European Union Reference
Laboratory for Crustacean Diseases
Cefas Weymouth Laboratory, Barrack Road, Weymouth, Dorset DT4 8UB, United Kingdom

# PROFICIENCY TEST 2011

# Detection of White Spot Syndrome Virus in *Litopenaeus vannamei* pleopods

**EURL Ring Trial Reference Number: CRL11003**

**Sample numbers: CRL11003-1**

**CRL11003-2**

**CRL11003-3**

**CRL11003-4**

| | |
|---|---|
| **Distribution Date:** | **8th September 2011** |
| **Report Date:** | **4th January 2012** |
| **Report compiled by:** | **K. Bateman** |

## 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 White Spot Syndrome Virus (WSSV) in accordance with EC Directive 2006/88.

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

Viral inoculates of WSSV were originally obtained from the OIE reference laboratory at the University of Arizona, USA. The OIE isolate of WSSV (UAZ 00-173B) was generated in *L. vannamei* from an original outbreak in *F. chinensis* in China in 1995. Subsequent passages of this isolate into naïve *L. vannamei* held at the Cefas Weymouth laboratory have demonstrated continued infectivity of this isolate.

There are currently no crustacean cell lines available; WSSV infected shrimp carcasses were prepared by direct intramuscular injection of WSSV inoculum into specific pathogen free (SPF) *L. vannamei* at a rate of 10 μl $g^{1}$ shrimp weight. Water temperature was held constant at 24˚C. Shrimp were monitored throughout the day for five days, dead and moribund shrimp were removed from the experimental tanks; pleopods were fixed in ethanol for molecular analysis, two pleopods per tube, 5 tubes per shrimp. SPF shrimp provided tissues for WSSV negative samples, pleopods were fixed in ethanol for molecular analysis, two pleopods per tube, 5 tubes per shrimp.

Prior to distribution the EURL tested pleopods from each individual shrimp to ensure a satisfactory titre in the tissue and homogeneity of content of sample. Tissues were prepared and tested according to PCR protocols accredited under ISO 17025 standards.

Multiple NRLs received pleopods from the same shrimp.

## Distribution

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 18 participants within three days.

## Expected Results

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

The table below highlights the expected results:

| Sample Number | Expected Result |
|---|---|
| **CRL11003-1** | **+ve** |
| **CRL11003-2** | **-ve** |
| **CRL11003-3** | **+ve** |
| **CRL11003-4** | **-ve** |

## Actual Results

- 10 laboratories correctly identified all four samples.
- 3 laboratories correctly identified three samples.
- 1 laboratory correctly identified two samples.
- 4 laboratories did not submit any results.

The following methods were used by the participants:

- 11 laboratories used nested PCR
- 3 laboratories used real-time PCR

## General Comments

NRLs which did not correctly diagnose all materials were requested to re-analyse original samples and fresh material was sent by the EURL for further analysis. Three laboratories correctly identified all material upon re-analysis.

The results presented in this report were presented and discussed at the 3rd Annual Meeting of National Reference Laboratories for Crustacean Diseases, 15th November 2011in Venice.

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4th January 2012