



European Union Reference Laboratory for Crustacean Diseases

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PROFICIENCY TEST 2012

Detection of White Spot Syndrome Virus in Lenticules

EURL Ring Trial Reference Number: EURL12004

Sample numbers: EURL12004-1

EURL12004-2

EURL12004-3

EURL12004-4

Distribution Date:	27/09/12
Report Date:	29/10/12
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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 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. SPF shrimp provided tissues for WSSV negative samples. Shrimp were confirmed as WSSV positive and WSSV 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. Tissues were prepared and tested according to PCR protocols accredited under ISO 17025 standards.

All NRLs received lenticule discs from the same batch.

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 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:

Lenticule Reference	Expected Results	
	Nested PCR ¹	Real-time PCR ²
EURL12004-1	WSSV Negative	WSSV Negative
EURL12004-2	WSSV Positive	WSSV Positive Ct 32
EURL12004-3	WSSV Positive	WSSV Positive Ct 33
EURL12004-4	WSSV Positive	WSSV Positive Ct 34

¹ OIE recommended technique (Lo *et al.*, 1996)

² Durand and Lightner, 2002

Actual Results

- 15 laboratories correctly identified all four samples.
- 1 laboratory correctly identified two samples.
- 1 laboratory did not submit any results.

The following methods were used by the participants:

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

General Comments

Fresh material was sent by EURL to NRL which did not correctly diagnose all materials, NRL was requested to re-analyse new samples. Laboratory correctly identified all material upon re-analysis.

The results presented in this report will be presented and discussed at the 4th Annual Meeting of National Reference Laboratories for Crustacean Diseases, 1st November 2012 in Weymouth.

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29th October 2012