

Community Reference Laboratory (CRL) Proficiency Testing Schemes

Report on the *Vibrio parahaemolyticus* ring trial 2009

CRL ring trial reference number: RT 29

Sample numbers: RT29.1; RT29.2; RT29.3; RT29.4

Contents

1	Introduction	2
2	Proficiency testing samples	2
2.1	Sample preparation and distribution	2
2.2	Quality control at dispatch	2
3	Results	2
3.1	Confidentiality of results	2
3.2	Reference results	2
3.3	Analysis of results	3
4	Summary	3
5	References	4

Tables

Table 1.	Source of <i>Vibrio</i> spp. strains	2
Table 2.	<i>Vibrio</i> spp. reference range and PCR results	2
Table 3.	Participants' results for RT29.1 Vial 1	5
Table 4.	Participants' results for RT29.2 Vial 2	6
Table 5.	Participants' results for RT29.3 Vial 3	7
Table 6.	Participants' results for RT29.4 Vial 4	8

Appendices

Methods used for enumeration and detection of the pathogenic markers	9
--	---

Figures

Figure 1.	<i>toxR</i> PCR	10
Figure 2.	<i>tdh</i> PCR	10
Figure 3.	<i>trh</i> PCR	10
Figure 4.	Participants' mean quantitative cfu/g for vial 1	11
Figure 5.	Participants' mean quantitative cfu/g for vial 2	11
Figure 6.	Participants' mean quantitative cfu/g for vial 3	12
Figure 7.	Participants' mean quantitative cfu/g for vial 4	12

1. Introduction

In March 2009 the CRL organised a distribution of freeze-dried cultures for the enumeration and determination of potential pathogenic *V. parahaemolyticus*.

2. Proficiency testing samples

2.1 Sample preparation and distribution

Four samples from the Cefas *Vibrio* spp. reference strain bank (Table 1) were streaked onto non-selective marine agar (MA). Plates were incubated for 18-24hrs at 30±2°C. Following visual purity checks 2-5 colony forming units (cfu) were inoculated into 2 x 100ml alkaline salt peptone water (ASPW) and incubated for 18-24hrs at 30±2°C. After incubation samples were centrifuged at 2000rpm for 20min. The supernatant was discarded and the pellets were resuspended in 15ml of mist desiccant containing ASPW. Each strain was designated a sample code, Vial 1 to 4. Aliquots of 0.2ml were added to sterile vials and freeze dried. Once freeze-drying was complete vials were stored at 3±2°C until distribution. Samples were packaged according to IATA regulations and distributed to twenty-two participating laboratories by Worldnet shipping on the 9th March 2009. On receipt, participants were requested to store the samples at 3±2°C prior to analysis during the week commencing the 16th March 2009.

Table 1: Source of *Vibrio* spp. strains

Sample ID	Reference bank designation	Source of strain	Presence of <i>tdh/trh</i>	Reference bank code
RT29. 1	<i>V. parahaemolyticus</i>	Clinical	<i>tdh</i> only	V05/65
RT29.2	<i>V. parahaemolyticus</i>	Clinical	<i>tdh</i> and <i>trh</i>	V05/14
RT29. 3	<i>V. alginolyticus</i>	Reference	No	NCTC 12160
RT29.4	<i>V. parahaemolyticus</i>	Environmental	<i>trh</i> only	V05/70

2.2 Quality control at dispatch

Samples were tested prior to distribution to confirm the reference strain bank designation using CRL standard procedures for detection of *V. parahaemolyticus*, with additional biochemical tests to confirm the identification of *V. alginolyticus*. In addition, polymerase chain reaction (PCR) was used to detect *toxR* and the haemolysin genes *tdh* and *trh* following the methods of Kim *et al* (1999) and Tada *et al* (1992) with minor modifications.

3.0 Results

3.1 Confidentiality of results

Each laboratory was provided with a personal identification number to preserve anonymity.

3.2 Reference results

Reference analyses were performed by the CRL on 4 randomly selected vials per batch to determine the species type and levels (cfu/g). The reference results are summarised in Table 2 and included in Figures 4 - 7. The presence of *tdh* and *trh* was determined using PCR as described above (Figure 1 - 3).

Table 2: *Vibrio* spp. ring trial expected reference range and PCR results

Sample ID	Reference range cfu/g	<i>toxR</i>	<i>tdh</i>	<i>trh</i>
RT29. 1	2.6 x 10 ⁵ – 2.2 x 10 ⁶	+	+	-
RT29. 2	2.2 x 10 ³ – 3.2 x 10 ⁵	+	+	+
RT29. 3	8.0 x 10 ⁴ – 1.2 x 10 ⁶	-	-	-
RT29. 4	5.6 x 10 ⁵ – 1.9 x 10 ⁶	+	-	+

3.3 Analysis of results

Twenty-two laboratories returned results to the CRL. Nine (41%) used methods that enabled detection of *tdh* and *trh* genes. Participants' results are shown in Tables 3 to 8 presumptive false positive, negative and uncertain results are identified in yellow, red and grey respectively. Results are provided for information only. No performance assessments were carried out. Laboratories reporting quantitative results are shown in Figures 4 - 7 with reference results included.

3.3.1 RT29.1 Vial 1 [CRL expected result *V. parahaemolyticus* present; *tdh* positive and *trh* negative]

Fourteen laboratories (64%) identified *V. parahaemolyticus* in both replicates. Two laboratories identified *V. parahaemolyticus* in a single replicate. Laboratories 22, 35, 76, 83, 85 and 113 reported the absence of *V. parahaemolyticus* with 1 identifying *V. vulnificus*. Nine laboratories carried out tests for *tdh* and/or *trh*. Three detected *tdh* positive and *trh* negative. Laboratory 44 reported the absence of *tdh* and laboratories 17, 19, 33, 68 and 137 reported the presence of *trh*. Nine laboratories reported quantitative results within the range of $2.0 \times 10^2 - 4.7 \times 10^5$.

3.3.2 RT29.2 Vial 2 [CRL expected result *V. parahaemolyticus* present; *tdh* and *trh* positive].

Seventeen laboratories (77%) identified *V. parahaemolyticus* in both replicates. One laboratory identified *V. parahaemolyticus* in a single replicate. Laboratories 76, 85 and 113 reported the absence of *V. parahaemolyticus*. Eleven laboratories applied tests for *tdh* and/or *trh* with 5 detecting *tdh* and *trh* positive. Laboratories 7, 32, 35 and 68 reported the absence of *trh* and laboratory 44 reported the absence of *tdh* and *trh*. Thirteen laboratories reported quantitative results within the range of $5.0 \times 10^0 - 4.2 \times 10^5$.

3.3.3 RT29.3 Vial 3 [CRL expected result *V. parahaemolyticus* not detected (sample *V. alginolyticus* NCTC12160); *tdh* and *trh* negative]

Eight laboratories (36%) identified the absence of *V. parahaemolyticus* in both replicates, 5 laboratories reported the presence of *V. alginolyticus*. Three laboratories identified the absence of *V. parahaemolyticus* in a single replicate of which 2 identified *V. alginolyticus*. Laboratories 22, 33, 68, 85, 124, 137 and 140 reported the presence of *V. parahaemolyticus*. Nine laboratories applied tests for *tdh* and/or *trh* with 5 reported absence of *tdh* and *trh* negative. Laboratories 35, 68 and 137 reported the presence of *trh*. Nine laboratories reported quantitative results within the range of $1.5 \times 10^2 - 1.2 \times 10^6$.

3.3.4 RT29.4 Vial 4 [CRL expected result *V. parahaemolyticus* present, *tdh* negative, *trh* positive]

Eighteen laboratories (82%) identified *V. parahaemolyticus* in both replicates. One laboratory identified *V. parahaemolyticus* in a single replicate. Laboratory 76 reported the absence of *V. parahaemolyticus*. Eleven laboratories applied tests for *tdh* and/or *trh* with 9 reporting *tdh* negative and *trh* positive. Laboratory 44 reported the absence of *trh*. Thirteen laboratories reported quantitative results within the range of $2.0 \times 10^2 - 4.6 \times 10^6$.

4 Summary

Of the 22 laboratories returned results for this ring trial. Forty one % of laboratories returned results corresponding to CRL expected results for presence / absence of *V. parahaemolyticus* in the 4 vials received. Eight laboratories detected the presence of *V. alginolyticus* in vial 3. A number of laboratories reported *V. parahaemolyticus* in vials where the presence of this organism was not anticipated. Further analysis of a limited number of reference vials stored at the CRL were unable to identify cross contamination of vial 3 using cultural and PCR methods. However the potential for laboratory cross contamination cannot be ruled out. Quantitative results were returned by between 13 and 14 laboratories dependent upon sample. Levels of *Vibrio* spp. were highly variable with over 4 log₁₀ variation in participants resulted observed.

Eleven laboratories used various methods that enabled detection of *tdh* and *trh* (see Appendix I). The majority of laboratories applying these tests assigned the presence or absence of both *tdh* and *trh* in accordance with the CRL tested results. One laboratory (Lab 44) reported duplicate *tdh* negative results for

vials 1 and 2, and 3 laboratories (Lab 7, 32 and 44) reported *trh* as negative in vials 2 and 4. Overall the recognition of the *tdh* gene by laboratories applying the test was in accordance with CRL predicted and participants' results; overall accuracy rate ≈95%.

A number of laboratories (7, 12, 19, 32, 33, 35, 44, 68 and 137) reported *trh* results that differed from those anticipated by the CRL. These results fell into two categories, laboratories that did not detect the *trh* gene in vials 2 and 4, and those that detected *trh* in vials 1 and 3. For vials 2 and 4 most laboratories undertaking the tests assigned presence of *trh* genes in accordance with CRL predicted results; accuracy rate ≈76%. It has been reported that *trh* genes cluster into two main subgroups, *trh1* and *trh2*, which share 84% identity (Kishishita *et al* 1992). In addition Gonzalez-Escalona *et al* (2006) reported a *trh*-like gene of *V. alginolyticus* isolated from Alaskan oysters that shared 98% sequence homology with the *trh2* gene of *V. parahaemolyticus*. Methods of Tada *et al* (1995) used to characterise reference materials at the CRL prior to distribution detect *trh1* only. Subsequent tests on stored material appropriate for detection of *trh2* (Nordstrom *et al* 2006; Gonzalez-Escalona *et al* 2006) were negative however its presence cannot be ruled out and thus it was not possible to assign accuracy rates of *trh* detection.

Following analysis of results, and after discussion at the 8th workshop of NRL in Spain 2009 participants were requested to provide information on the methods used in RT29. Where information was provided it was noted that a number of method of standard methods (ISO, NMKL, BAM) and non- standard methods were in use for identification and enumeration of *V. parahaemolyticus*. Similarly a wide variety of published and laboratory developed procedures were used for detection of pathogenic principles. These are summarized in Appendix I.

The outcomes of this ring trial highlight the urgent need for standardization of fit-for-purpose methods for the detection and enumeration of potentially pathogenic *Vibrio* spp. for use in European bivalve shellfish.

5 References

- Gonzalez-Escalona N., Blackstone G. M. And DePaola A. 2006) Characterisation of a *Vibrio alginolyticus* strain, isolated from Alaskan oysters carrying a hemolysin gene similar to the thermostable direct hemolysin-related hemolysin gene (*trh*) of *Vibrio parahaemolyticus*. *Applied and Environmental Microbiology* 72, 12 7925-7929.
- Kim, Y.B., J. Okuda, C. Matsumoto, N. Takahashi, S. Hashimoto, and M. Nishibuchi. 1999. Identification of *V. parahaemolyticus* strains at the species level by PCR targeted to the *ToxR* gene. *Journal of Clinical Microbiology*, 37:1173-1177.
- Nordstrom J.L., Rangdale R.E. *, Vickery M.C.L., Phillips A.M.B., Watt S and DePaola A. 2006 Identification and enumeration of potentially virulent *Vibrio parahaemolyticus* using an alkaline phosphatase labeled DNA probe targeting *trh*" *Journal of Food Protection*, 69:2770-2772.
- Tada J, Ohashi T, Nishimura N, Shirasaki Y, Ozaki H, Fukushima S, Takano J, Nishibuchi M, and Takeda Y. 1992. Detection of the thermostable direct haemolysin gene (*tdh*) and the thermostable direct haemolysin-related haemolysin gene (*trh*) of *V. parahaemolyticus* by polymerase chain reaction. *Mol.Cell Probe* 6: 477-487.
- McCarthy S, DePaola A, Kaysner C, Hill W and Cook D. 2000. Direct plating procedure for the enumeration of total and pathogenic *Vibrio parahaemolyticus* in oyster meats. FDA/Gulf Coast Seafood Laboratory protocol Vp-ISSC-3. issue 4/03/00.

Table 3. Participants' results for RT29.1 Vial 1

[expected result *V. parahaemolyticus* present; *tdh* positive and *trh* negative]

Lab ID	Replicate 1			Replicate 2				
	Sample result	tdh	trh	cfu/g	Sample result	tdh	trh	cfu/g
7	<i>V. parahaemolyticus</i>	+	-	1.2 x 10 ⁵	<i>V. parahaemolyticus</i>	+	-	2.5 x 10 ⁵
10	<i>V. parahaemolyticus</i>	+	-	4.7 x 10 ⁵	<i>V. parahaemolyticus</i>	+	-	4.7 x 10 ⁵
17	<i>V. parahaemolyticus</i>	+	+	1.7 x 10 ³	<i>V. parahaemolyticus</i>	+	+	NE
19	<i>V. parahaemolyticus</i>	+	+	>100	<i>V. parahaemolyticus</i>	+	+	>100
22	No <i>V. parahaemolyticus</i> present	NE	NE	NE	No <i>V. parahaemolyticus</i> present	NE	NE	NE
32	<i>V. parahaemolyticus</i>	+	-	2.9 x 10 ⁵	<i>V. parahaemolyticus</i>	+	-	1.9 x 10 ⁵
33	<i>V. parahaemolyticus</i>	+	+	NE	<i>V. parahaemolyticus</i>	+	+	NE
35	<i>V. vulnificus</i>	NE	NE	<10	<i>V. parahaemolyticus</i>	+	-	<10
44	<i>V. parahaemolyticus</i>	-	-	6.2 x 10 ²	<i>V. parahaemolyticus</i>	-	NE	6.6 x 10 ²
48	<i>V. parahaemolyticus</i>	NE	NE	4.6 x 10 ²	<i>V. parahaemolyticus</i>	NE	NE	4.6 x 10 ²
68	<i>V. parahaemolyticus</i>	+	+	1.0 x 10 ⁵	<i>V. parahaemolyticus</i>	+	+	8.0 x 10 ⁴
76	Other species	NE	NE	NE	Other species	NE	NE	NE
83	Negative	NE	NE	NE	Negative	NE	NE	NE
85	<i>V. parahaemolyticus</i> not detected	NE	NE	NE	<i>V. parahaemolyticus</i> not detected	NE	NE	NE
89	-	NE	NE	NE	-	NE	NE	NE
90	<i>V. parahaemolyticus</i>	NE	NE	7.0 x 10 ⁴	<i>V. parahaemolyticus</i>	NE	NE	2.0 x 10 ⁵
98	<i>V. parahaemolyticus</i>	NE	NE	3.9 x 10 ⁵	<i>V. parahaemolyticus</i>	NE	NE	3.3 x 10 ⁵
113	No <i>V. parahaemolyticus</i>	NE	NE	NE	NE	NE	NE	NE
115	<i>V. parahaemolyticus</i>	NE	NE	NE	NE	NE	NE	NE
124	<i>V. parahaemolyticus</i>	NE	NE	NE	NE	NE	NE	NE
137	<i>V. parahaemolyticus</i>	+	+	7.2 x 10 ²	<i>V. parahaemolyticus</i>	+	+	5.6 x 10 ²
140	<i>V. parahaemolyticus</i>	NE	NE	>11000	<i>V. parahaemolyticus</i>	NE	NE	>11000

NE – Not examined

Yellow denotes false positive, Red denotes false negative, Grey denotes uncertain result (see summary above)

Table 4. Participants' results for RT29.2 Vial 2

 [expected result *V. parahaemolyticus* present; *tdh* and *trh* positive].

Lab ID	Replicate 1			Replicate 2				
	Sample result	tdh	trh	cfu/g	Sample result	tdh	trh	cfu/g
7	<i>V. parahaemolyticus</i>	+	-	3.7 x 10 ⁴	<i>V. parahaemolyticus</i>	+	-	5.6 x 10 ³
10	<i>V. parahaemolyticus</i>	+	+	3.7 x 10 ³	<i>V. parahaemolyticus</i>	+	+	3.7 x 10 ³
17	<i>V. parahaemolyticus</i>	+	+	7.3 x 10 ²	<i>V. parahaemolyticus</i>	+	+	NE
19	<i>V. parahaemolyticus</i>	+	+	>100	<i>V. parahaemolyticus</i>	+	+	>100
22	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
32	<i>V. parahaemolyticus</i>	+	-	4.2 x 10 ⁵	<i>V. parahaemolyticus</i>	+	+	3.6 x 10 ⁵
33	<i>V. parahaemolyticus</i>	+	+	NE	<i>V. parahaemolyticus</i>	+	+	NE
35	<i>V. parahaemolyticus</i>	+	+	9.2 x 10 ³	<i>V. parahaemolyticus</i>	+	-	4.3 x 10 ³
44	<i>V. parahaemolyticus</i>	-	-	2.9 x 10 ²	<i>V. parahaemolyticus</i>	-	-	3.1 x 10 ²
48	<i>V. parahaemolyticus</i>	NE	NE	1.1 x 10 ³	<i>V. parahaemolyticus</i>	NE	NE	2.4 x 10 ³
68	<i>V. parahaemolyticus</i>	+	-	2.7 x 10 ⁴	<i>V. parahaemolyticus</i>	+	-	2.4 x 10 ⁴
76	Other species	NE	NE	NE	Other species	NE	NE	NE
83	Positive	+	NE	NE	Positive	+	NE	NE
85	<i>V. parahaemolyticus</i> not detected	NE	NE	NE	<i>V. parahaemolyticus</i> not detected	NE	NE	NE
89	Not detected	NE	NE	<1	Not detected	NE	NE	<1
90	<i>V. parahaemolyticus</i>	NE	NE	2.0 x 10 ³	<i>V. parahaemolyticus</i>	NE	NE	8.0 x 10 ²
98	<i>V. parahaemolyticus</i>	NE	NE	5.6 x 10 ⁴	<i>V. parahaemolyticus</i>	NE	NE	1.1 x 10 ⁵
113	<i>V. parahaemolyticus</i> not detected	NE	NE	NE	NE	NE	NE	NE
115	<i>V. parahaemolyticus</i>	NE	NE	NE	NE	NE	NE	NE
124	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
137	<i>V. parahaemolyticus</i>	+	+	<10	<i>V. parahaemolyticus</i>	+	+	<10
140	Presumptive <i>V. parahaemolyticus</i>	NE	NE	>11000	Presumptive <i>V. parahaemolyticus</i>	NE	NE	2.4 x 10 ³

NE – Not examined

Red denotes false negative, Grey denotes uncertain result (see summary above)

Table 5. Participants' results for RT29.3 Vial 3

 [expected result *V. parahaemolyticus* not detected (sample *V. alginolyticus*); *tdh* and *trh* negative]

Lab ID	Replicate 1			Replicate 2				
	Sample result	tdh	trh	cfu/g	Sample result	tdh	trh	cfu/g
7	Non <i>V. parahaemolyticus</i>	-	-	3.0 x 10 ³	Non <i>V. parahaemolyticus</i>	-	-	1.3 x 10 ⁴
10	<i>V. alginolyticus</i>	-	-	3.8 x 10 ⁵	<i>V. alginolyticus</i>	-	-	3.8 x 10 ⁵
17	<i>V. parahaemolyticus</i>	-	+	<10	<i>V. parahaemolyticus</i>	-	+	<10
19	No <i>V. parahaemolyticus</i> , <i>Past. Multocida</i>	-	-	>100	No <i>V. parahaemolyticus</i> , <i>Past. Multocida</i>	-	-	>100
22	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
32	<i>V. alginolyticus</i>	-	-	1.0 x 10 ⁶	<i>V. alginolyticus</i>	-	-	1.2 x 10 ⁶
33	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
35	<i>V. alginolyticus</i>	NE	NE	2.9 x 10 ³	<i>V. parahaemolyticus</i>	-	+	<1000
44	<i>V. alginolyticus</i>	-	-	1.1 x 10 ⁴	<i>V. alginolyticus</i>	-	-	1.0 x 10 ⁴
48	<i>V. alginolyticus</i>	NE	NE	<300	<i>V. alginolyticus</i>	NE	NE	<300
68	<i>V. parahaemolyticus</i> + <i>Vibrio</i> spp.	-	+	1.2 x 10 ⁵	<i>V. parahaemolyticus</i> + <i>Vibrio</i> spp.	-	+	1.1 x 10 ⁵
76	Other species	NE	NE	NE	Other species	NE	NE	NE
83	Positive	-	NE	NE	Positive	-	NE	NE
85	<i>V. parahaemolyticus</i>	NE	NE	<20	<i>V. parahaemolyticus</i>	NE	NE	<20
89	-	NE	NE	NE	-	NE	NE	NE
90	<i>V. alginolyticus</i>	NE	NE	>1000	<i>V. alginolyticus</i>	NE	NE	>1000
98	<i>V. alginolyticus</i> and <i>V. parahaemolyticus</i>	NE	NE	2.8 x 10 ⁵	<i>V. alginolyticus</i> and <i>V. parahaemolyticus</i>	NE	NE	5.4 x 10 ⁵
113	<i>V. parahaemolyticus</i> not detected	NE	NE	NE	NE	NE	NE	NE
115	<i>V. alginolyticus</i>	NE	NE	NE	NE	NE	NE	NE
124	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
137	<i>V. parahaemolyticus</i>	-	+	<10	<i>V. parahaemolyticus</i>	-	+	<10
140	<i>V. parahaemolyticus</i>	NE	NE	<3	<i>V. parahaemolyticus</i>	NE	NE	<3

NE – Not examined

Yellow denotes false positive, Grey denotes uncertain result (see summary above)

Table 6. Participants' results for RT29.4Vial 4

 [expected result *V. parahaemolyticus* present, *tdh* negative, *trh* positive]

Lab ID	Replicate 1			Replicate 2				
	Sample result	tdh	trh	cfu/g	Sample result	tdh	trh	cfu/g
7	<i>V. parahaemolyticus</i>	-	+	1.2 x 10 ⁵	<i>V. parahaemolyticus</i>	-	+	1.6 x 10 ⁶
10	<i>V. parahaemolyticus</i>	-	+	4.9 x 10 ⁶	<i>V. parahaemolyticus</i>	-	+	4.9 x 10 ⁶
17	<i>V. parahaemolyticus</i>	-	+	4.2 x 10 ³	<i>V. parahaemolyticus</i>	-	+	4.2 x 10 ³
19	<i>V. parahaemolyticus</i>	-	+	>100	<i>V. parahaemolyticus</i>	-	+	>100
22	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
32	<i>V. parahaemolyticus</i>	-	+	1.7 x 10 ⁶	<i>V. parahaemolyticus</i>	-	+	1.5 x 10 ⁶
33	<i>V. parahaemolyticus</i>	-	+	NE	<i>V. parahaemolyticus</i>	-	+	NE
35	<i>V. parahaemolyticus</i>	-	+	2.2 x 10 ⁴	<i>V. parahaemolyticus</i>	-	+	2.0 x 10 ⁴
44	<i>V. parahaemolyticus</i>	-	-	2.9 x 10 ⁴	<i>V. parahaemolyticus</i>	-	-	3.0 x 10 ⁴
48	<i>V. parahaemolyticus</i>	NE	NE	1.1 x 10 ⁴	<i>V. parahaemolyticus</i>	NE	NE	1.1 x 10 ⁴
68	<i>V. parahaemolyticus</i>	-	+	9.0 x 10 ⁵	<i>V. parahaemolyticus</i>	-	+	8.5 x 10 ⁵
76	Other species	NE	NE	NE	Other species	NE	NE	NE
83	Positive	-	NE	NE	Positive	-	NE	NE
85	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
89		NE	NE	<1		NE	NE	<1
90	<i>V. parahaemolyticus</i>	NE	NE	1.0 x 10 ⁴	<i>V. parahaemolyticus</i>	NE	NE	1.0 x 10 ⁴
98	<i>V. parahaemolyticus</i>	NE	NE	5.5 x 10 ⁵	<i>V. parahaemolyticus</i>	NE	NE	4.6 x 10 ⁵
113	<i>V. parahaemolyticus</i>	NE	NE	NE	NE	NE	NE	NE
115	NE	NE	NE	NE	NE	NE	NE	NE
124	<i>V. parahaemolyticus</i>	NE	NE	NE	<i>V. parahaemolyticus</i>	NE	NE	NE
137	<i>V. parahaemolyticus</i>	-	+	9.0 x 10 ³	<i>V. parahaemolyticus</i>	-	+	5.9 x 10 ³
140	<i>V. parahaemolyticus</i>	NE	NE	>11000	<i>V. parahaemolyticus</i>	NE	NE	>11000

NE – Not examined

Red denotes false negative

Appendix I. Methods used for enumeration and detection of the pathogenic markers of *V. parahaemolyticus*.

Lab ID	Enumeration of <i>vibrio</i> species	Detection of pathogenic markers
7	ISO 8914 and direct plating ⁴	Cefas PCR SOP ¹
10	ISO/TS 21872 - Part 1	Cefas PCR SOP and hybridisation ⁷
17	-	-
19	Cefas SOP 1333	-
22	ISO/TS 21872 - Part 1	-
32	Cefas SOP 1333 ³	-
33	Cefas SOP 1333 ³	PCR target <i>toxR</i> , <i>tdh</i> and <i>trh</i> genes ²
35	Direct plating ⁴	Cefas PCR SOP ¹
44	NMKL method no.156	-
48	ISO/TS 21872	-
68	FDA Bacteriological Analytical Manual	PCR target <i>toxR</i> , <i>tdh</i> and <i>trh</i> genes ⁶
76	SP-VG-M006	-
83	NMKL method no.156	-
85	-	-
89	FDA Bacteriological Analytical Manual	-
90	ISO/TS 21872 and direct plating ⁴	-
98	ISO/TS 21872 - Part 1 and 2	-
113	ISO/TS 21872 - Part 2	Real-time PCR ⁵
115	ISO/TS 21872 - Part 1	-
124	ISO/TS 21872 - Part 1	-
137	ISO/TS 21872 - Part 1	Real-time PCR target <i>toxR</i> , <i>tdh</i> , <i>trh1</i> and <i>trh2</i> genes ²
140	FDA Bacteriological Analytical Manual	-

¹ Derived from Kim *et al* (1999) *toxR* and *tdh* , Tada *et al* (1999) for *trh*

² No further information supplied

³ Derived from ISO TS 21872-1

⁴ Direct plating onto TCBS followed by biochemical confirmation of sucrose negative colonies

⁵ Derived from Bej *et al* (1999) for *tdh*

⁶ Derived from Bej *et al* (1999) for *tlh* and Kim *et al* 1999) for *toxR*. *Trh* and *tdh* positives derived from Tada *et al* (1992) sequences

R2-R6 for *trh* gene and the sequences D1-D2 for *tdh* gene.

⁷ Derived from Kim *et al* (1999) *toxR* and *tdh* , Tada *et al* (1999) for *trh* , Nordstrom *et al* (2006) and McCarthy *et al* (2000).

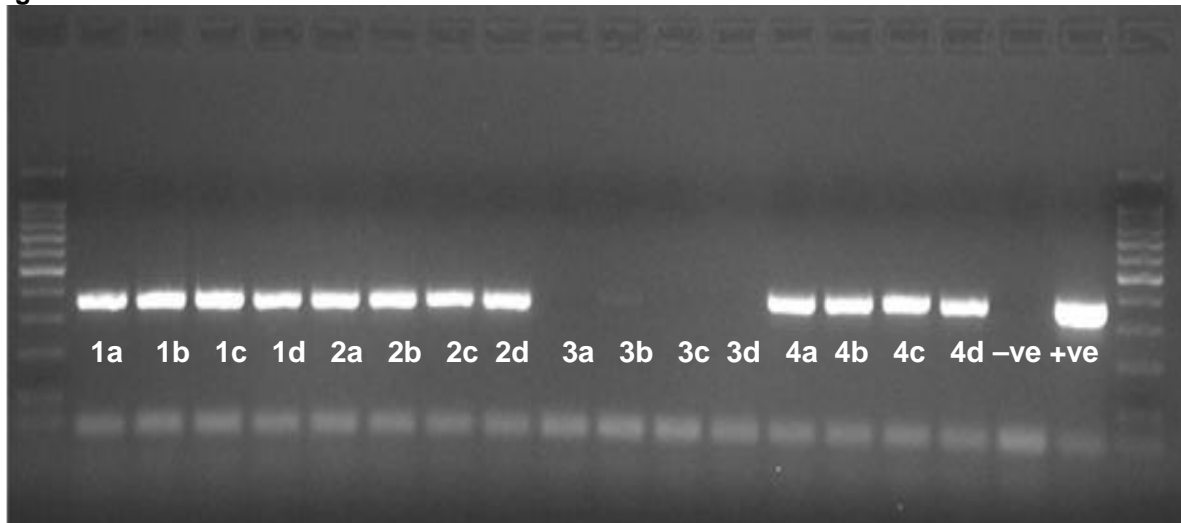
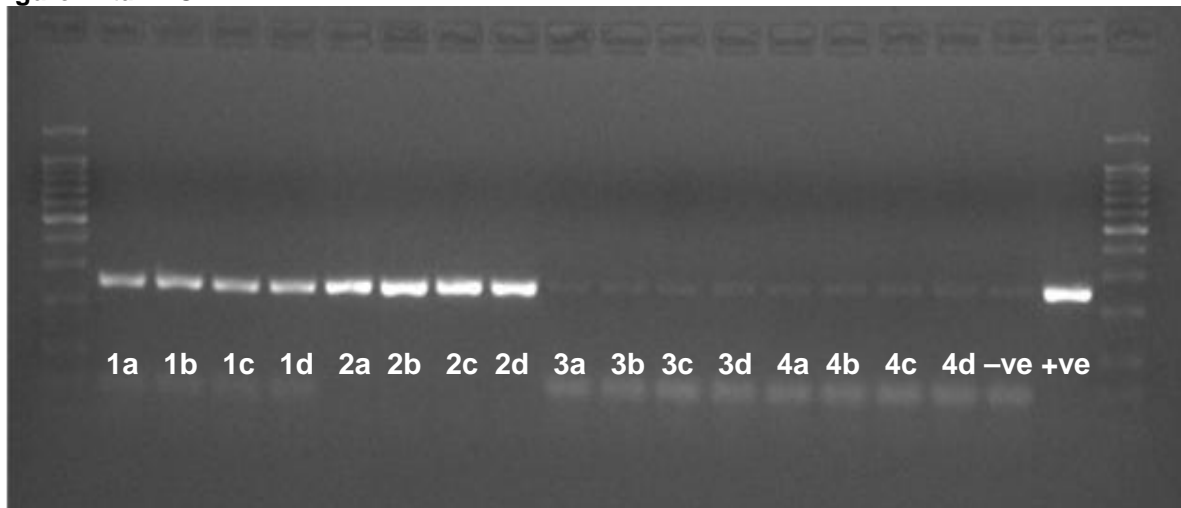
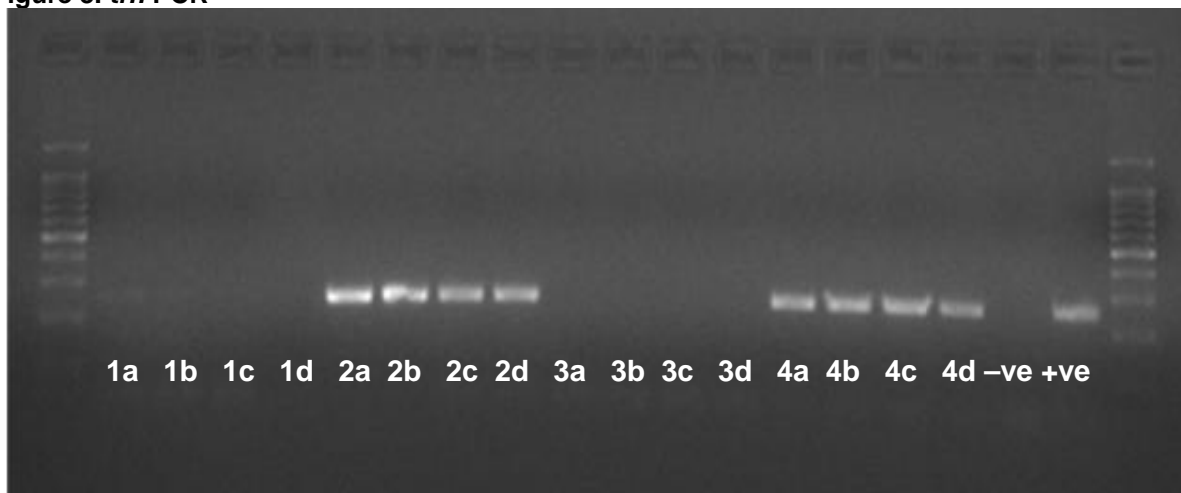
Figure 1. Tox R PCR**Figure 2. *tdh* PCR****Figure 3. *trh* PCR**

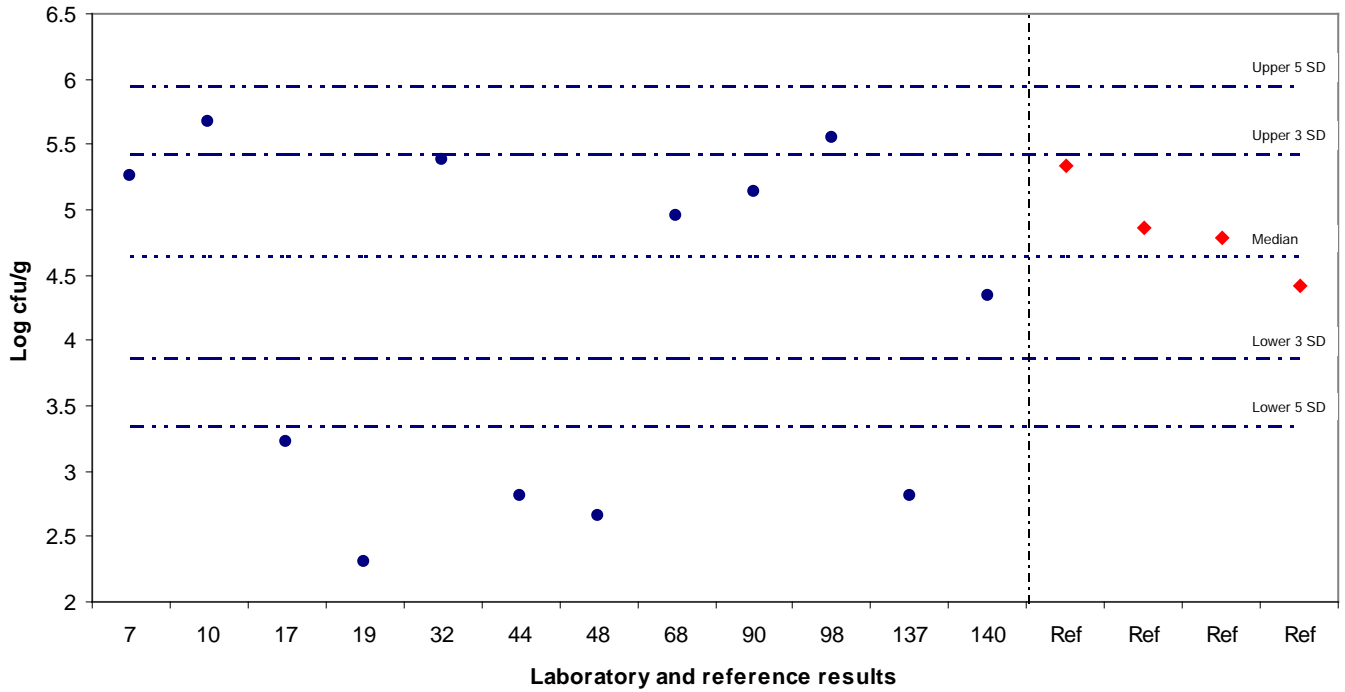
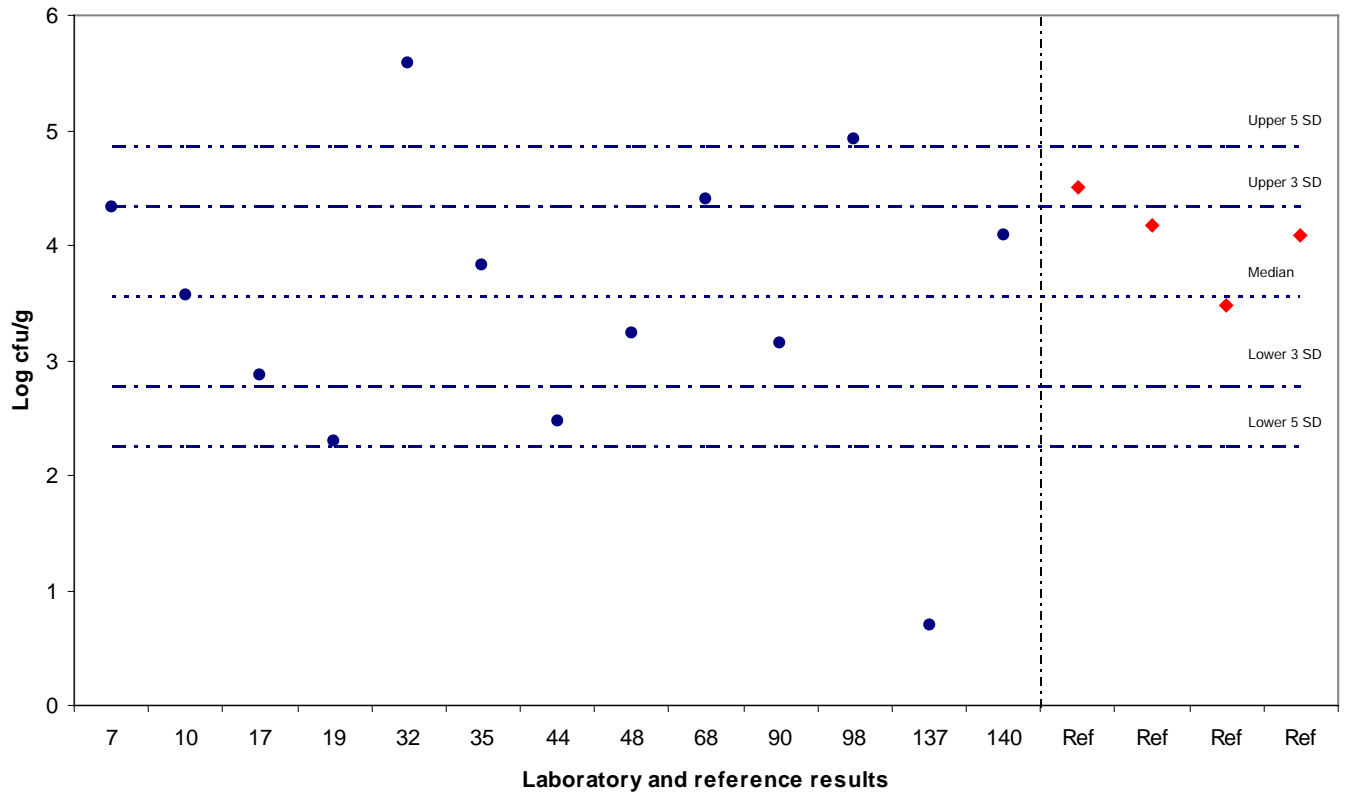
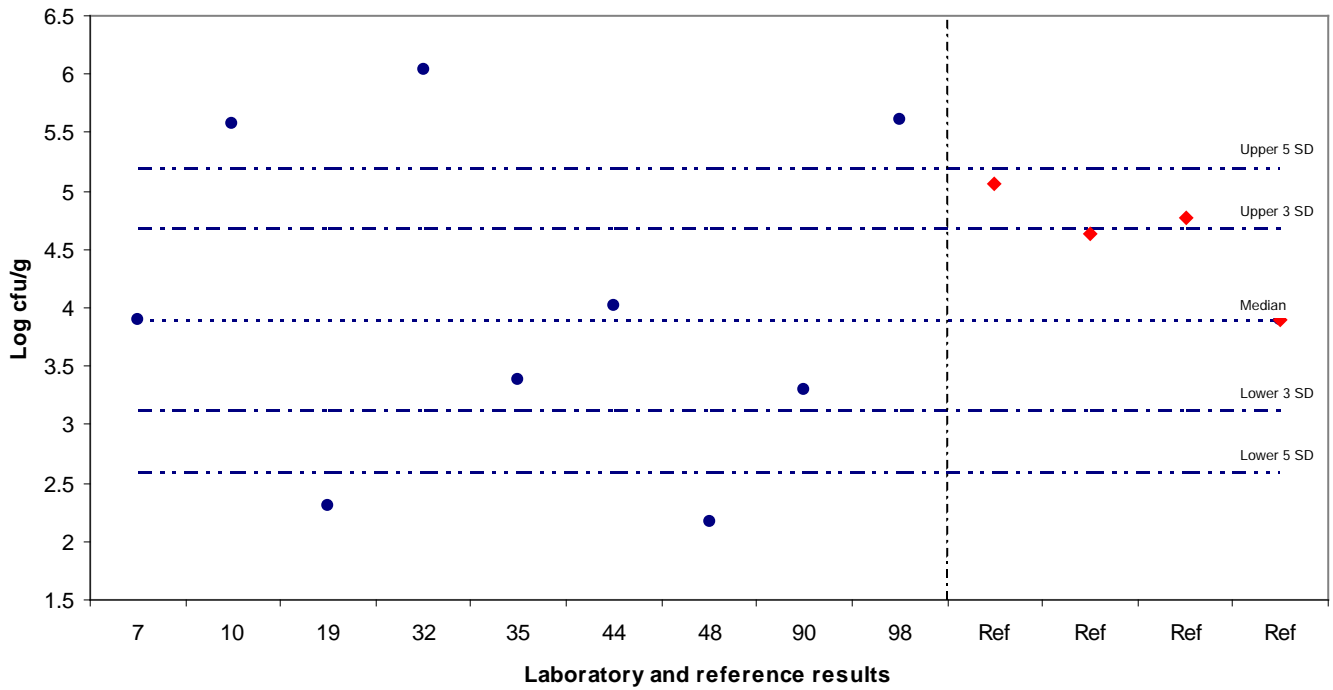
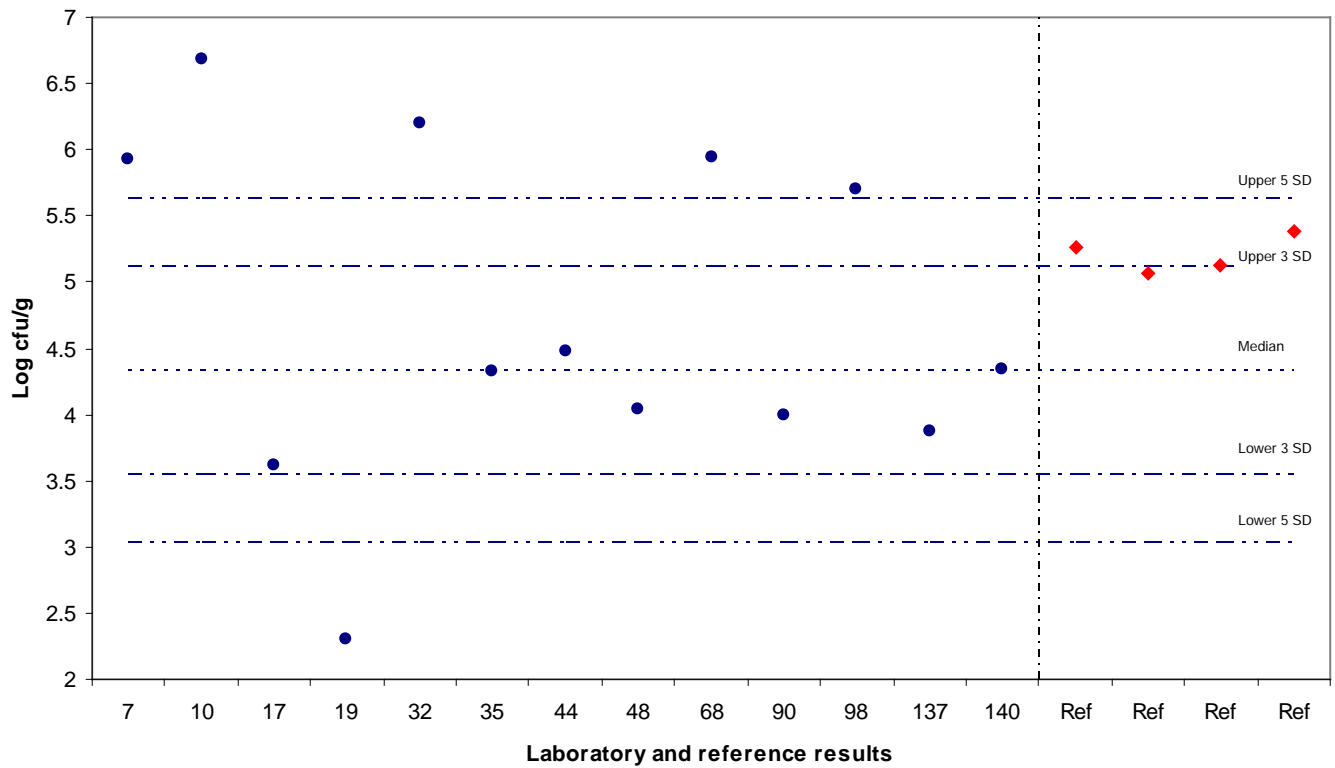
Figure 4. Participants' mean quantitative (cfu/g) for vial 1.

Figure 5. Participants' mean quantitative (cfu/g) for vial 2.


Figure 6. Participants' mean quantitative (cfu/g) for vial 3.

Figure 7. Participants' mean quantitative (cfu/g) for vial 4.


About us

Cefas is a multi-disciplinary scientific research and consultancy centre providing a comprehensive range of services in fisheries management, environmental monitoring and assessment, and aquaculture to a large number of clients worldwide.

We have more than 500 staff based in 2 laboratories, our own ocean-going research vessel, and over 100 years of fisheries experience.

We have a long and successful track record in delivering high-quality services to clients in a confidential and impartial manner.
(www.cefas.co.uk)

Cefas Technology Limited (CTL) is a wholly owned subsidiary of Cefas specialising in the application of Cefas technology to specific customer needs in a cost-effective and focussed manner.

CTL systems and services are developed by teams that are experienced in fisheries, environmental management and aquaculture, and in working closely with clients to ensure that their needs are fully met.
(www.cefastechnology.co.uk)

Head office

**Centre for Environment,
Fisheries & Aquaculture Science
Pakefield Road, Lowestoft,
Suffolk NR33 0HT UK**

Tel +44 (0) 1502 56 2244

Fax +44 (0) 1502 51 3865

Web www.cefas.co.uk

Customer focus

With our unique facilities and our breadth of expertise in environmental and fisheries management, we can rapidly put together a multi-disciplinary team of experienced specialists, fully supported by our comprehensive in-house resources.

Our existing customers are drawn from a broad spectrum with wide ranging interests. Clients include:

- international and UK government departments
- the European Commission
- the World Bank
- Food and Agriculture Organisation of the United Nations (FAO)
- oil, water, chemical, pharmaceutical, agro-chemical, aggregate and marine industries
- non-governmental and environmental organisations
- regulators and enforcement agencies
- local authorities and other public bodies

We also work successfully in partnership with other organisations, operate in international consortia and have several joint ventures commercialising our intellectual property.

**Centre for Environment,
Fisheries & Aquaculture Science
Weymouth Laboratory,
Barrack Road, The Nothe, Weymouth,
Dorset DT4 8UB**

Tel +44 (0) 1305 206600

Fax +44 (0) 1305 206601

