

DTU



Inter-laboratory proficiency tests for crustacean diseases 2019 and 2020

Participating countries

2019

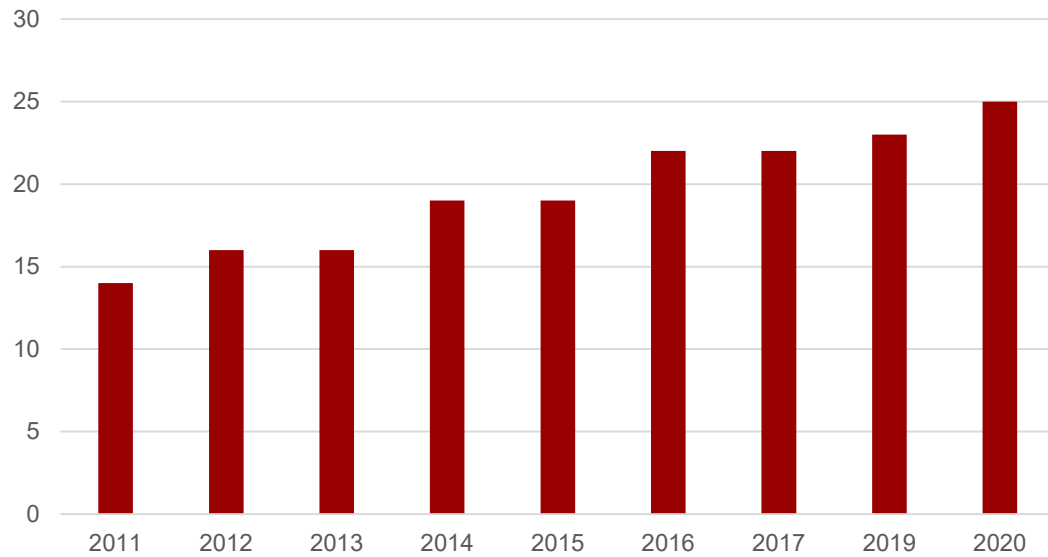
- WSSV test: 23 laboratories including 20 EU NRLs

2020

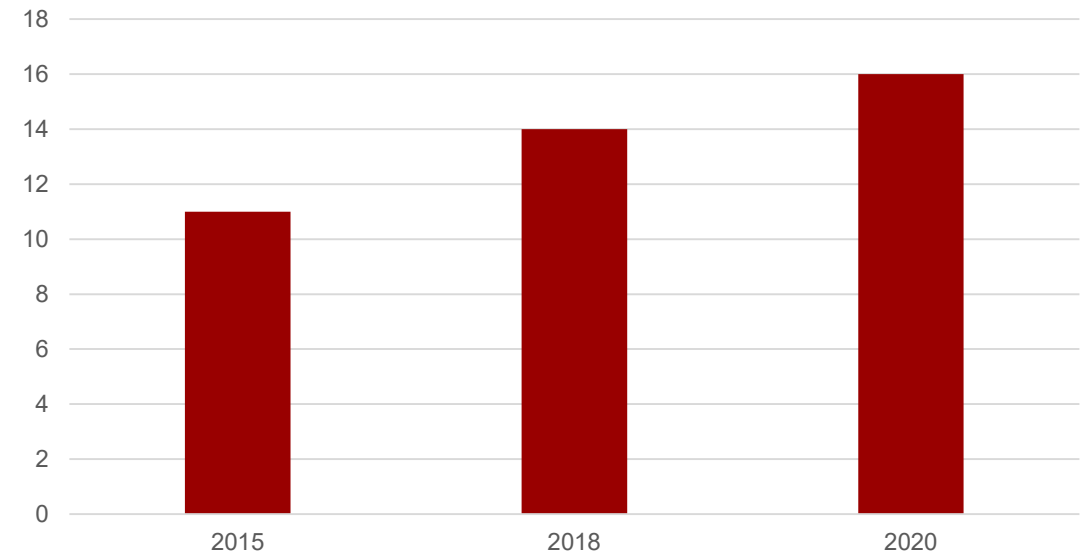
- WSSV test: 25 laboratories including 18 EU NRLs
- TSV + YHV: 16 laboratories including 12 EU NRLs

Participation in crustacean proficiency tests

Participation in WSSV PT

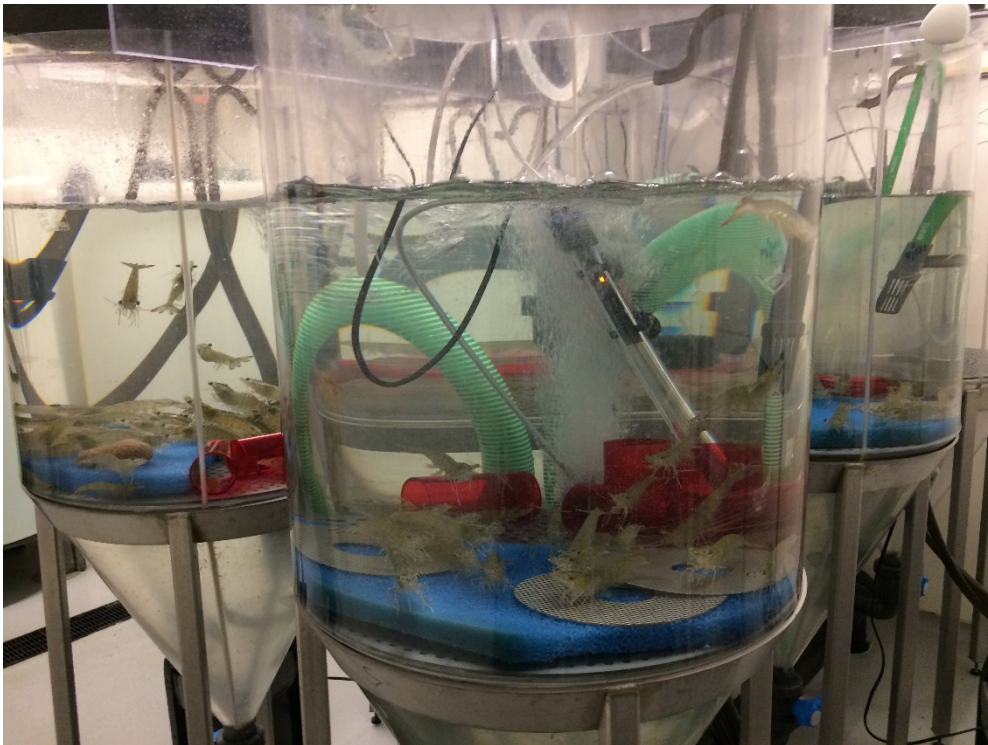


Participation in TSV/YHV PT

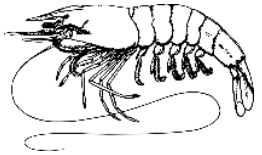


Materials

- 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



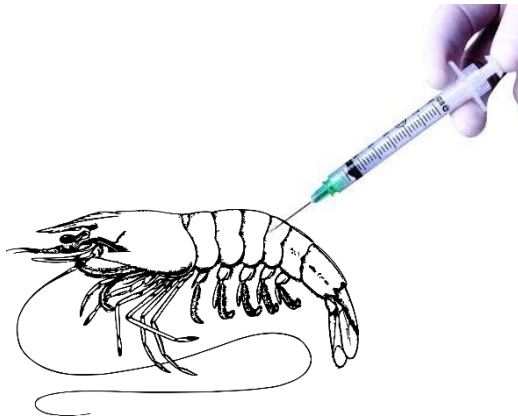
Centrifuges 3000g 30 min



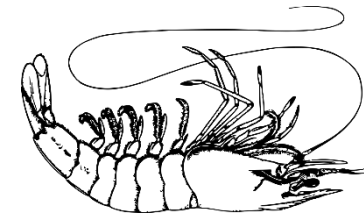
Supernatant stored at -20°C



Diluted and sterile filtered

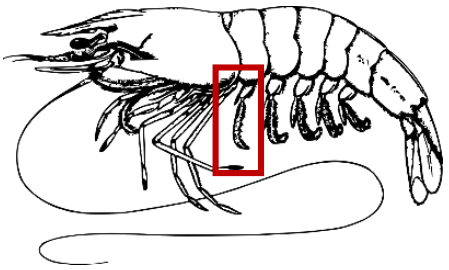


Live animals injected with ca. 100 µl extract



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)
- TSV tested with qPCR (Tang et al. 2004)
- YHV tested with nested PCR (Mohr et al. 2015)

Results 2019 - WSSV

Results were received from all 23 participating laboratories.

- 22 laboratories correctly diagnosed all samples, 6/6 (100%)
- 1 laboratory correctly diagnosed 4/6 samples (66%)

The following methods were used by the participants:

- 13 laboratories used nested PCR methods (Lo et al. 1996)
- 8 laboratories used real time PCR (Durand & Lightner 2002)
- 2 laboratories used both methods
- 3 laboratories verified the identity of at least one of the obtained PCR products by sequencing.

Results 2019 - WSSV

	Shrimp ID	Pleopod ID					
		A	B	C	D	E	
Non-inoculated Shrimp	19-5656-1	EURL	1	2	3	5	
	19-5656-2	EURL		6	7	8	
	19-5656-3	EURL	9	11	12	13	
	19-5656-4	EURL	14	15	16	18	
	19-5656-5	EURL	19	20	21	22	
	19-5656-6	EURL	23	24	26	27	
	19-5656-8	EURL	1	2	3	5	
	19-5656-9	EURL		6	7	8	
	19-5656-10	EURL	9	11	12	13	
	19-5656-11	EURL	14	15	16	18	
	19-5656-12	EURL	19	20	21	22	
	19-5656-13	EURL	23	24	26	27	
	19-5656-15	EURL	1	2	3	5	
	19-5656-16	EURL		6	7	8	
	19-5656-17	EURL	9	11	12	13	
	19-5656-18	EURL	14	15	16	18	
	19-5656-19	EURL	19	20	21	22	
	19-5656-20	EURL	23	24	26	27	
	Shrimp inoculated with WSSV	19-5656-49	EURL	1	2	3	5
		19-5656-50	EURL		6	7	8
19-5656-51		EURL	9	11	12	13	
19-5656-52		EURL	14	15	16	18	
19-5656-53		EURL	19	20	21	22	
19-5656-54		EURL	23	24	26	27	
19-5656-56		EURL	1	2	3	5	
19-5656-57		EURL		6	7	8	
19-5656-58		EURL	9	11	12	13	
19-5656-59		EURL	14	15	16	18	
19-5656-60		EURL	19	20	21	22	
19-5656-61		EURL	23	24	26	27	
19-5656-63		EURL	1	2	3	5	
19-5656-64		EURL		6	7	8	
19-5656-65		EURL	9	11	12	13	
19-5656-66		EURL	14	15	16	18	
19-5656-67		EURL	19	20	21	22	
19-5656-68		EURL	23	24	26	27	

Results 2019 - WSSV

	Shrimp ID	Pleopod ID					
		A	B	C	D	E	
Non-inoculated Shrimp	19-5656-1	EURL	1	2	3	5	
	19-5656-2	EURL		6	7	8	
	19-5656-3	EURL	9	11	12	13	
	19-5656-4	EURL	14	15	16	18	
	19-5656-5	EURL	19	20	21	22	
	19-5656-6	EURL	23	24	26	27	
	19-5656-8	EURL	1	2	3	5	
	19-5656-9	EURL		6	7	8	
	19-5656-10	EURL	9	11	12	13	
	19-5656-11	EURL	14	15	16	18	
	19-5656-12	EURL	19	20	21	22	
	19-5656-13	EURL	23	24	26	27	
	19-5656-15	EURL	1	2	3	5	
	19-5656-16	EURL		6	7	8	
	19-5656-17	EURL	9	11	12	13	
	19-5656-18	EURL	14	15	16	18	
	19-5656-19	EURL	19	20	21	22	
	19-5656-20	EURL	23	24	26	27	
	Shrimp inoculated with WSSV	19-5656-49	EURL	1	2	3	5
		19-5656-50	EURL		6	7	8
19-5656-51		EURL	9	11	12	13	
19-5656-52		EURL	14	15	16	18	
19-5656-53		EURL	19	20	21	22	
19-5656-54		EURL	23	24	26	27	
19-5656-56		EURL	1	2	3	5	
19-5656-57		EURL		6	7	8	
19-5656-58		EURL	9	11	12	13	
19-5656-59		EURL	14	15	16	18	
19-5656-60		EURL	19	20	21	22	
19-5656-61		EURL	23	24	26	27	
19-5656-63		EURL	1	2	3	5	
19-5656-64		EURL		6	7	8	
19-5656-65		EURL	9	11	12	13	
19-5656-66		EURL	14	15	16	18	
19-5656-67		EURL	19	20	21	22	
19-5656-68		EURL	23	24	26	27	

Nested PCR

Nested PCR

Results were received from all 25 participating laboratories.

- 20 laboratories correctly diagnosed all samples, 5/5 (100 %).
- 4 laboratories correctly diagnosed 4/5 samples (80 %).
- 1 laboratory correctly diagnosed 0/5 samples (0 %).

The following methods were used by the participants:

- 12 laboratories used nested PCR methods (Lo et al. 1996)
- 10 laboratories used real time PCR (Durand & Lightner 2002)
- 3 laboratories used both methods
- 3 laboratories verified the identity of at least one of the obtained PCR products by sequencing.

Results 2020 – WSSV

	Shrimp ID	Pleopod ID				
		A	B	C	D	E
Non-inoculated Shrimp	19-5656-10	EURL	1	2	3	5
	19-5656-11	EURL	6	7	8	9
	19-5656-12	EURL	11	12	13	14
	19-5656-13	EURL	15	16	18	19
	19-5656-14	EURL	20	21	22	23
	19-5656-15	EURL	24	25	26	27
	19-5656-16	EURL	28	1	2	3
	19-5656-17	EURL	5	6	7	8
	19-5656-18	EURL	9	11	12	13
	19-5656-19	EURL	14	15	16	18
	19-5656-22	EURL	19	20	21	22
	19-5656-23	EURL	23	24	25	26
	19-5656-24	EURL	27	28	1	2
	19-5656-25	EURL	3	5	6	7
	19-5656-26	EURL	8	9	11	12
	19-5656-27	EURL	13	14	15	16
19-5656-28	EURL	18	19	20	21	
19-5656-29	EURL	22	23	24	25	
19-5656-30	EURL	26	27	28		
Shrimp inoculated with WSSV	19-5656-74	EURL	1	2	3	5
	19-5656-75	EURL	6	7	8	9
	19-5656-76	EURL	11	12	13	14
	19-5656-77	EURL	15	16	18	19
	19-5656-78	EURL	20	21	22	23
	19-5656-79	EURL	24	25	26	27
	19-5656-80	EURL	28	1	2	3
	19-5656-81	EURL	5	6	7	8
	19-5656-82	EURL	9	11	12	13
	19-5656-83	EURL	14	15	16	18
	19-5656-84	EURL	19	20	21	22
19-5656-85	EURL	23	24	25	26	
19-5656-86	EURL	27	28			

Results 2020 – WSSV

	Shrimp ID	Pleopod ID							
		A	B	C	D	E			
Non-inoculated Shrimp	19-5656-10	EURL		1	2	3	5	Nested PCR	
	19-5656-11	EURL		6	7	8	9		
	19-5656-12	EURL		11	12	13	14		
	19-5656-13	EURL		15	16	18	19		
	19-5656-14	EURL		20	21	22	23		
	19-5656-15	EURL		24	25	26	27		
	19-5656-16	EURL		28	1	2	3		Nested PCR
	19-5656-17	EURL		5	6	7	8		
	19-5656-18	EURL		9	11	12	13		Nested PCR
	19-5656-19	EURL		14	15	16	18		
	19-5656-22	EURL		19	20	21	22		
	19-5656-23	EURL		23	24	25	26		qPCR
	19-5656-24	EURL		27	28	1	2		
	19-5656-25	EURL		3	5	6	7		
	19-5656-26	EURL		8	9	11	12		
19-5656-27	EURL		13	14	15	16			
19-5656-28	EURL		18	19	20	21			
19-5656-29	EURL		22	23	24	25			
19-5656-30	EURL		26	27	28		Nested PCR		
19-5656-74	EURL		1	2	3	5			
19-5656-75	EURL		6	7	8	9			
19-5656-76	EURL		11	12	13	14			
19-5656-77	EURL		15	16	18	19			
19-5656-78	EURL		20	21	22	23			
19-5656-79	EURL		24	25	26	27			
19-5656-80	EURL		28	1	2	3			
19-5656-81	EURL		5	6	7	8			
19-5656-82	EURL		9	11	12	13			
19-5656-83	EURL		14	15	16	18			
19-5656-84	EURL		19	20	21	22			
19-5656-85	EURL		23	24	25	26			
19-5656-86	EURL		27	28					

Results 2020 – TSV/YHV

Results were received from all 16 participating laboratories.

- 11 laboratories correctly diagnosed all samples, 6/6 (100 %)
- 5 laboratories correctly diagnosed 5/6 samples (83.3 %)

The following methods were used by the participants to diagnose TSV:

- 8 laboratories used real time PCR
- 8 laboratories used single PCR

The following methods were used by the participants to diagnose YHV:

- 8 laboratories used nested PCR
- 6 laboratories used single PCR
- 2 laboratories used real time PCR

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

Results 2020 – TSV/YHV

	Shrimp ID	Pleopod ID				
		A	B	C	D	E
Non-inoculated Shrimp	20-854-51	EURL	3	7	9	11
	20-854-52	EURL	12	13	14	18
	20-854-53	EURL	19	20	21	23
	20-854-54	EURL	24	26	27	28
	20-854-55	EURL	3	7	9	11
	20-854-56	EURL	12	13	14	18
	20-854-57	EURL	19	20	21	23
	20-854-58	EURL	24	26	27	28
Shrimp inoculated with TSV	20-854-102	EURL	3	7	9	11
	20-854-103	EURL	12	13	14	18
	20-854-107	EURL	19	20	21	23
	20-854-111	EURL	24	26	27	28
	20-854-116	EURL	3	7	9	11
	20-854-117	EURL	12	13	14	18
	20-854-123	EURL	19	20	21	23
	20-854-131	EURL	24	26	27	28
Shrimp inoculated with YHV	20-854-151	EURL	3	7	9	11
	20-854-152	EURL	12	13	14	18
	20-854-153	EURL	19	20	21	23
	20-854-154	EURL	24	26	27	28
	20-854-155	EURL	3	7	9	11
	20-854-156	EURL	12	13	14	18
	20-854-157	EURL	19	20	21	23
	20-854-158	EURL	24	26	27	28

Results 2020 – TSV/YHV

	Shrimp ID	Pleopod ID				
		A	B	C	D	E
Non-inoculated Shrimp	20-854-51	EURL	3	7	9	11
	20-854-52	EURL	12	13	14	18
	20-854-53	EURL	19	20	21	23
	20-854-54	EURL	24	26	27	28
	20-854-55	EURL	3	7	9	11
	20-854-56	EURL	12	13	14	18
	20-854-57	EURL	19	20	21	23
	20-854-58	EURL	24	26	27	28
Shrimp inoculated with TSV	20-854-102	EURL	3	7	9	11
	20-854-103	EURL	12	13	14	18
	20-854-107	EURL	19	20	21	23
	20-854-111	EURL	24	26	27	28
	20-854-116	EURL	3	7	9	11
	20-854-117	EURL	12	13	14	18
	20-854-123	EURL	19	20	21	23
	20-854-131	EURL	24	26	27	28
Shrimp inoculated with YHV	20-854-151	EURL	3	7	9	11
	20-854-152	EURL	12	13	14	18
	20-854-153	EURL	19	20	21	23
	20-854-154	EURL	24	26	27	28
	20-854-155	EURL	3	7	9	11
	20-854-156	EURL	12	13	14	18
	20-854-157	EURL	19	20	21	23
	20-854-158	EURL	24	26	27	28

Single PCR (points to row 20-854-57)

qPCR (points to row 20-854-102)

Single PCR (points to row 20-854-102)

Single PCR (points to row 20-854-117)

Nested PCR (points to row 20-854-151)

Conclusions

- No clear pattern between PCR method and false results
- Low viral load of TSV infected shrimps may cause problems of false negatives
- Most false results caused by cross-contamination of samples
- Nested PCR may increase the risk of getting false positives

Recommendations

- Use separate rooms for nucleic acid extraction, master mix preparation, PCR setup and gel electrophoresis to decrease risk of cross contamination of samples
- Try to avoid nested PCR procedures
- If nested PCR is used, consider to only do second round PCR on samples negative in the first round PCR. Also consider using single tubes instead of strips to enable physical separation of samples
- Consider to use non-amplified positive control to decrease risk of cross-contamination
- Consider to include an extra PCR with host gene specific primers (e.g. EF1a) to check the efficiency of RNA/DNA extraction
- Consider to use artificial positive control with introduced SNPs

Next Inter-laboratory proficiency test

- Will most likely be send out in May/June
- Will most likely concern WSSV, TSV and YHV
- We are considering to use FTA cards instead of pleopods to have better control of virus load

Questions?