

DTU



Inter-laboratory proficiency tests for crustacean diseases 2023

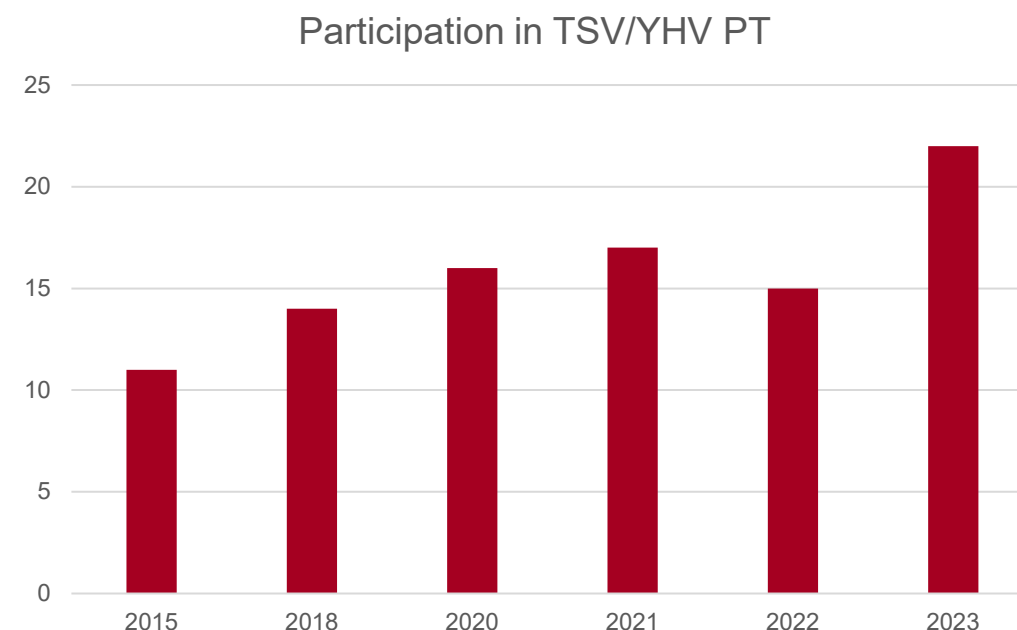
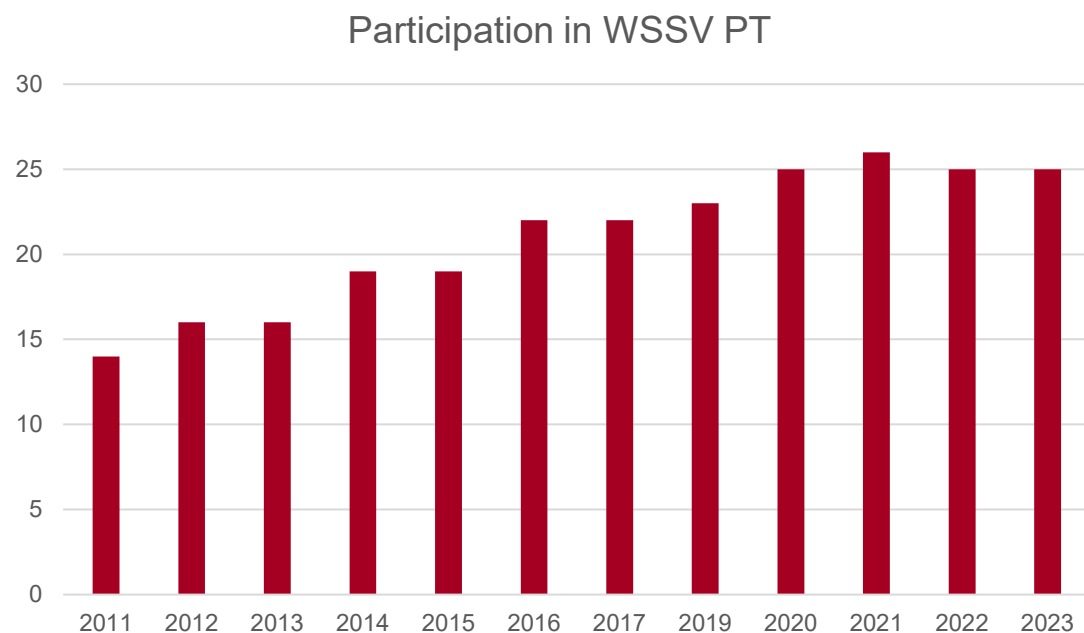
Change of format

- Due to low participation rate in the list A diseases, it was emphasized to the NRLs that participation in the ILPT is a mandatory task given by the legislation.
- Derogations are possible.
- Format change to one panel including pathogens of all listed diseases (WSSV/TSV/YHV1) rather than having two panels (WSSV + TSV/YHV1).
- All samples applied on FTA cards
- High concentration and low concentration samples included for all pathogens.

Participating countries

- 25 laboratories including 18 EU NRLs

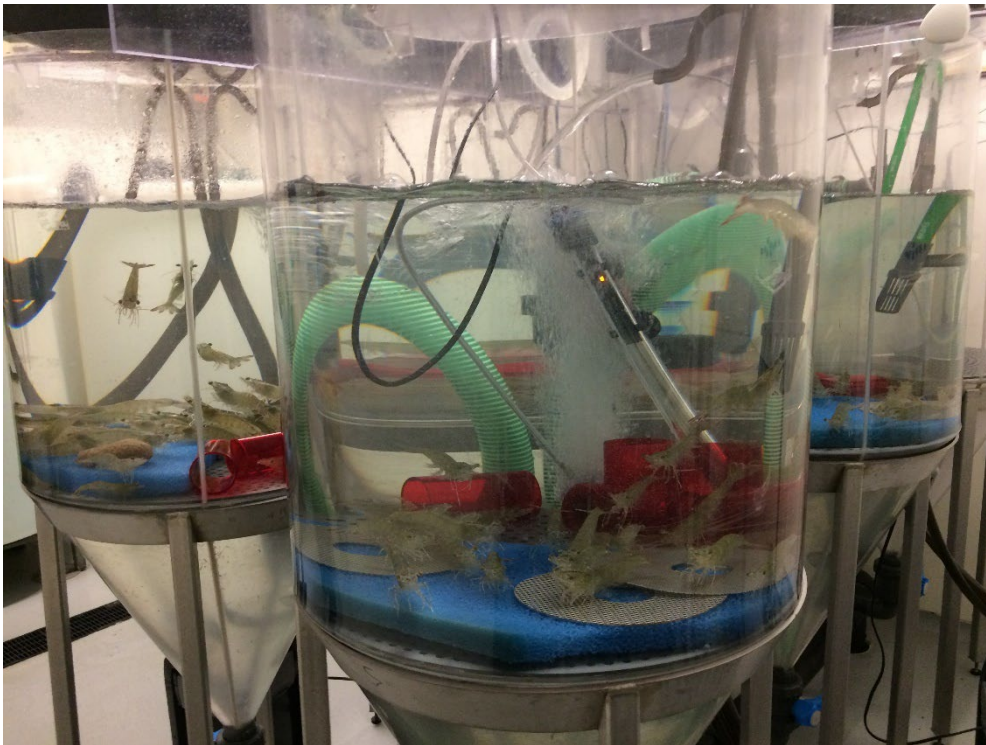
Participation in crustacean proficiency tests



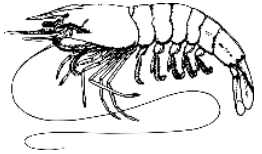
Three countries have derogated the TSV/YHV1 diagnostic task

Materials

- Protocols and infected shrimp tissue kindly provided by the former EURL (CEFAS)
- Shrimp (*P. vannamei*) kindly provided by, Förde Garnelen in Kiel, Germany



Inoculation procedure



Infected shrimp



Grind in PBS with sand



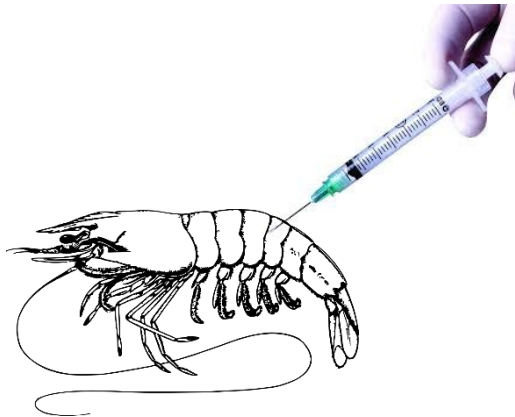
Centrifuges 3000g 30 min



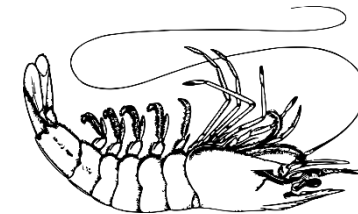
Supernatant stored at -20°C



Diluted and sterile filtered

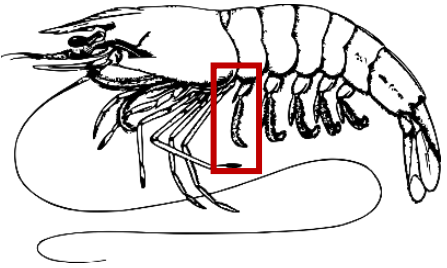


Live animals injected
with ca. 100 µl extract



Dead and moribund animals
Collected and pleopods removed

Testing methods



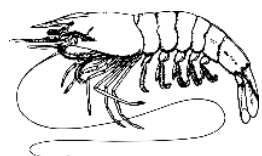
First pair of pleopods
tested by EURL



DNA/RNA extracted on
Indimag 48s

- WSSV tested with qPCR (Durand & Lightner 2002)
- TSV tested with qPCR (Tang et al. 2004)
- YHV tested with conv. PCR (Mohr et al. 2015) + qPCR (ACDP unpublished method)

Materials for WSSV, TSV and YHV1



Infected shrimp



Grind in PBS with sand



Centrifuges 3000g 30 min



Supernatant stored at -20°C

FTA cards

- Supernatant applied to FTA cards



-FTA Cards contain chemicals that:

- lyse cells
- denature proteins
- protect nucleic acids from nucleases, oxidation and UV damage.
- inactivate organisms, including blood-borne pathogens, and prevent the growth of bacteria and other microorganisms.

-Cards can be shipped at ambient temperature.

-For DNA, cards can be stored at room temperature, for RNA they should preferably be stored frozen.

Materials for WSSV, TSV and YHV1



DNA/RNA extracted
on Indimag 48s

- WSSV tested with qPCR (Durand & Lightner 2002)
- TSV tested with qPCR (Tang et al. 2004)
- YHV tested with conv. PCR (Mohr et al. 2015) + qPCR (ACDP unpublished method)

Expected results

Sample ID	Virus
Sample 01	TSV, low concentration
Sample 02	Negative
Sample 03	Negative
Sample 04	WSSV, high concentration
Sample 05	YHV1, low concentration
Sample 06	YHV1, high concentration
Sample 07	WSSV, low concentration
Sample 08	TSV, high concentration

Results 2023 – TSV/YHV

Results were received from all 25 participating laboratories.

- 20 laboratories correctly diagnosed all samples, 8/8 (100 %)
- 5 laboratories correctly diagnosed 7/8 samples (87.5 %)

All incorrect answers are false negatives in low concentration samples

Laboratory	Method YHV	Method WSSV	S-01	S-02	S-03	S-04	S-05	S-06	S-07	S-08	Score
EURL	qPCR/RT-PCR	qPCR	TSV (28.7)	-ve	-ve	WSSV (20.0)	YHV1 (low)	YHV1 (high)	WSSV (28.9)	TSV (23.0)	
A	RT-PCR	Nested PCR	TSV	-ve	-ve	WSSV	YHV1	YHV1	-ve	TSV	7/8
B	RT-PCR	qPCR	TSV (28.86)	-ve	-ve	WSSV (21.15)	-ve	YHV1	WSSV (34.5)	TSV (22.81)	7/8
C	RT-qPCR	qPCR	TSV (29.3)	-ve	-ve	WSSV (21, 20.4)	-ve	YHV1 (32.9)	WSSV (32.9, 32.4)	TSV (24.1)	7/8
D	RT-PCR	qPCR	TSV (37.5)	-ve	-ve	WSSV (23)	-ve	YHV1	WSSV (31.5)	TSV (29)	7/8
E	RT-PCR	qPCR	TSV	-ve	-ve	WSSV (22.32)	YHV1	YHV1	-ve	TSV	7/8

Methods

The following methods were used to diagnose WSSV:

- 14 laboratories used qPCR
- 5 laboratories used nested PCR
- 6 laboratories used both qPCR and PCR

The following methods were used to diagnose TSV:

- 12 laboratories used qPCR
- 9 laboratories used single PCR
- 1 laboratory used both qPCR and PCR

The following methods were used to diagnose YHV:

- 2 laboratories used qPCR
- 5 laboratories used nested PCR
- 14 laboratories used single PCR
- 1 laboratory used both qPCR and PCR

3 laboratories verified the identity of at least one of the obtained PCR products by sequencing.

Methods

Laboratory Code	DNA/RNA Extraction Method	PCR Kit
EURL	IndiMag Pathogen Kit with INDIMAG robot	TaqPath™ 1-Step RT-qPCR Master Mix; Qiagen OneStep RT-PCR kit
1	MagNA Pure 24 Total NA isolation Kit	Qiagen HotStarTaq DNA Polymerase
2	QIAamp cador pathogen kit	Bioline MyTaq mix; Qiagen One step RT PCR kit
3	IndiMag Pathogen kit with BioSprint 96 Workstation	Go Taq Hot Start Green; MultiScribe Reverse Transcriptase; AgPath-ID One-Step RT-PCR kit
5	QIAamp PowerFecal Pro DNA Kit using QIAcube platform	iTaq Universal Probes mastermix
6	IndiMag Pathogen Kit	PerfeCTa qPCR Toughmix; KiCqStart One-Step RT-PCR Readymix
7	Qiagen DNA Mini Kit; Qiagen RNeasy Mini Kit	Super-Script One-Step RT-PCR with Platinum Tag; QuantiNova Pathogen + IC Kit
8	NucleoMag VET Kit with KingFisher Flex magnetic particle processor	QuantiTect Probe PCR Kit; QIAGEN OneStep RT-PCR Kit
9	DNeasy Blood & Tissue Kit; QIAamp Viral RNA Mini Kit	One-Step Probe PCR Mix, One-Step RT PCR Mix, 5xHOT FIREPol Mix
11	MagMax CORE Kit with KingFisher Flex magnetic particle processor	One Step RT-PCR Kit; Platinum PCR SuperMix Kit; QuantiNova Pathogen + IC Kit
12	Qiagen DNA Tissue–Biorobot Advanced XL Qiagen RNA Tissue–Biorobot Advanced XL	Promega M-MLV Reverse transcriptase; Promega Go Taq G2 Flexi DNA Polymerase
13	Biomerieux NucliSENS® easyMAG®	Taqman® reverse transcription reagent kit; SYBR.GR Master mix

14	MagMax CORE Kit with KingFisher Flex magnetic particle processor	SuperScript™ III One-Step RT-PCR System with Platinum™ Tag DNA Polymerase; Luminaris Probe qPCR Master Mix low ROX; Tag PCR Master Mix Kit
15	QIAamp DNA Mini Kit with Qiacube robot	TaqMan® Universal PCR Master Mix
16	GoTaq® Probe qPCR Master Mix; Qiagen One Step RT-PCR; Amplifyme Probe One Step Universal RTqPCR Mix	innuPREP AniPath DNA/RNA Kit
18	IndiMag Pathogen Kit with INDIMAG robot	QuantiTect Probe PCR Kit; QIAGEN OneStep RT-PCR Kit
19	RNeasy Mini Kit	Platinum Tag DNA Polymerase; Qiagen OneStep RT-PCR Enzyme Mix
20	QIAamp DNA Mini Kit; QIAamp RNA Mini Kit	QIAGEN OneStep RT-PCR Kit; Qiagen QuantiTect Probe RT-PCR one-step kit; DNA QPCR – Perfecta qPCR ToughMix UNG ROX
21	DNeasy kit for Blood and Tissue using QIAcube platform; RNeasy Plus Kit for Tissue using QIAcube platform	QuantiTect Probe RT-PCR OneStep kit; Qiagen OneStep RT-PCR Kit; Perfecta qPCR ToughMix UNG ROX
22	DNeasy Blood&Tissue Kit; RNeasy Mini Kit	Qiagen Multiplex PCR Kit; Qiagen quantitect probe rt-pcr kit; Qiagen one-step RT PCR Kit
23	IndiMag Pathogen Kit with INDIMAG robot	TaqPath 1-step RT-qPCR Mastermix; Qiagen OneStep RT-PCR kit; Luna® Universal Probe qPCR Mastermix
24	Idexx Real PCR DNA/RNA spin Column Kit; Jena Bioscience Total RNA Purification Kit	AmpliTag Gold DNA Polymerase kit; SuperScript III One-Step RT-PCR System with Platinum Tag DNA Polymerase

Conclusions

- Low concentration virus samples allows for better evaluation of test sensitivity.
- Test results comparable to last year. WSSV errors false negatives rather than false positives.
- No clear effect of PCR methods on performance.
- Almost no labs use the same methods (so difficult to draw any conclusions).

Next Inter-laboratory proficiency test

- Will most likely be send out in April 2024
- Will most likely concern WSSV, TSV and YHV
- Will most likely again be based on FTA cards instead of pleopods in order to have better control of virus load

Questions?