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 2026 for identification of White spot syndrome virus (WSSV), Taura syndrome virus (TSV) and Yellowhead virus genotype 1 (YHV1)

Organised by the

European Union Reference Laboratory for Fish and Crustacean Diseases,
DTU Aqua - National Institute of Aquatic Resources,
Technical University of Denmark,
Lyngby, Denmark

Distribution Date:	16/03/2026
Report Date:	28/05/2026
Report compiled by:	Shyam K Uthaman

PT Reg.No. 515
Member of EA MLA

Table of Contents

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 26 laboratories including 20 NRLs of EU Member States and 6 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.

C-ILPT

9 FTA cards containing various concentration of EU listed crustacean pathogens - WSSV, TSV, YHV1 were delivered to all NRLs in the EU Member States including Denmark and likewise to the NRLs in Australia, Norway, Montenegro, Switzerland, and the United Kingdom (Scotland, England and Wales) respectively. Figure 1 shows the worldwide distribution of the participating NRLs.

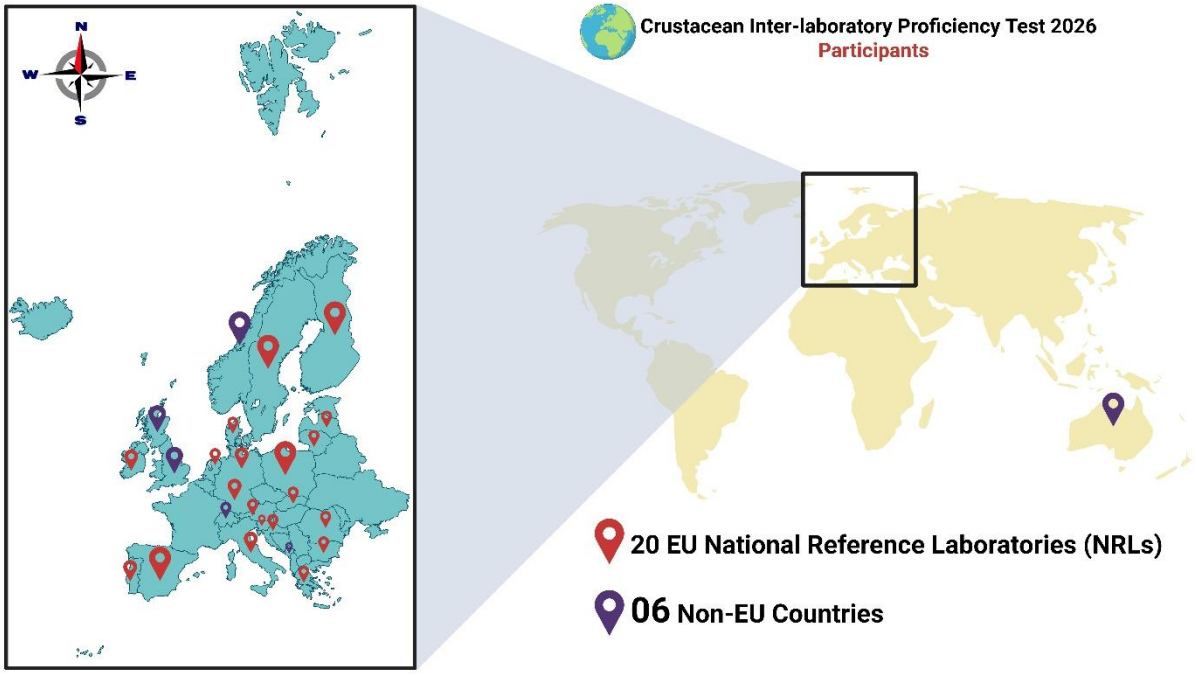

Figure 1. Map showing the distribution of C-ILPT 2026 participant laboratories.

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 this isolate 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 µL 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 1-1.5 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 µL) for 30 min at room with occasional vortexing. 200 µL 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

YHV1 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.

YHV1 conventional PCR

Based on Mohr et al. (2015).

First round RT-PCR: 5 µL template RNA was added to a PCR tube containing: 5 µL Qiagen OneStep RT-PCR kit buffer (Qiagen), 1.5 µL forward primer (10 µM), 1.5 µL reverse primer (10 µM), 1 µL of dNTP (10 mM each), 1 µL of Enzyme Mix and 10 µL 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 µL template DNA was added to a PCR tube containing: 10 µL Luna® Universal Probe qPCR Master Mix (New England Biolabs), 0.8 µL forward primer (10 µM), 0.8 µL reverse primer (10 µM), 0.4 µL TaqMan Probe (10 µM) and 6 µL molecular grade water. The PCR profile is one cycle of 94°C for 15 minutes, followed by 50 cycles of 94°C for 15 seconds and 60°C for 60 seconds.

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 1 negative card 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 3 cards adsorbed with YHV1 at three different concentrations.

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	YHV1 UAZ 99-294, low concentration dilution 1:500
Sample 02	TSV UAZ 00-273, low concentration, dilution factor 1:400
Sample 03	Negative (SPF Shrimp homogenate at 1:20 dilution)
Sample 04	WSSV UAZ 00-173B, high concentration dilution factor 1:100
Sample 05	Negative (cell culture media)
Sample 06	TSV UAZ 00-273, high concentration dilution factor 1:200
Sample 07	WSSV UAZ 00-173B, low concentration dilution factor 1:500
Sample 08	YHV1 UAZ 99-294, medium concentration dilution 1:100
Sample 09	YHV1 UAZ 99-294, high concentration dilution 1:10

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. 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 YHV1 and TSV. In this case, the maximum score that can be obtained is 4 points.

Results were received from all 26 participating laboratories

- 25 laboratories correctly diagnosed all samples (100 %)
- 1 laboratory had 1 underperformance (8/9; 88,89%)
    - This laboratory had a negative detection for S07 (WSSV), laboratory could not detect the WSSV low titer even though they correctly detect High titer sample
- 3 out of 26 laboratories tested only for WSSV, and therefore could obtain a maximum score of 4

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

All the statistical analysis and graphical representations were done using Microsoft Excel, OriginPro 2024b.

Résumé and concluding remarks C-ILPT

100% of the packages reached the destination country eleven days after dispatch. One participant received the package after 15 days due to internal delays and wrong delivery to the address mentioned.

Only 1 of the participants scored 88.89% success rate. Suggestions to improve on underperformance will be provided individually to the laboratory. The error rate of the results received in 2026 was comparable to previous proficiency tests from 2023 and 2024, but lower than that of 2025. The erroneous result consists of false negative test.

The underperformance experienced by the laboratory can potentially be related to reduced sensitivity of the implemented diagnostic procedure as it occurred in the sample with low concentration of WSSV.

In the current year Crustacean ILPT, we requested participants to fill the results in the updated version of 'spreadsheet result report form' considering the feedback received from the participants. The EURL was able to collect data related to many relevant aspects of the ring test 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 2-15.

Mean Ct values between before and after preparation of the FTA cards samples have been tabulated in Table 5.

A total of 23 laboratories conducted probe-based qPCR testing for the detection of White Spot Syndrome Virus (WSSV) in both sample sets S04 and S07. For S04, 17 laboratories reported cycle threshold (Ct) values within the expected range, indicated in magenta. One laboratory—specifically Labs 23 — showed high Ct values, marked in black, while four laboratories reported low Ct values, represented in yellow. (Fig. 9)

In the case of S07, one laboratory reported an incorrect detection, identifying the sample as negative. Thirteen laboratories had Ct values within the expected range, indicated in blue. Four laboratories—Labs 13, 15, 19 and 23—exhibited high Ct values (black), and four laboratories showed low Ct values (yellow). (Fig. 10)

A total of 16 laboratories performed probe-based qPCR testing for the detection of Taura Syndrome Virus (TSV) in both sample sets S02 and S06. For S02, 15 laboratories performed well, with Ct values falling within the expected range, indicated in cyan. One laboratory—Labs 6 —was identified as underperforming, marked in black (Fig. 11)

In the case of S06, 12 laboratories reported Ct values within the expected range, indicated in violet. Two laboratories—Labs 6 and 19 —had high Ct values (black), and two laboratories reported low Ct values (yellow). (Fig. 12)

A total of eight laboratories performed probe-based qPCR testing for the detection of Yellow Head Virus genotype 1 (YHV1) in sample sets S01, S08 and S09. In low titer sample (S01), six laboratories reported Ct values within the expected range, indicated in magenta, while one laboratory—Lab 17—showed a high Ct value, marked in black and one laboratory (Lab 18) reported low Ct value, highlighted in yellow (Fig. 13). Similar pattern, in medium (S08) and high (S09), lab 17 and lab 18 reported Ct values outside the expected range as set by other 8 laboratories. (Fig. 14 & 15)

Table 2 – Result overview of the ILPT for crustacean diseases 2026

C-ILPT26	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	SCORE	
CORRECT DETCETION →	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1		
	Low	Low	SPF	High	Blank	High	Low	Medium	High		
Laboratory code	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Virus identification	Out of 9	%
1	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
2	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
3	0	0	Negative	WSSV	Negative	0	WSSV	0	0	4/4	100
4	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
5	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
6	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
7	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
8	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
9	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
10	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
11	0	0	Negative	WSSV	Negative	0	WSSV	0	0	4/4	100
12	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
13	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
14	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
15	0	0	Negative	WSSV	Negative	0	WSSV	0	0	4/4	100
16	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
17	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
18	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
19	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100
20	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	9/9	100

21	YHV1	TSV	Negative	WSSV	Negative	TSV	0	YHV1	YHV1	**8/9**	**88,88**
22	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	**9/9**	**100**
23	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	**9/9**	**100**
24	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	**9/9**	**100**
25	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	**9/9**	**100**
26	YHV1	TSV	Negative	WSSV	Negative	TSV	WSSV	YHV1	YHV1	**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 2026

Country Code	Accreditation	Accredited for			Tested			No. Region Labs	Conducting ILPT	Any Other Pathogen	Date of FTA card arrival	Sequencing	Internal Control
		WSSV	TSV	YHV1	WSSV	TSV	YHV1	No/Nil	Yes/No	Name			
1	ISO/IEC 17025	qPCR/PCR/Seq	-	-	qPCR/PCR	RT-qPCR/RT-PCR/Seq	RT-qPCR/RT-PCR/Seq	7	Yes	Nil	25.03.26	Yes	EF1-a
2	No Answer	No Answer	No Answer	No Answer	qPCR	RT-qPCR	RT-PCR	No	No	Nil	No Answer	No	Nil
3	ISO/IEC 17025: 2017	No	No	No	qPCR	-	-	5	Yes (Fish)	Nil	25.03.26	No	rRNA
4	ISO/IEC 17025: 2017	qPCR/PCR/Seq	RT-PCR/Seq	RT-PCR/Seq	qPCR	RT-PCR/Seq	RT-PCR/Seq	No	No	Crayfish Plague	18.03.26	Yes	Nil
5	ISO 17025	Histo/PCR/Seq	Histo/RT-PCR/Seq	Histo/RT-PCR/Seq	qPCR/PCR	RT-qPCR/RT-PCR	RT-qPCR/RT-PCR	No	No	*Nil*	18.03.26	Yes	Nil
6	ISO EN 17025	qPCR	-	-	qPCR	RT-PCR	RT-PCR	2	No	Nil	27.03.26	No	rRNA
7	No data	No data	No data	NO data	qPCR	qRT-PCR	RT-PCR	No answer	No answer	Nil	02.04.26	No	Any Other
8	No	No	No	No	PCR	RT-PCR	RT-PCR	No	No	Nil	18.03.26	No	Nil
9	ISO/IEC 17025:2017 HR	qPCR	Nil	Nil	qPCR	qRT-PCR	RT-PCR	No	No answer	Nil	18.03.26	No	Nil
10	ISO 17025 & ISO 17043	qPCR	RT-PCR	qRT-PCR	PCR/qPCR	RT-PCR/qRT-PCR	RT-PCR/qPCR	No	Yes	Nil	18.03.26	No	EF1a
11	ISO EN 17025	Nil	Nil	Nil	qPCR	No	No	No	No	*Aphanomyces astaci* Culture	26.03.26	No	Any Other
12	ISO EN 17025	PCR/qPCR/seq	RT-PCR	RT-PCR	PCR/Seq/qPCR	RT-PCR/Seq	RT-PCR/Seq	Yes	Yes	Nil	19.03.26	Yes	Nil
13	ISO EN 17025	PCR/Seq/qPCR	RT-PCR/Seq	RT-PCR/Seq	PCR/qPCR	RT-PCR	RT-PCR	No	No	*Aphanomyces astaci* qPCR Probe	23.03.26	Yes	Nil
14	ISO EN 17025	Nil	Nil	Nil	qPCR	RT-PCR	RT-PCR	No	No	Nil	18.03.26	No	Nil
15	ISO EN 17025	PCR/Seq/qPCR	Nil	Nil	qPCR/Seq	No	No	No	No	Nil	19.03.26	Yes	rRNA
16	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	18.03.26	Yes	Nil
17	ISO 17025	PCR	qRT-PCR	RT-PCR	qPCR	qRT-PCR	RT-PCR	No	No	Nil	18.03.26	No	Nil
18	ISO 17025	Nil	Nil	Nil	qPCR	qRT-PCR	qRT-PCR	No	No	Nil	19.03.26	No	Beta Actin
19	ISO EN 17025:2018-02	PCR/qPCR	RT-PCR/qRT-PCR	RT-PCR	PCR/qPCR	RT-PCR/qRT-PCR	RT-PCR	No	No	Nil	19.03.26	No	Any Other
20	No	Nil	Nil	Nil	PCR	qRT-PCR	RT-PCR/qRT-	No	No	Nil	18.03.26	No	Anyother

							PCR						
21	ISO EN 17025	Nil	Nil	Nil	qPCR	RT-PCR	RT-PCR	Yes, no data	Yes	Nil	18.03.26	No	Nil
22	ISO EN 17025	Nil	Nil	Nil	qPCR	qRT-PCR	RT-PCR	No	No	Nil	18.03.26	No	Any Other
23	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	18.03.26	Yes	Any Other
24	ISO EN 17025	PCR/Seq	Nil	Seq	PCR/Seq	qRT-PCR	RT-PCR/Seq	No	No	Nil	18.03.26	Yes	Nil
25	ISO EN 17025:2005	qPCR	Nil	Nil	qPCR	qRT-PCR	RT-PCR	No	No	Nil	18.03.26	Yes	Any Other
26	No	Nil	Nil	Nil	qPCR	qRT-PCR	qRT-PCR	No	No	Nil	31.03.26	No	Nil

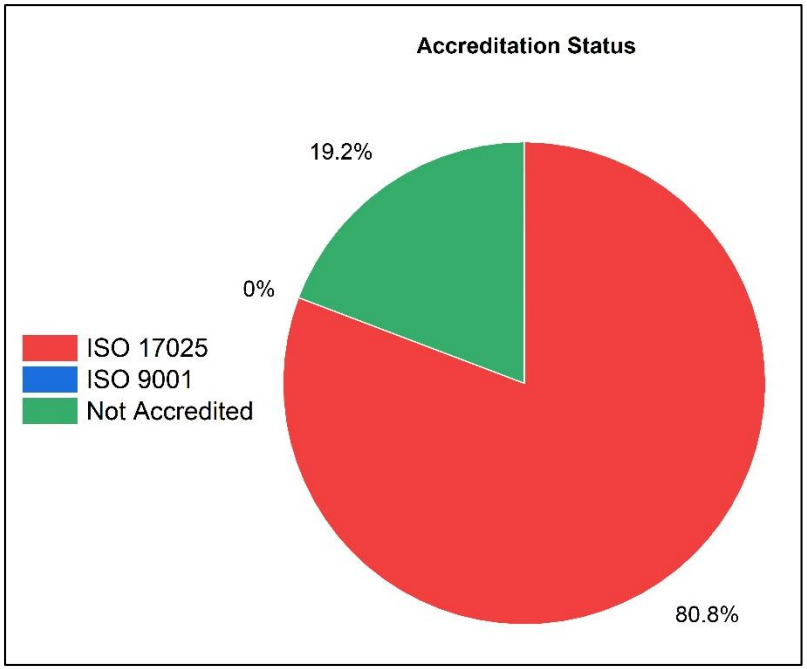

Fig. 2. Accreditation of the participating laboratories in ILPT26

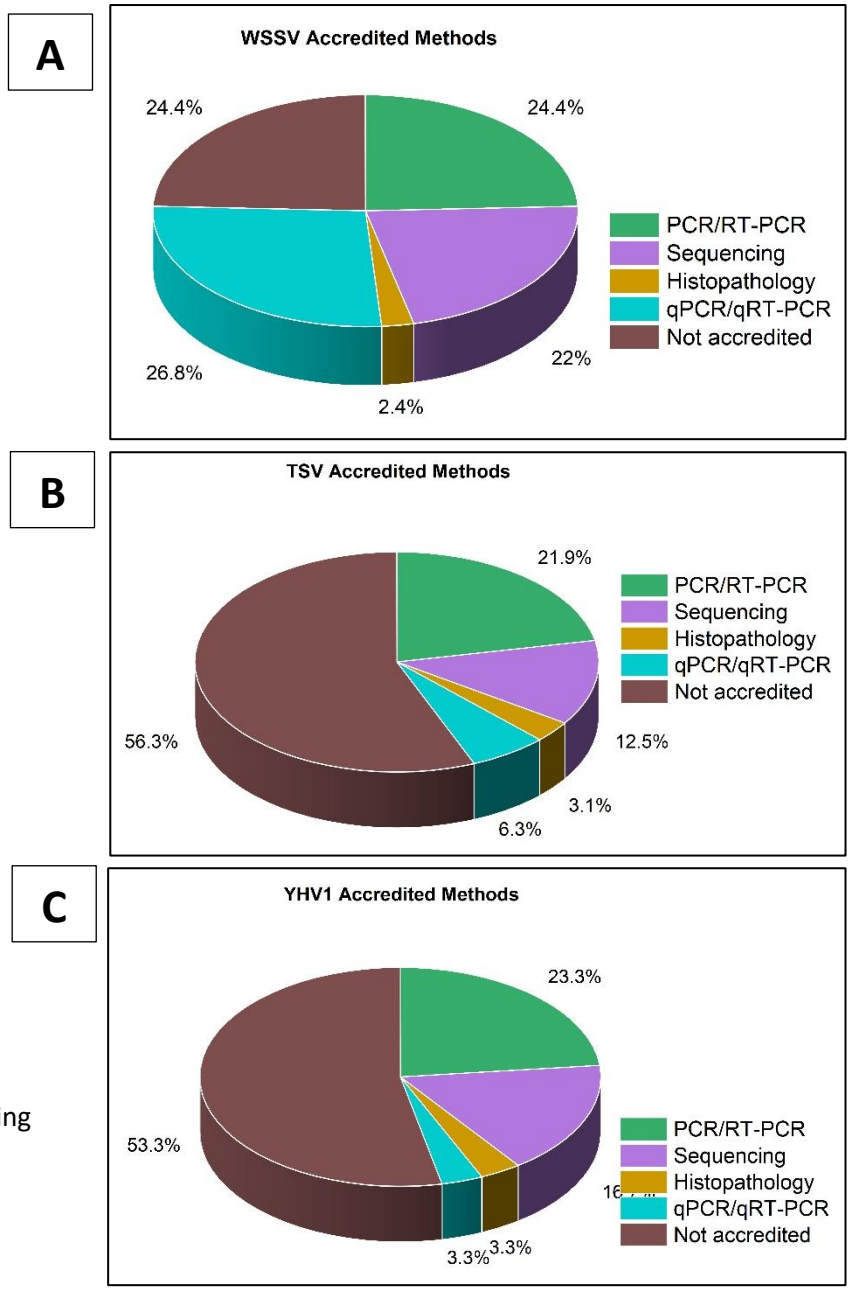

Fig. 3. Accredited methods for the listed viruses by the participating laboratories in ILPT26 – (**A**) WSSV; (**B**) TSV & (**C**) YHV1

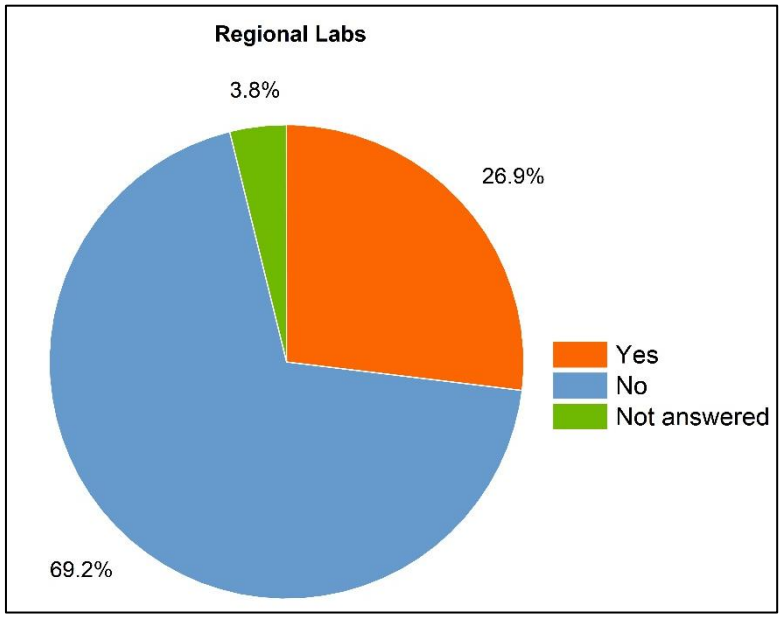

Fig. 4. Number of regional labs under participating laboratories in ILPT26

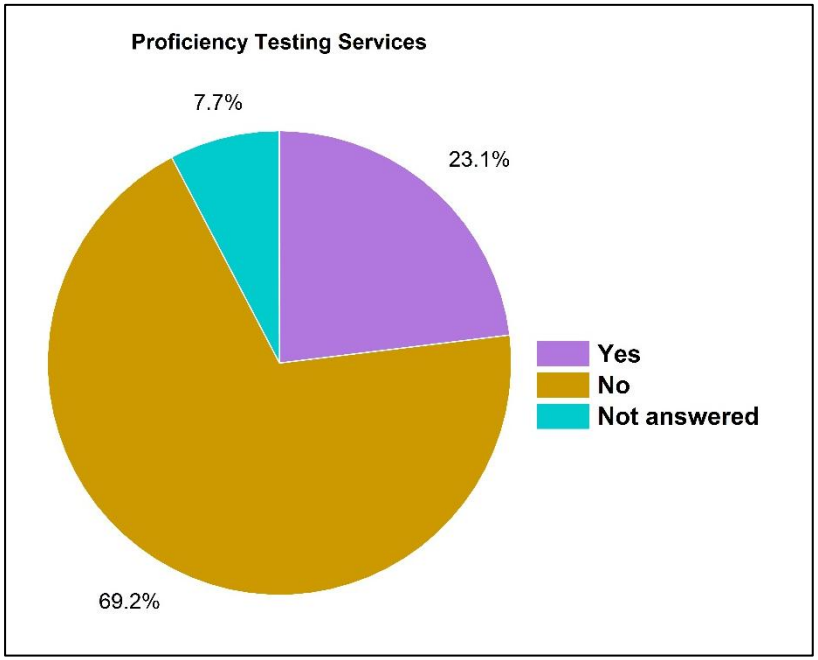

Fig. 5. Provision of Proficiency services to the regional labs under participating laboratories in ILPT26

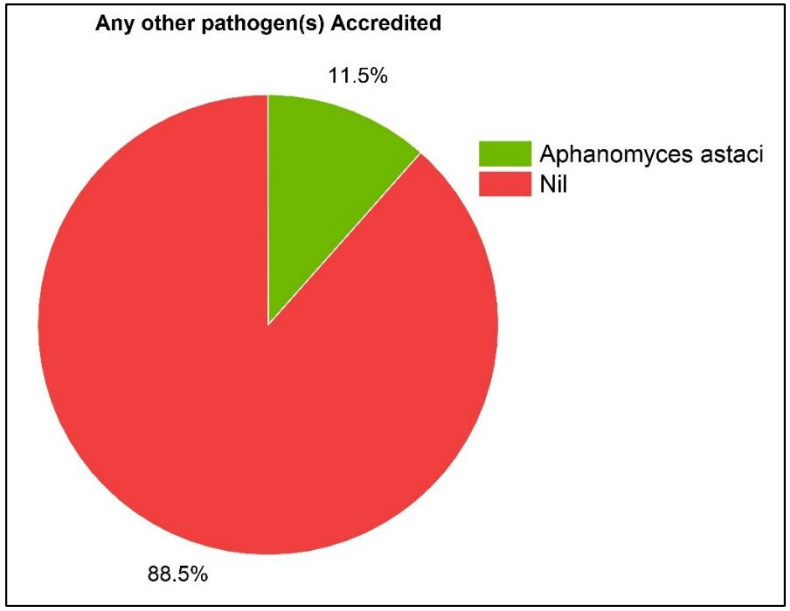

Fig. 6. Details of the other accredited pathogen for analysis in participated labs

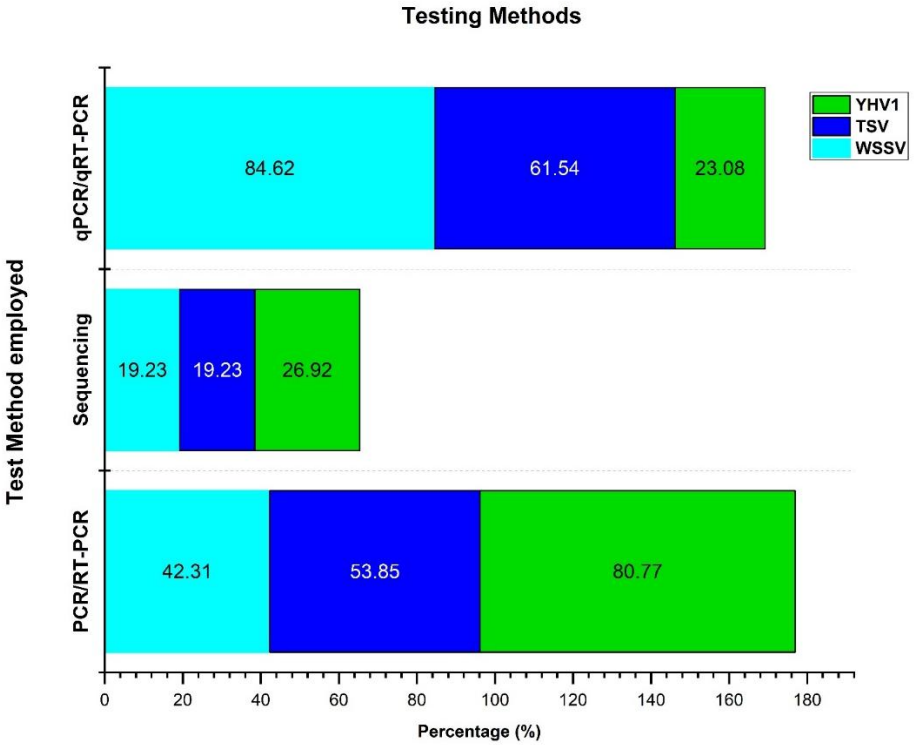

Fig. 7. Use of testing methods by the participated labs for the analysis of FTA card under ILPT26

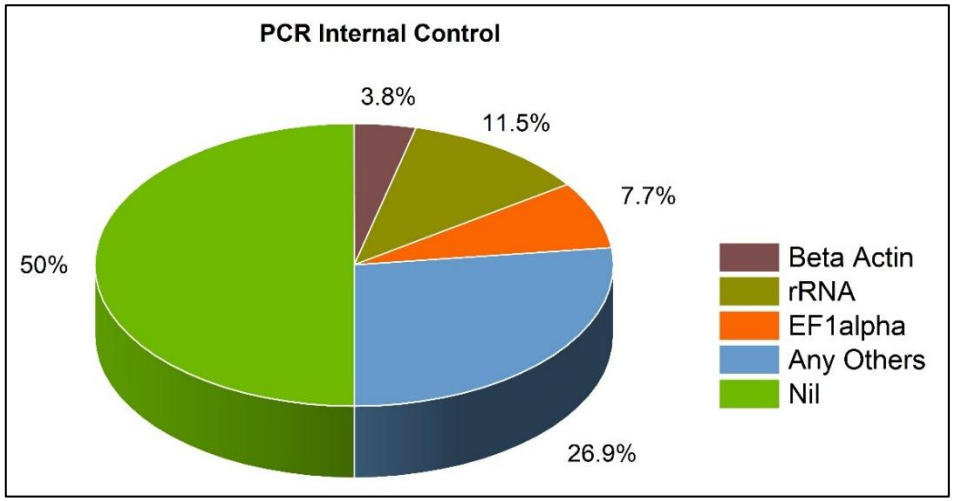

Fig. 8. Use of internal controls for the PCR/qPCR by the participated labs under ILPT26

Table 4 – Ct values overview of the ILPT for crustacean diseases 2026

C-ILPT26 CORRECT DETCETION →	Sample 1		Sample 2		Sample 4		Sample 6		Sample 7		Sample 8		Sample 9		SCORE	
	YHV1		TSV		WSSV		TSV		WSSV		YHV1		YHV1			
	Low		Low		High		High		Low		Medium		High			
Laboratory code	Virus identification	Ct value	Virus identification	Ct value	Virus identification	Ct Value	Virus identification	Ct value	Virus identification	Ct value	Virus identification	Ct value	Virus identification	Ct value	Out of 9	%
1	YHV1	26,79	TSV	29,98	WSSV	29,99	TSV	30,26	WSSV	32,77	YHV1	24,2	YHV1	21,86	9/9	100
2	YHV1	0	TSV	29,42	WSSV	27,65	TSV	29,41	WSSV	30,68	YHV1	0	YHV1	0	9/9	100
3	0	0	0	0	WSSV	27,7	0	0	WSSV	30,4	0	0	0	0	4/4	100
4	YHV1	0	TSV	0	WSSV	29,93	TSV	0	WSSV	32,19	YHV1	0	YHV1	0	9/9	100
5	YHV1	30,214	TSV	31,96	WSSV	29,971	TSV	30,272	WSSV	31,834	YHV1	26,264	YHV1	23,244	9/9	100
6	YHV1	0	TSV	36,91	WSSV	32,75	TSV	36,24	WSSV	35,07	YHV1	0	YHV1	0	9/9	100
7	YHV1	0	TSV	31,39	WSSV	31,53	TSV	31,57	WSSV	34,2	YHV1	0	YHV1	0	9/9	100
8	YHV1	0	TSV	0	WSSV	0	TSV	0	WSSV	0	YHV1	0	YHV1	0	9/9	100
9	YHV1	0	TSV	30,8	WSSV	28	TSV	30,05	WSSV	30,56	YHV1	0	YHV1	0	9/9	100
10	YHV1	28,19	TSV	31,78	WSSV	30,14	TSV	30,57	WSSV	32,47	YHV1	25,26	YHV1	22,74	9/9	100
11	0	0	0	0	WSSV	30,03	0	0	WSSV	32,65	0	0	0	0	4/4	100
12	YHV1	0	TSV	0	WSSV	30.29	TSV	0	WSSV	32.7	YHV1	0	YHV1	0	9/9	100
13	YHV1	0	TSV	0	WSSV	33,19	TSV	0	WSSV	35,79	YHV1	0	YHV1	0	9/9	100
14	YHV1	0	TSV	0	WSSV	30,74	TSV	0	WSSV	34,13	YHV1	0	YHV1	0	9/9	100
15	0	0	0	0	WSSV	33,6	0	0	WSSV	35,86	0	0	0	0	4/4	100
16	YHV1	0	TSV	0	WSSV	0	TSV	0	WSSV	0	YHV1	0	YHV1	0	9/9	100
17	YHV1	35,24	TSV	30,19	WSSV	29,79	TSV	30,27	WSSV	32,64	YHV1	31,75	YHV1	27,71	9/9	100
18	YHV1	24,35	TSV	28,52	WSSV	29,31	TSV	27,69	WSSV	31,95	YHV1	21,84	YHV1	18,71	9/9	100
19	YHV1	0	TSV	34,3	WSSV	33,5	TSV	33,64	WSSV	36,44	YHV1	0	YHV1	0	9/9	100

20	YHV1	26,73	TSV	28,47	WSSV	0	TSV	28,17	WSSV	0	YHV1	24,96	YHV1	20,69	**9/9**	**100**
21	YHV1	0	TSV	0	WSSV	0	TSV	0	0	0	YHV1	0	YHV1	0	**8/9**	**88,88**
22	YHV1	0	TSV	29,58	WSSV	31,45	TSV	28,38	WSSV	34,21	YHV1	0	YHV1	0	**9/9**	**100**
23	YHV1	27,39	TSV	28,151	WSSV	34,918	TSV	29,885	WSSV	35,92	YHV1	25,649	YHV1	22,946	**9/9**	**100**
24	YHV1	0	TSV	25,78	WSSV	27,7	TSV	24,74	WSSV	28,92	YHV1	0	YHV1	0	**9/9**	**100**
25	YHV1	0	TSV	28,38	WSSV	30,00	TSV	27,31	WSSV	32,15	YHV1	0	YHV1	0	**9/9**	**100**
26	YHV1	28,81	TSV	28,75	WSSV	32,97	TSV	26,91	WSSV	35,03	YHV1	27,38	YHV1	23,49	**9/9**	**100**

Table 5 – Mean Ct Values comparison before and after preparation of the FTA cards

Sample No	Sample description	Before the FTA card preparation		After the FTA card preparation	
		Replicate Ct	Mean Ct value	Replicate Ct	Mean Ct value
S01	YHV1 1:500	20,07	20,06	30,36	30,24
				30,36	
		20,05		29,99	
				29,90	
				30,57	
S02	TSV 1:400	22,92	22,67	34,47	34,94
				34,67	
		22,39		35,18	
				35,21	
				35,17	
S03	Negative SPF 1:20		-		-
S04	WSSV 1:100	24,08	23,99	30,53	31,13
				31,59	
		23,89		31,23	
				31,08	
				31,24	
S05	Negative Cell culture media	-	-	-	-
S06	TSV 1:200	23,01	22,95	34,57	34,37
				34,33	
				34,41	
		22,89		34,47	

				34,08	
S07	WSSV 1:500	25,97	26,06	33,19	33,92
				33,82	
				35,10	
		26,14		34,03	
				33,49	
S08	YHV1 1:100	18,02	17,98	27,70	27,59
				27,59	
				26,86	
		17,93		27,69	
				28,14	
S09	YHV1 1:10	14,28	14,35	23,08	23,92
				23,96	
				24,77	
		14,41		23,64	
				24,18	

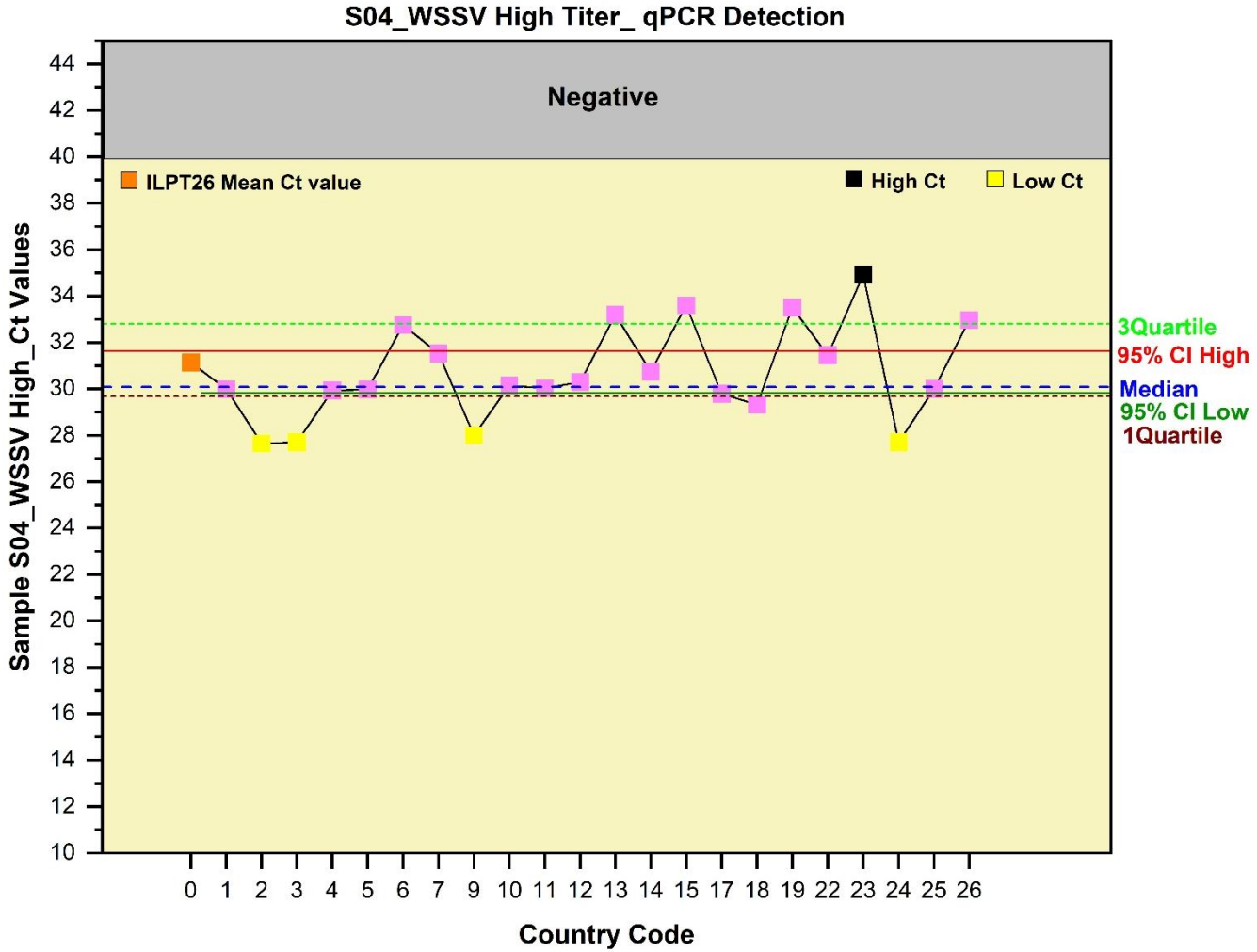

Fig. 9. Sample S04_WSSV High Titer – Result qPCR Ct value distribution
Median – 30,085; 95% CI High – 31,63; 95% CI Low – 29,83; 25% Quartile – 29,67; 75% Quartile – 32,81
Mean Ct value of the sample tested at EURL indicated in ■

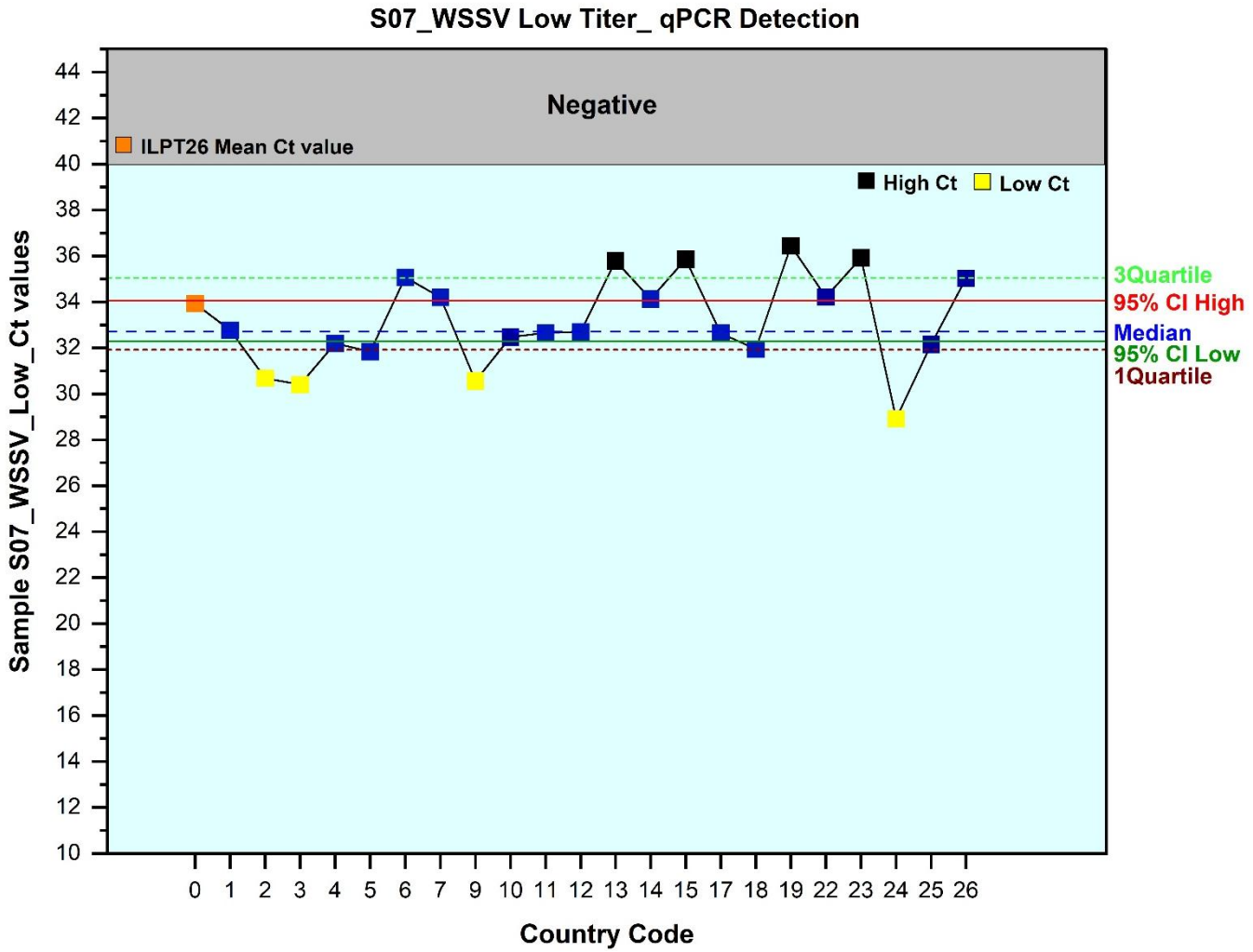

Fig. 10. Sample S07_WSSV Low Titer – Result qPCR Ct value distribution
Median – 32,710; 95% CI High – 34,06; 95% CI Low – 32,28; 25% Quartile – 31,92; 75% Quartile – 35,04
Mean Ct value of the sample tested at EURL indicated in ■

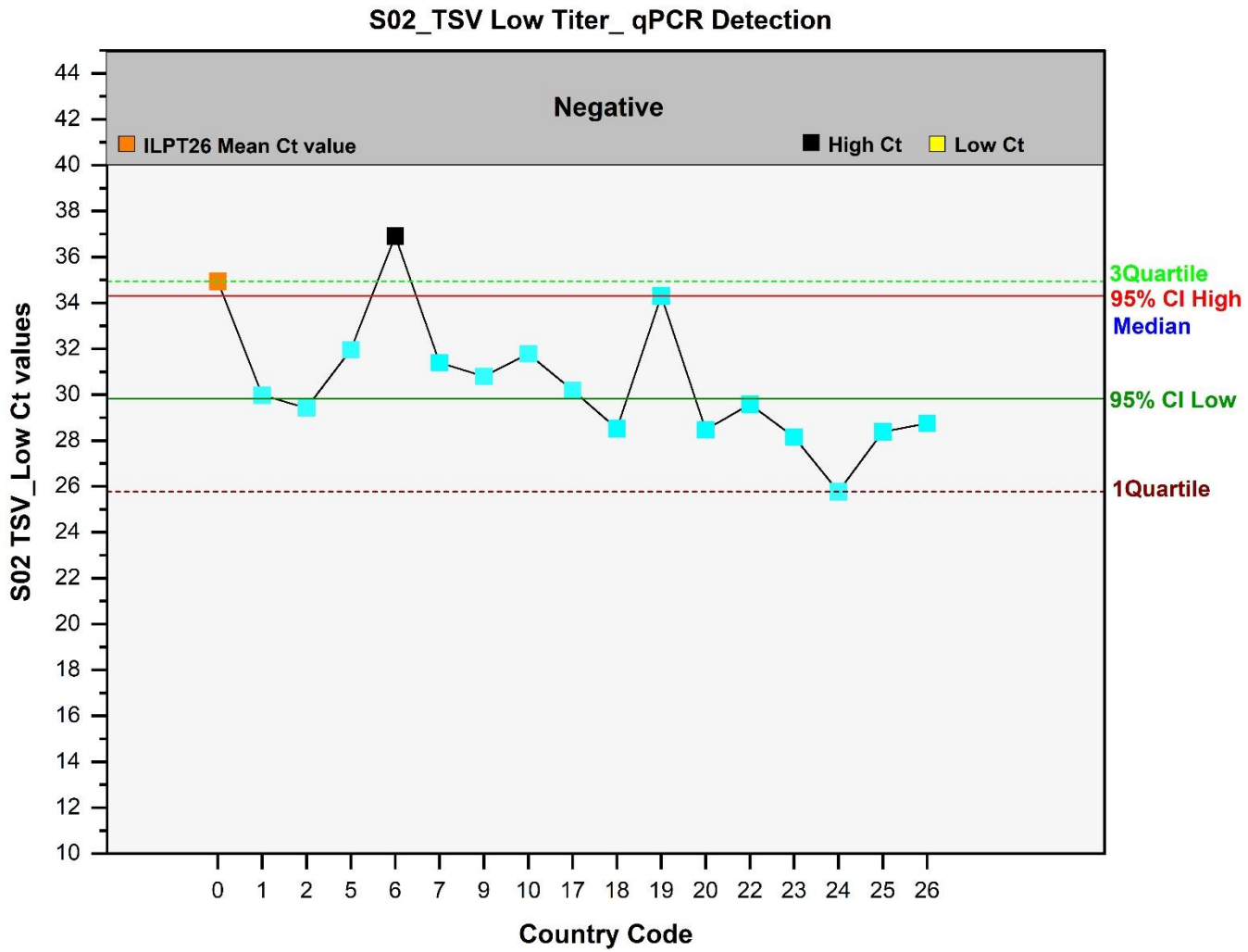

Fig. 11. Sample S02_TSV Low Titer – Result qPCR Ct value distribution
Median – 34,30; 95% CI High – 34,30; 95% CI Low – 29,04; 25% Quartile – 25,78; 75% Quartile – 34,94
Mean Ct value of the sample tested at EURL indicated in ■

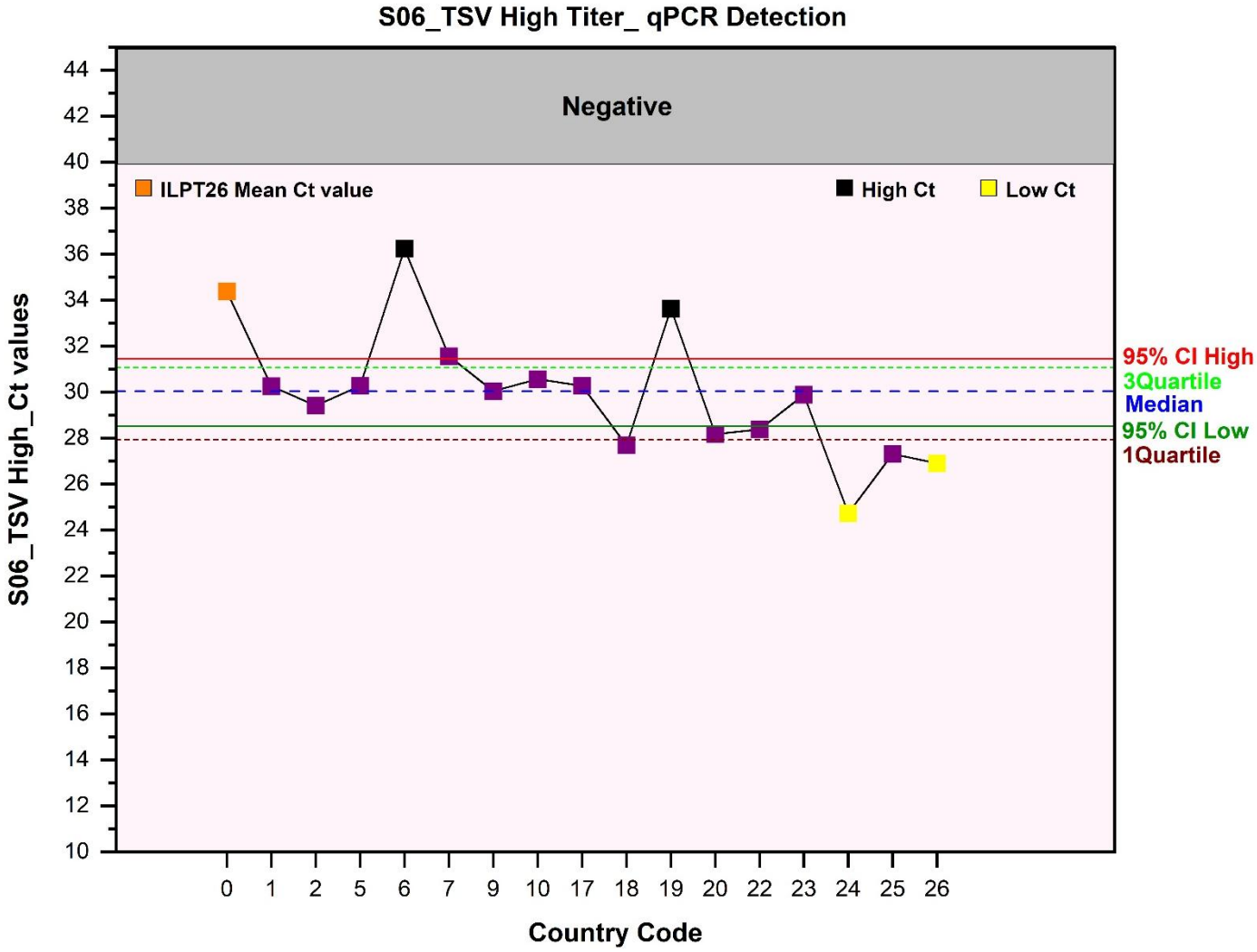

Fig. 12. Sample S06_TSV High Titer – Result qPCR Ct value distribution
Median – 30,050; 95% CI High – 31,45; 95% CI Low – 28,52; 25% Quartile – 27,93; 75% Quartile – 31,07
Mean Ct value of the sample tested at EURL indicated in ■

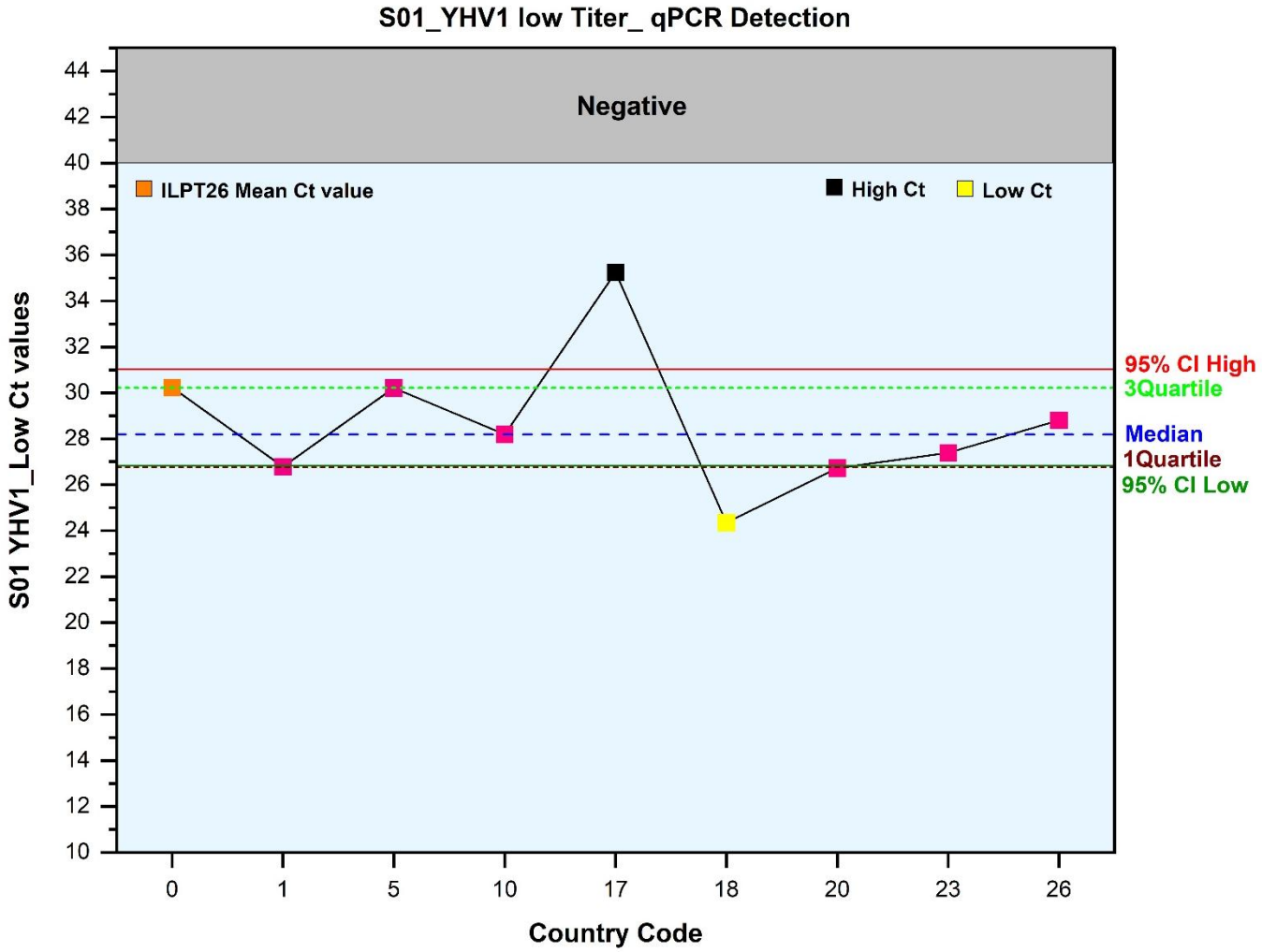

Fig. 13. Sample S01_YHV1 Low Titer – Result qPCR Ct value distribution
Median – 28,19; 95% CI High – 31,03; 95% CI Low – 26,29; 25% Quartile – 26,76; 75% Quartile – 30,23
Mean Ct value of the sample tested at EURL indicated in ■

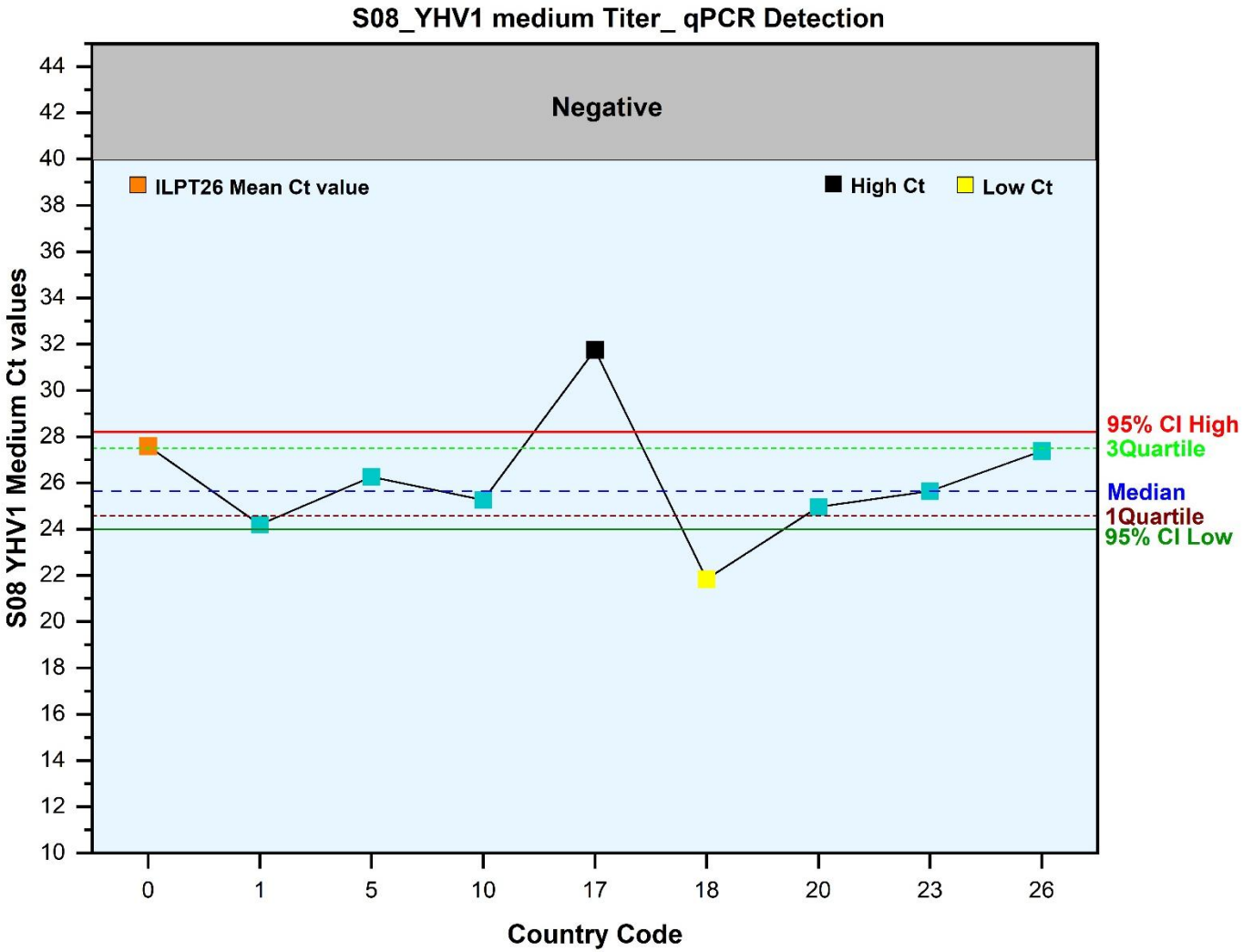

Fig. 14. Sample S08_YHV1 Medium Titer – Result qPCR Ct value distribution
Median – 25,649; 95% CI High – 28,20; 95% CI Low – 24,00; 25% Quartile – 24,58; 75% Quartile – 27,49
Mean Ct value of the sample tested at EURL indicated in ■

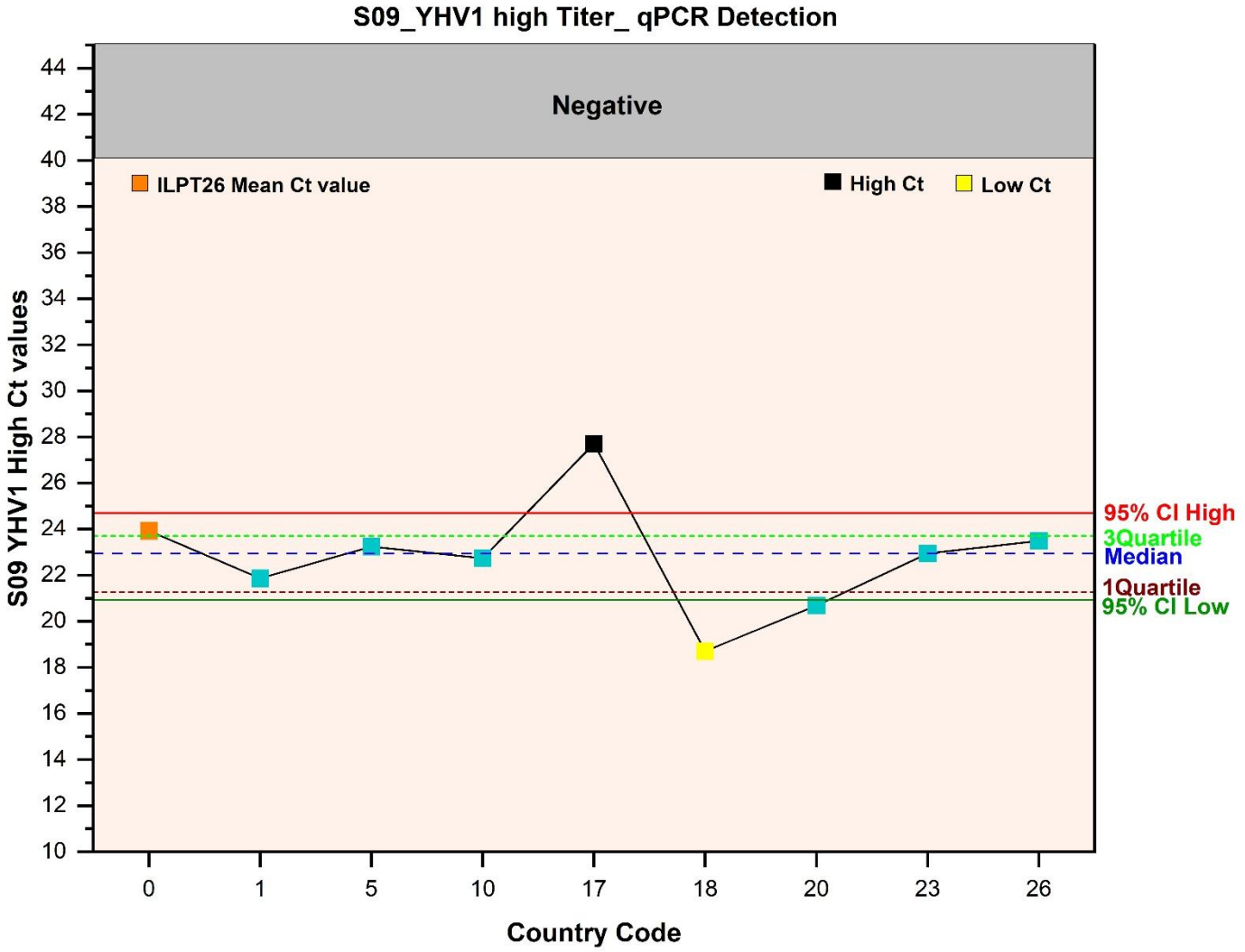

Fig. 15. Sample S09_YHV1 High Titer – Result qPCR Ct value distribution
Median – 22,946; 95% CI High – 24,70; 95% CI Low – 20,93; 25% Quartile – 23,71; 75% Quartile – 21,28
Mean Ct value of the sample tested at EURL indicated in ■

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.

Shyam K Uthaman, Niccolò Vendramin, Argelia Cuenca, Charlotte Bjørner Larsen and Thomas Weise
European Union Reference Laboratory for Fish and Crustacean Diseases
Technical University of Denmark, June 2026

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