CERTIFICATION

AOAC Research Institute

Performance Tested Methods^{SM}

Certificate No.

022301

The AOAC Research Institute hereby certifies the method known as:

Thermo Scientific™ SureTect™ Vibrio cholerae, Vibrio parahaemolyticus and Vibrio vulnificus PCR Assay

manufactured by

Oxoid Ltd. part of Thermo Fisher Scientific
Wade Road
Basingstoke
Hampshire, RG248PW

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*^{SM} Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*^{SM} certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date	October 10, 2024
Expiration Date	December 31, 2025

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SUBMITTING COMPANY
Oxoid Ltd. part of Thermo Fisher Scientific
Wade Road
Basingstoke
Hampshire, RG248PW

METHOD NAME
Thermo Scientific™ SureTect™ Vibrio cholerae, Vibrio parahaemolyticus and Vibrio vulnificus PCR Assay

CATALOG NUMBERS
A56837

INDEPENDENT LABORATORY(IES)
*Mérieux NutriSciences, Silliker® Food Science Center
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APPLICABILITY OF METHOD
Analytes – *Vibrio cholerae, Vibrio parahaemolyticus* and *Vibrio vulnificus*.

Matrixes – Up to 50 g raw tuna, raw mussels, green lipped mussel extract, salmon roll with cream cheese and up to 125 g cooked shrimp

Performance claims – The study data were unable to find a significant difference between the SureTect Vibrio PCR Assay and the U. S. Food and Drug Administration Bacteriological Analytical Manual (BAM), Chapter 9 (2004), *Vibrio* (2) reference method for raw mussels, green lipped mussel extract, and cooked shrimp. The SureTect method detected significantly more positive results for raw tuna and salmon roll with cream cheese than the BAM method. The study data were unable to find a significant difference between the SureTect Vibrio PCR Assay and the ISO 21872-1:2017 Microbiology of the food chain – Horizontal method for the determination of Vibrio spp. – Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus (3) reference method for raw tuna and raw mussels.

ORIGINAL CERTIFICATION DATE
February 6, 2023

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2025.

METHOD MODIFICATION RECORD
1. **December 2024 Level 1**
2. **January 2024 Level 2**

SUMMARY OF MODIFICATION
1. **Editorial/clerical changes.**
2. **Addition of automated lysis procedure and PCR setup procedure.**

Under this AOAC *Performance Tested Methods^{SM}* License Number, 022301 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The SureTect Vibrio PCR Assay method is used in conjunction with either the Applied Biosystems™ 7500 Fast Real-Time Food Safety PCR Instrument with Applied Biosystems RapidFinder™ Express Software (version 2.0 or higher) or the Applied Biosystems QuantStudio™ 5 Real-Time Food Safety PCR instrument with Thermo Scientific™ RapidFinder Analysis Software (version 1.1 or higher) for the multiplex detection of *V. cholerae, V. parahaemolyticus* or *V. vulnificus* in seafood samples.
The SureTect Vibrio PCR Assay is supplied as a kit containing all necessary reagents to conduct the sample lysis, including pre-filled Lysis Tubes and lyophilized PCR pellets as well as all necessary PCR reagents (target-specific primers, dye-labelled probes, and PCR master mix components) to easily conduct the PCR analysis PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The oligonucleotides target unique DNA sequences, including three unique targets for *V. cholerae, V. parahaemolyticus* and *V. vulnificus*. If any of the strains are present, the target DNA sequences will be amplified and the increasing fluorescent signal generated will be detected by the 7500 Fast or the QuantStudio PCR Instrument and interpreted by the respective software.
In addition to detecting any target DNA, the PCR pellets contain probes, primers and DNA templates for an internal positive control (IPC). During PCR cycling, the IPC template is amplified regardless of the presence of any target DNA. The probe used for the IPC, which is labelled with a different colored fluorescent dye to the probes used within the assay to detect target DNA, can be detected by either the 7500 Fast or the QuantStudio 5 PCR Instrument through a separate dye channel. If there is no presence of target DNA, the presence of the IPC amplification curve indicates that the PCR process has occurred successfully.
The PCR probes used in the SureTect Vibrio PCR Assay are based on TaqMan® PCR technology. Results are achieved approximately 80 minutes after loading the prepared sample into either PCR instrument and are displayed via the appropriate instrumentational software on the attached computer screen as simple positive or negative symbols with an attached PCR amplification plot that is easily accessible for review. All results interpreted by the software can be reported, stored, printed and downloaded as required by the user.

DISCUSSION OF THE VALIDATION STUDY (1)

The SureTect Vibrio PCR assay method successfully detected all spiked target organisms in 50 g of raw tuna, 50 g of raw mussels, 50 g of salmon roll with cream cheese, 50 g of green lipped mussel extract and 125 g of cooked shrimp. The candidate method tested 50 g portions compared to 25 g portions for the ISO and BAM (detection principle) reference method. POD analysis of the data showed no statistically significant differences between the candidate method and the ISO reference method but did demonstrate significant differences in favor of the candidate method when compared to the BAM method for raw tuna and salmon roll with cream cheese. Furthermore, the matrix data demonstrated the high sensitivity and robust performance of the candidate method considering the differing test portion sizes but uniform spiking levels. POD analysis also showed no statistically significant differences between presumptive positives and confirmed positives for the candidate method for any of the matrixes, except raw tuna in which more presumptive positives than confirmed positives were produced (18 presumptive positive results with 8 confirmed).

The challenge with the raw tuna matrix was likely due to the high level of background flora present. It is well documented that raw tuna has a high-level of background flora which greatly complicates the isolation of suspect colonies, due to overgrowth of non-target organisms. The APC count for raw tuna was high and similar to that for salmon roll with cream cheese where culture confirmation was also difficult following the initial study design. The salmon roll with cream cheese was triple inoculated with all three target organisms and only *V. parahaemolyticus* was culturally confirmed. *Vibrio parahaemolyticus* has a faster growth rate compared to both *V. cholerae* (which was the spike organism in raw tuna) and *V. vulnificus*. As mentioned in the multiplex spike study section above, *Vibrio* spp. are well documented for secreting extracellular protein effectors in competition with both other *Vibrio* spp. and other background organisms that may be present (18, 19). The salmon roll with cream cheese was spiked with three *Vibrio* targets compared to raw tuna meaning there was a higher level of effector proteins likely combating growth of background flora. This, in combination with the faster growth rate of *V. parahaemolyticus* compared to *V. cholerae,* is likely why confirmation of *V. parahaemolyticus* in the salmon roll with cream cheese was not overly challenging compared to confirming the *V. cholerae* in raw tuna. There was also comparable confirmation performance to the ISO reference method in which only seven positives were confirmed, the BAM method failed to detect and confirm any positives. Therefore, the SureTect Vibrio PCR Assay method is a highly sensitive method able to detect potential samples at risk when culture confirmation fails due to interference from high levels of background flora that the PCR technology negates.

For cooked shrimp no statistically significant differences were seen between the candidate versus reference and presumptive vs confirmed results. However, there was an important technical challenge encountered during the study that must be noted. The cooked shrimp matrix routinely returned PCR positives for the unspiked targets (matrix was spiked with *V. vulnificus* and routinely returned *V. parahaemolyticus* and *V. cholerae* positive results). Initial thoughts were that natural contamination had occurred, but this was deemed unlikely in this cooked, ready-to-eat matrix. In addition, culturing of the samples also failed to return any suspect *V. parahaemolyticus* or *V. cholerae* colonies. Review of the PCR amplification plots showed that the non-spiked organisms' amplification typically had very late C_t values (33-40). Coupled with the failure to isolate these strains on agar after enrichment led to the conclusion that these amplifications were likely from dead cell DNA. *Vibrio* spp. are typically ubiquitous within shrimp and prior to cooking, levels high enough for detection are likely to be present. Given that *Vibrio* spp. are Gram-negative, the cooking/boiling process easily denatures the bacterial cell wall allowing free floating DNA to be released into the matrix. In addition, if dead cells are present but DNA has not been released, the lysis procedure of the SureTect Vibrio PCR Assay workflow will break down any remaining dead *Vibrio* cell walls to release the DNA. During PCR this free-floating/dead cell DNA is amplified leading to a registered positive by the software.

To confirm the presence of dead cells rather than low level contamination, as the workflow does not include a free DNA wash step, test portions were prepared and tested at 0, 16 and 24 h of enrichment to compare C_t values. Portions were also plated at 0, 16 and 24 h to ensure that no suspect colonies were present. The C_t values for all non-spiked amplifications were constant at all timepoints with no suspect growth seen on any of the culture media plates. Had the positive PCR call been due to low level contamination, after 16 and 24 h of incubation the C_t value would decrease to show an increase in the cell load present in the portion due to cell division during incubation, but as this did not occur, the call was due to the presence of dead cells.

To negate this effect, a post-enrichment 1-in-10 dilution was added to the instructions for use that diluted out the dead cell DNA to lower the C_t value. This does not risk screening out low-level-contaminated samples since 16 h of incubation would typically result in a sufficiently high cell load to trigger a positive PCR result.

The salmon roll investigation study demonstrated the natural competition that exists between different *Vibrio* strains and established the challenge of culture confirming dual or triple-inoculated test portions. The results show that the kit is a consistent and capable multiplex assay and was able to easily detect the presence of all three spiked strains, whereas the culture confirmation struggled due to difference in growth rates, natural competition and in a few cases breakthrough growth of background flora.

The inclusivity and exclusivity studies correctly detected all 155 inclusivity isolates tested and excluded all 50 exclusivity isolates tested, highlighting the specificity of the method.

The real time stability study results, and consequential POD analysis, demonstrated no significant differences between kit lots, showing that manufacture and performance are equivalent between kit lots demonstrating no overall degradation of the product over time, supporting the shelf-life statement.

In the robustness study no statistically significant differences were seen between the nominal and test conditions for the later enrichment timepoint, demonstrating that typical small parameter deviations that might occur when performed by an end user do not impact assay performance. For the 7 h timepoint there were no statistically significant differences, but the POD confidence interval was very close to the limit for equivalence, with notably less positives at 7 h compared to the nominal conditions. This means that samples must be incubated for the minimum time specified.

Table 2: Inclusivity results of Thermo Scientific SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay. (1)

No.	*Vibrio* species	Source	Origin	SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay result[a]		
				V. cholerae	*V. parahaemolyticus*	*V. vulnificus*
1	*Vibrio cholerae*	RDCC[b] 3437	Unknown	+	-	-
2	*Vibrio cholerae*	RDCC 5794	Cholerae Res.Cent.Calcutta 16, India.CRC11025/64	+	-	-
3	*Vibrio cholerae*	RDCC 5797	Cholerae Res.Cent.Calcutta 16, India.CRC8351/64	+	-	-
4	*Vibrio cholerae*	RDCC 6136	Cholerae Ref. Lab.,Colindale/Mr Donaldson	+	-	-
5	*Vibrio cholerae*	RDCC 6269	N.I.H. Bethesola U.S.A. Dr Smith Ref 569/B RPF	+	-	-
6	*Vibrio cholerae*	RDCC 6372	Maryland via Dr Nogy.	+	-	-
7	*Vibrio cholerae*	RDCC 6771	NCTC Collindale labelled	+	-	-
8	*Vibrio cholerae*	RDCC 6772	NCTC Collindale labelled	+	-	-
9	*Vibrio cholerae*	RDCC 6844	Dr Carl Miller N.I.H. via Dr H L Smith jnr Vibrio Ref Lab Jefferson Med. College Philadelphia	+	-	-
10	*Vibrio cholerae*	RDCC 6846	Dr R.O. Thomson.1972 drying ex CN1269	+	-	-
11	*Vibrio cholerae*	RDCC 6857	N.I.H. via W.R.L.	+	-	-
12	*Vibrio cholerae*	RDCC 7179	Dr H.L.Smith jr, Jeff. Univ. Phil. 19107 originally labelled Lankford & Burrows rough strain CA385	+	-	-
13	*Vibrio cholerae*	RDCC 7181	S.A. Inst. of Medical Res. via Dr McIllmurray.Ref C23962/75 It possesses a heat sensitive somatic antigen which agglutinates slowly with B-W polyvalent cholera serum, destroyed by heating. No agglutination is obtained with monospecific ina	+	-	-
14	*Vibrio cholerae*	RDCC 7184	P.H.L. Maidstone via Dr McIllmurray.3405 (NoCA385)	+	-	-
15	*Vibrio cholerae*	RDCC 7189	D.H.E.W Bethesda,Maryland via Dr Novotny WRL Ref41	+	-	-
16	*Vibrio cholerae*	RDCC 7190	D.H.E.W Bethesda,Maryland via Dr Novotny WRL ref 35-A-3	+	-	-
17	*Vibrio cholerae*	RDCC 8299	PHL Preston Hall Hosp. Maidstone. via Mr C.Gaywood ref 1035	+	-	-
18	*Vibrio cholerae*	RDCC 8301	PHL Preston Hall Hosp. Maidstone. via Mr C.Gaywood ref 1037	+	-	-
19	*Vibrio cholerae*	RDCC 8302	P.H.L.S. Maidstone ex Australia ref RD107	+	-	-
20	*Vibrio cholerae*	RDCC 9127	recieved from Mike Gaston	+	-	-
21	*Vibrio cholerae*	RDCC 9442	QC048/2	+	-	-
22	*Vibrio cholerae*	RDCC 9444	QC048/4	+	-	-
23	*Vibrio cholerae*	RDCC 3636	G.H.Turner WRL CN2005 passaged in mice	+	-	-
24	*Vibrio cholerae*	CCUG[c] 66155	Human eye, Västerås, Sweden	+	-	-
25	*Vibrio cholerae*	CCUG 60231	Human ear, Täby, Sweden	+	-	-
26	*Vibrio cholerae*	MH[d] 4444	Thermo Fisher Australia	+	-	-
27	*Vibrio cholerae*	NCTC[e] 11348	Human faeces	+	-	-
28	*Vibrio cholerae*	MH 4880	THL - Thailand	+	-	-
29	*Vibrio cholerae*	MH 4882	THL - Thailand	+	-	-
30	*Vibrio cholerae*	MH 4885	THL - Thailand	+	-	-
31	*Vibrio cholerae*	NCTC 12945	Cholera patient, India: Madras	+	-	-
32	*Vibrio cholerae*	NCTC 4693	Unknown	+	-	-
33	*Vibrio cholerae*	NCTC 4715	Unknown	+	-	-
34	*Vibrio cholerae*	NCTC 5395	Human, pilgrim of the 1983 haj	+	-	-
35	*Vibrio cholerae*	NCTC 6561	34-D10	+	-	-
36	*Vibrio cholerae*	NCTC 7254	Cholera epidemic, Egypt	+	-	-
37	*Vibrio cholerae*	NCTC 8023	NCTC Collindale labelled -5- Inaba	+	-	-
38	*Vibrio cholerae*	NCTC 9420	Unknown	+	-	-
39	*Vibrio cholerae*	NCTC 9421	Unknown	+	-	-
40	*Vibrio cholerae*	NCTC10256	Human, rice water stool	+	-	-
41	*Vibrio cholerae*	MH 4881	Unknown	+	-	-
42	*Vibrio cholerae*	CECT[f] 659	Water sample, India	+	-	-
43	*Vibrio cholerae*	MH 4886	Unknown	+	-	-
44	*Vibrio cholerae*	CECT 658	Water sample, Bangladesh	+	-	-
45	*Vibrio cholerae*	CECT 652	Man	+	-	-
46	*Vibrio cholerae*	CECT 569	Man, India	+	-	-
47	*Vibrio cholerae*	CECT 552	Pilgrim to Mecca	+	-	-
48	*Vibrio cholerae*	CECT 513	Unknown	+	-	-
49	*Vibrio cholerae*	CECT 8265	Human feces, UK	+	-	-
50	*Vibrio cholerae*	MH 4883	Unknown	+	-	-
51	*Vibrio cholerae*	NCTC 8021	Unknown	+	-	-
52	*Vibrio cholerae*	CECT 655	Water, Dacca, Bangladesh	+	-	-
53	*Vibrio cholerae*	MH 4884	Unknown	+	-	-
54	*Vibrio cholerae*	MH1201	Unknown	+	-	-
55	*Vibrio parahaemolyticus*	MH 3522	Marshfield labs - USA	-	+	-

56	*Vibrio parahaemolyticus*	CCUG 43365	Japan	-	+	-
57	*Vibrio parahaemolyticus*	CCUG 51447	Human leg, fasciitis, Göteborg, Sweden	-	+	-
58	*Vibrio parahaemolyticus*	MH 4624	Thailand	-	+	-
59	*Vibrio parahaemolyticus*	CCM[g] 5937	Mussels, Czechoslovakia	-	+	-
60	*Vibrio parahaemolyticus*	ATCC[h] 17802	Shirasu food poisoning, Japan	-	+	-
61	*Vibrio parahaemolyticus*	ATCC 43996	Cockles, England	-	+	-
62	*Vibrio parahaemolyticus*	RDCC 5847	UK Southwest, C. gigas	-	+	-
63	*Vibrio parahaemolyticus*	RDCC 5848	UK Southwest, Nereididae Ragworm	-	+	-
64	*Vibrio parahaemolyticus*	RDCC 5849	UK Southwest, Nereididae Ragworm	-	+	-
65	*Vibrio parahaemolyticus*	RDCC 5850	UK Southwest, M. edulis	-	+	-
66	*Vibrio parahaemolyticus*	RDCC 5863	Galicia Spain	-	+	-
67	*Vibrio parahaemolyticus*	RDCC 5868	Worm	-	+	-
68	*Vibrio parahaemolyticus*	RDCC 5869	Galicia Spain	-	+	-
69	*Vibrio parahaemolyticus*	RDCC 5870	Galicia Spain	-	+	-
70	*Vibrio parahaemolyticus*	RDCC 5872	Southampton, UK	-	+	-
71	*Vibrio parahaemolyticus*	RDCC 5873	Southampton, UK	-	+	-
72	*Vibrio parahaemolyticus*	RDCC 5874	Anre, UK. C. gigas	-	+	-
73	*Vibrio parahaemolyticus*	RDCC 5875	Weymouth, UK. P. maximus	-	+	-
74	*Vibrio parahaemolyticus*	RDCC 5876	Santiago de Composterla, Spain. Human	-	+	-
75	*Vibrio parahaemolyticus*	RDCC 5877	Santiago de Composterla, Spain. Human	-	+	-
76	*Vibrio parahaemolyticus*	RDCC 5878	Santiago de Composterla, Spain. Human	-	+	-
77	*Vibrio parahaemolyticus*	RDCC 5879	Santiago de Composterla, Spain. Human	-	+	-
78	*Vibrio parahaemolyticus*	RDCC 5880	Universidad de Satiago de Compostela, Spain. Clinical stool sample	-	+	-
79	*Vibrio parahaemolyticus*	RDCC 5881	UK Southwest	-	+	-
80	*Vibrio parahaemolyticus*	RDCC 5882	UK Southwest	-	+	-
81	*Vibrio parahaemolyticus*	RDCC 5883	UK Southwest, M. edulis	-	+	-
82	*Vibrio parahaemolyticus*	RDCC 5884	UK Southwest, C. gigas	-	+	-
83	*Vibrio parahaemolyticus*	RDCC 5885	Fawey, Wems. O. edulis	-	+	-
84	*Vibrio parahaemolyticus*	RDCC 5887	C. gigias	-	+	-
85	*Vibrio parahaemolyticus*	RDCC 5888	Larne lough. C. gigas	-	+	-
86	*Vibrio parahaemolyticus*	RDCC 5889	Daleford, M. edulis	-	+	-
87	*Vibrio parahaemolyticus*	RDCC 5890	Limosa pre dep. M. edulis	-	+	-
88	*Vibrio parahaemolyticus*	RDCC 5891	River Thames. Eriocheir sinensis	-	+	-
89	*Vibrio parahaemolyticus*	RDCC 5892	River Thames. Eriocheir sinensis	-	+	-
90	*Vibrio parahaemolyticus*	RDCC 5893	D. Gladwell. O. edulis	-	+	-
91	*Vibrio parahaemolyticus*	RDCC 5894	Helford River. M. edulis	-	+	-
92	*Vibrio parahaemolyticus*	RDCC 5895	River Lee Area London, Eirocheir sinensis	-	+	-
93	*Vibrio parahaemolyticus*	RDCC 5896	Newtons Bay, water sample	-	+	-
94	*Vibrio parahaemolyticus*	RDCC 5898	River Thames, water sample	-	+	-
95	*Vibrio parahaemolyticus*	RDCC 5899	River Thames, water sample	-	+	-
96	*Vibrio parahaemolyticus*	VP[i] 1	Unknown	-	+	-
97	*Vibrio parahaemolyticus*	VP4	Unknown	-	+	-
98	*Vibrio parahaemolyticus*	VP13	Faeces, Far East	-	+	-
99	*Vibrio parahaemolyticus*	VP17	Faeces, Thailand	-	+	-
100	*Vibrio parahaemolyticus*	VP33	Sea, UK	-	+	-
101	*Vibrio parahaemolyticus*	VP34	Cockles, UK	-	+	-
102	*Vibrio parahaemolyticus*	VP67	Unknown	-	+	-
103	*Vibrio parahaemolyticus*	VP71	Unknown	-	+	-
104	*Vibrio parahaemolyticus*	VP87	Unknown	-	+	-
105	*Vibrio vulnificus*	MH 7445	Unknown	-	-	+
106	*Vibrio vulnificus*	CCM 2840	Human Leg, USA	-	-	+
107	*Vibrio vulnificus*	ATCC 29307	Blood, USA	-	-	+
108	*Vibrio vulnificus*	RDCC 1268	Isolated seafood, Japan. Environmental strain	-	-	+
109	*Vibrio vulnificus*	RDCC 2887	Isolated seafood, Japan. Environmental strain	-	-	+
110	*Vibrio vulnificus*	RDCC 2889	Isolated seafood, Japan. Environmental strain	-	-	+
111	*Vibrio vulnificus*	RDCC 2890	Isolated seafood, Japan. Environmental strain	-	-	+
112	*Vibrio vulnificus*	RDCC 2891	Isolated seafood, Japan. Environmental strain	-	-	+
113	*Vibrio vulnificus*	RDCC 2892	Isolated seafood, Japan. Environmental strain	-	-	+
114	*Vibrio vulnificus*	RDCC 2893	Isolated seafood, Japan. Environmental strain	-	-	+
115	*Vibrio vulnificus*	RDCC 5025	Unknown	-	-	+
116	*Vibrio vulnificus*	RDCC 5855	Bristol Channel, water discharge	-	-	+
117	*Vibrio vulnificus*	RDCC 5856	Bristol Channel, water discharge	-	-	+
118	*Vibrio vulnificus*	RDCC 5857	Bristol Channel, beach control, water	-	-	+
119	*Vibrio vulnificus*	RDCC 5858	Bristol Channel, beach control, water	-	-	+
120	*Vibrio vulnificus*	RDCC 5861	Bristol Channel, water intake	-	-	+
121	*Vibrio vulnificus*	RDCC 5862	Bristol Channel, water intake	-	-	+
122	*Vibrio vulnificus*	RDCC 5871	Southampton, UK	-	-	+
123	*Vibrio vulnificus*	CCUG 15887	Unknown	-	-	+

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124	*Vibrio vulnificus*	CCUG 16395	Blood	-	-	+
125	*Vibrio vulnificus*	CCUG 38297	Human blood, 74-year-old man, repeated isolations	-	-	+
126	*Vibrio vulnificus*	CCUG 38430	Unknown	-	-	+
127	*Vibrio vulnificus*	CCUG 38521	Human wound, fishing hook in thumb	-	-	+
128	*Vibrio vulnificus*	CCUG 39349	Scampi	-	-	+
129	*Vibrio vulnificus*	CCUG 45996	Human blood, 90-year-old woman	-	-	+
130	*Vibrio vulnificus*	CCUG 46876	Human blood, 86-year-old man	-	-	+
131	*Vibrio vulnificus*	CCUG 46877	Human wound, hand, 86-year-old man	-	-	+
132	*Vibrio vulnificus*	CCUG 47319	Human biopsy, necrotizing fascitis, 69-year-old woman	-	-	+
133	*Vibrio vulnificus*	CCUG 47321	Human blood, necrotizing fascitis	-	-	+
134	*Vibrio vulnificus*	CCUG 48492	Human soft tissue injury, handling marine- animals	-	-	+
135	*Vibrio vulnificus*	MH 7446	Unknown	-	-	+
136	*Vibrio vulnificus*	CCM 2838	Ulcer of cornea, Virginia, USA	-	-	+
137	*Vibrio vulnificus*	ATCC 27562	Human Blood, Florida, USA	-	-	+
138	*Vibrio vulnificus*	RDCC 2886	Unknown	-	-	+
139	*Vibrio vulnificus*	RDCC 5851	Bristol Channel, water intake	-	-	+
140	*Vibrio vulnificus*	CCUG 15886	Human leg ulcer	-	-	+
141	*Vibrio vulnificus*	CCUG 38429	Eels, diseased, pond-culture	-	-	+
142	*Vibrio vulnificus*	CECT 4602	Diseased eel from fish farm	-	-	+
143	*Vibrio vulnificus*	CECT 4608	Tank water from a fish farm	-	-	+
144	*Vibrio vulnificus*	CECT 4862	Disease eel, *Anguilla japonica*, Japan (ATCC 33149)	-	-	+
145	*Vibrio vulnificus*	CECT 4863	Leg wound, Rhode Island, USA (ATCC 33817)	-	-	+
146	*Vibrio vulnificus*	CECT 5167	Human blood, Japan	-	-	+
147	*Vibrio vulnificus*	CECT 4865	Vibriosis affected shrimps, Taiwan	-	-	+
148	*Vibrio vulnificus*	CECT 4866	Human blood, Austrailia	-	-	+
149	*Vibrio vulnificus*	CECT 4867	Diseased eel	-	-	+
150	*Vibrio vulnificus*	CECT 4868	Diseased eel, Norway	-	-	+
151	*Vibrio vulnificus*	CECT 4869	Diseased eel, Belgium	-	-	+
152	*Vibrio vulnificus*	CECT 5168	Human blood, USA	-	-	+
153	*Vibrio vulnificus*	CECT 7029	Internal organ of diseased eel, Denmark	-	-	+
154	*Vibrio vulnificus*	CECT 5198	Liver vibriosis of diseased *Anguilla anguilla* (eel), Spain	-	-	+
155	*Vibrio vulnificus*	CECT 5689	Internal organs of diseased eel, Spain	-	-	+

[a]Results identical for both QuantStudio 5 and 7500 Fast.
[b]RDCC = Research and Development Culture Collection 1, Thermo Fisher Scientific, Basingstoke, UK
[c]CCUG = Culture Collection University of Gothenburg, Göteborg, Sweden.
[d]MH = Research and Development Culture Collection 2, Thermo Fisher Scientific, Basingstoke, UK
[e]NCTC = National Collection of Type Cultures, Health Protection Agency, London, UK
[f]CECT = Spanish Type Culture Collection, Valencia, Spain.
[g]CCM = Czech Collection of Microorganisms, Kralovopolska, Czech Republic.
[h]ATCC = American Type Culture Collection, Manassas, VA, USA.
[i]VP = Research and Development Culture Collection 3, Thermo Fisher Scientific, Basingstoke, UK

Table 3: Exclusivity results of Thermo Scientific SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay. (1)

No.	Organism	Source	Origin	SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay result[a]		
				V. cholerae	*V. parahaemolyticus*	*V. vulnificus*
1	*Acinetobacter iwoffii*	RDCC[b] 2962	Unknown	-	-	-
2	*Actinobacillus pleuropneumoniae*	RDCC 4998	Unknown	-	-	-
3	*Aeromonas hydrophilia*	NCTC[c] 7810	Frog	-	-	-
4	*Candida albicans*	RDCC 0434	Unknown	-	-	-
5	*Citrobacter freundii*	NCTC 8581	Red Leg Tree Frog	-	-	-
6	*Citrobacter koseri*	ATCC[d] 27026	Throat swab	-	-	-
7	*Cronobacter sakazakii*	ATCC 12868	Unknown	-	-	-
8	*Edwardsiella tarda*	NCTC 10396	Human feces	-	-	-
9	*Enterococcus faecalis*	NCTC 12697	Unknown	-	-	-
10	*Escherichia coli*	ATCC 10536	Unknown	-	-	-
11	*Klebsiella oxytoca*	ATCC 49131	Clinical isolate	-	-	-
12	*Klebsiella pneumoniae*	NCTC 7427	Unknown	-	-	-
13	*Klebsiella pneuoniae*	ATCC 700603	Urine, human, Virginia, USA	-	-	-
14	*Listeria innocua*	NCTC 11288	Brain of cow	-	-	-
15	*Listeria ivonovii*	NCTC 11846	Sheep	-	-	-
16	*Listeria monocytogenes*	ATCC 13932	Spinal fluid, child, meningitis, Germany	-	-	-
17	*Listeria seeligeri*	ATCC 35967	Soil, Germany	-	-	-
18	*Listeria welshimeri*	NCTC 11857	Compost	-	-	-
19	*Pasteurella multocida*	ATCC 43137	Pig	-	-	-
20	*Pediococcus sp*	ATCC 33316	Dried beer yeast	-	-	-
21	*Plesiomonas shigelloides*	NCTC 10360	Unknown	-	-	-

22	*Proteus mirabilis*	NCTC 10975	Human urine	-	-	-
23	*Proteus spp.*	RDCC 0237	Unknown	-	-	-
24	Salmonella ser *Typhimurium*	ATCC 14028	4-week-old chickens - heart and liver pool	-	-	-
25	*Streptococcus pyogenes*	RDCC 0624	Unknown	-	-	-
26	*Vibrio metschnikovii*	NCTC 8443	Bird	-	-	-
27	*Vibrio alginolyticus*	RDCC 6102	Mussels, UK	-	-	-
28	*Vibrio alginolyticus*	RDCC 6103	Cockles, UK	-	-	-
29	*Vibrio alginolyticus*	RDCC 6104	Whelks, UK	-	-	-
30	*Vibrio anguillarum*	RDCC 6107	Seafood	-	-	-
31	*Vibrio anguillarum*	RDCC 6108	Seawater	-	-	-
32	*Vibrio anguillarum*	RDCC 6111	USA	-	-	-
33	*Vibrio fluvialis*	RDCC 6113	UK	-	-	-
34	*Vibrio fluvialis*	RDCC 6116	Cat, Yugoslavia	-	-	-
35	*Vibrio furnissii*	RDCC 6122	R Medway, UK	-	-	-
36	*Vibrio furnissii*	RDCC 6123	Kenya	-	-	-
37	*Vibrio furnissii*	RDCC 6124	Feces, UK	-	-	-
38	*Vibrio metschnikovii*	RDCC 6129	Unknown	-	-	-
39	*Vibrio harveyi*	RDCC 6131	Marine, Yugoslavia	-	-	-
40	*Vibrio metschnikovii*	RDCC 6139	Porton Down, UK	-	-	-
41	*Vibrio fluvialis*	RDCC 6145	Man, Dar Es Salaam	-	-	-
42	*Vibrio mimicus*	VM[e]30	Unknown	-	-	-
43	*Vibrio mimicus*	VM31	Prawns, Malaysia	-	-	-
44	*Vibrio mimicus*	VM12	Anacostia River, USA	-	-	-
45	*Vibrio mimicus*	VM13	Anacostia River, USA	-	-	-
46	*Vibrio mimicus*	VM18	Prawns, Thailand	-	-	-
47	*Vibrio mimicus*	VM24	Dacca	-	-	-
48	*Vibrio natriegens*	CECT 526 T	Salt Marsh Mud, US	-	-	-
49	*Vibrio diazotrophicus*	CECT 627 T	Gastrointestinal tract of sea urchin, Canada	-	-	-
50	*Vibrio proteolyticus*	CECT 630 T	Intestine of wood-boring isopod	-	-	-

[a]Results identical for QuantStudio 5 and 7500 Fast
[b]RDCC= Research and Development Culture Collection 1, Thermo Fisher Scientific, Basingstoke, UK
[c]NCTC = National Collection of Type Cultures, Health Protection Agency, London, UK
[d]ATCC = American Type Culture Collection, Manassas, VA, USA.
[e]VM = Research and Development Culture Collection 3, Thermo Fisher Scientific, Basingstoke, UK
[f]CECT = Spanish Type Culture Collection, Valencia, Spain.

Table 5. Thermo Scientific SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay, Candidate vs. FDA/BAM Chapter 9 Reference – POD Results (1)

Matrix	Timepoint	Strain	MPN[a]/ Test Portion	N[b]	Candidate[c]			Reference			dPOD$_C$[g]	95% CI[h]
					x[d]	POD$_C$[e]	95% CI	x	POD$_R$[f]	95% CI		
Raw tuna (50 g)	16 h	*Vibrio cholerae* ATCC[i] 14033	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.00 (0.00, 0.00)	20	8	0.40	0.22, 0.61	0	0.00	0.00, 0.16	0.40	0.16, 0.61
			0.00 (0.00, 0.00)	5	3	0.60	0.23, 0.89	0	0.00	0.00, 0.43	0.60	0.03, 0.89
Salmon roll with cream cheese (50 g)	8 h and 20 h[k]	*Vibrio parahaemolyticus* ATCC 27519	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.16 (0.07, 0.40)	20	9	0.45	0.26, 0.66	2	0.10	0.03, 0.30	0.35	0.07, 0.58
			0.28 (0.07, 1.10)	5	3	0.60	0.23, 0.89	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
Raw mussels (50 g)	16 h	*Vibrio parahaemolyticus* ATCC 43996	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.10 (0.03, 0.32)	20	8	0.40	0.22, 0.61	3	0.15	0.05, 0.40	0.25	-0.03, 0.49
			0.78 (0.32, 1.91)	5	3	0.60	0.23, 0.88	2	0.40	0.12, 0.77	0.20	-0.32, 0.60
Green lipped mussel extract powder (50 g)	16 h	*Vibrio cholerae* ATCC 14033	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.76, 1.83)	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.25, 0.25
			4.80 (2.51, 9.20)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cooked shrimp (125 g)	16 h	*Vibrio vulnificus* ATCC 33147	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.06 (0.66, 1.69)	20	14	0.70	0.48, 0.86	12	0.60	0.39, 0.78	0.10	-0.18, 0.36
			9.26 (3.80, 22.6)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b]N = Number of test portions.
[c]Results were identical for analysis conducted on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument.
[d]x = Number of positive test portions.
[e]POD$_C$ = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.
[g]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i]ATCC = American Type Culture Collection, Manassas, VA.
[j]N/A = Not applicable.
[k]= Results the same for both timepoints.

Table 6. Thermo Scientific SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay, Candidate vs. ISO 21872-1:2017 Reference – POD Results (1)

Matrix	Strain	Timepoint	MPN[a]/ Test Portion	N[b]	Candidate[c]			Reference			$dPOD_C$[g]	95% CI[h]
					X[d]	POD_C[e]	95% CI	X	POD_R[f]	95% CI		
Raw tuna (50 g)	*Vibrio cholerae* ATCC[i] 14033	16 h	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.33 (0.13, 0.59)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	-0.23, 0.32
			0.75 (0.31, 1.83)	5	3	0.60	0.23, 0.89	1	0.20	0.00, 0.62	0.40	-0.16, 0.75
Raw mussels (50 g)	*Vibrio parahaemolyticus* ATCC 43996	16 h	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.29 (0.13, 0.53)	20	8	0.40	0.22, 0.61	4	0.20	0.08, 0.42	0.20	-0.08, 0.45
			0.41 (0.13, 1.30)	5	3	0.60	0.23, 0.89	2	0.40	0.12, 0.77	0.20	-0.32, 0.60

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b]N = Number of test portions.
[c]Results were identical for analysis conducted on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument.
[d]x = Number of positive test portions.
[e]POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f]POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[g]$dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[h]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i]ATCC = American Type Culture Collection, Manassas, VA.
[j]N/A = Not applicable.

Table 7. Thermo Scientific SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay, Presumptive vs. Confirmed–FDA/BAM Chapter 9 and ISO 21872-1:2017 POD Results (1)

Matrix	Timepoint	Strain	MPN[a]/ Test Portion	N[b]	Presumptive[c]			Confirmed[f]			$dPOD_{CP}$[h]	95% CI[i]
					X[d]	POD_{CP}[e]	95% CI	X	POD_{CC}[g]	95% CI		
Raw tuna (50 g)	16 h	*Vibrio cholerae* ATCC[j] 14033	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.00 (0.00, 0.00)	20	18	0.90	0.70, 0.97	8	0.40	0.22, 0.61	0.50	0.25, 0.76[l]
			0.00 (0.00, 0.00)	5	5	1.00	0.57, 1.00	3	0.60	0.23, 0.89	0.40	-0.21, 1.01
Salmon roll with cream cheese (50 g)	8 h	*Vibrio parahaemolyticus* ATCC 27519	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.16 (0.07, 0.40)	20	11	0.55	0.34, 0.74	9	0.45	0.26, 0.66	0.10	-0.08, 0.28
			0.28 (0.07, 1.10)	5	5	1.00	0.57, 1.00	3	0.60	0.23, 0.89	0.40	-0.21, 1.01
Salmon roll with cream cheese (50 g)	20 h	*Vibrio parahaemolyticus* ATCC 27519	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.16 (0.07, 0.40)	20	13	0.65	0.43, 0.82	9	0.45	0.26, 0.66	0.20	-0.02, 0.42
			0.28 (0.07, 1.10)	5	5	1.00	0.57, 1.00	3	0.60	0.23, 0.89	0.40	-0.21, 1.01
Raw mussels (50 g)	16 h	*Vibrio parahaemolyticus* ATCC 43996	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			0.10 (0.03, 0.32)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
			0.78 (0.32, 1.91)	5	3	0.60	0.23, 0.89	3	0.60	0.23, 0.89	0.00	-0.47, 0.47
Green lipped mussel extract (50 g)	16 h	*Vibrio cholerae* ATCC 14033	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.17 (0.76, 1.83)	20	16	0.80	0.58, 0.92	16	0.80	0.58, 0.92	0.00	-0.13, 0.13
			4.80 (2.51, 9.20)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cooked shrimp (125 g)	16 h QS5	*Vibrio vulnificus* ATCC 33147	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.06 (0.66, 1.69)	20	16	0.80	0.58, 0.91	14	0.70	0.00	0.10	-0.08, 0.28
			9.26 (3.80, 22.6)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Cooked shrimp (125 g)	16 h 7500 FAST	*Vibrio vulnificus* ATCC 33147	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
			1.06 (0.66, 1.69)	20	15	0.75	0.53, 0.89	14	0.70	0.48, 0.86	0.05	-0.11, 0.21
			9.26 (3.80, 22.6)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

[a]MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b]N = Number of test portions.
[c]Unless otherwise indicated results were identical for analysis conducted on the QuantStudio 5 and 7500 Fast PCR Instruments.
[d]x = Number of positive test portions.
[e]POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f]Results obtained following the alternative confirmation were identical to results obtain from confirmation by FDA/BAM Chapter 9 and ISO 21872:1:2017 reference method.
[g]POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[h]$dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[i]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j]ATCC = American Type Culture Collection, Manassas, VA.
[k]N/A = Not applicable.
[l]Confirmation challenge due to high level of background flora. Culture media performance was equivalent to ISO 21872:1:2017 reference method performance.

DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2024 (4)

The comparison study was selected to evaluate the automated procedure as it allowed for an accurate and precise comparison of the performance between the manual and automated lysis and PCR setup procedures without interference from other parts of the method, such as the enrichment. The study followed a paired study design with a post enrichment spike to assess the performance of the lysis and PCR setup procedures specifically.

Comparison studies above the LOD of the PCR assays showed that the difference in average C_t values were always ±1.5 cycles when comparing the automated and manual procedures. At the LOD, the numbers of positives per dilution for each assay-matrix combination was statistically comparable when comparing the automated procedure to the manual.

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