Campylobacter jejuni*	Turkey	36	*Campylobacter jejuni*	Turkey
12	*Campylobacter jejuni*	Turkey	37	*Campylobacter jejuni*	Turkey
13	*Campylobacter jejuni*	Turkey	38	*Campylobacter jejuni*	Turkey
14	*Campylobacter jejuni*	Turkey	39	*Campylobacter jejuni*	Turkey
15	*Campylobacter coli*	Turkey	40	*Campylobacter lari*	Collection
16	*Campylobacter coli*	Turkey	41	*Campylobacter fetus*	Collection
17	*Campylobacter jejuni*	Turkey	42	*Campylobacter fetus*	Collection
18	*Campylobacter coli*	Turkey	43	*C. upsaliensis*	Collection
19	*Campylobacter jejuni*	Poultry	44	*C. jejuni doylei*	Collection
20	*Campylobacter coli*	Poultry	45	*C. lari*	Collection
21	*Campylobacter jejuni*	Poultry	46	*C. lari*	Collection
22	*Campylobacter jejuni*	Poultry	47	*C. lari subsp lari*	Collection
23	*Campylobacter jejuni*	Poultry	48	*C. jejuni doylei*	Collection
24	*Campylobacter jejuni*	Poultry	49	*Campylobacter lari*	Hospital
25	*Campylobacter jejuni*	Poultry	50	*C. upsaliensis*	Hospital

Table E. Complete Exclusivity List

	Organisms	*Source*
1	*Bacillus cereus*	Egg
2	*Bacillus mycoïdes*	Collection
3	*Bacillus pumilus*	Custard
4	*Enterobacter agglomerans*	Porc breath
5	*Enterobacter cloacae*	Environmental surface
6	*Enterobacter amnigenus*	Ham
7	*Klebsiella oxytoca*	Collection
8	*Hafnia alvei*	Ground beef
9	*Pseudomonas aeruginosa*	Fish filet
10	*Pseudomonas putida*	Collection
11	*Pseudomonas putida*	Collection
12	*Pseudomonas fluorescens*	Vegetable
13	*Proteus mirabilis*	Meat
14	*Staphylococcus aureus*	CIP 7625
15	*Staphylococcus epidermidis*	Smoked salmon
16	*Aeromonas hydrophila*	Collection
17	*Acinetobacter baumanii*	Minced pork meat
18	*Escherichia coli*	Persil
19	*Salmonella hadar*	Poultry
20	*Citrobacter freundii*	Vegetable
21	*Shigella flexneri*	Collection
22	*Escherichia hermanii*	Feed
23	*Pseudomonas fluorescens*	Mineral water
24	*Acinetobacter calcoaeceticus*	Collection
25	*A. cryo*	Collection
26	*Arcobacter butzleri*	Collection
27	*Proteus vulgaris*	Collection
28	*Helicobacter pylori*	Clinical
29	*Helicobacter pylori*	Clinical
30	*Vibrio parahaermolyticus*	Collection

AFNOR NF Validation Study

Certificate No: BIO 12/29 – 05/10

Certification date: May 2010

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

Reference Method: ISO 10272-1 Standard, Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method.

Independent Expert Laboratory Study

Inclusivity/exclusivity (Tables D and E): The inclusivity studies demonstrated that the VIDAS CAM method could detect all 52 *Campylobacter* strains tested. For the exclusivity testing, 32 non-*Campylobacter* strains tested negative by the VIDAS CAM method.

Relative sensitivity (Tables F and G): The purpose of these tests was to evaluate the performance of the VIDAS CAM method with respect to the ISO 10272 reference method, on test portions naturally and artificially contaminated with *Campylobacter,* for the categories falling within the scope. The study evaluated 418 test portions that were inoculated with a variety of *Campylobacter* strains and analyzed at a level to achieve approximately 50% positive test portions with the following breakdown: poultry – 64, other meat – 146, environmental – 208. The results demonstrated that there are no statistically significant differences between the alternative method (96.4%) and the reference method (87.2%) when analyzing sensitivity values.

Relative level of detection (Table H): 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 CAM 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 (0.2-2.4 cfu/25g).

Inter-laboratory Study (Tables I and J): Poultry minced meat (25g) was analyzed by eleven laboratories in this inter-laboratory study. The matrix was artificially contaminated with *Campylobacter* at two levels: a high level of 26 CFU/25g and a low level of 4.4 CFU/25g. 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 CAM and the ISO 10272 reference method due to different test portion enrichments for each method. All test portions were confirmed following plating onto both CFA and mCCDA. The results demonstrated that there are no statistically significant differences between the alternative method (92.2%) and the reference method (43.1%) when analyzing sensitivity values.

Table F. AFNOR Independent Expert Laboratory Study Summary

	Matrix	Enrichment broth	Test portion size	Number Positive	Number Negative	Total
Poultry	Raw, frozen	CFB	25g	15	14	29
	Prepared with poultry, raw or cooked	CFB	25g	4	10	14
	Rinsed carcasses	CFB	25mL	8	0	8
	Skin of poultry neck	CFB	25g	9	4	13
				36	**28**	**64**
Other meat	Raw meat	CFB	25g	20	27	47
	Raw, flavored meat	CFB	25g	7	12	19
	Read-made meal	CFB	25g	6	10	16
				33	**49**	**146**
Environment	Various waters	CFB	25mL	8	11	19
	Surface samples	CFB	sponge or swab	13	12	25
	Residues & scraps	CFB	25g	9	9	18
				30	**32**	**208**
			TOTALS	**99**	**109**	**418**

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

	PA	NA	ND	PD	N	Relative Sensitivity, %	
						VIDAS CAM	Reference
Confirmed using CFA	83	109	2	14	208	98	85.9
Confirmed using mCCDA	80	112	5	11	208	94.8	88.5
	PA	**NA**	**ND**	**PD**	**N**		
Isolated on CFA	**83**	**109**	**2**	**14**	**208**		
Poultry	31	28	0	5	64		
Other meat	28	49	1	4	82		
Environmental	24	32	1	5	62		
Isolated on CFA	**80**	**112**	**5**	**11**	**208**		
Poultry	28	29	3	4	64		
Other meat	28	50	1	3	82		
Environmental	24	33	1	4	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 H. AFNOR Independent Expert Laboratory Study Data Summary – Relative Level of Detection Limit

Matrix	Test portion size	Strain	Relative detection level (CFU/25g or 375g) with confidence interval[a] LOD_{50}	
			Reference Method	VIDAS CAM Method
Poultry meat	25g	*C. jejuni*	0.9 [0.3, 2.4]	0.7 [0.3, 1.8]
Pork	25g	*C. jejuni*	0.9 [0.4, 1.8]	0.9 [0.4, 1.8]
Process water	25mL	*C. coli*	0.3 [0.2, 0.4]	0.3 [0.2, 0.4]

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

Table I. AFNOR Inter-laboratory Study – Testing Results

Level	# positive/total	
	VIDAS CAM	Reference
Level 0 (0 CFU/25g)	5/88	15/88
Low level (4.4 CFU/25g)	78/88	23/88
High level (26 CFU/25g)	85/88	41/88

Table J. AFNOR Inter-laboratory Study – Relative Sensitivity

Level	PA	NA	ND	PD	N	Relative Sensitivity, %	
						VIDAS CAM	Reference
Level 0 (0 CFU/25g)	64	83	14	103	264	92.2	43.1
Low level (4.4 CFU/25g)							
High level (26 CFU/25g)							

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 CAM method performance parameters. See also inter-laboratory study.

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

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

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

Positive deviation – Result for the VIDAS CAM 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.

For more information, please contact your local bioMérieux Account Representative.

www.bioMerieux-usa.com/phage