DECIDE WITH CONFIDENCE

FOOD SAFETY

VIDAS® UP *E. coli* O157 (Including H7) (ECPT)
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 *E. coli* O157 (including H7) (ECPT) test method. The VIDAS ECPT 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 ECPT method has been certified NF VALIDATION (AFNOR) as an alternative method for the detection of *E. coli* O157 (including H7) through the completion of an independent single laboratory study and a multi-laboratory study. All of the validation studies demonstrated that the VIDAS ECPT method was statistically equivalent to the corresponding reference method for the matrices tested with 95% confidence.

BIOMÉRIEUX INDUSTRY

AOAC Performance Tested Method: PTM#060903

In June 2009, the VIDAS ECPT Assay was granted *Performance Tested Method*℠ (PTM) status by the AOAC Research Institute for the detection of *E. coli* O157 (including H7) in selected foods. The VIDAS ECPT method was validated according to AOAC Microbiology Guidelines (2002). Results of the validation study demonstrated the ability of the VIDAS ECPT method to: 1) detect 55 different *E. coli* O157 (including H7) strains; 2) correctly show negative results for 42 non-*E. coli* O157 strains; 3) support a shelf life of 12 months from date of manufacturing and quality of the test kit; 4) perform appropriately after varied protocol parameters including enriched sample boiling time (2, 5, 10 min), sample temperature after boiling (10, 25, 50°C) and sample volume analyzed by VIDAS (450, 500, 550µL); and 5) demonstrate no statistically significant differences when compared to a reference method for the following matrices: ground beef (25, 75, 375g), beef trim (25, 75, 375g) bagged lettuce (25g), fresh spinach (25g) and irrigation water (25 mL). Independent testing included a method comparison study for ground beef (25g). A matrix extension was approved in January 2011 adding frozen cookie dough (25g) to the existing PTM certification.

AFNOR – NF Validation: Certificate No: BIO 12/25 – 05/09

In May 2009, the VIDAS ECPT Assay was certified NF VALIDATION as an alternative analysis method for the detection of E. coli O157 (including H7) in raw beef (25g and 375g). This validation was obtained by comparison with the reference method described in the international standard ISO 16654 according to the standard EN ISO 16140 (2003). In the single laboratory study, the results showed a relative sensitivity level of 96.3% for the VIDAS ECPT method. 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 ECPT and the ISO 16654 reference method. All presumptive positive test portions were confirmed following direct plating on selective and chromogenic agar as well as using VIDAS ICE followed by plating on selective and chromogenic agar. The results showed a relative sensitivity level of 98.3% for the VIDAS ECPT method. A matrix extension was approved in December 2009 adding additional foods and environmental matrices to the existing AFNOR certification.

PERFORMANCE DOCUMENT VIDAS® UP *E. coli* O157 (Including H7) (ECPT)

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VIDAS® UP *E. coli* O157 (including H7) (ECPT)

Catalog Number - REF 30 122

Contents of the VIDAS ECPT Kit

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

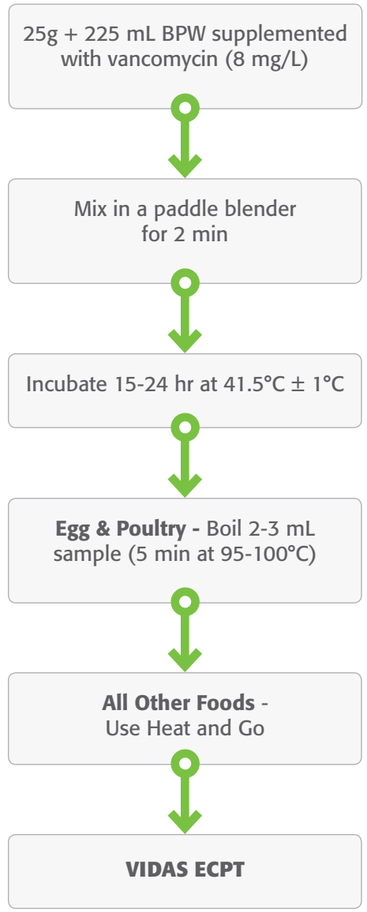
Principle of the Assay

VIDAS® ECPT is an automated assay based on the ELFA technique (Enzyme-Linked Fluorescent Assay) for use on the VIDAS family of instruments. The Solid Phase Receptacle (SPR), serves as the solid phase as well as the pipetting device. The interior of the SPR is coated with recombinant phage tail fiber protein for the capture of *E. coli* O157 including H7. Reagents for the assay are ready-to-use and pre-dispensed 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 *E. coli* O157 including H7 present are captured by the recombinant phage protein coating the interior of the SPR. Unbound components are eliminated during the washing steps. Alkaline phosphatase conjugate is then cycled in and out of the SPR and will bind to any *E. coli* O157 including H7 which are themselves bound to the phage protein on 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 this substrate into a fluorescent product (4-Methyl- umbelliferone) 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® UP *E. coli* O157 (Including H7) (ECPT)

Figure 1. Flow diagrams showing the VIDAS ECPT standard protocol

VIDAS ECPT Standard Protocol – 25g



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 90s 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 PTM	AFNOR
Method Developer	●	
Independent	●	●
Collaborative		●

AOAC PTM Validation Study

PTM Certification#: 060903

PTM Certified: June 2009

PTM Matrix Extension Certified: January 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 ECPT and appropriate reference method. Primary enrichments for each method were confirmed using CT-SMAC Agar and chromID® O157:H7 as well as VIDAS ICE followed by plating onto CT-SMAC Agar and chromID® O157:H7.

A matrix extension study was performed on one matrix, frozen cookie dough (25g). Twenty replicates at one inoculation level (0.2-2 cfu/25g) and 5 uninoculated replicates were tested by both the VIDAS ECPT and US-FDA reference method. Primary enrichments for each method were confirmed using CT-SMAC Agar and chromID® O157:H7 as well as VIDAS ICE followed by plating onto CT-SMAC Agar and chromID® O157:H7.

In the method comparison studies there were no significant differences between the VIDAS ECPT method and the reference methods using unpaired Chi-square or the POD test at 5% level for the majority of the matrices evaluated. The two matrices that showed a significant difference, with the VIDAS ECPT method resulting in a higher number of positive test portions, beef trim (25g) and bagged lettuce (25g).

Inclusivity/Exclusivity (Tables D and E): The inclusivity studies demonstrated that the VIDAS ECPT method could detect all 55 *E. coli* O157 strains in two different enrichment conditions: buffered peptone water (BPW) and BPW plus vancomycin, cefixime and cefsulodine (VCC). For the exclusivity testing, all 42 non-*E. coli* O157 strains tested negative by the VIDAS ECPT method.

Lot-to-lot/Stability: Stability and lot-to-lot variation of the VIDAS ECPT method was evaluated over 12 months at 2-8°C using *E. coli* O157 and non-*E. coli* O157 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 (2, 5, 10 min), sample temperature after boiling (10, 25, 50°C) and sample volume analyzed by VIDAS (450, 500, 550µL). There were no differences seen in 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
Ground beef	25g	*E. coli* O157:H7 BMX 104322	USDA/FSIS MLG 5.04
Ground beef - Independent	25g	*E. coli* O157:H7 ATCC 43895	USDA/FSIS MLG 5.04
Ground beef	75g	*E. coli* O157:H7 ATCC 35150	USDA/FSIS MLG 5.04
Ground beef	375g	*E. coli* O157:H7 ATCC 35150	USDA/FSIS MLG 5.04
Beef trim	25g	*E. coli* O157:H7 ATCC 700728	USDA/FSIS MLG 5.04
Beef trim	75g	*E. coli* O157:H7 ATCC 35150	USDA/FSIS MLG 5.04
Beef trim	375g	*E. coli* O157:H7 ATCC 35150	USDA/FSIS MLG 5.04
Bagged lettuce	25g	*E. coli* O157:H7 FSC-CC 435	USFDA BAM Chapter 4a
Fresh spinach	25g	*E. coli* O157:H7 FSC-CC 1825	USFDA BAM Chapter 4a
Irrigation water	25mL	*E. coli* O157:H7 FSC-CC 1912	USFDA BAM Chapter 4a

Table C. AOAC PTM Method Comparison Study Results

INTERNAL LABORATORY DATA	Test portion size	Enrichment broth	VIDAS ECPT		Reference	X^2	Sensitivity, %	Specificity, %	False pos, %	False neg, %	dPOD CI	
			Presumptive	Confirmed							P vs C[a]	C vs R[b]
Ground beef	25g	BPW	16	16	11	2.78	100	100	0	0	0 [-0.25, 0.25]	0.25 [-0.04, 0.49]
	75g	BPW + Vanco (8 mg/L)	10	10	13	0.90	100	100	0	0	0 [-0.28, 0.28]	-0.15 [-0.41, 0.25]
	375g	BPW + Vanco (8 mg/L)	11	10	11	0.10	100	93	7	0	0.05 [-0.24, 0.33]	-0.05 [-0.33, 0.24]
Beef Trim	25g	BPW	20	20	13	8.27	100	100	0	0	0 [-0.16, 0.16]	0.35 [0.12, 0.57]
	75g	BPW + Vanco (8 mg/L)	14	14	14	0.00	100	100	0	0	0 [-0.27, 0.27]	0 [-0.27, 0.27]
	375g	BPW + Vanco (8 mg/L)	9	9	8	0.10	100	100	0	0	0 [-0.28, 0.28]	0.05 [-0.24, 0.33]
Bagged lettuce	25g	BPW + Vanco (8 mg/L)	11	12	5	3.66	92	100	0	8	-0.05 [-0.33, 0.24]	0.35 [0.04, 0.58]
Fresh spinach	25g	BPW + Vanco (8 mg/L)	12	12	12	0.00	100	100	0	0	0 [-0.28, 0.28]	0 [-0.28, 0.28]
Irrigation water	25mL	BPW + Vanco/ Cefixime/ Cefsulodin	15	15	17	0.60	100	100	0	0	0 [-0.26, 0.26]	-0.10 [-0.34, 0.15]
Frozen cookie dough	25g	BPW + Vanco (8 mg/L)	19	19	17	1.08	100	100	0	0	0 [-0.19, 0.19]	0.10 [-0.11, 0.31]
INDEPENDENT LABORATORY STUDY	**Test portion size**	**Enrichment broth**	**VIDAS ECPT**		**Reference**	**X^2**	**Sensitivity, %**	**Specificity, %**	**False pos, %**	**False neg, %**	**dPOD CI**	
			Presumptive	**Confirmed**							**P vs C[a]**	**C vs R[b]**
Ground beef	25g	BPW	6	6	3	1.26	100	100	0	0	0 [-0.27, 0.27]	0.15 [-0.11, 0.39]

[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

	Code[a]	Organisms	Source		Code[a]	Organisms	Source
1	ENV 6352	E. coli O157:H7	Unknown	52	Ec 11	E. coli O157:H7	Clinical origin
2	ENV 36153	E. coli O157:H7	Ground beef	53	Ec 12	E. coli O157:H7	Clinical origin
3	ENV 448	E. coli O157:H7	Beef	54	Ec 22	E. coli O157:H7	ATCC 43888 (human feces)
4	ENV BIO	E. coli O157:H7	Unknown	55	Ec 24	E. coli O157:H7	Collection P1446
5	ENV B 76	E. coli O157:H7	Unknown	56	Ec 25	E. coli O157:H7	Collection P1524
6	ENV B 77	E. coli O157:H7	Unknown	57	Ec 30	E. coli O157:H7	Milk
7	ENV FCH 6	E. coli O157:H7	Unknown	58	Ec 31	E. coli O157:H7	ATCC 43895 (hamburger)
8	ENV LS 24	E. coli O157:H7	Unknown	59	Ec 40	E. coli O157:H7	ATCC 35150 (human origin)
9	ENV LS 29	E. coli O157:H7	Human	60	Ec 41	E. coli O157:H7	ATCC 43894 (human origin)
10	ENV LZ 30	E. coli O157:H7	Unknown	61	Ec 42	E. coli O157:H7	ATCC 43890 (human feces)
11	ENVLS 240	E. coli O157:H7	Unknown	62	Ec 43	E. coli O157:H7	ATCC 43889 (human origin)
12	ENV 380214	E. coli O157:H7	Unknown	63	Ec 44	E. coli O157:H7	ATCC 46197
13	ENV 39239	E. coli O157:H7	Unknown	64	Ec 45	E. coli O157:H7	Collection
14	ENV B182	E. coli O157:H7	Unknown	65	Ec 46	E. coli O157:H7	Collection
15	ENV B184	E. coli O157:H7	Unknown	66	Ec 47	E. coli O157:H7	Collection
16	ENV 05117C	E. coli O157:H7	Unknown	67	Ec 48	E. coli O157:H7	Collection
17	ENV 42197	E. coli O157:H7	Unknown	68	Ec 49	E. coli O157:H7	Collection
18	ENV V9	E. coli O157:H7	Unknown	69	Ec 50	E. coli O157:H7	Collection
19	ENV 166	E. coli O157b	Environmental	70	Ec 52	E. coli O157:H7	Clinical origin
20	ENV LS27	E. coli O157c	Food	71	Ec 55	E. coli O157:H7	Environment
21	ENV LS3	E. coli O157:H7	Human feces	72	Ec 56	E. coli O157:H7	Environment
22	ENA 33-35V	E. coli O157:H7	Bovine feces	73	Ec 57	E. coli O157:H7	Clinical origin
23	ENV B 68	E. coli O157:H7	Bovine slaughter	74	Ec 58	E. coli O157:H7	Clinical origin
24	ENV B177	E. coli O157:H7	Bovine slaughter	75	Ec 59	E. coli O157:H7	Clinical origin
25	ENV B188	E. coli O157:H7	Bovine slaughter	76	Ec 61	E. coli O157:H7	Clinical origin
26	ENV 143	E. coli O157:H7	Environmental	77	EC 63	E. coli O157:H7	Slaughterhouse environment
27	ENV 185	E. coli O157:H7	Environmental	78	Ec 65	E. coli O157:H7	Slaughterhouse environment
28	ENV LS 2	E. coli O157:H7	Food	79	Ec 66	E. coli O157:H7	Slaughterhouse environment
29	ENV LS 4	E. coli O157:H7	Human feces	80	EC 67	E. coli O157:H7	Slaughterhouse environment
30	ENV LS23	E. coli O157:H7	Sliced meat	81	EC 68	E. coli O157:H7	Slaughterhouse environment
31	ENV LS25	E. coli O157:H7	Ground beef	82	EC 69	E. coli O157:H7	Feces
32	ENV LS28	E. coli O157:H7	Food	83	EC 70	E. coli O157:H7	Clinical origin
33	ENV LS32	E. coli O157:H7	Human	84	EC 72	E. coli O157:H7	Slaughterhouse environment
34	ENV LS 56	E. coli O157:H7	Human feces	85	EC 73	E. coli O157:H7	Collection
35	ENV MKB	E. coli O157:H7	Ground beef	86	EC 74	E. coli O157:H7	Feces of bovine
36	ENV R33-9	E. coli O157:H7	Bovine feces	87	EC 75	E. coli O157:H7	Clinical origin
37	ENV 435	E. coli O157:H7	Beef	88	EC 76	E. coli O157:H7	Clinical origin
38	ENV 267	E. coli O157:H7	Unknown	89	EC 77	E. coli O157:H7	Clinical origin
39	ENV 258 ID1	E. coli O157:H7	Unknown	90	EC 78	E. coli O157:H7	Clinical origin
40	ENV 258 ID3	E. coli O157:H7	Unknown	91	EC 79	E. coli O157:H7	Clinical origin
41	BM 98.12.916	E. coli O157:H7	Unknown	92	EC 80	E. coli O157:H7	Chopped meat of beef
42	BM 98.12.924	E. coli O157:H7	Unknown	93	EC 81	E. coli O157:H7	Pork
43	BM 98.12.927	E. coli O157:H7	Unknown	94	EC 82	E. coli O157:H7	Beef
44	BM 261	E. coli O157:H7	Unknown	95	EC 83	E. coli O157:H7	Cider
45	ATCC 43888	E. coli O157:H7	Human feces	96	MEU 29	E. coli O157:H4	Clinical origin
46	Ec 3	E. coli O157:H7	Feces	97	Ec 51	E coli O157 H7-	Feces
47	Ec 5	E. coli O157:H7	Collection	98	Ec 53	E coli O157 H7-	Environment
48	Ec 6	E. coli O157:H7	Collection	99	Ec 54	E coli O157 H7-	Environment
49	Ec 7	E. coli O157:H7	Environment	100	Ec 60	E coli O157 H7-	Collection
50	Ec 9	E. coli O157:H7	Clinical origin	101	Ec 62	E coli O157 H7-	Environment
51	Ec 10	E. coli O157:H7	Clinical origin				

[a] Numbers 1-45 were evaluated in the PTM Study, 46-101 were evaluated in the AFNOR study
[b] Non motile uida(+) ; stx2(+) ; eae (+)
[c] Non motile uida(+) ; stx1(+) ; stx2(+) ; eae (+)

Table E. Complete Exclusivity List

	Organisms[a]	Source		Organisms	Source
1	*E. coli* O166:H28	Wastewater slurry	47	*E. coli* O121:H19	Clinical origin
2	*E. coli* O113 :H4	Treatment clarifier	48	*E. coli* O156	Clinical origin
3	*E. coli* O2 :H27	Plant slurry pit	49	*E. coli* O113:H21	Clinical origin
4	*E. coli* O76 :H19	Plant aerator	50	*E. coli* O153:H25	Clinical origin
5	*E. coli* O141 :H4	Pig feces	51	*E. coli* O103:H2	Clinical origin
6	*E. coli* Ont :H19	Pig feces	52	*E. coli* O26:H11	Clinical origin
7	*E. coli* O121 :H-	Pig feces	53	*E. coli* O111:H8	Clinical origin
8	*E. coli* O174:H21	Herd manure	54	*E. coli* O111	Clinical origin
9	*E. coli* O8:H19	Herd manure	55	*E. coli* O111:B4	Clinical origin
10	*E. coli* O129 :H25	Raw milk cheese	56	*E. coli* O103:H3	Clinical origin
11	*E. coli* O22 :H8	Raw milk cheese	57	*E. coli* O111:B4	Clinical origin
12	*E. coli* O145	Unknown	58	*E. coli* O55:H7	Collection CIP 105228
13	*E. coli* O26	Cheese	59	*E. coli* O26:H11	Clinical origin
14	*E. coli* O26	Animal Feed	60	*E. coli* O26:H11	Clinical origin
15	*E. coli* O26	Water	61	*E. coli* O111	Clinical origin
16	*E. coli* O55 :H7	Unknown	62	*E. coli* O103	Clinical origin
17	*E. coli* O111 :H8	Human Feces	63	*E. coli* O111	Clinical origin
18	*E. coli* O103 :H8	Feces	64	*E. coli* O26	Clinical origin
19	*Citrobacter freundii*	Unknown	65	*E. coli* O111	Clinical origin
20	*Citrobacter freundii*	Unknown	66	*E. coli* O111	Clinical origin
21	*Hafnia alvei*	Unknown	67	*E. coli* O111	Clinical origin
22	*Proteus valgaris*	Unknown	68	*Salmonella Urbana*	Beef
23	*E. sakazakii*	Unknown	69	*Salmonella Soeranga*	Soya
24	*Salmonella Enteritidis*	Unknown	70	*Salmonella Hilversar*	Collection
25	*Salmonella Hadar*	Unknown	71	*E coli*	Parsley
26	*Salmonella Muenchen*	Unknown	72	*E coli*	"Crépinette"
27	*Salmonella Newport*	Unknown	73	*E coli*	"Crépineaux"
28	*Salmonella Typhimurium*	Animal tissue	74	*E coli*	Pork's(Pig) kidney
29	*Salmonella Worthington*	Unknown	75	*E coli*	Sausage meat
30	*Salmonella Sinburry*	Unknown	76	*E coli*	Pork's liver
31	*Salmonella Tallahasee*	Unknown	77	*E coli*	Sausage of calf
32	*S. aureus*	Pleural fluid	78	*E coli*	Reblochon
33	*S. aureus*	Unknown	79	*E coli*	Spinach
34	*Klebsiella oxytoca*	Seafood	80	*E coli*	Tomme cheese
35	*Enterobacter cloacae*	Pork sausage	81	*E coli*	Tomato
36	*Salmonella bongori*	Unknown	82	*E coli*	Celeriac in remoulade dressing
37	*Klebsiella pneumoniae*	Unknown	83	*E coli*	Vanilla cream
38	*Serratia liquefaciens*	Milk	84	*E coli*	Chipolata
39	*Shigella boydii*	Unknown	85	*Citrobacter freundii*	Vegetables
40	*Shigella dysenteriae*	Human	86	*Citrobacter freundii*	Meat product
41	*Shigella flexneri*	Human feces	87	*Citrobacter freundii*	Fish
42	*Shigella sonnei*	Unknown	88	*Citrobacter freundii*	Milk
43	*E. coli* O139:K82	Clinical origin	89	*Citrobacter diversus*	Dried herbs
44	*E. coli* O128:B12	Clinical origin	90	*Hafnia alvei*	Minced meat
45	*E. coli* O26:H11	Clinical origin	91	*Hafnia alvei*	Pork's spinal column
46	*E. coli* O91:H21	Clinical origin	92	*Escherichia hermanii*	Collection

[a]Numbers 1-42 were evaluated in the PTM Study, 43-92 were evaluated in the AFNOR study

PERFORMANCE DOCUMENT VIDAS® UP *E. coli* O157 (Including H7) (ECPT)

AFNOR NF Validation Study

Certificate No: BIO 12/25 – 05/09

Certification date: May 2009

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

Reference Method: ISO 16654 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157

Independent Expert Laboratory Study

Inclusivity/exclusivity (Tables D and E): The inclusivity studies demonstrated that the VIDAS ECPT method could detect all 56 *E. coli* O157 strains tested after enrichment in both BPW and BPW+VCC. For the exclusivity testing, 50 non-*E. coli* O157 strains tested negative by the VIDAS ECPT method.

Relative sensitivity (Tables F and G): The purpose of these tests was to evaluate the performance of the VIDAS ECPT method with respect to the ISO 16654 reference method, on test portions naturally and artificially contaminated with *E. coli* O157, for the categories falling within the scope. The study evaluated 496 test portions that were inoculated with a wide variety of *E. coli* O157 strains and analyzed at a level to achieve approximately 50% positive test portions with the following breakdown: raw beef and raw veal – 166, raw milk/raw milk cheese – 76, raw vegetables – 61, environmental – 63, other (including raw meats) – 100. The results demonstrated that there are no statistically significant differences between the alternative method (average of 95.4% for all protocols) and the reference method (average of 98.2%) 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 ECPT method, for the studied categories. The results demonstrated that there are no statistically significant differences between the alternative method (0.2-1.6 cfu/25g) and the reference method (0.2-1.6 cfu/25g).

Inter-laboratory Study (Tables I and J): Ground beef (25g) was analyzed by eleven laboratories in this inter-laboratory study. The matrix was artificially contaminated with *E. coli* O157 at two levels: a high level of 46 CFU/25g and a low level of 4.3 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 ECPT and the ISO 16654 reference method due to different test portion enrichments for each method. All test portions were confirmed following plating onto CT-SMAC Agar and chromID® O157:H7 as well as VIDAS ICE followed by plating onto CT-SMAC Agar and chromID® O157:H7. The results demonstrated that there are no statistically significant differences between the alternative method (98.3%) and the reference method (99.4%) when analyzing sensitivity values.

Table F. AFNOR Independent Expert Laboratory Study Summary

Matrix	Protocol Type	Enrichment broth	Test portion size	Number Positive	Number Negative	Total
Raw beef and raw veal	Specific 1	1/10 w/o vancomycin	25g	40	31	71
Raw beef and raw veal	Specific 2	1/10 with vancomycin	25g	30	34	64
Raw beef and raw veal	Specific 3	1/4 with vancomycin	50-375g	30	31	61
Raw milk, raw milk cheese	Specific 4	1/10 with vancomycin, cefixime, cefsulodin	25g	46	30	76
Environmental	Specific 4	1/10 with vancomycin, cefixime, cefsulodin	25g	32	31	63
Raw vegetables	Specific 5	1/10 with vancomycin	25g	30	31	61
Other products (including raw meats)	General	1/10 with vancomycin	25g	70	30	100
			TOTALS	**278**	**218**	**496**

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

	PA	NA	ND	PD	N	Relative Sensitivity, %	
						VIDAS ECPT	Reference
Raw beef and raw veal - 375g	29	31	1	0	61	96.7	100
Raw beef and raw veal - 25g (w/o vancomycin)	38	31	1	1	71	97.5	97.5
Raw beef and raw veal - 25g (w/ vancomycin)	23	34	2	5	64	93.3	83.3
Raw dairy products	44	30	1	1	76	97.8	97.8
Fresh vegetable products	29	31	1	0	61	96.7	100
Environmental	32	31	0	0	63	100	100
Other Products	64	31	3	2	100	95.7	97.1
ALL PRODUCTS	**229**	**189**	**9**	**8**	**435**	**96.3**	**96.7**

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 ECPT Method
Ground beef (6-24 hr)	25g	*E. coli* O157:H7	0.4 [0.2 - 0.8]	0.4 [0.3 - 0.7]
Ground beef (16 hr)	25g	*E. coli* O157:H7	0.8 [0.5 - 1.4]	0.8 [0.5 - 1.2]
Ground beef	50-375g	*E. coli* O157:H7	0.6 [0.4 - 0.8]	0.6 [0.4 - 0.8]
Paté	25g	*E. coli* O157:H7	0.9 [0.5 - 1.6]	0.5 [0.2 - 1.2]
Raw milk	25mL	*E. coli* O157:H7	0.6 [0.4 - 1.0]	0.5 [0.3 - 0.8]
Process water	25mL	*E. coli* O157:H7	0.7 [0.4 - 1.2]	0.7 [0.4 - 1.1]
Raw spinach	25g	*E. coli* O157:H7	0.7 [0.4 - 1.3]	0.9 [0.6 - 1.6]

[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 ECPT	Reference
Level 0 (0 CFU/25g)	0/88	1/88
Low level (4.3 CFU/25g)	86/88	87/88
High level (46 CFU/25g)	88/88	88/88

Table J. AFNOR Inter-laboratory Study – Relative Sensitivity

Level	PA	NA	ND	PD	N	Relative Sensitivity, %	
						VIDAS	Reference
Level 0 (0 CFU/25g)	-	87	1	-	88	98.3	99.4
Low level (4.3 CFU/25g)	86	-	-	2	88		
High level (46 CFU/25g)	87	-	-	1	88		

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

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

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

Presumptive result – The qualitative response from the presumptive phase of the VIDAS ECPT 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.

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

Positive deviation – Result for the VIDAS ECPT 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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