

DTU



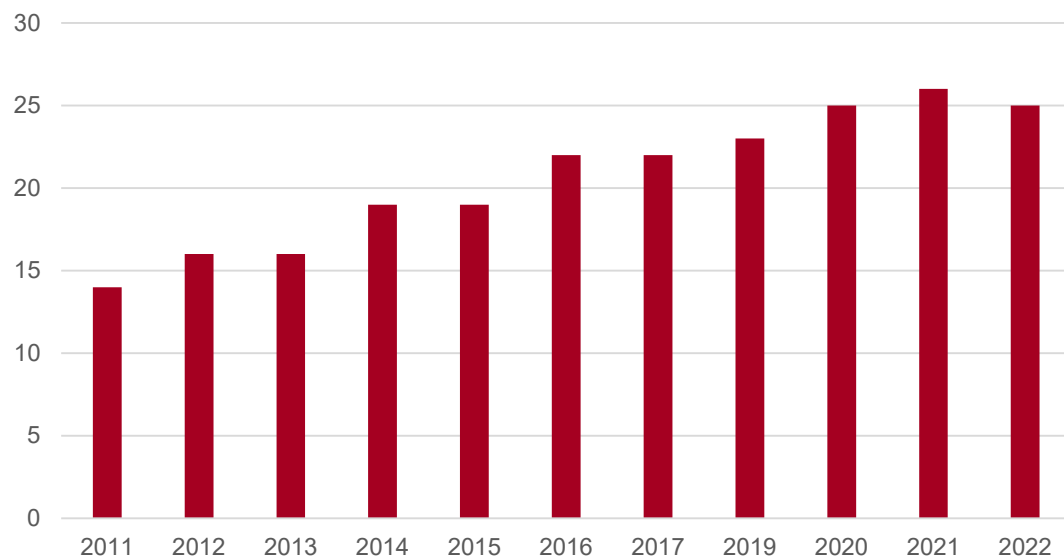
# Inter-laboratory proficiency tests for crustacean diseases 2022

# Participating countries

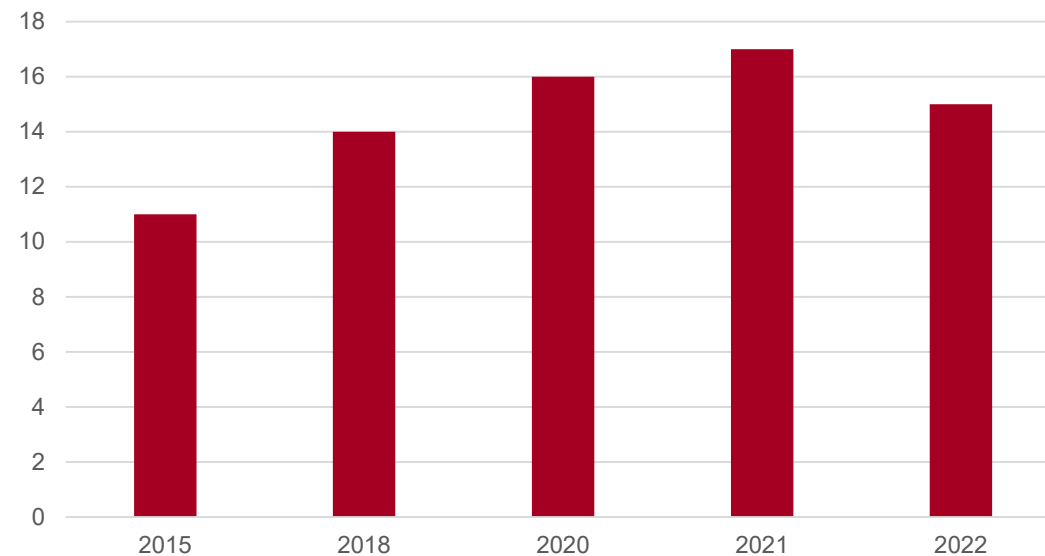
- WSSV test: 25 laboratories including 18 EU NRLs
- TSV + YHV: 15 laboratories including 11 EU NRLs

# Participation in crustacean proficiency tests

Participation in WSSV PT

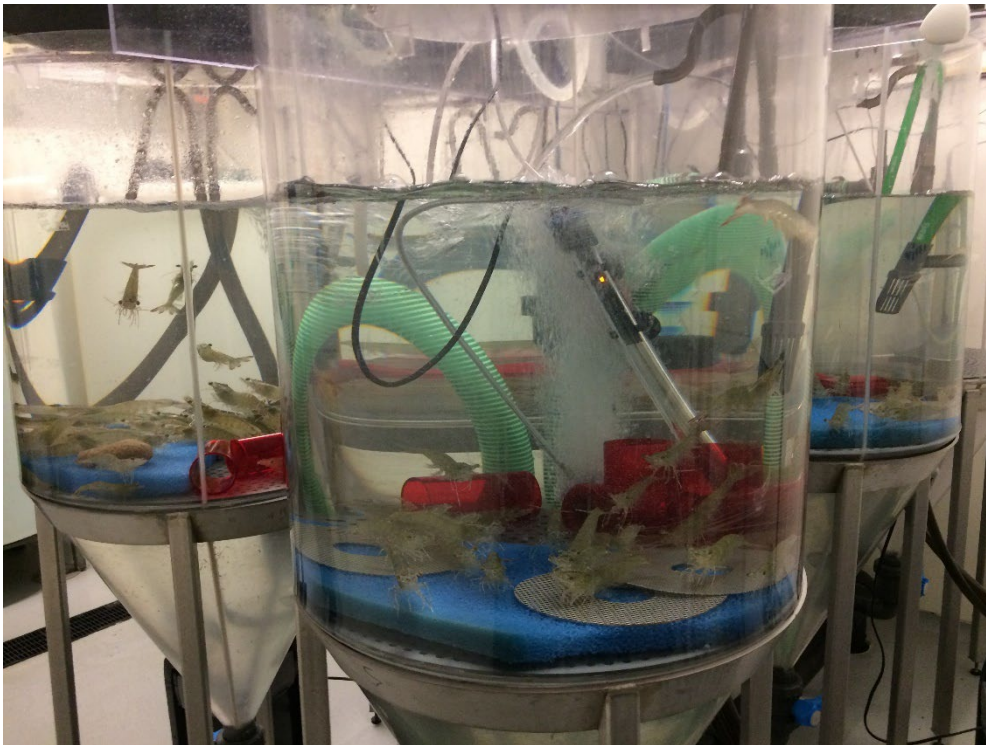


Participation in TSV/YHV PT

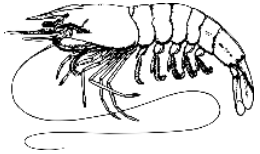


# Materials

- 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



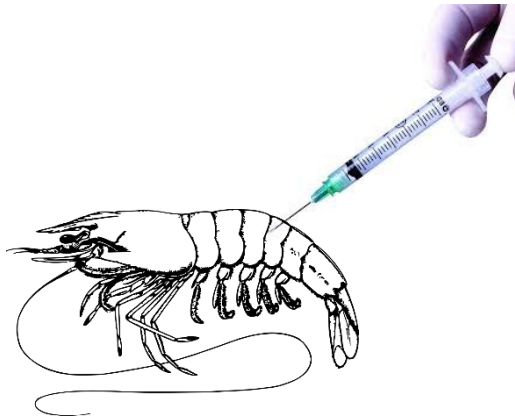
Centrifuges 3000g 30 min



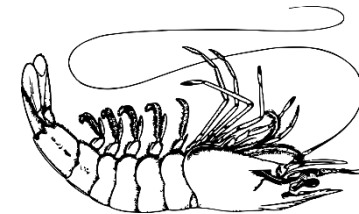
Supernatant stored at -20°C



Diluted and sterile filtered

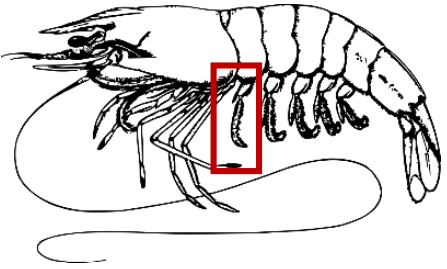


Live animals injected  
with ca. 100 µl extract



Dead and moribund animals  
Collected and pleopods removed

# Testing methods

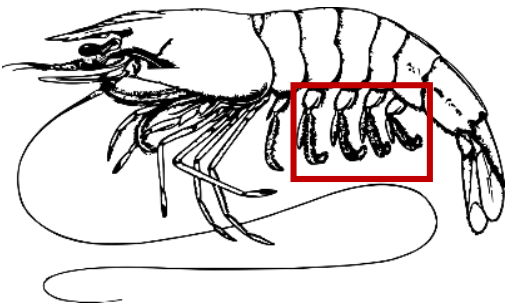


First pair of pleopods  
tested by EURL



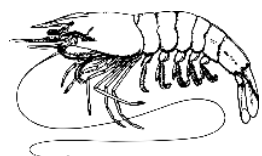
DNA/RNA extracted on  
Indimag 48s

- WSSV tested with qPCR (Durand & Lightner 2002)



Second to fifth pair of pleopods used in test

# Materials for 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.

# Content of PT-2021 TSV/YHV1

6 x

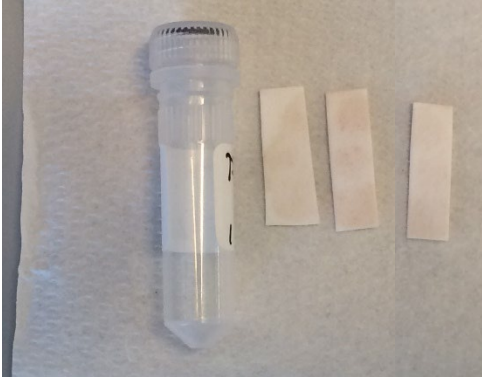


Strips of FTA cards containing shrimp homogenate (spf, TSV or YHV1)

All three strips are similar

# Content of PT-2021 TSV/YHV1

6 x

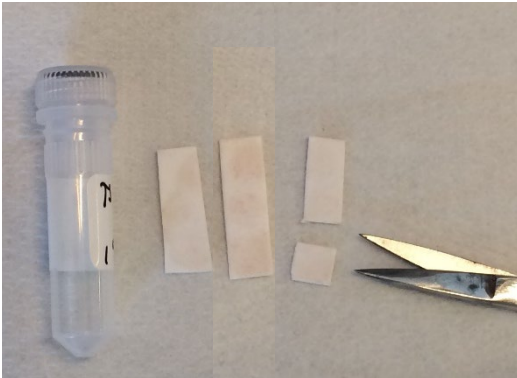


Strips of FTA cards containing shrimp homogenate (spf, TSV or YHV1)

All three strips are similar

Cut piece of one strip from each tube

6 x



# Content of PT-2021 TSV/YHV1

6 x

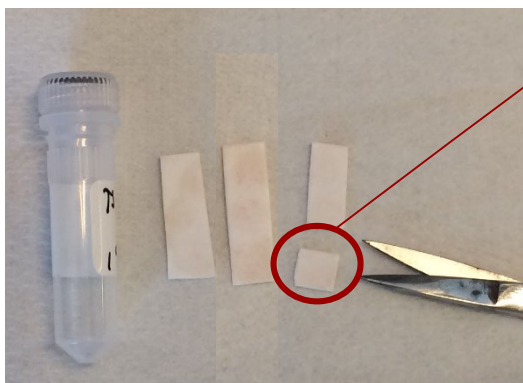


Strips of FTA cards containing shrimp homogenate (spf, TSV or YHV1)

All three strips are similar

Cut piece of one strip from each tube

6 x



## Expected results - WSSV

Sample ID	Sample type	WSSV infection status
Sample XX-001	P. vannamei pleopods in EtOH	Negative
Sample XX-002	P. vannamei pleopods in EtOH	Negative
Sample XX-003	P. vannamei pleopods in EtOH	Positive (UAZ 00-173B)
Sample XX-004	P. vannamei pleopods in EtOH	Negative
Sample XX-005	P. vannamei pleopods in EtOH	Positive (UAZ 00-173B)

# Results 2022 - WSSV

Results were received from all 25 participating laboratories.

- 23 laboratories correctly diagnosed all samples, 5/5 (100%)
- 2 laboratory correctly diagnosed 4/5 samples (80%)

False positives

Most likely due to cross contamination

Lab 14: Nested PCR

Lab 24: qPCR (CT =31.1)

	Shrimp ID	Pleopod ID				
		A	B	C	D	E
Non-inoculated Shrimp	19-5656-7	EURL	1	2	3	5
	20-5656-14	EURL	6	7	8	9
	20-5656-21	EURL	11	12	13	14
	20-854-9	EURL	15	16	18	19
	20-854-25	EURL	20	21	22	23
	20-854-59	EURL	24	25	26	27
	20-854-60	EURL	28	1	2	3
	20-854-61	EURL	5	5	7	8
	20-854-62	EURL	9	11	12	13
	20-854-63	EURL	14	15	16	18
	20-854-65	EURL	19	20	21	22
	20-854-67	EURL	23	24	25	26
	20-854-68	EURL	27	28	1	2
	20-854-69	EURL	3	5	6	7
	20-854-70	EURL	8	9	11	12
	20-854-71	EURL	13	14	15	16
	20-854-72	EURL	18	19	20	21
	20-854-73	EURL	22	23	24	25
	20-854-74	EURL	26	27	28	
Shrimp inoculated with WSSV	19-5656-33	EURL	1	2	3	5
	19-5656-34	EURL	6	7	8	9
	19-5656-35	EURL	11	12	13	14
	19-5656-36	EURL	15	16	18	19
	19-5656-37	EURL	20	21	22	23
	19-5656-38	EURL	24	25	26	27
	19-5656-39	EURL	28	1	2	3
	19-5656-40	EURL	5	6	7	8
	19-5656-41	EURL	9	11	12	13
	19-5656-42	EURL	14	15	16	18
	19-5656-43	EURL	19	20	21	22
	19-5656-44	EURL	23	24	25	26
	19-5656-45	EURL	27	28		

# Methods used - WSSV

- 15 laboratories used real time PCR (Durand & Lightner 2002) – CT values: 12.1 – 21.0
- 7 laboratories used nested PCR methods (Lo et al. 1996)
- 3 laboratories used both methods
- 3 laboratories verified the identity of at least one of the obtained PCR products by sequencing.

Laboratory Code	DNA Extraction Method	PCR Kit
<b>EURL</b>	IndiMag Pathogen Kit with INDIMAG robot	Luna® Universal Probe qPCR Master Mix (NEB)
<b>1</b>	MagNA Pure 24 total NA kit	HotStarTaq DNA Polymerase (Qiagen)
<b>2</b>	QIAamp cadof Pathogen Mini Kit with Qiacube robot	Red Master Mix (Bioline)
<b>3</b>	IndiMag Pathogen kit with BioSprint 96 Workstation	PCRBIO HS Taq Mix Red (PCRBIO SYSTEMS)
<b>5</b>	QIAamp power fecal DNA kit	QuantStudio 5 Real-Time PCR System (Applied Biosystems)
<b>6</b>	IndiMag Pathogen kit with Maelstrom-9600 (TANBead) robot	PerfeCTa qPCR ToughMix Low Rox (QuantaBio)
<b>7</b>	Qiagen DNA Mini Kit	Platinum Taq DNA Polymerase kit (Invitrogen) & QuantiNova Pathogen + IC Kit (Qiagen)
<b>8</b>	InviMag Universal Kit (INVITEK) with KingFisher Flex	QuantiTect Probe PCR Kit (Qiagen)
<b>9</b>	Qiagen DNEasy Blood & Tissue Kit	5xHOT FIREPol Mix (Solis BioDyne)
<b>11</b>	QiaAmp Viral RNA Mini Kit	Platinum PCR SuperMix Kit (Invitrogen) & QuantiNova Pathogen + IC Kit (Qiagen)
<b>12</b>	Qiagen EZ1 DNA Tissue Kit	GoTaq® G2 Flexi DNA Polymerase (Promega)
<b>13</b>	Biomerieux Easy Mag	TaqMan Fast Universal PCR mix
<b>14</b>	NucleoSpin Tissue Kit (Macherey-Nagel)	Taq PCR Master Mix Kit (Qiagen)
<b>15</b>	QIAamp DNA Mini Kit with Qiacube robot	Real Time Taqman® Universal master mix (Life Technologies Ltd)
<b>16</b>	innuPREP AniPath DNA/RNA Kit – IPC16	GoTaq® Probe qPCR Master Mix (Promega)
<b>18</b>	Cador Pathogen for INDIMAG with INDIMAG robot	QuantiTect Probe PCR kit (Qiagen)
<b>19</b>	QIAamp DNA Mini Kit	Platinum Taq DNA Polymerase (Invitrogen)
<b>20</b>	QIAamp DNA Mini-Kit	QIAGEN OneStep RT-PCR Kit
<b>21</b>	Qiagen DNEasy Blood & Tissue Kit with Qiacube	Perfecta qPCR ToughMix UNG ROX (VWR)
<b>22</b>	Qiagen DNEasy Blood & Tissue Kit	Qiagen Multiplex PCR Kit
<b>23</b>	IndiMag Pathogen Kit with INDIMAG robot	Luna® Universal Probe qPCR Master Mix (NEB)
<b>24</b>	The Real PCR DNA/RNA spin Column Kit from Idexx	AmpliTaq Gold DNA Polymerase kit (Invitrogen)
<b>25</b>	Qiagen DNEasy Blood & Tissue Kit	GoTaq® Probe qPCR Master Mix (Promega)
<b>26</b>	Indical IndiMag Pathogen kit with Thermo Scientific KingFisher Flex	NZYTaq II Green Mix (NZYTech)
<b>27</b>	Roche High Pure Viral Nucleic Acid Kit	QuantiTect Probe PCR Kit (Qiagen)
<b>28</b>	QIAamp Viral RNA Mini Kit	TaqMan Universal PCR Master Mix (Applied Biosystems).

# Expected results – TSV/YHV1

Sample ID	Sample type	TSV	YHV1
Sample XX-006	P. vannamei homogenate on FTA cards	Negative	Negative
Sample XX-007	P. vannamei homogenate on FTA cards	Negative	Positive
Sample XX-008	P. vannamei homogenate on FTA cards	Negative	Negative
Sample XX-009	P. vannamei homogenate on FTA cards	Negative	Positive
Sample XX-010	P. vannamei homogenate on FTA cards	Positive	Negative
Sample XX-011	P. vannamei homogenate on FTA cards	Positive	Negative



# Results 2022 – TSV/YHV

Results were received from all 15 participating laboratories.

- 12 laboratories correctly diagnosed all samples, 6/6 (100 %)
- 2 laboratories correctly diagnosed 5/6 samples (83 %)
- 1 laboratory correctly diagnosed 4/6 samples (67 %)

All are false negatives

Laboratory Code	Method TSV	Method YHV	XX-001	XX-002	XX-003	XX-004	XX-005	XX-006	Score
<b>EURL</b>	qPCR	Single PCR	-ve	YHV	-ve	YHV	TSV (22.9)	TSV (22.9)	
<b>A</b>	Single PCR	Nested PCR	-ve	YHV	-ve	YHV	TSV	-ve	5/6
<b>B</b>	Single PCR	Single PCR	-ve	YHV	-ve	YHV	TSV	-ve	5/6
<b>C</b>	qPCR	Single PCR	-ve	-ve	-ve	-ve	TSV (31)	TSV (31)	4/6

# Methods – TSV/YHV

The following methods were used to diagnose TSV:

- 8 laboratories used real time PCR – CT 22.1 - 31
- 5 laboratories used single PCR
- 2 laboratories used both real time PCR and single PCR

The following methods were used to diagnose YHV:

- 2 laboratories used real time PCR
- 3 laboratories used nested PCR
- 9 laboratories used single PCR
- 1 laboratory used both real time PCR and single PCR

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

Laboratory Code	DNA Extraction Method	PCR Kit
EURL	IndiMag Pathogen Kit with INDIMAG robot	TagPath™ 1-Step RT-qPCR Master Mix; Qiagen OneStep RT-PCR kit
3	IndiMag Pathogen kit with BioSprint 96 Workstation	PCRBIO HS Taq Mix Red; MultiScribe Reverse Transcriptase; AgPath-ID One-Step RT-PCR kit
7	RNA isolation kit from A&A Biotechnology; Qiagen RNeasy Mini Kit	Super-Script One-Step RT-PCR with Platinum Taq; QuantiNova Pathogen + IC Kit
9	QiaAmp Viral RNA Mini Kit (Qiagen)	One-Step Probe PCR Mix
11	QiaAmp Viral RNA Mini Kit	One Step RT-PCR Kit; Platinum PCR SuperMix Kit; QuantiNova Pathogen + IC Kit
12	Qiagen EZ1 RNA tissue kit	Promega M-MLV Reverse transcriptase; Promega Go Taq® G2 Flexi DNA Polymerase
13	Biomerieux NucliSENS® easyMAG®	Tagman® 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™ Taq DNA Polymerase
19	RNeasy Mini Kit	Qiagen OneStep RT-PCR Enzyme Mix
20	NucleoSpin RNA Kit	QIAGEN OneStep RT-PCR Kit; Superscript III Platinum Taq Polymerase
21	RNeasy Mini Kit using QIAcube platform	QuantiTect Probe RT-PCR OneStep kit; Qiagen OneStep RT-PCR Kit
23	IndiMag Pathogen Kit with INDIMAG robot	TagPath 1-step RT-qPCR Mastermix; Qiagen Onestep RT-PCR kit
24	PureLink RNA Mini Kit	SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase
25	QIAGEN RNeasy Plus Kit	QIAGEN OneStep RT-PCR Kit
26	Indical IndiMag Pathogen kit with KingFisher Flex system	Qiagen One-Step RT-PCR kit
28	QIAamp Viral RNA Mini Kit	AgPath-ID™ One-Step RT-PCR Reagents

# Conclusions

- WSSV test good performance – false positives, most likely caused by cross-contamination
- TSV/YHV1 test lower performance than last year – false negatives in both tests
- 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 2023
- Will most likely concern WSSV, TSV and YHV
- We are considering to use FTA cards instead of pleopods also for WSSV in order to have better control of virus load

# Questions?