

# CERTIFICATION

## AOAC Research Institute Performance Tested Methods<sup>SM</sup>

Certificate No. 022301

The AOAC Research Institute hereby certifies the method known as:

### Thermo Scientific<sup>™</sup> SureTect<sup>™</sup> 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 in the AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated in the applicability of the method. 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*<sup>SM</sup> 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.

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Bradley A. Stawick, Senior Director Signature for AOAC Research Institute Issue Date Expiration Date January 25, 2024 December 31, 2024

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METHOD NAME Thermo Scientific <sup>™</sup> SureTect <sup>™</sup> Vibrio cholerae, Vibrio parahaemolyticus and Vibrio vulnificus PCR Assay	CATALOG NUMBERS A44253, A56837
	APPLICABILITY OF METHOD
*Mérieux NutriSciences, Silliker® Food Science Center 3600 Eagle Nest Drive Crete, Illinois 60417	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), <i>Vibrio</i> (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 2024.
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

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#### PRINCIPLE OF THE METHOD (1)

The SureTect Vibrio PCR Assay method is used in conjunction with either the Applied Biosystems<sup>™</sup> 7500 Fast Real-Time Food Safety PCR Instrument with Applied Biosystems RapidFinder<sup>™</sup> Express Software (version 2.0 or higher) or the Applied Biosystems QuantStudio<sup>™</sup> 5 Real-Time Food Safety PCR Instrument with Thermo Scientific<sup>™</sup> 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<sup>®</sup> 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 Ct 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 Gramnegative, 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<sub>t</sub> 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 SureTe	ct Vibrio cholerae, V. parahaemolyticus and V. vulnificus P	CR Assay. (1	1)	
			Su	reTect Vibrio cholerae	, V.	
				parahaemo	olyticus and V. vulnificu	s PCR Assay
					result <sup>a</sup>	
				<i>V</i> .	<i>V</i> .	<i>V.</i>
No.	Vibrio species	Source	Origin	cholerae	parahaemolyticus	vulnificus
1	Vibrio cholerae	RDCC <sup>b</sup> 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	+	-	-
1	Vibrio cholerge	RDCC 6136	Cholerae Ref Lab. Colindale/Mr Donaldson	+	_	
	Vibrio cholorgo	RDCC 6360	NILL Bothosola II S.A. Dr. Smith Bof E60/B BDE		-	
5	Vibrio cholerge	RDCC 0203	N.I.II. Bettlesola U.S.A. DI Shifti Kei 505/B KFI	+	-	-
0		RDCC 0372	Iviaryianu via Drivogy.	+	-	-
/	Vibrio cholerae	RDCC 6771	NCTC Collindale labelled	+	-	-
8	Vibrio cholerae	RDCC 6772	NCTC Collindale labelled	+	-	-
٥	Vibrio cholarga		Dr Carl Miller N.I.H. via Dr H L Smith jnr Vibrio Ref Lab	-	_	
5	vibrio cholerae	NDCC 0044	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.	+	-	-
			Dr H.L.Smith ir. Jeff. Univ. Phil. 19107 originally labelled			
12	Vibrio cholerae	RDCC 7179	Lankford & Burrows rough strain CA385	+	-	-
			S A Inst of Medical Res via Dr McIllmurray Ref			
			C23962/75 It nossesses a heat sensitive comptic antigen			
12	Vibrio cholarga	PDCC 7191	which agglutinates slowly with P-W polyvalent cholora	-	_	
15	VIDITO CITOTETUE	KDCC / 101	which agglutinates slowly with B-W polyvalent choiera	Ŧ	-	-
			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 cholerge	RDCC 7190	D.H.E.W Bethesda, Maryland via Dr Novotny WRL ref 35-	+	_	_
10	vibrio cholerde	RDCC / 190	A-3	Ŧ	-	-
47			PHL Preston Hall Hosp. Maidstone. via Mr C.Gaywood			
1/	Vibrio cholerae	RDCC 8299	ref 1035	+	-	-
			PHI Preston Hall Hosp. Maidstone, via Mr C.Gaywood			
18	Vibrio cholerae	RDCC 8301	ref 1037	+	-	-
10	Vibria abalarga	DDCC 9202	DILLS Maidstone av Australia ref DD107			
19		RDCC 8302	P.H.L.S. Malustone ex Australia fer RD107	+	-	-
20		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 <sup>c</sup> 66155	Human eye, Västerås, Sweden	+	-	-
25	Vibrio cholerae	CCUG 60231	Human ear, Täby, Sweden	+	-	-
26	Vibrio cholerae	MH <sup>d</sup> 4444	Thermo Fisher Australia	+	-	-
27	Vibrio cholerge	NCTC <sup>e</sup> 11348	Human faeces	+	_	-
20	Vibrio cholerge	MH 4880	THL_Thailand	· ·		
20	Vibrio cholerge	NILL 4992	THL Theiland	+	-	-
29		IVITI 4882		+	-	-
30	Vibrio cholerae	MH 4885	IHL - 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 cholerge	NCTC 8023	NCTC Collindale Jabelled -5- Inaba	+		
20	Vibrio cholorgo	NCTC 0420				
38	Vibrio cholerae	NCTC 9420		+	-	-
39	Vibrio cholerae	NCTC 9421	Unknown	+	-	-
40	Vibrio cholerae	NCTC10256	Human, rice water stool	+	-	-
41	Vibrio cholerae	MH 4881	Unknown	+	-	-
42	Vibrio cholerae	CECT <sup>f</sup> 659	Water sample, India	+	-	-
43	Vibrio cholerae	MH 4886	Unknown	+	-	-
44	Vibrio cholerae	CECT 658	Water sample. Bangladesh	+	-	-
45	Vibrio cholerae	CECT 652	Man	+	-	_
15	Vibrio cholerge	CECT 560	Man India	+		
40			IVIdII, IIIUId		-	-
4/			Pilgrim to iviecca	+	-	-
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 Jahs - USA	_	+	_
55	i isino paramacinoryticas	1111 3322				

#### Thermo Scientific SureTect<sup>™</sup> Vibrio, AOAC Research Institute *Performance Tested Methods<sup>™</sup>* certification number 022301

S2         Weire parabemolyticus         CUES 5417         Human (E., Sotteorp, Sweden)         Image of the second se	56	Vibrio parahaemolyticus	CCUG 43365	Japan	-	+	-
Set         Where parahemery/cost         M-M-4624         The lated         Image         I	57	Vibrio parahaemolyticus	CCUG 51447	Human leg. fasciitis, Göteborg, Sweden	-	+	-
Sol         Weire parabemorytical         CCM 9392         Mutacis, General optimiza, Japan         -         -           61         Weire parabemorytical         ACC 4396         Codes, England         -         -         -           63         Weire parabemorytical         ACC 4396         UK 5000000000000000000000000000000000000	58	Vibrio parabaemolyticus	MH 4624	Thailand	_	+	-
0         Work parathemotylica         ATCC '1 202         Sharas fod pointing para         >         >         >           0         Work parathemotylica         DOC 5447         UK Southwest, Eiglas         -         +         -           0         Work parathemotylica         DOC 5447         UK Southwest, Nerediate Rayourn         -         +         -           64         Work parathemotylica         DOC 5458         UK Southwest, Nerediate Rayourn         -         +         -           65         Work parathemotylica         DOC 5458         UK Southwest, Nerediate Rayourn         -         +         -           66         Work parathemotylica         DOC 5458         UK Southwest, Nerediate Sapin         -         +         -           67         Work parathemotylica         DOC 5450         Galca Sapin         -         +         -           70         Work parathemotylica         DOC 5451         Southampton, UK         +         -         -           71         Work parathemotylica         DOC 5471         Southampton, UK         +         -         -           74         Work parathemotylica         DOC 5471         Southampton, UK         +         -         -         -           74	50	Vibrio parahaemolyticus	CCM8 5937	Mussels Czechoslovakia	_	+	
Bit         The Control         Contro <thcontrol< th="">         Control         <thc< td=""><td>60</td><td>Vibrio parahaomolyticus</td><td>ATCCh 17902</td><td>Shirasu food poisoning Japan</td><td>_</td><td></td><td>-</td></thc<></thcontrol<>	60	Vibrio parahaomolyticus	ATCCh 17902	Shirasu food poisoning Japan	_		-
B.         Work parabetenychics         ALC. Figs         LOBINS, Edges         I         I         I           64         Work parabetenychics         RECC 3848         UK Suthivesk, Merdiale Rageworn         I         I         I           64         Work parabetenychics         RECC 3848         UK Suthivesk, Merdiale Rageworn         I         I         I         I           66         Work parabetenychics         RECC 3848         UK Suthivesk, Merdiale Rageworn         I <td>00</td> <td>Vibrio purunaemolyticus</td> <td>ATCC 17802</td> <td></td> <td>-</td> <td>Ŧ</td> <td>-</td>	00	Vibrio purunaemolyticus	ATCC 17802		-	Ŧ	-
6.1         Work parabasens/rices         RUCC 5847         UK Southwesk, Neerdidae Rayworn         -         +         -           6.1         Work parabasens/rices         RUCC 5868         UK Southwesk, Neerdidae Rayworn         -         +         -           6.1         Work parabasens/rices         RUCC 5868         UK Southwesk, Neerdidae Rayworn         -         +         -           6.1         Work parabasens/rices         RUCC 5868         Worn         -         +         +           6.1         Work parabasens/rices         RUCC 5863         Worn         -         +         +           6.1         Work parabasens/rices         RUCC 5873         Southampton, UK         -         +         +           6.1         Work parabasens/rices         RUCC 5873         Southampton, UK         -         +         +           7.1         Work parabasens/rices         RUCC 5873         Southampton, UK         -         +         +           7.2         Work parabasens/rices         RUCC 5873         Southampton, UK         -         +         +           7.3         Work parabasens/rices         RUCC 5873         Southawest, Anduin         -         +         +           7.4         Work parabase	61	Vibrio paranaemolyticus	ATCC 43996	Cockles, England	-	+	-
63         Withis parahaemalytics         RDCC 3848         UK Southwest, Neerdidae Ragworm          +-         +-           64         Withis parahaemalytics         RDCC 3840         UK Southwest, Meddiak Ragworm          +-         +-           65         Withis parahaemalytics         RDCC 3840         UK Southwest, Meddiak Spain          +-         +-           66         Withis parahaemalytics         RDCC 3840         Galaca Spain          +-         +-           67         Withis parahaemalytics         RDCC 3873         Southamaptics         +-         +-         +-           68         Withis parahaemalytics         RDCC 3873         Southamaptics         +-         +-         +-         +-           71         Withis parahaemalytics         RDCC 3873         Southamaptics         Hore         +-	62	Vibrio parahaemolyticus	RDCC 5847	UK Southwest, C. gigas	-	+	-
64         Wihn parchaemolytics         RDCC 5880         UK Southwest, M. edilate Rayworn              65         Wihn parchaemolytics         RDCC 5880         Galica Spain              66         Wihn parchaemolytics         RDCC 5880         Galica Spain	63	Vibrio parahaemolyticus	RDCC 5848	UK Southwest, Nereididae Ragworm	-	+	-
65         Within partheemorphicus         RDCC 5880         UK Suuthwest, M edulis              67         Within partheemorphicus         RDCC 5880         Galicia Spain              68         Within partheemorphicus         RDCC 5880         Galicia Spain               69         Within partheemorphicus         RDCC 5871         Southampton, UK	64	Vibrio parahaemolyticus	RDCC 5849	UK Southwest, Nereididae Ragworm	-	+	-
66         Whini parahaemphytus         NDCC 5883         Galica Spain              68         Whon parahaemphytus         NDCC 5880         Galica Spain              70         Whon parahaemphytus         NDCC 5870         Galica Spain	65	Vibrio parahaemolyticus	RDCC 5850	UK Southwest, M. edulis	-	+	-
67         Wire parahearnolyticus         RECC 5880         Galicia Spain             68         Wolz parahearnolyticus         RECC 5870         Galicia Spain             70         Wolz parahearnolyticus         RECC 5872         Southampton, UK             71         Wolz parahearnolyticus         RECC 5872         Southampton, UK             72         Wolz parahearnolyticus         RECC 5875         Santago de Composteria, Spain, Human             73         Wolz parahearnolyticus         RECC 5876         Santago de Composteria, Spain, Human             74         Wolz parahearnolyticus         RECC 5878         Santago de Composteria, Spain, Human             74         Wolz parahearnolyticus         RECC 5878         Santago de Composteria, Spain, Human             75         Wolz parahearnolyticus         RECC 5878         Santago de Composteria, Spain, Human	66	Vibrio parahaemolyticus	RDCC 5863	Galicia Spain	-	+	-
68         Whrip partheemplyticus         PDCC 58/0         Galles Spain         -         +         +           70         White partheemplyticus         PDCC 58/7         Southampton, UK         -         +         +           71         White partheemplyticus         PDCC 58/7         Southampton, UK         -         +         +           72         White partheemplyticus         PDCC 58/7         Southampton, UK         -         +         -           73         White partheemplyticus         RDCC 58/7         Santilago de Composteria, Spain. Human         -         -           74         White partheemplyticus         RDCC 58/7         Santilago de Composteria, Spain. Human         -         -           75         White partheemplyticus         RDCC 58/7         Santilago de Composteria, Spain. Human         -         -           78         White partheemplyticus         RDCC 58/8         Santilago de Composteria, Spain. Clinical         -         -           79         White partheemplyticus         RDCC 58/8         UK Southwest         -         -         -           79         White partheemplyticus         RDCC 58/8         UK Southwest, M. eduls         -         -           70         White partheemplyticus         RD	67	Vibrio parahaemolyticus	RDCC 5868	Worm	-	+	-
69         Watering parcheomorphics         RPCC 5870         Galles Sprin            10         Watering parcheomorphics         RPCC 5877         Southampton, UK             11         Watering parcheomorphics         RPCC 5877         Southampton, UK             12         Watering parcheomorphics         RPCC 5875         Mean parcheomorphics            13         Watering parcheomorphics         RPCC 5876         Santtago de Compostenti, Spain, Human            14         Watering parcheomorphics         RPCC 5877         Santtago de Compostenti, Spain, Human            15         Watering parcheomorphics         RPCC 5879         Santtago de Compostenti, Spain, Human            17         Watering parcheomorphics         RPCC 5882         Universidad de Satago de Compostenti, Spain, Human            18         Watering parcheomorphics         RPCC 5882         UK Southwest             19         Watering parcheomorphics         RPCC 5887         UK Southwest, Medulis             10         Watering parcheomorphics         RPCC 5887         UK Southwest, Medulis             12         Wate	68	Vibrio parahaemolyticus	RDCC 5869	Galicia Spain	-	+	-
0         vibris parabempirization         NDC 5873         Southampton, UK         0         0           11         Vibris parabempirization         NDC 5873         Southampton, UK         0         0           12         Vibris parabempirization         NDC 5873         Southampton, UK         0         0           13         Vibris parabempirization         NDC 5875         Southampton, UK         0         0           14         Vibris parabempirization         NDC 5877         Southago de Compostella, Spain, Human         0         0           15         Vibris parabempirization         NDC 5877         Santiago de Compostella, Spain, Human         0         0           16         Vibris parabempirization         NDC 5878         Santiago de Compostella, Spain, Clinical         0           17         Vibris parabempirization         RDC 5882         UK Southwest, Gigga         0         0           18         Vibris parabempirization         RDC 5883         UK Southwest, M. edulis         0         0           18         Vibris parabempirization         RDC 5883         UK Southwest, Gigga         0         0           18         Vibris parabempirization         RDC 5883         UK Southwest, Gigga         0         0	69	Vibrio parahaemolyticus	RDCC 5870	Galicia Spain	_	+	
Nume         Nume <th< td=""><td>70</td><td>Vibrio parahaamaluticus</td><td>RDCC 5870</td><td>Southampton LIK</td><td></td><td></td><td></td></th<>	70	Vibrio parahaamaluticus	RDCC 5870	Southampton LIK			
11         Water parahaemolytics         RUCC 58/3         Southamplan, SA         -           12         Water parahaemolytics         RUCC 58/3         Water parahaemolytics         +         +           13         Water parahaemolytics         RUCC 58/3         Sartiago de Composteria, Spain, Human         +         +           14         Water parahaemolytics         RUCC 58/7         Sartiago de Composteria, Spain, Human         +         +           15         Water parahaemolytics         RUCC 58/7         Sartiago de Composteria, Spain, Human         +         +           16         Water parahaemolytics         RUCC 58/2         Sartiago de Composteria, Spain, Human         +         +           17         Water parahaemolytics         RUCC 58/2         UR Southwest         -         +         -           18         Water parahaemolytics         RUCC 58/2         UR Southwest         -         +         -           18         Water parahaemolytics         RUCC 58/2         UR Southwest         -         +         -           18         Water parahaemolytics         RUCC 58/2         UR Southwest         -         +         -           18         Water parahaemolytics         RUCC 58/2         UR Southwest         -	70	Vibrio parahaomolyticus	RDCC 5872	Southampton, UK	-	т .	-
12       Waho particlemolyticus       HDC 5874       Anre U.C. 2 gas       -       +       -         13       Waho particlemolyticus       RDCC 5875       Sartiago de Compostela, Spain. Human       -       +       -         14       Waho particlemolyticus       RDCC 5875       Sartiago de Compostela, Spain. Human       -       +       -         17       Waho particlemolyticus       RDCC 5875       Sartiago de Compostela, Spain. Human       -       +       -         18       Waho particlemolyticus       RDCC 5880       Universidad de Satago de Compostela, Spain. Human       -       +       -         19       Waho particlemolyticus       RDCC 5880       Universidad de Satago de Compostela, Spain. Human       -       +       -         10       Waho particlemolyticus       RDCC 5880       UK Southwest       -       +       -         14       Waho particlemolyticus       RDCC 5883       UK Southwest       -       +       -         15       Waho particlemolyticus       RDCC 5883       Lame lough. C gigas       -       +       -         16       Waho particlemolyticus       RDC 5883       Lame lough. C gigas       -       +       -         17       Waho particlemolyticus       RDC 5883	71		RDCC 5873	Southampton, UK	-	+	-
73         Ware paraheemolyticus         RDCC 5875         Weymouth, UK, P. maximus	/2	Vibrio parahaemolyticus	RDCC 5874	Anre, UK. C. gigas	-	+	-
74     Warb paraheemolytics     RDCC 5876     Santiago de Composteria, Spain, Human     -     +     -       75     Warb paraheemolytics     RDCC 5878     Santiago de Composteria, Spain, Human     -     +     -       76     Warb paraheemolytics     RDCC 5878     Santiago de Composteria, Spain, Human     -     +     -       78     Warb paraheemolytics     RDCC 5883     Universidad de Satiago de Compostela, Spain, Human     -     +     -       79     Warb paraheemolytics     RDCC 5883     UK Southwest     -     +     -       81     Warb paraheemolytics     RDCC 5883     UK Southwest, Gigas     -     +     -       83     Warb paraheemolytics     RDCC 5883     Lure longh, Ciggas     -     +     -       84     Warb paraheemolytics     RDCC 5883     Larne longh, Ciggas     -     +     -       84     Warb paraheemolytics     RDCC 5883     Larne longh, Ciggas     -     +     -       85     Warb paraheemolytics     RDCC 5883     Larne longh, Ciggas     -     +     -       86     Warb paraheemolytics     RDCC 5893     Diaderd, Heduis     -     +     -       87     Warb paraheemolytics     RDCC 5893     River Thames, Eincheri sinensis     -     <	73	Vibrio parahaemolyticus	RDCC 5875	Weymouth, UK. P. maximus	-	+	-
75         Whrie parcheemolytics         RDCC 5877         Santiago de Composteria, Spain, Human         -         +         -           77         Whrie parcheemolytics         RDCC 5879         Santiago de Composteria, Spain, Human         -         +         -           78         Whrie parcheemolytics         RDCC 5880         Universidad é statago de Composteria, Spain, Clinical         -         +         -           79         Whrie parcheemolytics         RDCC 5881         UK Southwest         -         +         -           80         Whrie parcheemolytics         RDCC 5883         UK Southwest, Me duils         -         +         -           81         Whrie parcheemolytics         RDCC 5884         UK Southwest, Me duils         -         +         -           82         Whrie parcheemolytics         RDCC 5884         UK Southwest, Me duils         -         +         -           83         Whrie parcheemolytics         RDCC 5881         Linewa parce der, M. eduils         -         +         -           84         Whrie parcheemolytics         RDCC 5882         Dualeford, M. eduils         -         +         -           85         Whrie parcheemolytics         RDCC 5892         Ruer Thamess, Ericheler sinensis         -	74	Vibrio parahaemolyticus	RDCC 5876	Santiago de Composterla, Spain. Human	-	+	-
76         Whrie parcheemolytics         RDCC 5878         Santage de Composterle, Spain, Human         -         +         -           77         Wink parcheemolytics         RDCC 5878         Santage de Composterle, Spain, Linical Stol sample         -         +         -           78         Wink parcheemolytics         RDCC 5881         Uliversidad de Satago de Composterle, Spain, Clinical Stol sample         -         +         +         +           79         Wink parcheemolytics         RDCC 5881         Ul Southwest, M. eduis         -         +         +         +           81         Wink parcheemolytics         RDCC 5883         Ul Southwest, M. eduis         -         +         +         +           82         Wink parcheemolytics         RDCC 5887         C. gigas         -         +         +         +           83         Wink parcheemolytics         RDCC 5883         Larne lough, C. gigas         -         +         +         +           84         Wink parcheemolytics         RDCC 5893         Daleford, M. eduis         -         +         +         +           85         Wink parcheemolytics         RDCC 5893         River Thames, Enchelr sinensis         +         +         +         +           86 <td>75</td> <td>Vibrio parahaemolyticus</td> <td>RDCC 5877</td> <td>Santiago de Composterla, Spain. Human</td> <td>-</td> <td>+</td> <td>-</td>	75	Vibrio parahaemolyticus	RDCC 5877	Santiago de Composterla, Spain. Human	-	+	-
77         Vibrio paraheemolyticus         RDCC 3879         Santiago de Compostela, Spain. Clinical stalogo de Compostela, Spain. Clinicalogo de Compostela, Spain. Clinicalogo stalogo de Compostela, Sp	76	Vibrio parahaemolyticus	RDCC 5878	Santiago de Composterla, Spain. Human	-	+	-
78         Vibrio paraheemolyticus         NDCC 5880         Universidal de Satago de Compostela, Spain. Clinical stol sample         -         +           79         Vibrio paraheemolyticus         NDCC 5881         UK Southwest         -         +           80         Wibrio paraheemolyticus         NDCC 5881         UK Southwest.         +         +           81         Wibrio paraheemolyticus         NDCC 5881         UK Southwest.         +         +           82         Wibrio paraheemolyticus         NDCC 5881         UK Southwest.         +         +           83         Wibrio paraheemolyticus         RDCC 5884         UK Southwest.         +         +           84         Wibrio paraheemolyticus         RDCC 5887         C. gigas         -         +         -           84         Wibrio paraheemolyticus         RDCC 5880         Daleford, M. edulis         +         +         -           85         Vibrio paraheemolyticus         RDCC 5891         River Thames. Frichel's inensis         +         +         -           88         Vibrio paraheemolyticus         RDCC 5893         D. Giadwell. O. edulis         +         +         -           90         Vibrio paraheemolyticus         RDCC 5893         River Thames. Yache's	77	Vibrio parahaemolyticus	RDCC 5879	Santiago de Composterla, Spain. Human	-	+	-
18         Vario parchaemolyticus         RDCC 5880         Vario Standar         Image: Standard				Universidad de Satiago de Compostela. Spain. Clinical			
79         Vibrio parahaemolyticus         RDCC 5881         UK Southwest         -         +           80         Vibrio parahaemolyticus         RDCC 5882         UK Southwest, M. eduis         -         +         -           81         Vabrio parahaemolyticus         RDCC 5883         UK Southwest, M. eduis         -         +         -           82         Vibrio parahaemolyticus         RDCC 5887         C. gigas         -         +         -           84         Vibrio parahaemolyticus         RDCC 5887         C. gigas         -         +         -           85         Vibrio parahaemolyticus         RDCC 5880         Larne lough, C. gigas         -         +         -           86         Vibrio parahaemolyticus         RDCC 5893         Daleford, M. eduis         -         +         -           87         Vibrio parahaemolyticus         RDCC 5893         D. Gladwell. O. eduis         -         +         -           88         Vibrio parahaemolyticus         RDCC 5893         RWer Thames, Eriocheri sinensis         -         +         -           91         Vibrio parahaemolyticus         RDCC 5893         RWer Thames, water sample         -         +         -           92         Vibrio parahae	78	Vibrio parahaemolyticus	RDCC 5880	stool sample	-	+	-
12         12         12         12         14           10         Vibrio parahaemolyticus         RDCC 5883         UK Southwest, M. edulis         -         +         -           11         Vibrio parahaemolyticus         RDCC 5883         UK Southwest, C. gigas         -         +         -           12         Vibrio parahaemolyticus         RDCC 5887         C. gigas         -         +         -           13         Vibrio parahaemolyticus         RDCC 5887         C. gigas         -         +         -           14         Vibrio parahaemolyticus         RDCC 5889         Daleford, M. edulis         -         +         -           15         Vibrio parahaemolyticus         RDCC 5893         River Thames. Encher sinensis         -         +         -           16         Vibrio parahaemolyticus         RDCC 5893         River Thames. Encher sinensis         -         +         -           17         Vibrio parahaemolyticus         RDCC 5893         River Thames. Encher sinensis         -         +         -           16         Vibrio parahaemolyticus         RDCC 5893         River Thames, water sample         -         +         -           17         Vibrio parahaemolyticus         RDCC 5898 <td>79</td> <td>Vibrio parabaemolyticus</td> <td>RDCC 5881</td> <td></td> <td>_</td> <td>+</td> <td>_</td>	79	Vibrio parabaemolyticus	RDCC 5881		_	+	_
Non-Dynamic production         Non-Construction         Construction         Construction           10         White parahaemolyticus         RDCC 5883         UUS Southwest, C. gigas         -         +         -           82         White parahaemolyticus         RDCC 5883         LUS Southwest, M. Eduils         -         +         -           84         White parahaemolyticus         RDCC 5883         Lare lough, C. gigas         -         +         -           84         White parahaemolyticus         RDCC 5883         Lare lough, C. gigas         -         +         -           85         White parahaemolyticus         RDCC 5890         Limosa pre dep. M. eduils         -         +         -           87         White parahaemolyticus         RDCC 5893         D. Gladwell O. eduils         -         +         -           88         White parahaemolyticus         RDCC 5893         D. Gladwell O. eduils         -         +         -           91         White parahaemolyticus         RDCC 5895         River Thames, Eriocher is inensis         -         +         -           92         White parahaemolyticus         RDCC 5895         River Thames, water sample         -         +         -           93         White pa	80	Vibrio parahaemolyticus	RDCC 5882				
1         Winking parahaemolyticus         RDCC 5884         UK Southwest, C. gigas         -         +         -           83         Winking parahaemolyticus         RDCC 5885         Fawey, Werns, O. edulis         -         +         -           84         Winking parahaemolyticus         RDCC 5887         C. gigias         -         +         -           85         Winking parahaemolyticus         RDCC 5889         Daleford, M. edulis         -         +         -           86         Winking parahaemolyticus         RDCC 5889         Daleford, M. edulis         -         +         -           87         Vibrio parahaemolyticus         RDCC 5892         River Thames, Eriocheri sinensis         -         +         -           88         Vibrio parahaemolyticus         RDCC 5892         River Thames, Eriocheri sinensis         -         +         -           90         Vibrio parahaemolyticus         RDCC 5893         River Thames, Eriocheri sinensis         -         +         -           91         Wibrio parahaemolyticus         RDCC 5895         River Thames, Water sample         -         +         -           92         Wibrio parahaemolyticus         RDCC 5898         River Thames, Water sample         -         +         <	01		RDCC 5882	UK Southwest	-	+	-
B2         Winking parthaemolyticus         NOLC 3884         Unk SouthWest, C. gigiss	02		RDCC 5883		-	+	-
B3         Vibrio parahaemolyticus         RDCC 5885         Fawey, Werns. O. coluits         -         +         +           84         Wibrio parahaemolyticus         RDCC 5887         C. gigias         -         +         -           85         Wibrio parahaemolyticus         RDCC 5888         Larne lough. C. gigas         -         +         -           86         Wibrio parahaemolyticus         RDCC 5889         Daleford, M. edulis         -         +         -           87         Wibrio parahaemolyticus         RDCC 5891         River Thames. Enrocher i sinensis         -         +         -           88         Wibrio parahaemolyticus         RDCC 5892         River Thames. Enrocher i sinensis         -         +         -           90         Wibrio parahaemolyticus         RDCC 5893         River Thames, set rample         -         +         -           91         Wibrio parahaemolyticus         RDCC 5898         River Thames, water sample         -         +         -           92         Wibrio parahaemolyticus         VP4         Unknown         +         +         -           93         Wibrio parahaemolyticus         VP4         Unknown         +         +         -           94	82	Vibrio parahaemolyticus	RDCC 5884	UK Southwest, C. gigas	-	+	-
84         Whrie paraheemolyticus         RDCC 5887         C. gigas        +	83	Vibrio parahaemolyticus	RDCC 5885	Fawey, Wems. O. edulis	-	+	-
85     Vibrio paraheemolyticus     RDCC 5888     Larne lough. C. gigas     -     +     -       86     Vibrio paraheemolyticus     RDCC 5890     Linnesa pre dep. M. edulis     -     +     -       87     Vibrio paraheemolyticus     RDCC 5891     River Thames, Eriocheir sinensis     -     +     -       88     Vibrio paraheemolyticus     RDCC 5892     River Thames, Eriocheir sinensis     -     +     -       90     Vibrio paraheemolyticus     RDCC 5893     D. Gladwell. O. edulis     -     +     -       91     Vibrio paraheemolyticus     RDCC 5893     River Thames, water sample     -     +     -       92     Vibrio paraheemolyticus     RDCC 5898     River Thames, water sample     -     +     -       93     Vibrio paraheemolyticus     RDCC 5898     River Thames, water sample     -     +     -       94     Vibrio paraheemolyticus     VD1     Unknown     -     +     -       95     Vibrio paraheemolyticus     VP1     Unknown     -     +     -       97     Vibrio paraheemolyticus     VP1     Unknown     -     +     -       98     Vibrio paraheemolyticus     VP17     Faeces, Fae fast     -     +       90     Vib	84	Vibrio parahaemolyticus	RDCC 5887	C. gigias	-	+	-
86         Vibrio paraheemolyticus         RDCC 5889         Daleford, M. edulis         -         +         -           87         Vibrio paraheemolyticus         RDCC 5891         River Thames, Eriocheir sinensis         -         +         -           88         Vibrio paraheemolyticus         RDCC 5891         River Thames, Eriocheir sinensis         -         +         -           89         Vibrio paraheemolyticus         RDCC 5893         D. Gladwell. O. edulis         -         +         -           91         Vibrio paraheemolyticus         RDCC 5895         River Thames, water sample         -         +         -           92         Vibrio paraheemolyticus         RDCC 5895         River Thames, water sample         -         +         -           93         Vibrio paraheemolyticus         RDCC 5898         River Thames, water sample         -         +         -           94         Vibrio paraheemolyticus         VP1         Unknown         -         +         -           95         Vibrio paraheemolyticus         VP13         Faeces, Far East         -         +         -           96         Vibrio paraheemolyticus         VP13         Faeces, Far East         -         +         -           <	85	Vibrio parahaemolyticus	RDCC 5888	Larne lough. C. gigas	-	+	-
17         Vibrio parahaemolyticus         RDCC 5890         Limosa pre dep. M. edulis         -         +         -           88         Vibrio parahaemolyticus         RDCC 5891         River Thames. Eriocheir sinensis         -         +         -           90         Vibrio parahaemolyticus         RDCC 5892         River Thames. Eriocheir sinensis         -         +         -           91         Vibrio parahaemolyticus         RDCC 5893         D. Gladwell.O. edulis         +         +         -           92         Vibrio parahaemolyticus         RDCC 5895         River Thames, water sample         +         +         -           93         Vibrio parahaemolyticus         RDCC 5898         River Thames, water sample         -         +         -           94         Vibrio parahaemolyticus         RDCC 5898         River Thames, water sample         -         +         -           95         Vibrio parahaemolyticus         VP11         Unknown         -         +         -           96         Vibrio parahaemolyticus         VP12         Faeces, Thatland         -         +         -           97         Vibrio parahaemolyticus         VP13         Faeces, Thatland         -         +         -	86	Vibrio parahaemolyticus	RDCC 5889	Daleford, M. edulis	-	+	-
88         Vibrio parahaemolyticus         RDCC 5891         River Thames. Eriocheir sinensis         +         +           89         Whrio parahaemolyticus         RDCC 5892         River Thames. Eriocheir sinensis         -         +         -           90         Whrio parahaemolyticus         RDCC 5893         D. Gladwell. O. edulis         -         +         -           91         Whrio parahaemolyticus         RDCC 5895         River Thames, water sample         -         +         -           93         Vibrio parahaemolyticus         RDCC 5896         River Thames, water sample         -         +         -           94         Vibrio parahaemolyticus         RDCC 5899         River Thames, water sample         -         +         -           95         Vibrio parahaemolyticus         VP1         Unknown         -         +         -           96         Vibrio parahaemolyticus         VP13         Faeces, Far East         -         +         -           97         Vibrio parahaemolyticus         VP13         Faeces, Far East         -         +         -           98         Vibrio parahaemolyticus         VP13         Gaeces, Far East         -         +         -           100         Vibrio	87	Vibrio parahaemolyticus	RDCC 5890	Limosa pre dep. M. edulis	-	+	-
89       Wibrio parahaemolyticus       RDCC 5892       River Thames, Eriocheir sinensis       -       +         90       Wibrio parahaemolyticus       RDCC 5893       D. Gladwell. O. edulis       -       +         91       Wibrio parahaemolyticus       RDCC 5895       River Lee Area London, Eriocheir sinensis       -       +         92       Wibrio parahaemolyticus       RDCC 5895       River Lee Area London, Eriocheir sinensis       -       +       -         93       Wibrio parahaemolyticus       RDCC 5898       River Thames, water sample       -       +       -         94       Wibrio parahaemolyticus       RDCC 5899       River Thames, water sample       -       +       -         95       Wibrio parahaemolyticus       VP1       Unknown       -       +       -         96       Wibrio parahaemolyticus       VP13       Faeces, Far East       -       +       -         97       Vibrio parahaemolyticus       VP33       Sea, UK       -       +       -         100       Vibrio parahaemolyticus       VP34       Cockles, UK       -       +       -         108       Vibrio parahaemolyticus       VP34       Cockles, UK       -       +       -         10	88	Vibrio parahaemolyticus	RDCC 5891	River Thames. Eriocheir sinensis	-	+	-
Data parahaemolyticus         PRCC 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         VP1         Unknown         -         +         -           96         Vibrio parahaemolyticus         VP1         Unknown         -         +         -           97         Vibrio parahaemolyticus         VP13         Faeces, Far East         -         +         -           98         Vibrio parahaemolyticus         VP13         Faeces, LK         -         +         -           100         Vibrio parahaemolyticus         VP34         Cockles, UK         -         +         -           101         Vibrio parahaemolyticus         VP47         Unknown         -	89	Vibrio parahaemolyticus	RDCC 5892	Biver Thames, Eriocheir sinensis	-	+	-
Solution Processing         Note of the second	00	Vibrio parahaemolyticus	RDCC 5802	D Gladwell O edulis			
1         With During Productions         NCC 3934         The Hold Net Net W. Eduits         -         +         -           92         Wibrie parahemolyticus         RDCC 5896         River Lee Area London, Ericcheir sinensis         -         +         -           93         Wibrie parahemolyticus         RDCC 5896         Newtons Bay, water sample         -         +         -           94         Vibrie parahemolyticus         RDCC 5898         River Thames, water sample         -         +         -           95         Wibrie parahemolyticus         VP1         Unknown         -         +         -           96         Wibrie parahemolyticus         VP1         Unknown         -         +         -           97         Wibrie parahemolyticus         VP13         Faeces, Far East         -         +         -           98         Wibrie parahemolyticus         VP17         Faeces, Thailand         -         +         -         -           100         Wibrie parahemolyticus         VP34         Cockles, UK         -         +         -         -           101         Wibrie parahemolyticus         VP67         Unknown         -         +         -           102         Wib	90	Vibrio parahaomolyticus	RDCC 5893	Lielford Divor M. adulia	-	т	-
32         Ward barandemolyticus         RUCC Says         RWPL Lée Aréa London, Elfocner sinnesis         -         +         -           33         Wibrio parahaemolyticus         RDCC Says         Never Thames, water sample         -         +         +           34         Wibrio parahaemolyticus         RDCC Says         River Thames, water sample         -         +         +           35         Wibrio parahaemolyticus         RDCC Says         River Thames, water sample         -         +         -           36         Wibrio parahaemolyticus         VP1         Unknown         -         +         -           37         Vibrio parahaemolyticus         VP1         Unknown         -         +         -           38         Wibrio parahaemolyticus         VP13         Faeces, Far East         -         +         -           30         Vibrio parahaemolyticus         VP17         Faeces, Far East         -         +         -         -           30         Vibrio parahaemolyticus         VP17         Unknown         -         +         -         -         -           30         Vibrio parahaemolyticus         VP11         Unknown         -         +         -         -         + </td <td>91</td> <td></td> <td>RDCC 5894</td> <td>Hellord River, Ivi, edulis</td> <td>-</td> <td>+</td> <td>-</td>	91		RDCC 5894	Hellord River, Ivi, edulis	-	+	-
33       Vibrio paraheemolyticus       RDCC 5896       Newtons Bay, water sample       -       +       -         95       Vibrio paraheemolyticus       RDCC 5899       River Thames, water sample       -       +       -         96       Vibrio paraheemolyticus       VP1       Unknown       -       +       -         97       Vibrio paraheemolyticus       VP1       Unknown       -       +       -         98       Vibrio paraheemolyticus       VP13       Faeces, Far East       -       +       -         99       Vibrio paraheemolyticus       VP17       Faeces, Thailand       -       +       -         100       Vibrio paraheemolyticus       VP33       Sea, UK       -       +       -         101       Vibrio paraheemolyticus       VP34       Cockles, UK       -       +       -         103       Vibrio paraheemolyticus       VP71       Unknown       -       +       -         103       Vibrio valinificus       CM 240       Human Leg, USA       -       +       +         104       Vibrio valinificus       CM 2840       Human Leg, USA       -       +       +         105       Vibrio valinificus       RDCC 1268 </td <td>92</td> <td>Vibrio paranaemolyticus</td> <td>RDCC 5895</td> <td>River Lee Area London, Eirocheir sinensis</td> <td>-</td> <td>+</td> <td>-</td>	92	Vibrio paranaemolyticus	RDCC 5895	River Lee Area London, Eirocheir sinensis	-	+	-
94     Vibrio parahaemolyticus     RDCC 5898     River Thames, water sample     -     +       95     Vibrio parahaemolyticus     RDCC 5899     River Thames, water sample     -     +       96     Vibrio parahaemolyticus     VP1     Unknown     -     +       97     Vibrio parahaemolyticus     VP4     Unknown     -     +       98     Vibrio parahaemolyticus     VP13     Faeces, Far East     -     +       100     Vibrio parahaemolyticus     VP33     Sea, UK     -     +       101     Vibrio parahaemolyticus     VP34     Cockles, UK     -     +       101     Vibrio parahaemolyticus     VP34     Cockles, UK     -     +       102     Vibrio parahaemolyticus     VP71     Unknown     -     +       103     Vibrio parahaemolyticus     VP71     Unknown     -     +       104     Vibrio valnificus     VP71     Unknown     -     +       105     Vibrio valnificus     MT745     Unknown     -     +       106     Vibrio valnificus     ATCC 29307     Blood, USA     -     -       107     Vibrio valnificus     RDCC 1268     Isolated seafood, Japan. Environmental strain     -     +       108	93	Vibrio parahaemolyticus	RDCC 5896	Newtons Bay, water sample	-	+	-
95       Vibrio parahaemolyticus       RDCC 5899       River Thames, water sample       -       +       -         96       Vibrio parahaemolyticus       VP1       Unknown       -       +       -         97       Vibrio parahaemolyticus       VP1       Unknown       -       +       -         98       Wibrio parahaemolyticus       VP13       Faeces, Far East       -       +       -         99       Vibrio parahaemolyticus       VP17       Faeces, Thalland       -       +       -         100       Vibrio parahaemolyticus       VP34       Cockles, UK       -       +       -         101       Vibrio parahaemolyticus       VP34       Cockles, UK       -       +       -         102       Vibrio parahaemolyticus       VP77       Unknown       -       +       -         103       Vibrio parahaemolyticus       VP77       Unknown       -       +       -         105       Vibrio vulnificus       MP445       Unknown       -       +       -         106       Vibrio vulnificus       ATCC 29307       Blood, USA       -       -       +         107       Vibrio vulnificus       RDCC 1268       Isolated seafood	94	Vibrio parahaemolyticus	RDCC 5898	River Thames, water sample	-	+	-
96Vibrio parahaemolyticusVP1Unknown-+97Vibrio parahaemolyticusVP4Unknown-+98Vibrio parahaemolyticusVP13Faeces, Far East-+99Vibrio parahaemolyticusVP17Faeces, Far East-+100Vibrio parahaemolyticusVP33Sea, UK-+-101Vibrio parahaemolyticusVP34Cockles, UK-+-102Vibrio parahaemolyticusVP77Unknown-+-103Vibrio parahaemolyticusVP71Unknown-+-104Vibrio parahaemolyticusVP71Unknown-+-105Vibrio parahaemolyticusVP71Unknown-+-106Vibrio vulnificusCCM 2840Human Leg, USA+107Vibrio vulnificusRDCC 1268Isolated seafood, Japan. Environmental strain-++108Vibrio vulnificusRDCC 2887Isolated seafood, Japan. Environmental strain+110Vibrio vulnificusRDCC 2889Isolated seafood, Japan. Environmental strain+111Vibrio vulnificusRDCC 2892Isolated seafood, Japan. Environmental strain+118Vibrio vulnificusRDCC 2893Isolated seafood, Japan. Environmental strain+119Vibrio vulnificusRDCC 2855Bristol Channel,	95	Vibrio parahaemolyticus	RDCC 5899	River Thames, water sample	-	+	-
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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       Wbrio parahaemolyticus       VP71       Unknown       -       +       -         104       Vibrio parahaemolyticus       VP87       Unknown       -       +       -         105       Vibrio vulnificus       MH 7445       Unknown       -       +       +         106       Vibrio vulnificus       RDCC 1268       Isolated seafood, Japan. Environmental strain       -       +         108       Vibrio vulnificus       RDCC 2887       Isolated seafood, Japan. Environmental strain       -       +         110       Vibrio vulnificus       RDCC 2891       Isolated seafood, Japan. Environmental strain       -       +         1111       Vibrio vulnificus       RDCC	97	Vibrio parahaemolyticus	VP4	Unknown	-	+	-
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113Vibrio VulnificusNDCC 2892Isolated seafood, Japan. Environmental strain+114Vibrio vulnificusRDCC 2893Isolated seafood, Japan. Environmental strain+115Vibrio vulnificusRDCC 5025Unknown+116Vibrio vulnificusRDCC 5855Bristol Channel, water discharge+117Vibrio vulnificusRDCC 5856Bristol Channel, water discharge+118Vibrio vulnificusRDCC 5857Bristol Channel, water discharge+119Vibrio vulnificusRDCC 5858Bristol Channel, beach control, water+120Vibrio vulnificusRDCC 5861Bristol Channel, water intake+121Vibrio vulnificusRDCC 5862Bristol Channel, water intake+122Vibrio vulnificusRDCC 5871Southampton, UK+123Vibrio vulnificusCCUG 15887Linknown+	112	Vibrio vulnificus	RDCC 2091	Isolated soafood Japan Environmental strain	-	-	
114Vibrio vulnificusRDCC 2893Isolated searood, Japan. Environmental strain+115Vibrio vulnificusRDCC 5025Unknown+116Vibrio vulnificusRDCC 5855Bristol Channel, water discharge+117Vibrio vulnificusRDCC 5856Bristol Channel, water discharge+118Vibrio vulnificusRDCC 5857Bristol Channel, water discharge+119Vibrio vulnificusRDCC 5858Bristol Channel, beach control, water+120Vibrio vulnificusRDCC 5861Bristol Channel, water intake+121Vibrio vulnificusRDCC 5862Bristol Channel, water intake+122Vibrio vulnificusRDCC 5871Southampton, UK+123Vibrio vulnificusCCUG 15887Unknown+	113			Isolated seafood, Japan, Environmental Strain	-	-	+
115Vibrio vulnificusRDCC 5025Unknown+116Vibrio vulnificusRDCC 5855Bristol Channel, water discharge+117Vibrio vulnificusRDCC 5856Bristol Channel, water discharge+118Vibrio vulnificusRDCC 5857Bristol Channel, water discharge+119Vibrio vulnificusRDCC 5858Bristol Channel, beach control, water+120Vibrio vulnificusRDCC 5861Bristol Channel, water intake+121Vibrio vulnificusRDCC 5862Bristol Channel, water intake+122Vibrio vulnificusRDCC 5871Southampton, UK+123Vibrio vulnificusCCUG 15887Unknown+	114		RDCC 2893	Isolated seatood, Japan. Environmental strain	-	-	+
116Vibrio vulnificusRDCC 5855Bristol Channel, water discharge-+117Vibrio vulnificusRDCC 5856Bristol Channel, water discharge+118Vibrio vulnificusRDCC 5857Bristol Channel, beach control, water+119Vibrio vulnificusRDCC 5858Bristol Channel, beach control, water+120Vibrio vulnificusRDCC 5861Bristol Channel, water intake+121Vibrio vulnificusRDCC 5862Bristol Channel, water intake+122Vibrio vulnificusRDCC 5871Southampton, UK+123Vibrio vulnificusCCUG 15887Unknown+	115	Vibrio vulnificus	RDCC 5025	Unknown	-	-	+
117Vibrio vulnificusRDCC 5856Bristol Channel, water discharge+118Vibrio vulnificusRDCC 5857Bristol Channel, beach control, water+119Vibrio vulnificusRDCC 5858Bristol Channel, beach control, water+120Vibrio vulnificusRDCC 5861Bristol Channel, water intake+121Vibrio vulnificusRDCC 5862Bristol Channel, water intake+122Vibrio vulnificusRDCC 5871Southampton, UK+123Vibrio vulnificusCCUG 15887Unknown+	116	Vibrio vulnificus	RDCC 5855	Bristol Channel, water discharge	-	-	+
118Vibrio vulnificusRDCC 5857Bristol Channel, beach control, water+119Vibrio vulnificusRDCC 5858Bristol Channel, beach control, water+120Vibrio vulnificusRDCC 5861Bristol Channel, water intake+121Vibrio vulnificusRDCC 5862Bristol Channel, water intake+122Vibrio vulnificusRDCC 5871Southampton, UK+123Vibrio vulnificusCCUG 15887Unknown+	117	Vibrio vulnificus	RDCC 5856	Bristol Channel, water discharge	-	-	+
119Vibrio vulnificusRDCC 5858Bristol Channel, beach control, water+120Vibrio vulnificusRDCC 5861Bristol Channel, water intake+121Vibrio vulnificusRDCC 5862Bristol Channel, water intake+122Vibrio vulnificusRDCC 5871Southampton, UK+123Vibrio vulnificusCCUG 15887Unknown+	118	Vibrio vulnificus	RDCC 5857	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     Lukrown     -     +	119	Vibrio vulnificus	RDCC 5858	Bristol Channel, beach control, water	-	-	+
121     Vibrio vulnificus     RDCC 5862     Bristol Channel, water intake     -     +       122     Vibrio vulnificus     RDCC 5871     Southampton, UK     -     +       123     Vibrio vulnificus     CCUG 15887     Luknown     -     +	120	Vibrio vulnificus	RDCC 5861	Bristol Channel, water intake	-	-	+
122     Vibrio vulnificus     RDCC 5871     Southampton, UK     -     +       123     Vibrio vulnificus     CCUG 15887     Unknown     -     +	121	Vibrio vulnificus	RDCC 5862	Bristol Channel, water intake	-	-	+
123 Vibrio vulnificus CCUG 15887 Unknown	122	Vibrio vulnificus	RDCC 5871	Southampton, UK	-	-	+
	123	Vibrio vulnificus	CCUG 15887	Unknown	-	-	+

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		А				
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	-	-	+

<sup>a</sup>Results identical for both QuantStudio 5 and 7500 Fast.

<sup>b</sup>RDCC = Research and Development Culture Collection 1, Thermo Fisher Scientific, Basingstoke, UK

<sup>c</sup>CCUG = Culture Collection University of Gothenburg, Göteborg, Sweden.

<sup>d</sup>MH = Research and Development Culture Collection 2, Thermo Fisher Scientific, Basingstoke, UK

<sup>e</sup>NCTC = National Collection of Type Cultures, Health Protection Agency, London, UK

<sup>f</sup>CECT = Spanish Type Culture Collection, Valencia, Spain.

<sup>g</sup>CCM = Czech Collection of Microorganisms, Kralovopolska, Czech Republic.

<sup>h</sup>ATCC = American Type Culture Collection, Manassas, VA, USA.

<sup>i</sup>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)

	,			SureTect Vibri	o cholerae. V. parahaemolyticu	us and V. vulnificus
					PCR Assay result <sup>a</sup>	
No.	Organism	Source	Origin	V. cholerae	V. parahaemolyticus	V. vulnificus
1	Acinetobacter iwoffii	RDCC <sup>b</sup> 2962	Unknown	-	-	-
2	Actinobacillus pleuropneumoniae	RDCC 4998	Unknown	-	-	-
3	Aeromonas hydrophilia	NCTC <sup>c</sup> 7810	Frog	-	-	-
4	Candida albicans	RDCC 0434	Unknown	-	-	-
5	Citrobacter freundii	NCTC 8581	Red Leg Tree Frog	-	-	-
6	Citrobacter koseri	ATCC <sup>d</sup> 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	Listoria monocutogonos	ATCC 12022	Spinal fluid, child, meningitis,	-	-	-
10	Listena monocytogenes	ATCC 15952	Germany			
17	Listeria seeligeri	ATCC 35967	Soil, Germany	-	-	-
18	Listeria welshimeri	NCTC 11857	Compost	-	-	-
19	Pasteurella multocida	ATCC 43137	Pig	-	-	-
20	Pedioccus sp	ATCC 33316	Dried beer yeast	-	-	-
21	Plesiomonas shigelloides	NCTC 10360	Unknown	-	-	-

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22	Proteus mirabilis	NCTC 10975	Human urine	-	-	-
23	Proteus spp.	RDCC 0237	Unknown	-	-	-
24	Salmonolla sor Tunhimurium	ATCC 14028	4-week-old chickens - heart	-	-	-
24	Saimonena sei Typninnununi ATCC 1		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	VMe30	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	-	-	-
40	Vibria dissotranbique		Gastrointestinal tract of sea	-	-	-
49	vibrio diazotropriicas		urchin, Canada			
50	Vibrio protocluticus	CECT 620 T	Intestine of wood-boring	-	-	-
50			isopod			

<sup>a</sup>Results identical for QuantStudio 5 and 7500 Fast

<sup>b</sup>RDCC= Research and Development Culture Collection 1, Thermo Fisher Scientific, Basingstoke, UK

<sup>c</sup>NCTC = National Collection of Type Cultures, Health Protection Agency, London, UK

<sup>d</sup>ATCC = American Type Culture Collection, Manassas, VA, USA.

eVM = Research and Development Culture Collection 3, Thermo Fisher Scientific, Basingstoke, UK

<sup>f</sup>CECT = Spanish Type Culture Collection, Valencia, Spain.

Table 5. Thermo S	cientific Sure	ect Vibrio cholerae, V.	parahaemolyticus ar	nd V. v	ulnific	us PCR Assa	ay, Candidate v	/s. FD/	A/BAM Ch	apter 9 Refere	nce – POD	Results (1)
			MPN <sup>a</sup> /			Candid	ate <sup>c</sup>		Refere	ence		
Matrix	Timepoint	Strain	Test Portion	N <sup>b</sup>	Xd	PODc <sup>e</sup>	95% CI	х	POD <sub>R</sub> <sup>f</sup>	95% CI	$dPOD_{C^{g}}$	95% Cl <sup>h</sup>
			N/A <sup>j</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
Raw tuna	16 h	Vibrio cholerae	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
(50 g)	1011	ATCC <sup>i</sup> 14033	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	Q h and	Vibrio	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
with cream	8 n and 20 bk	parahaemolyticus	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
cheese (50 g)	2011	ATCC 27519	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
Deveryonale		Vibrio	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
(50 g)	16 h	parahaemolyticus	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
(50 g)		ATCC 43996	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			N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
mussel	16 h	Vibrio cholerae	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
extract powder (50 g)	-	ATCC 14033	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		Vibrio vulnificus	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
shrimp	16 h	ATCC 33147	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
(125 g)		ATCC 33147	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

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>Results were identical for analysis conducted on the Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument. <sup>d</sup>x = Number of positive test portions.

<sup>e</sup>POD<sub>C</sub> = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

<sup>f</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

<sup>g</sup>dPODc= Difference between the confirmed candidate method result and reference method confirmed result POD values.

<sup>h</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>ATCC = American Type Culture Collection, Manassas, VA.

 $^{j}N/A$  = Not applicable.

<sup>k</sup>= Results the same for both timepoints.

Table 6. There	Table 6. Thermo Scientific SureTect Vibrio cholerae, V. parahaemolyticus and V. vulnificus PCR Assay, Candidate vs. ISO 21872-1:2017 Reference – POD Results (1)											
			MPN <sup>a</sup> /		Candidate <sup>c</sup>				Refer	ence		
Matrix	Strain	Timepoint	Test Portion	N <sup>b</sup>	Xd	PODc <sup>e</sup>	95% CI	Х	POD <sub>R</sub> <sup>f</sup>	95% CI	apode	95% CI**
Devu turne	Vibrie ekolorea		N/A <sup>j</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
Kaw tuna $(50  \text{g})$		ATCCI 14022 16 h	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
(50 g)	ATCC 14033		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	Vibrio		N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
mussels	parahaemolyticus	16 h	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
(50 g)	ATCC 43996		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

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval. <sup>b</sup>N = Number of test portions.

<sup>c</sup>Results were identical for analysis conducted on the Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument. <sup>d</sup>x = Number of positive test portions.

<sup>e</sup>POD<sub>c</sub> = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

<sup>f</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

<sup>g</sup>dPOD<sub>c</sub>= Difference between the confirmed candidate method result and reference method confirmed result POD values.

<sup>h</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>i</sup>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)

			MPN <sup>a</sup> /			Presumptive <sup>c</sup>			Confirm	ned <sup>f</sup>		
Matrix	Timepoint	Strain	Test Portion	N <sup>b</sup>	Xd	POD <sub>CP</sub> <sup>e</sup>	95% CI	Х	POD <sub>CC</sub> <sup>g</sup>	95% CI	$dPOD_{CP}^h$	95% Cl <sup>i</sup>
David to the		Vibria abalarna	N/A <sup>k</sup>	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
$(50 \sigma)$	16 h		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 <sup>1</sup>
(50 g)		ATCC/ 14033	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		Vibrio	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
with cream	8 h	parahaemolyticus	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
cheese (50 g)		ATCC 27519	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		Vibrio	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
with cream	20 h	parahaemolvticus	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
cheese (50 g)		ATCC 27519	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		Vibrio	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
mussels (50	16 h	parahaemolyticus	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
g)		ATCC 43996	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			N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
lipped	1.5.1	Vibrio cholerae	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
mussel extract (50 g)	16 h	ATCC 14033	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	16 h	Vibrie undrifience	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
shrimp (125	10 11		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
g)	Q35	ATCC 33147	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	16 h	Vibrio vulnificus	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
shrimp (125	7500 FAST	ATCC 33147	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
g)	, 300 TAST	/// 22 3314/	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

<sup>a</sup>MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval. <sup>b</sup>N = Number of test portions.

<sup>c</sup>Unless otherwise indicated results were identical for analysis conducted on the QuantStudio 5 and 7500 Fast PCR Instruments.

<sup>d</sup>x = Number of positive test portions.

<sup>e</sup>POD<sub>CP</sub> = Candidate method presumptive positive outcomes divided by the total number of trials.

<sup>f</sup>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.

<sup>g</sup>POD<sub>CC</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>h</sup>dPOD<sub>CP</sub>= Difference between the candidate method presumptive result and candidate method confirmed result POD values.

95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>j</sup>ATCC = American Type Culture Collection, Manassas, VA.

kN/A = Not applicable.

Confirmation challenge due to high level of background flora. Culture media performance was equivalent to ISO 21872:1:2017 reference method performance.

#### Thermo Scientific SureTect<sup>™</sup> Vibrio, AOAC Research Institute *Performance Tested Methods*<sup>5M</sup> certification number 022301

#### **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  $\pm 1.5$  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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