European Community Reference laboratory
for monitoring bacteriological and viral
contamination of bivalve molluscs

Report on the *Vibrio parahaemolyticus* ring trial, 2008

CRL ring trial reference: RT 22 (*V. para* 2008)

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1. Introduction

Regulation (EC) No 882/2004 designates the Centre for Environment, Fisheries and Aquaculture Science at Weymouth U.K. as the Community Reference Laboratory (CRL) for monitoring the viral and bacteriological contamination of bivalve molluscs. Under Article 32 the CRL laboratory is responsible for organising comparative testing by National Reference Laboratories.

At the 6th annual workshop of NRLs Galway 2007, several resolutions were agreed relating to proficiency testing including:

> **Resolution 32.** NRLs requested that the CRL organise a further ring trial distribution for *V. parahaemolyticus* with the intent of providing samples that enabled enumeration and determination of potential pathogenicity principles.

2. Proficiency testing samples

2.1 Sample preparation

Six samples from the Cefas *Vibrio* spp. reference strain bank were streaked onto non-selective marine agar (MA). Plates were incubated for 18-24hrs at 30±2˚C. Each strain was designated a sample code, RT 22 A to F. Following visual purity checks 2-5 colony forming units (cfu) were transferred onto sterile (ENT) swabs and stabbed in semi-solid MA. Swabs were incubated for 18-24hrs at 30±2˚C. After incubation the inoculated swabs were stored at 3±2˚C until distribution. Each participating laboratory received 6 swabs, one replicate of each strain.

Note. RT 22 consisted of samples for presence/absence testing only.

2.2 Distribution of samples

The *V. parahaemolyticus* ring trial February 2008 was designated RT 22. Samples were packaged according to IATA regulations, UN3373 as biological samples, division 6.2 under the packing instruction code 650. All participating laboratories received a single box comprising of 6 separate sealed tubes (Samples RT 22 A to F). Samples were distributed by CitySprint Ltd at ambient temperature on week commencing the 25th February 2008. On receipt, participants were requested to store the samples at 3±2˚C prior to analysis during week commencing the 10th March 2008.

2.3 Quality control at dispatch

Samples were tested prior to distribution to verify conformation to the reference strain bank designation. Analyses were undertaken using CRL standard procedures for detection of *V. parahaemolyticus* (Cefas SOP 1333), with additional biochemical tests to confirm the identification of *V. hollisae* and *V. alginolyticus*. Standard operating procedures are available from the information centre of the CRL website (www.crlcefas.org). In addition, *V. parahaemolyticus* was confirmed using *toxR* (Kim *et al* 1999) and *tlh* (McCarthy *et al* 2000). The putative pathogenicity markers were confirmed by nucleic acid hybridisation (*tdh* (McCarthy *et al* 2000) and *trh* (Nordstrom *et al.* 2006)).

Table 1. Sample analysis prior to sample distribution

Sample ID	Reference bank designation	Presence of *tdh/trh*	Reference bank accession code
RT 22 A	*V. parahaemolyticus*	no	NCTC 10885
RT 22 B	*V. alginolyticus*	no	V05/007
RT 22 C	*V. parahaemolyticus*	*tdh* only	V05/067
RT 22 D	*V. parahaemolyticus*	*tdh* and *trh*	V05/014
RT 22 E	*V. hollisae*	no	V05/006
RT 22 F	*V. parahaemolyticus*	*trh* only	E154482

2.4 Confidentiality of results

Each laboratory participant was identified by a code to preserve anonymity.

2.5 Participation

Invitations inviting expressions of interest in the *V. parahaemolyticus* ring trial were sent to all designated MS NRLs, candidate countries laboratories, Norway, Iceland and to government laboratories in the U.S and Canada. Material was dispatched to twenty-two laboratories. Table 2 summarises the participation in RT 22.

Table 2. Participation in the *V. parahaemolyticus* ring trial distribution (RT 22)

Member State	Participation in RT 22	Results returned to CRL
Austria	Yes	Yes
Belgium & Luxembourg	Yes	Yes
Bulgaria	Yes	Yes
Czech Republic	No	-
Denmark	No	-
Estonia	Yes	Yes
Finland	No	-
France	No	-
Germany	Yes	Yes
Greece	Yes	Yes
Hungary	Yes	Yes
Ireland	No	-
Italy 1	Yes	Yes
Italy 2	Yes	Yes
Latvia	Yes	Yes
Lithuania	No	-
Netherlands	Yes	Yes
Poland	Yes	Yes
Portugal	Yes	Yes
Romania	No	-
Slovakia	Yes	Yes
Slovenia	Yes	Yes
Spain	No	-
Sweden	No	-
United Kingdom	Yes	Yes

Table 2 cont. Participation in the *V. parahaemolyticus* ring trial distribution (RT 22)

Accession/ Candidate country	Participation in RT 22	Results returned to CRL
Croatia	Yes	Yes
Turkey	Yes	Yes
ETFA		
Norway	Yes	Yes
Iceland	Yes	Yes
Third country		
Canada	Yes	Yes
USA	Yes	Yes

2.6 Reference results

Reference analyses were performed by the CRL. In brief, 5 replicate samples selected at random were analysed for the species type and the presence of *tdh* and *trh* genes using CRL standard methods. These analyses were carried out concurrently with participants' analyses and the results are shown in Table 3.

Table 3. Reference results for samples RT 22 A to RT 22 F

Sample ID	API identification	*tlh*	*toxR*	*tdh*	*trh*
RT 22 A	*V. parahaemolyticus*	+	+	-	-
RT 22 B	*V. alginolyticus*	-	-	-	-
RT 22 C	*V. parahaemolyticus*	+	+	+	-
RT 22 D	*V. parahaemolyticus*	+	+	+	+
RT 22 E	*V. hollisae*	-	-	-	-
RT 22 F	*V. parahaemolyticus*	+	+	-	+

3 Results

3.1 Distribution

All samples arrived at their destinations within 48 hr of dispatch. No participants reported that samples arrived in poor condition. Most laboratories (20) stored samples at 2-6˚C, 2 stored samples at room temperature prior to commencement of tests. Storage temperature appeared to have no effect on the results.

3.2 Analysis of results

Twenty-two laboratories returned results to the CRL. Eighteen (82%) used methods that enabled detection of the putative pathogenicity marker *tdh* and *trh* genes.

3.2.1 Sample RT 22 A [expected result *V. parahaemolyticus* present; *tdh* and *trh* negative]
All laboratories correctly identified *V. parahaemolyticus* in sample RT 22 A. Eighteen laboratories applied tests for *tdh* and/or *trh*. One laboratory (Lab 6) reported the presence of *tdh,* all other laboratories returned the anticipated results.

3.2.2. Sample RT 22 B [expected result *V. parahaemolyticus* not detected*, (*sample *V. alginolyticus)*; *tdh* and *trh* negative].
All laboratories correctly identified sample RT 22 B as *V. parahaemolyticus* not detected. Eleven laboratories correctly identified the presence of *V. alginolyticus*. One laboratory incorrectly identified the presence of *V. vulnificus.*

3.2.3 Sample RT 22 C [expected result *V. parahaemolyticus* present*, tdh* positive, *trh* negative]
All laboratories correctly identified *V. parahaemolyticus* in sample RT 22 C. All laboratories that applied tests to detect *tdh* correctly identified its presence. All laboratories returning results for *trh* correctly identified it as absent.

3.2.4 Sample RT 22 D [expected result *V. parahaemolyticus* present*, tdh* positive, *trh* positive]
Sixteen laboratories (73%) correctly identified sample RT 22 D as *V. parahaemolyticus.* All laboratories that undertook pathogenicity principle testing (n=13) correctly assigned sample RT 22 D as *tdh* and *trh* positive. Six laboratories (Lab 10, 14, 19, 21, 22 and 44) reported the absence of *V. parahaemolyticus* with 3 identifying *V. vulnificus* presence in sample RT 22 D.

3.2.5 Sample RT 22 E [expected result *V. parahaemolyticus* not detected *(*sample *V.hollisae), tdh* negative, *trh* negative]
Twenty laboratories that returned results reported the absence of *V. parahaemolyticus.* However, one laboratory (Lab 6) identified sample RT 22 E as *V. vulnificus.* One laboratory (Lab 10) reported the presence *V. parahaemolyticus* in sample RT 22 E. Three laboratories (Lab 10, 17 and 39) incorrectly reported the *tdh* and/or *trh* as positive.

3.2.6 Sample RT 22 F [expected result *V. parahaemolyticus* present*, tdh* negative, *trh* positive]
All laboratories that returned results correctly identified *V. parahaemolyticus* in sample RT 22 F*.* Lab 6 incorrectly reported the presence of *tdh.* Four laboratories (Lab 2, 22, 42 and 44) did not detect the presence of *trh.* All other laboratories that undertook pathogenicity principle testing correctly assigned sample RT 22 F as *trh* positive and *tdh* negative.

Participants' results are shown in Tables 4 to 9, false positive and negative results are identified in yellow and red respectively. Results are provided for information only. No performance assessments were carried out.

4 Summary

In general all laboratories performed well in this trial. A false negative rate of 6.8% was observed for identification of *V. parahaemolyticus*. All false negative species identification was associated with RT 22 D. RT 22 D was a clinical strain originating from a patient with *V. parahaemolyticus* associated gastroenteritis in South East Asia. It was interesting to note that three laboratories identified this sample as *V. vulnificus* illustrating the commonly experienced problems with misidentification of some *Vibrio* spp. strains. The false positive rate was less than 1%. Eighteen laboratories used methods that enabled detection of pathogenicity markers. The majority of laboratories applying these tests correctly assigned the presence or absence of both *tdh* and *trh*. Three laboratories (Lab 6, 17 and 39) reported false *tdh* positives, and two laboratories (Lab 10 and 17) reported *trh* in an intended *trh* negative sample. Four laboratories (Lab 2, 22, 42 and 44) failed to detect *trh* in one sample.

Table 4. Participants' results for sample RT 22 A
[expected result *V. parahaemolyticus* present; *tdh* and *trh* negative]

Lab ID	Sample result	*tdh*	*trh*
2	*V. parahaemolyticus*	-	-
6	*V. parahaemolyticus*	+	-
7	*V. parahaemolyticus*	-	-
9	*V. parahaemolyticus*	-	-
10	*V. parahaemolyticus*	-	-
13	*V. parahaemolyticus*	NE	NE
14	*V. parahaemolyticus*	NE	NE
15	*V. parahaemolyticus*	-	-
17	*V. parahaemolyticus*	-	-
19	*V. parahaemolyticus*	-	-
21	*V. parahaemolyticus*	NE	NE
22	*V. parahaemolyticus*	-	-
32	*V. parahaemolyticus*	-	-
33	*V. parahaemolyticus*	-	-
35	*V. parahaemolyticus*	-	-
39	*V. parahaemolyticus*	-	-
42	*V. parahaemolyticus*	-	-
44	*V. parahaemolyticus*	-	-
55	*V. parahaemolyticus*	-	-
68	*V. parahaemolyticus*	-	-
83	*V. parahaemolyticus*	-	NE
90	*V. parahaemolyticus*	NE	NE

NE – Not examined
Yellow denotes false positive

Table 5. Participants' results for sample RT 22 B
[expected result *V. parahaemolyticus* not detected, (sample *V. alginolyticus*); *tdh* and *trh* negative]

Lab ID	Sample result	*tdh*	*trh*
2	*V. alginolyticus*	-	-
6	*V. alginolyticus*	-	-
7	*V. parahaemolyticus* not detected	-	-
9	*V. alginolyticus*	-	-
10	*V. alginolyticus*	NE	NE
13	*V. alginolyticus*	NE	NE
14	*V. parahaemolyticus* not detected	NE	NE
15	*V. parahaemolyticus* not detected	-	-
17	*V. parahaemolyticus* not detected	-	-
19	*V. alginolyticus*	NE	NE
21	*Vibrio* spp. not detected	NE	NE
22	*V. parahaemolyticus* not detected	-	-
32	*V. alginolyticus*	-	-
33	*V. alginolyticus*	-	-
35	*V. alginolyticus*	NE	NE
39	*V. alginolyticus*	-	-
42	*V. vulnificus*	-	-
44	*V. parahaemolyticus* not detected	-	-
55	*V. parahaemolyticus* not detected	-	-
68	absence of growth	NE	NE
83	*V. parahaemolyticus* not detected	NE	NE
90	*V. alginolyticus*	NE	NE

Table 6. Participants' results for sample RT 22 C
[expected result *V. parahaemolyticus* present, *tdh* positive, *trh* negative]

Lab ID	Sample result	*tdh*	*trh*
2	*V. parahaemolyticus*	+	-
6	*V. parahaemolyticus*	+	-
7	*V. parahaemolyticus*	+	-
9	*V. parahaemolyticus*	+	-
10	*V. parahaemolyticus*	+	-
13	*V. parahaemolyticus*	NE	NE
14	*V. parahaemolyticus*	NE	NE
15	*V. parahaemolyticus*	+	-
17	*V. parahaemolyticus*	+	-
19	*V. parahaemolyticus*	+	-
21	*V. parahaemolyticus*	NE	NE
22	*V. parahaemolyticus*	NE	NE
32	*V. parahaemolyticus*	+	-
33	*V. parahaemolyticus*	+	-
35	*V. parahaemolyticus*	+	-
39	*V. parahaemolyticus*	+	-
42	*V. parahaemolyticus*	NE	NE
44	*V. parahaemolyticus*	NE	NE
55	*V. parahaemolyticus*	+	-
68	*V. parahaemolyticus*	+	-
83	*V. parahaemolyticus*	+	NE
90	*V. parahaemolyticus*	NE	NE

NE – Not examined
Red denotes false negative

Table 7. Participants' results for sample RT 22 D
[expected result *V. parahaemolyticus* present, *tdh* positive, *trh* positive]

Lab ID	Sample result	*tdh*	*trh*
2	*V. parahaemolyticus*	+	+
6	*V. parahaemolyticus*	+	+
7	*V. parahaemolyticus*	+	+
9	*V. parahaemolyticus*	+	+
10	*V. vulnificus*	NE	NE
13	*V. parahaemolyticus*	NE	NE
14	*V. parahaemolyticus* not detected	NE	NE
15	*V. parahaemolyticus*	+	+
17	*V. parahaemolyticus*	+	+
19	*V. vulnificus*	NE	NE
21	*V. vulnificus*	NE	NE
22	*V. parahaemolyticus* not detected	NE	NE
32	*V. parahaemolyticus*	+	+
33	*V. parahaemolyticus*	+	+
35	*V. parahaemolyticus*	+	+
39	*V. parahaemolyticus*	+	+
42	*V. parahaemolyticus*	NE	NE
44	*V. parahaemolyticus* not detected	NE	NE
55	*V. parahaemolyticus*	+	+
68	*V. parahaemolyticus*	+	+
83	*V. parahaemolyticus*	+	NE
90	*V. parahaemolyticus*	NE	NE

Table 8. Participants' results for sample RT 22 E
[expected result *V. parahaemolyticus* not detected (sample *V.hollisae*) *tdh* negative, *trh* negative]

Lab ID	Sample results	*tdh*	*trh*
2	No growth	NE	NE
6	*V. vulnificus*	-	-
7	*V. parahaemolyticus* not detected	-	-
9	*V. parahaemolyticus* not detected	-	-
10	*V. parahaemolyticus*	-	+
13	*Pasturella spp.*	NE	NE
14	*V. parahaemolyticus* not detected	NE	NE
15	*V. parahaemolyticus* not detected	-	-
17	*V. parahaemolyticus* not detected	+	+
19	*V. parahaemolyticus* not detected	NE	NE
21	*V. hollisae*	NE	NE
22	*V. parahaemolyticus* not detected	-	-
32	*Grimontia hollisae*	-	-
33	*V. parahaemolyticus* not detected	NE	NE
35	*Aeromonas* spp.	NE	NE
39	*V. hollisae*	+	-
42	*V. parahaemolyticus* not detected	-	-
44	*V. parahaemolyticus* not detected	-	-
55	*V. parahaemolyticus* not detected	-	-
68	No growth	NE	NE
83	*V. parahaemolyticus* not detected	NE	NE
90	*V. hollisae*	NE	NE

NE – Not examined
Yellow denotes false positive
Red denotes false negative

Table 9. Participants' results for sample RT 22 F
[expected result *V. parahaemolyticus* present, *tdh* negative, *trh* positive]

Lab ID	Sample results	*tdh*	*trh*
2	*V. parahaemolyticus*	-	-
6	*V. parahaemolyticus*	+	+
7	*V. parahaemolyticus*	-	+
9	*V. parahaemolyticus*	-	+
10	*V. parahaemolyticus*	-	+
13	*V. parahaemolyticus*	NE	NE
14	*V. parahaemolyticus*	NE	NE
15	*V. parahaemolyticus*	-	+
17	*V. parahaemolyticus*	-	+
19	*V. parahaemolyticus*	-	+
21	*V. parahaemolyticus*	NE	NE
22	*V. parahaemolyticus*	-	-
32	*V. parahaemolyticus*	-	+
33	*V. parahaemolyticus*	-	+
35	*V. parahaemolyticus*	-	+
39	*V. parahaemolyticus*	-	+
42	*V. parahaemolyticus*	-	-
44	*V. parahaemolyticus*	-	-
55	*V. parahaemolyticus*	-	+
68	*V. parahaemolyticus*	-	+
83	*V. parahaemolyticus*	-	NE
90	*V. parahaemolyticus*	NE	NE

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