

European Union Reference Laboratory for Fish and Crustacean Diseases NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK



EURL for Crustacean Diseases

Report of the Inter-laboratory proficiency test 2025 for identification of White spot syndrome virus (WSSV), Taura syndrome virus (TSV) and Yellowhead virus genotype 1 (YHV1)

Organised by the

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Introduction

A comparative test of diagnostic procedures for the detection of White spot syndrome virus (WSSV), Taura syndrome virus (TSV), and Yellowhead virus genotype 1 (YHV1) was provided by the European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases at DTU AQUA in accordance with EU Regulation (EU) 2017/625 § 94. The invitation to participate in this year's proficiency test was sent to 27 laboratories including 20 NRLs of EU Member States and 7 non-EU countries. All laboratories except one send the results.

Each laboratory was given a code number to ensure the anonymity of this report.

A coded version of the report is provided to all participants along with individual certificates where the code number of each participant is supplied. In this way each participating laboratory can compare its diagnostic performances with the overall performances. In the certificates, comments related to underperformances are included, if present.

An un-coded version of the report is sent to the European Commission.

Sample Preparation

Viral inoculates of WSSV (Isolate UAZ 00-173B), TSV (isolate UAZ 00-273), and YHV1 (isolate UAZ 99-294) were obtained from the Cefas laboratory in Weymouth, UK. These isolates were originally obtained from the WOAH reference laboratory at the University of Arizona, USA. Subsequent passages of these isolates into naïve *P. vannamei* held at DTU AQUA have demonstrated continued infectivity of these isolates.

Organ homogenate from infected *Penaeus vannamei* was tested by qPCR and RT-PCR (depending on the virus) and diluted in media culture (MEM).

To produce the FTA card test material, $65~\mu L$ of the diluted shrimp homogenate was applied to each circular FTA card area (QIAcard FTA Micro [Qiagen WB120210]), upon which the cards were dried for 2 hours at room temperature in a laminar flow chamber. Subsequently, the adsorbed cards were tested to ensure the expected pathogen was found in each batch. Five replicates of each sample were tested, and the batch was accepted only if cards gave consistent results for the viral species and the viral load, assessed by Ct values.

The samples were re-tested after the deadline by qPCR to ensure the stability of the material. All tests yielded the expected results.

Diagnostic methods

Extraction of nucleic acid (DNA and RNA) from FTA cards

A portion of FTA card with approximate dimensions of 5 x 5 mm was cut. The sample was incubated in an Eppendorf tube with TE buffer (200 μ L) for 30 min at room temperature with occasional vortexing. 200 μ L of the sample was used for automated DNA and RNA using the IndiMag Pathogen kit (Indical Bioscience) following manufacturer's instructions on an IndiMag 48s extraction robot.

TSV real-time PCR

Based on Tang et al. (2004).

5 μL template RNA was added to a PCR tube containing: 5 μL TaqPath[™] 1-Step RT-qPCR Master Mix, CG, 0.8 μL forward primer (10 μM), 0.8 μL reverse primer (10 μM), 0.4 μL hydrolysis Probe (10 μM) and 8 μL molecular grade water. The PCR profile was one cycle of 50°C for 15 minutes and 95°C for 2 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 60 seconds.

Primer sequences were,

TSV1004F: 5'-TTG-GGC-ACC-AAA-CGA-CAT-T-3',

TSV1075R: 5'-GGG-AGC-TT A-AAC-TGG-ACA-CAC-TGT-3',

Hydrolysis Probe TSV-P1: 5'-CAG-CAC-TGA-CGC-ACA-ATA-TTC-GAG-CAT-C-3' with fluorescent dyes 6-Carboxyfluorescein (6-FAM) on the 5' end and Black Hole Quencher (BHQ) on the 3' end.

A positive RT-qPCR control, consisting of a synthesized RNA fragment representing the TSV PCR amplicon was included

YHV real-time PCR

Based on WOAH manual (Moody, 2023)

5 μL template RNA was added to a PCR tube containing: 5 μL TaqPath™ 1-Step RT-qPCR Master Mix, CG, 1.8 μL forward primer (10 μM), 1.8 μL reverse primer (10 μM), 0.4 μL hydrolysis Probe (10 μM) and 6 μL molecular grade water. The PCR profile was one cycle of 25°C for 2 minutes, 50°C for 15 minutes and 95°C for 2 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds.

Primer sequences were,

YHV1-12-qF: 5'-AGT-CTA-CAG-TGC-TCT-GAT-CT-3',

YHV1-12-qR: 5'-GAT-TCT-TGA-AGC-GCA-TGA-GT-3',

Hydrolysis Probe YHV1-12-qPr: FAM-TCT-CAT-GTG/ZEN/TCA-TGATAT-TCT-CAA-GCG-AGT-IABkFQ.

A positive PCR control, consisting of an *in vitro* transcribed RNA fragment representing the YHV RT-q PCR amplicon was included.

YHV conventional PCR

Based on Mohr et al. (2015).

First round RT-PCR: 5 μ L template RNA was added to a PCR tube containing: 5 μ L Qiagen OneStep RT-PCR kit buffer (Qiagen), 1.5 μ L forward primer (10 μ M), 1.5 μ L reverse primer (10 μ M), 1 μ L of dNTP (10 mM each), 1 μ L of Enzyme Mix and 10 μ L molecular grade water. The PCR profile is one cycle of

50°C for 30 minutes and 95°C for 15 minutes, followed by 40 cycles of 94°C for 30 seconds, 58°C for 45 seconds, and 72°C for 45 seconds, followed by one cycle of 72°C for 7 minutes.

RT-PCR products were subsequently run on 2 % e-gels (Invitrogen).

Primer sequences were,

10F: 5'-CCG-CTA-ATT-TCA-AAA-ACT-ACG-3', 144R: 5'--AAG-GTG-TTA-TGT-CGA-GGA-AGT-3'.

WSSV real-time PCR

Based on Durand & Lightner (2002).

 $5~\mu L$ template DNA was added to a PCR tube containing: $10~\mu L$ Luna® Universal Probe qPCR Master Mix (New England Biolabs), $0.8~\mu L$ forward primer ($10~\mu M$), $0.8~\mu L$ reverse primer ($10~\mu M$), $0.4~\mu L$ TaqMan Probe ($10~\mu M$) and $6~\mu L$ molecular grade water. The PCR profile is one cycle of 94°C for 15~m minutes, followed by 50~cycles of 94°C for 15~cseconds and 60°C for 60~cseconds.

Primer sequences were,

WSS1011F: 5'-TGG-TCC-CGT-CCT-CAT-CTC-AG-3',

WSS1079R: 5'-GCT-GCC-TTG-CCG-GAA-ATT-A-3',

Hydrolysis Probe: 5'-AGC-CAT-GAA-GAA-TGC-CGT-CTA-TCA-CAC-A-3' with fluorescent dyes 6-Carboxyfluorescein (6-FAM) on the 5' end, Iowa Black FQ (IBFQ) on the 3' end and an internal ZEN quencher.

A positive PCR control, consisting of a synthesized gBlocks gene fragment representing the WSSV PCR amplicon was included.

Distribution

Each laboratory participating in the proficiency test received nine FTA cards, including 3 negative cards adsorbed with SPF shrimp homogenate and one with MEM, 2 cards adsorbed with WSSV at two different concentrations, 2 cards adsorbed with TSV at two different concentrations, and 1 card adsorbed with YHV1 at high concentration.

The test samples were sent out according to current international regulations for the shipment of diagnostic specimens UN 3373, "Biological substance, Category B". All proficiency tests were shipped by courier.

Proficiency test content and expected results

The proficiency test consisted of nine samples. The contents of the Proficiency Test are shown in Table 1.

Table 1. Expected results of the proficiency test.

Sample ID	Virus							
Sample 01	Negative (SPF Shrimp homogenate at 1:20 dilution)							
Sample 02	Negative (cell culture media)							
Sample 03	WSSV UAZ 00-173B, high concentration dilution factor 1:10							
Sample 04	TSV UAZ 00-273, high concentration dilution factor 1:10							
Sample 05	Negative (SPF Shrimp homogenate at 1:20 dilution)							
Sample 06	YHV UAZ 99-294, high concentration dilution 1:10							
Sample 07	Negative (SPF Shrimp homogenate at 1:20 dilution)							
Sample 08	TSV UAZ 00-273, low concentration, dilution factor 1:200							
Sample 09	WSSV UAZ 00-173B, low concentration dilution factor 1:100							

Results

Participants were asked to identify the content of each of the nine received FTA cards by the method used in their laboratory in accordance with EU Diagnostic manuals for the Crustacean listed diseases (https://www.eurl-fish-crustacean.eu/crustacean/diagnostic-manuals). Each correct answer accounted for one point, for a total of 9 points. Some laboratories are testing only for WSSV, as they have derogated their testing for YHV and TSV. In this case, the maximum score that can be obtained is 6 points.

Results were received from all 26 participating laboratories

- 22 laboratories correctly diagnosed all samples (100 %)
- 1 laboratory had 4 underperformances (5/9; 55,56%):

This laboratory had a dual detection for S05 (negative sample); TSV detected in S02 (Negative); YHV1 detection in S07 (Negative) and a NEGATIVE detection in S08 (TSV)

- 3 laboratories had 1 underperformance (8/9; 88,89%) 1 laboratory could not detect the WSSV low titer even though they correctly detect High titer sample; 1 laboratory detected YHV1 in samples 7 (Negative) and other in 1 (Negative)
- 3 out of 26 laboratories tested only for WSSV, and therefore could obtain a maximum score of 6
- 1 laboratory does not respond to the ILPT25

A detailed overview of the results is shown in Table 2 - 4.

Evaluation of results

The error rate of the results received in 2025 was comparable to previous proficiency tests from 2022 and 2023, but higher than that of 2024, in which 100% of the samples were correctly identified. All the erroneous results consist of false negative tests.

The laboratory with the lowest performance may have cross-contaminated samples during or after nucleic acid purification. It might have also caused due to mislabelling of the sample as the sample 2 detected as TSV that is Negative and sample 8 (TSV) detected as Negative. Another aspect to be considered to explain the low performances is mis-labelling the samples during the downstream processing. A new batch of the ILPT will be offered to this participant upon their interest so that they can reassess their procedures.

Sample 5 is a true Negative, but one laboratory had dual detection, and 4 laboratories had false positive detections in the true negative samples.

In the current year Crustacean ILPT, we requested participants to fill the results in the new 'spreadsheet result report form'. Thanks to the new spreadsheet it was possible for the EURL to collect data related to many relevant aspects of the ringtest including for example the use of internal controls for the PCR, accreditation status and accredited method for listed pathogens, regional labs etc. The detailed response analysis is provided in Table 2-4 and figure 1-12.

A total of 22 laboratories conducted probe-based qPCR testing for the detection of White Spot Syndrome Virus (WSSV) in both sample sets S03 and S09. For S03, 13 laboratories reported cycle threshold (Ct) values within the expected range, indicated in magenta. Five laboratories—specifically Labs 3, 9, 15, 24, and 27—showed high Ct values, marked in black, while four laboratories reported low Ct values, represented in yellow. (Fig. 8)

In the case of S09, one laboratory reported an incorrect detection, identifying the sample as negative. Thirteen laboratories had Ct values within the expected range, indicated in blue. Similar to S03, five laboratories—Labs 3, 9, 11, 24, and 27—exhibited high Ct values (black), and three laboratories showed low Ct values (yellow). (Fig. 9)

A total of 16 laboratories performed probe-based qPCR testing for the detection of Taura Syndrome Virus (TSV) in both sample sets S04 and S08. For S04, 11 laboratories performed well, with Ct values falling within the expected range, indicated in violet. Three laboratories—Labs 3, 13, and 27—were identified as underperforming, marked in black, while two laboratories showed less efficiency, represented in yellow. (Fig. 10)

In the case of S08, 10 laboratories reported Ct values within the expected range, indicated in cyan. Three laboratories—Labs 3, 11, and 27—had high Ct values (black), and three laboratories reported low Ct values (yellow). (Fig. 11)

A total of seven laboratories performed probe-based qPCR testing for the detection of Yellow Head Virus genotype 1 (YHV1). Among them, six laboratories reported Ct values within the expected range, indicated in magenta, while one laboratory—Lab 14—showed a high Ct value, marked in black. (Fig. 12)

Table 2 – Result overview of the ILPT for crustacean diseases 2025

C-ILPT25 CORRECT DETCETION	Sample 1 Negative	Sample 2 Blank	Sample 3 WSSV	Sample 4 TSV	Sample 5 Negative	Sample 6 YHV1	Sample 7 Negative	Sample 8 TSV	Sample 9 WSSV	sco	DRE
→	Blank	Blank	High	High	Blank	High	Blank	Low	Low		
Laboratory Code	Virus identification	Out of 9	%								
1	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
2	Negative	TSV	WSSV	TSV	Dual detection*	YHV1	YHV1	Negative	WSSV	5/9	55,56
3	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
4	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
5	Negative	Negative	WSSV	0	Negative	0	Negative	0	WSSV	6/6	100
6	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
7	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
8	0	0	WSSV	TSV	0	YHV1	0	TSV	WSSV	9/9	100
9	Negative	Negative	WSSV	0	Negative	0	Negative	0	WSSV	6/6	100
10	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
11	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
12	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
13	negative	negative	WSSV	TSV	negative	YHV1	negative	TSV	WSSV	9/9	100
14	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
15	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	Negative	8/9	88,89
16	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
17	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
18	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
19	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100

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20	Negative	Negative	WSSV	TSV	Negative	YHV1	YHV1	TSV	WSSV	8/9	88,89
21	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
22	YHV1	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	8/9	88,89
23	Negative	Negative	WSSV	0	Negative	0	Negative	0	WSSV	6/6	100
24	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
25	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100
26	0	0	0	0	0	0	0	0	0	NR	-
27	Negative	Negative	WSSV	TSV	Negative	YHV1	Negative	TSV	WSSV	9/9	100

Table 3 Overview of the methods used and other relevant information by the participant laboratories for pathogen detection in the ILPT for crustacean diseases 2025

Country	Accreditation	Acc	redited for			Tested		No. Region Labs	ILPT Pathogen		Date of FTA card arrival	Sequencing	Internal Control
Code		WSSV	TSV	YHV1	WSSV	TSV	YHV1	No/Nil	Yes/No	Name	caru arrivar		Control
1	17025	qPCR	RT-PCR	qRT-PCR	qPCR	qRT-PCR	RT-PCR	Not answered	Not answered	Nil	31-03-2025	No	Any Other
2	No	PCR	RT-PCR	RT-PCR	PCR	PCR	PCR	No	No	Nil	31-03-2025	No	Nil
3	No	Nil	Nil	Nil	qPCR	qRT-PCR	RT-PCR	No	Not answered	Nil	01-04-2025	No	Nil
4	ISO 17025 & ISO 17043	qPCR	RT-PCR	qRT-PCR	PCR/qPCR	RT-PCR/qRT-PCR	RT-PCR/qPCR	No	Yes	Nil	25-03-2025	No	Nil
5	ISO EN 17025	Nil	Nil	Nil	qPCR	No	No	No	No	Aphanomyces astaci Culture	01-04-2025	No	Any Other
6	ISO EN 17025	PCR	RT-PCR	RT-PCR	PCR/Seq/qPCR	RT-PCR/Seq	RT-PCR/Seq	Yes	Yes	Nil	45747	Yes	Nil
7	ISO EN 17025	PCR/Seq/qPCR	RT-PCR/Seq	RT- PCR/Seq	PCR/qPCR	RT-PCR	RT-PCR	No	No	A. astaci qPCR Probe	07-04-2025	Yes (no results)	Nil
8	ISO EN 17025	Nil	Nil	Nil	qPCR	RT-PCR	RT-PCR	No	No	Nil	31.03.2025	No	Nil
9	ISO EN 17025	PCR/Seq/qPCR	Nil	Nil	qPCR/Seq	No	No	No	No	Nil	No data	Yes	rRNA
10	UNI CEI EN ISO/IEC 17025:2018	PCR/Seq	RT-PCR/Seq	RT- PCR/Seq	PCR/Seq	RT-PCR/Seq	RT-PCR/Seq	1	No	Nil	31.03.2025	Yes	Nil
11	ISO 17025	Histo/PCR	Histo/qRT- PCR	Histo/RT- PCR	qPCR	qRT-PCR	RT-PCR	No	No	Nil	31.03.2025	No	Nil
12	ISO 17025	Nil	Nil	Nil	qPCR	qRT-PCR	qRT-PCR	No	No	Nil	31.03.2025	No	Beta Actin
13	ISO EN 17025	PCR/qPCR	RT-PCR/qRT- PCR	RT-PCR	PCR/qPCR	RT-PCR/qRT-PCR	RT-PCR	5	No	Nil	01.04.2025	No	Any Other
14	No	Nil	Nil	Nil	PCR	qRT-PCR	RT-PCR/qRT-PCR	No	No	Nil	31.03.2025	No	rRNA
15	ISO EN 17025	Nil	Nil	Nil	qPCR	RT-PCR	RT-PCR	Yes, no data	Yes	Nil	31.03.2025	No	Nil
16	ISO EN 17025	Nil	Nil	Nil	qPCR	qRT-PCR	RT-PCR	No	No	Nil	31.03.2025	No	Any Other

17	ISO EN 17025	qPCR	Nil	Nil	PCR/Seq/qPCR	RT- PCR/Seq/qRT- PCR	RT- PCR/Seq/qRT- PCR	Yes, not for Crustacean	Yes, not for Crustacean	Nil	31.03.2025	Yes	Any Other
18	ISO EN 17025	PCR/Seq	Nil	Seq	RT-PCR/Seq	qRT-PCR	RT-PCR/Seq	No	No	Nil	No Data	Yes	Nil
19	ISO EN 17025	qPCR	Nil	Nil	qPCR	qRT-PCR	RT-PCR	No	No	Nil	02.04.2025	Yes	Any Other
20	ISO 9001 Certification (Not for Crustaceans)	Nil	Nil	Nil	qPCR	qRT-PCR	qRT-PCR	No	No	Nil	01.04.2025	No	Nil
21	ISO/IEC 17025	PCR/Seq/qPCR	Nil	Nil	PCR/Seq/qPCR	RT-PCR/qRT-PCR	RT-PCR/qRT- PCR/Seq	7	Yes	Nil	01-04-2025	Yes	EF1a
22	ISO 17025	Nil	Nil	Nil	qPCR	qRT-PCR	RT-PCR	No	No	Nil	No Data	No	Nil
23	ISO EN 17025:2017	PCR	Nil	Nil	PCR	No	No	5	Yes (not Crustceans)	A. astaci qPCR	04.04.2025	No	rRNA
24	ISO EN 17025	PCR/Seq/qPCR	RT-PCR/Seq	RT- PCR/Seq	PCR/Seq/qPCR	RT-PCR/Seq	RT-PCR/Seq	No	No	<i>A. astaci</i> PCR/Seq	31.03.2025	Yes	Nil
25	ISO 17025	Histo/PCR/Seq	Histo/RT- PCR/Seq	Histo/RT- PCR/Seq	PCR/Seq/qPCR	RT- PCR/Seq/qRT- PCR	RT- PCR/Seq/qRT- PCR	No	No	Nil	02.04.2025	Yes	Nil
26						Results	not received						
27	ISO EN 17025	qPCR	Nil	Nil	qPCR	qRT-PCR	RT-PCR	2	No	Nil	03.04.2025	No	rRNA

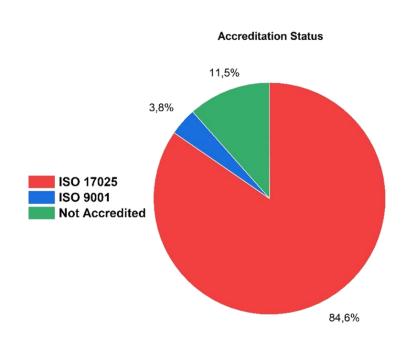


Fig. 1. Accreditation of the participating laboratories in ILPT25

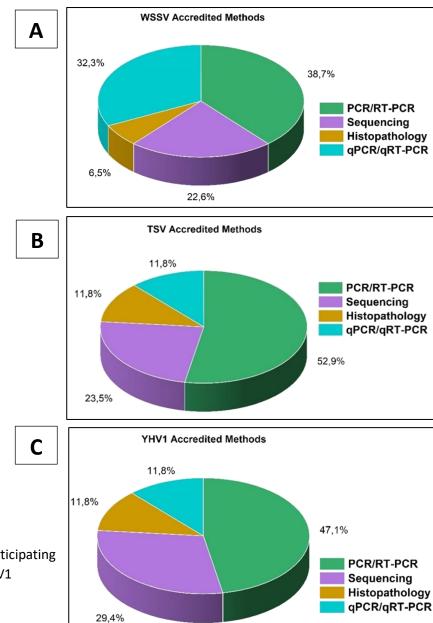


Fig. 2. Accredited methods for the listed viruses by the participating laboratories in ILPT25 – (**A**) WSSV; (**B**) TSV & (**C**) YHV1

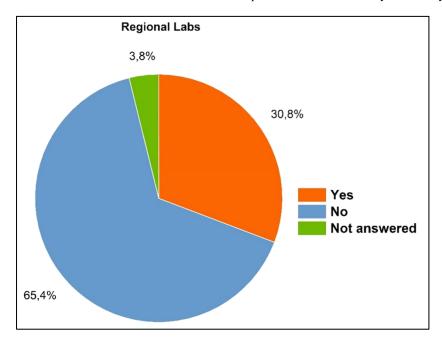
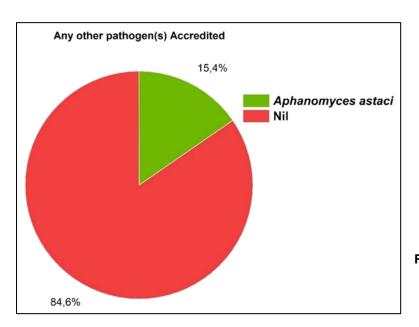


Fig. 3. Number of regional labs under participating laboratories in ILPT25



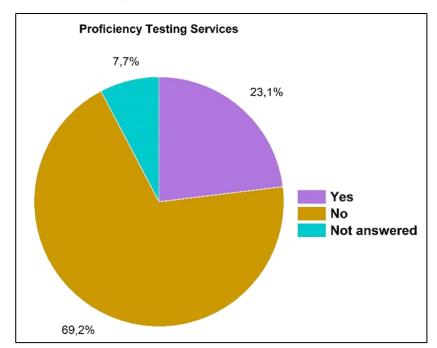
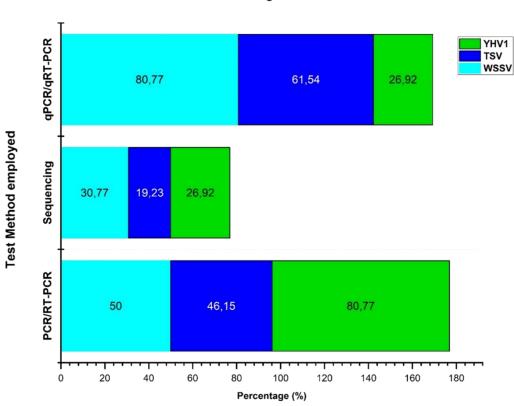


Fig. 4. Provision of Proficiency services to the regional labs under participating laboratories in ILPT25

Fig. 5. Details of the other accredited pathogen for analysis in participated labs

Testing Methods



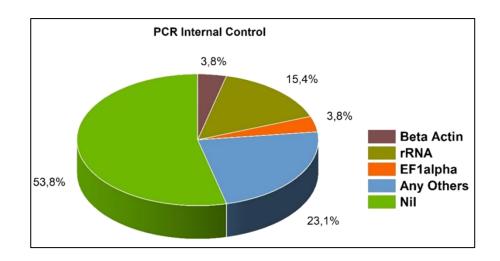


Fig. 6. Use of testing methods by the participated labs for the analysis of FTA card under ILPT25

Fig. 7. Use of internal controls for the PCR/qPCR by the participated labs under ILPT25

Table 4 – Ct values overview of the ILPT for crustacean diseases 2025

	Sample	3	Sample 4		Sample	6	Sample	8	Sample	9		
C-ILPT25	WSSV		TSV		YHV1		TSV		WSSV			
CORRECT DETCETION	UAZ 00-17	UAZ 00-173B		UAZ 00-273		UAZ 99-294		UAZ 00-273		73B	SCORE	% SCORE
→	Titer											
	High		High		High		Low		Low			
Laboratory Code	Virus identification	Ct Value										
1	WSSV	27,65	TSV	27,66	YHV1	0	TSV	31,16	WSSV	31,77	9/9	100
2	WSSV	0	TSV	0	YHV1	0	Negative	0	WSSV	0	5/9	55,56
3	WSSV	35,5	TSV	32,52	YHV1	0	TSV	38,57	WSSV	39	9/9	100
4	WSSV	28,05	TSV	24,77	YHV1	26,97	TSV	28,75	WSSV	30,82	9/9	100
5	WSSV	28,55	Negative	0	Negative	0	Negative	0	WSSV	32,7	6/6	100
6	WSSV	26,67	TSV	0	YHV1	0	TSV	0	WSSV	30,55	9/9	100
7	WSSV	30,43	TSV	0	YHV1	0	TSV	0	WSSV	33,68	9/9	100
8	WSSV	30,11	TSV	0	YHV1	0	TSV	0	WSSV	34,37	9/9	100
9	WSSV	31,9	0	0	0	0	0	0	WSSV	35,59	6/6	100
10	WSSV	0	TSV	0	YHV1	0	TSV	0	WSSV	0	9/9	100
11	WSSV	30,27	TSV	28,29	YHV1	0	TSV	34,05	WSSV	35,35	9/9	100
12	WSSV	26,98	TSV	23,04	YHV1	26,98	TSV	26,63	WSSV	30,33	9/9	100
13	WSSV	30,36	TSV	29,79	YHV1	0	TSV	33,17	WSSV	33,39	9/9	100
14	WSSV	0	TSV	27,01	YHV1	34,31	TSV	31,05	WSSV	0	9/9	100
15	WSSV	31,11	TSV	0	YHV1	0	TSV	0	Negative	0	8/9	88,89
16	WSSV	28,9	TSV	24,3	YHV1	0	TSV	31,7	WSSV	32,6	9/9	100
17	WSSV	29,17	TSV	26,28	YHV1	31,02	TSV	29,2	WSSV	31,23	9/9	100
18	WSSV	27,89	TSV	25,46	YHV1	0	TSV	25,64	WSSV	28,37	9/9	100

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19	WSSV	27,45	TSV	23,86	YHV1	0	TSV	27,32	WSSV	30,46	9/9	100
20	WSSV	28,33	TSV	23,54	YHV1	29,73	TSV	25,91	WSSV	31,65	8/9	88,89
21	WSSV	25,96	TSV	23,86	YHV1	26,67	TSV	27,73	WSSV	29,85	9/9	100
22	WSSV	25,72	TSV	25,52	YHV1	0	TSV	28,28	WSSV	29,92	8/9	88,89
23	WSSV	0	Negative	0	Negative	0	Negative	0	WSSV	0	6/6	100
24	WSSV	32,25	TSV	0	YHV1	0	TSV	0	WSSV	35,56	9/9	100
25	WSSV	29,38	TSV	25,03	YHV1	30,21	TSV	31,16	WSSV	32,85	9/9	100
26	0	0	0	0	0	0	0	0	0	0	NR	-
27	WSSV	32,22	TSV	34,06	YHV1	0	TSV	39,37	WSSV	35,67	9/9	100

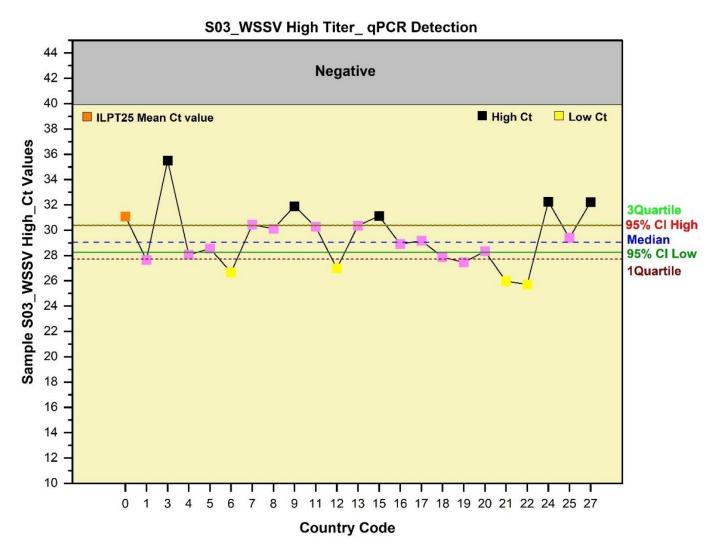


Fig. 8. Sample S03_WSSV High Titer – Result qPCR Ct value distribution Median – 29,035; 95% CI High – 30,36; 95% CI Low – 28,26; 25% Quartile – 27,71; 75% Quartile – 30,41

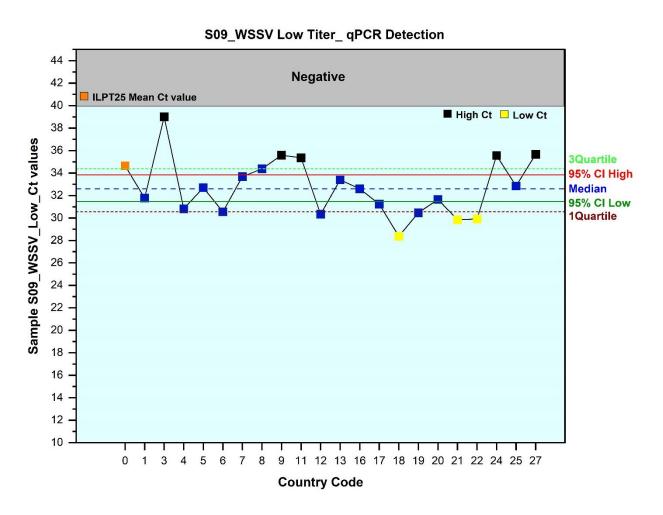


Fig. 9. Sample S09_WSSV Low Titer – Result qPCR Ct value distribution Median – 32,60; 95% CI High – 33,83; 95% CI Low – 31,48; 25% Quartile – 30,55; 75% Quartile – 34,37

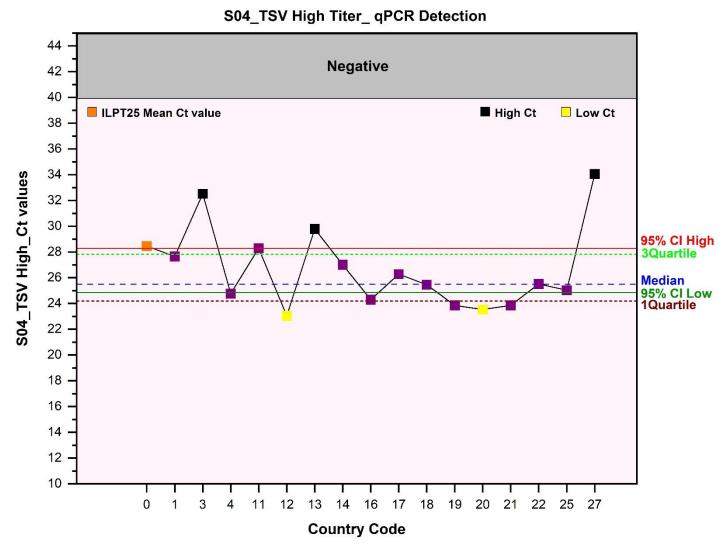


Fig. 10. Sample S04_TSV High Titer – Result RT-qPCR Ct value distribution Median – 25,49; 95% CI High – 28,28; 95% CI Low – 24,85; 25% Quartile – 24,19; 75% Quartile – 27,82

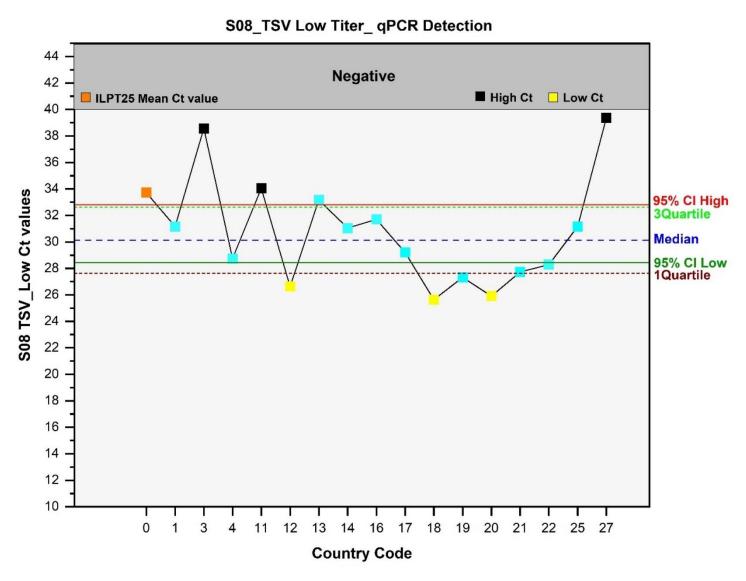


Fig. 11. Sample S08_TSV Low Titer – Result RT-qPCR Ct value distribution Median – 30,125; 95% CI High – 32,80; 95% CI Low – 28,42; 25% Quartile – 27,63; 75% Quartile – 32,07

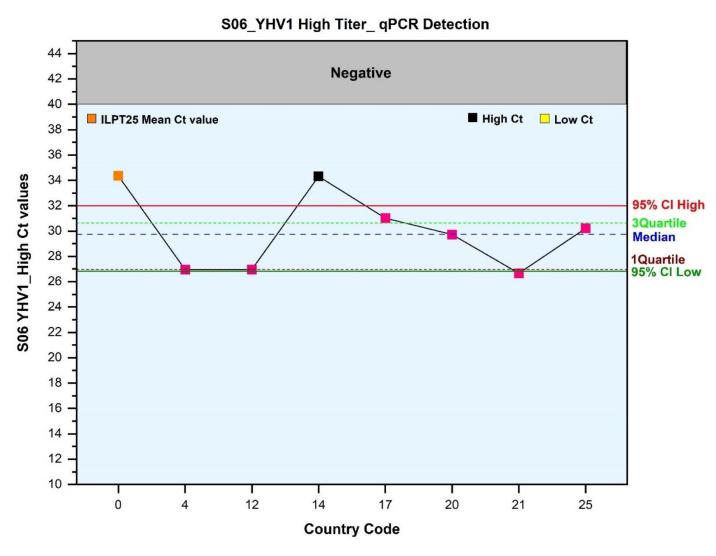


Fig. 12. Sample S06_YHV1 High Titer – Result RT-qPCR Ct value distribution Median – 29,73; 95% CI High – 32,80; 95% CI Low – 28,42; 25% Quartile – 26,98; 75% Quartile – 30,62

The EURL provides the annual proficiency test, collates the data, and processes the figures so that individual laboratories can see how they fare in comparison to the other participants. It is up to the individual laboratories to assess if they perform according to their own expectations and standards.

We take the opportunity to provide comments to participants regarding submitted results if relevant. Furthermore, we encourage all participants to contact us with any questions concerning the test or any other diagnostic matters.

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References

- Durand, S. V. and Lightner, D. V. (2002) 'Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp', Journal of Fish Diseases, 5, 381-389. doi: 10.1046/j.13652761.2002.00367.x.
- Lo, C. F., Leu, J. H., Ho, C. H., Chen, C. H., Peng, S. E., Chen, Y. T., Chou, C. M., Yeh, P. Y., Huang, C. J., Chou, H. Y., Wang, C. H. and Kou, G. H. (1996) 'Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction', Diseases of Aquatic Organisms. 25, 133–141. doi: 10.3354/dao025133.
- Mohr P.G., Moody N.J.G., Hoad J., Williams L.M., Bowater R.O., Cummins D.M., Cowley J.A. & Crane M.STJ. (2015). New yellow head virus genotype (YHV7) in giant tiger shrimp *Penaeus monodon* indigenous to northern Australia. *Diseases of Aquatic Organisms*, 115, 263–268.
- Tang K.F.J., Wang J. & Lightner D.V. (2004). Quantitation of Taura syndrome virus by real-time RT-PCR with a TaqMan assay. *Journal of Virological Methods*, 115, 109–114.

WOAH Manual CHAPTER 2.2.10. INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1