European Union Reference Laboratory for Crustacean Diseases

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TSV/YHV PROFICIENCY TEST 2015

Detection of Taura Syndrome Virus (TSV) and Yellowhead Virus (YHV) in Lenticules

EURL Ring Trial Reference Number: EURL15006

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Introduction

This scheme is intended to provide proficiency testing samples for National Reference Laboratories (NRLs) undertaking examination of crustacean tissues for the presence/absence of Taura Syndrome Virus (TSV) and Yellowhead Virus (YHV) in accordance with EC Directive 2006/88. The invitation to participate in this year's proficiency test was sent to 23 NRL's in 21 Member States. Samples were sent to 11 NRL's in 10 Member States, 12 NRL's declined to take part in this trial.

This Proficiency test was organised by the European Union Reference laboratory (EURL) for Crustacean Diseases.

Further information can be obtained via the EURL website (www.crustaceancrl.eu)

Sample Preparation

Shrimp were confirmed as either TSV or YHV positive and TSV/YHV negative (SPF) by nested PCR techniques. These shrimp were homogenised and the homogenate used to inoculate a lenticulating fluid at various dilutions. This fluid was then aliquoted into 25µl drops which formed the lenticule discs. Prior to distribution the EURL tested 10% of lenticule discs produced to ensure a satisfactory titre in the tissue and homogeneity of content of sample.

Methods

The dessication filter was removed from the tubes containing the Lenticules and 1ml of molecular grade water was added and left for 5 minutes at room temperature. The sample was vortexed until the lenticule was fully dissolved and 50µl of this was added to 250µl RLT buffer. Total RNA was extracted from the samples using an EZ1 RNA Mini kit and EZ1 Advanced XL BioRobot® (Qiagen) following manufactures' instructions. A reverse transcription was completed following the extraction. Reactions were performed in 20µl reaction mix consisting of 4µl of extracted RNA, 1µl of RNasin® (40u/µl), 1µl dNTPs (25mM), 1µl of reverse primer (TSV or YHV, see Table 1), 8µl of Molecular biology grade water, 1µl M-MLV reverse transcriptase (200u/µl) and 4µl M-MLV reverse transcriptase 5x reaction buffer. Amplifications were performed using the following reverse transcription thermal cycler program on a Peltier PTC-225 thermal cycler: 37°C for 1 hour and held at 4°C.

TSV

PCR reactions were performed on each RNA extract using the OIE recommended TSV primer sets (Nunan *et al.* 1998), see Table 1. Reactions were performed in 50µl reaction mix consisting of 1 X Green Go Taq buffer, 2.5mM $MgCl_2$, 0.25mM dNTPs, 5 pmol each of the forward and reverse primer, 0.25 units Go Taq Flexi (Promega), and 2.5µl RNA extract. Amplifications were performed using the following TSV thermal cycler program on a Peltier PTC-225 thermal cycler: 40 cycles of 94°C for 45 seconds, 60°C for 45 seconds, followed by 60°C for 7 minutes and held at 4°C.

Amplification products were resolved on 2% agarose gels stained with ethidium bromide and visualised using a UV illuminator. A 231bp product was shown for positive samples following PCR.

YHV

Separate first and second round (Nested) PCR reactions were performed on each RNA extract using the OIE recommended YHV primer sets (Wijegoonawardane *et al.*, 1998), see Table 1. Reactions were performed in 50µl reaction mix consisting of 1 X Green Go Taq buffer, 2.5mM $MgCl_2$, 0.25mM dNTPs, 5 pmol each of the forward and reverse primer, 0.25 units Go Taq Flexi (Promega), and 2.5µl RNA extract. Amplifications were performed using the following YHV thermal cycler program on a Peltier PTC-225 thermal cycler: 95°C for 1 minute, 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, followed by 72°C for 7 minutes and held at 4C. The above process was repeated but using 2.5µl of the 1st round PCR product instead of RNA extract.

Amplification products were resolved on 2% agarose gels stained with ethidium bromide and visualised using a UV illuminator. A 359bp product was shown for positive samples in the first round PCR and a 147bp product in the second round.

Table 1. TSV and YHV PCR primers (Nunan *et al.*, 1998; Wijegoonawardane *et al.*, 1998)

Primer name	Sequence
1st Round	
TSV 9992F	5'-AAGTAGACAGCCGCGCTT
TSV 9195R	5'-TCAATGAGAGCTTGGTCC
YHV F1ab pool	5'-ATCGTCGTCAGCTACCGCAATACTGC 5'-ATCGTCGTCAGYTAYCGTAACACCGC
YHV R1ab pool	5'-TCTTCRCGTGTGAACACYTTCTTRGC 5'-TCTGCGTGGGTGAACACCTTCTTGGC
2nd Round	
YHV YC-F2ab	5'-CGCTTCCAATGTATCTGYATGCACCA 5'-CGCTTYCARTGTATCTGCATGCACCA
YHV YC-R2ab	5'-RTCDGTGTACATGTTTGAGAGTTTGTT 5'-GTCAGTGTACATATTGGAGAGTTTRTT

Quality Control

The replicate lenticules of each batch (negative control and dilutions of TSV and YHV) were tested for both TSV and YHV infections. Negative control and YHV dilutions produced consistent results. TSV dilutions were shown to have a co-infection with YHV in some but not all of the lenticules tested. It was agreed that this batch was adequate to test the laboratories ability to identify TSV infections, labs

were scored as correctly identifying the sample if they identified TSV or TSV and YHV.

Distribution

Samples were sent to 11 NRL's in 10 Member States. The test was sent out according to current international regulations for shipment of diagnostic specimens UN 3373, "Biological substance, Category B". All proficiency tests were handled by courier and were delivered to all participants within three days.

All NRLs received lenticule discs from the same batch.

Expected Results

Participants were asked to identify the content of each tube by the method used in their laboratory.

Table 2. Expected results of the Proficiency Test

Sample ID	Sample Type	TSV[1]	YHV[2]
RA15006-1	Lenticule disc	Positive	Positive
RA15006-2	Lenticule disc	Negative	Negative
RA15006-3	Lenticule disc	Negative	Positive
RA15006-4	Lenticule disc	Negative	Positive

[1] Nunan *et al.*, 1998)

[2] Wijegoonawardane *et al.*, 1998

Actual Results

Results were received from all 11 laboratories, all laboratories correctly identified all samples 4/4 (100%)

Table 3. Proficiency test results submitted by the individual laboratories

Laboratory Code	RA15005-1	RA15005-2	RA15005-3	RA15005-4
EURL	TSV and YHV	-ve	YHV	YHV
1				
2				
3	TSV and YHV	-ve	YHV	YHV
4				
5				
6				
7	TSV	-ve	YHV	YHV
8				
9	TSV and YHV	-ve	YHV	YHV
10				
11	TSV and YHV	-ve	YHV	YHV
12	TSV	-ve	YHV	YHV
13	TSV	-ve	YHV	YHV
14	TSV and YHV	-ve	YHV	YHV
15				
16				
17				
18				
19	TSV and YHV	-ve	YHV	YHV
20	TSV	-ve	YHV	YHV
21	TSV	-ve	YHV	YHV
22				
23				
24				
25				

Grey boxes indicate that the lab did not take part in the testing

General Comments

The results presented in this report were discussed at the 7th Annual Meeting of National Reference Laboratories for Crustacean Diseases, 4th – 5th November 2015 in Weymouth, UK.

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15th January 2016

References

Nunan, L.M., Poulos, B.T. & Lightner D.V. (1998). Reverse transcription polymerase chain reaction (RT-PCR) used for the detection of Taura Syndrome Virus (TSV) in experimentally infected shrimp. *Dis. Aquat. Org.,* **34**, 87–91.

Wijegoonawardane, P.K.M., Cowley, J.A. & Walker, P.J. (2008). Consensus RT-nested PCR to detect yellow head virus genotypes in penaeid shrimp. *J. Virol. Methods*, **153**, 168–175.