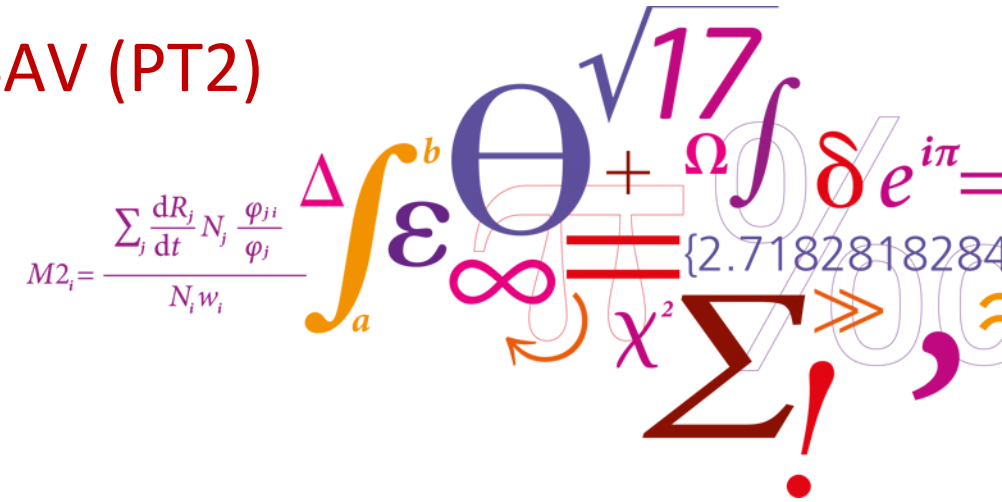
European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Inter-Laboratory Proficiency Test 2019 for identification of VHSV, IHNV, EHNV SVCV and IPNV (PT1) and identification of CyHV-3 (KHV), ISAV and SAV (PT2)



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DANAK

PT Reg. no.: 515

Content

- Proficiency test 1, PT1
- Proficiency test 2, PT2
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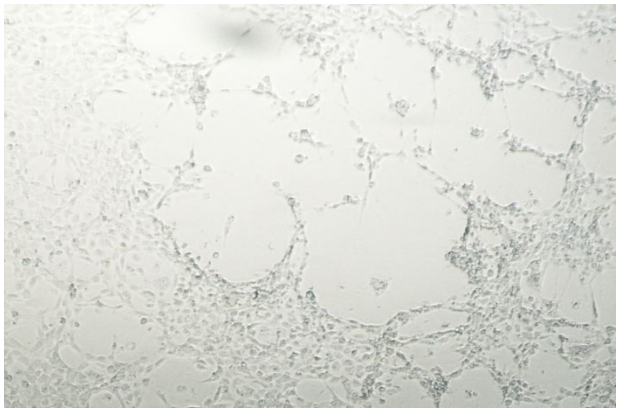
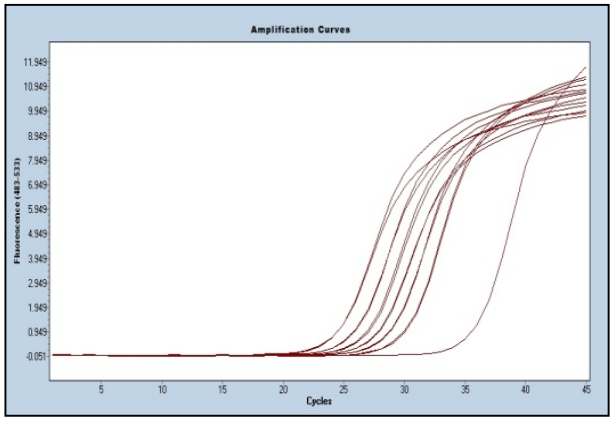


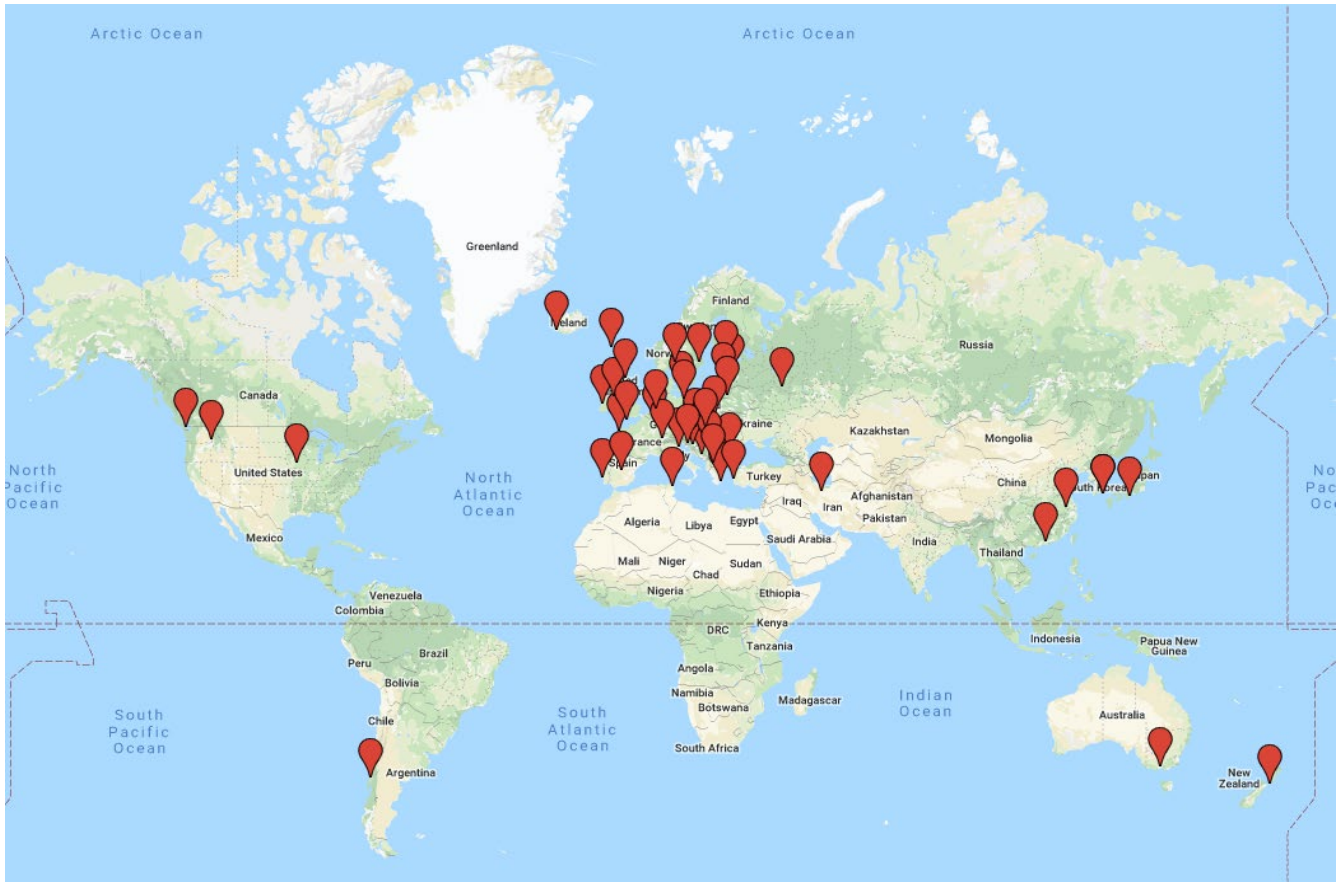


PT1 and PT2 was shipped to 49 laboratories in 2019

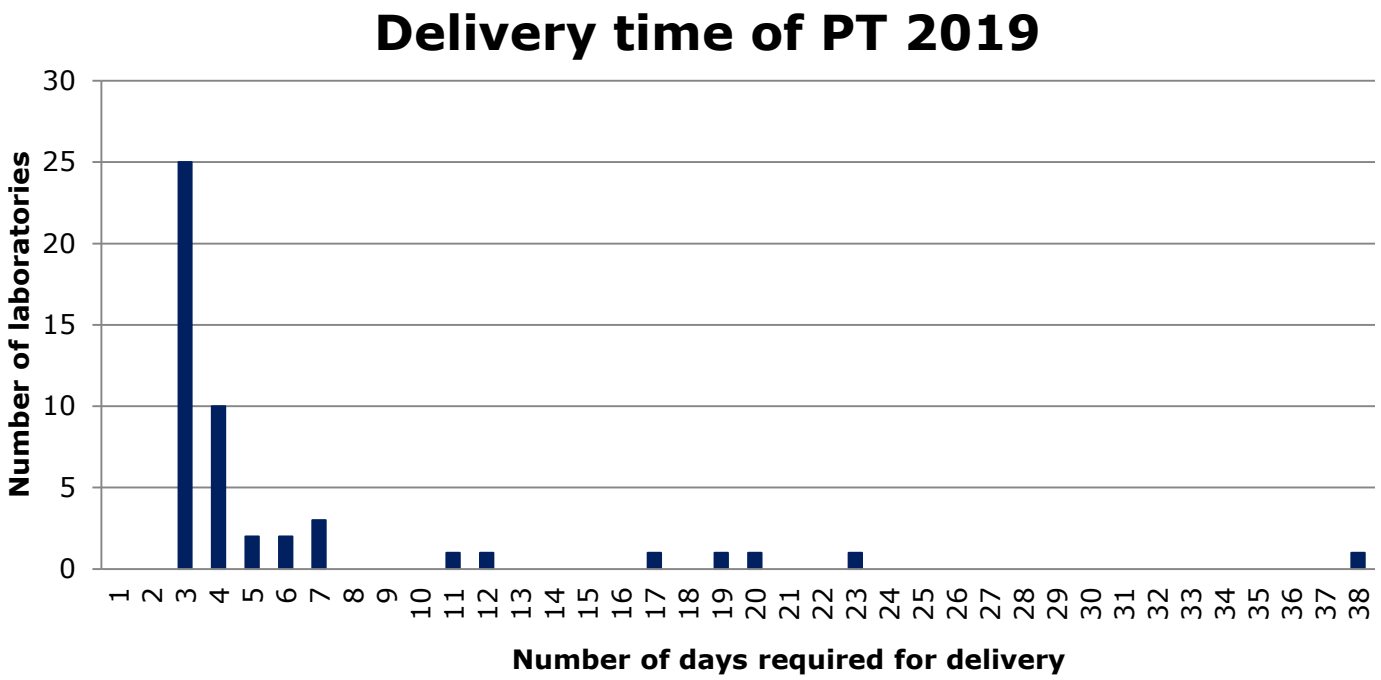
All NRL's for Fish Diseases in EU Member States

NRL's in:

Australia
Canada
Chile
Faroe Islands
Iceland
Iran
Japan
New Zealand
Norway
P.R. China (2)
Republic of Korea (2)
Russia
Switzerland
Turkey
USA (2)



Distribution of PT1 and PT2



86% of the tests were delivered within the first week

96% within three weeks.

PT1: Content of ampoules

Five ampoules containing virus/ lyophilised tissue culture supernatant

Code	Isolate
Ampoule I:	**VHSV DK-9695377, Genotype Ia + IHNV 32/87, Genotype E**
Ampoule II:	**ECV 562/92**
Ampoule III:	**VHSV 4p101, Genotype IIIa**
Ampoule IV:	**SVCV DK-203273, Genotype 1a**
Ampoule V:	**IPNV strain Sp, Genogroup 5**

Testing PT1

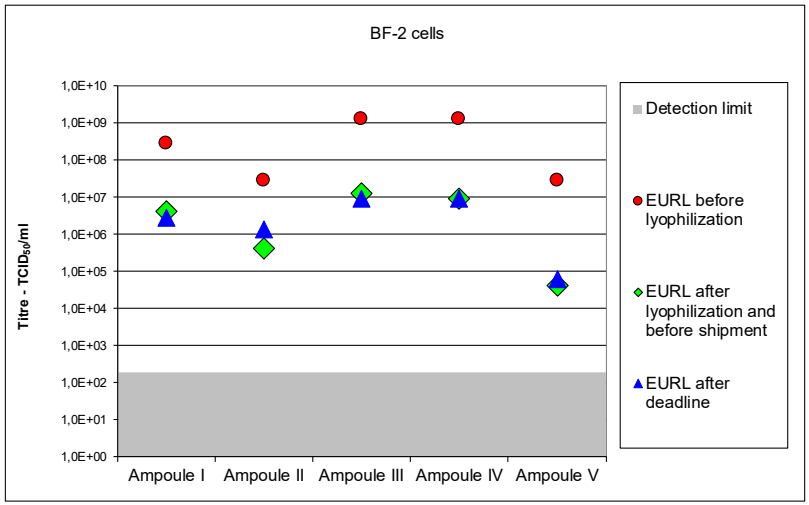
- The proficiency test was prepared under DS/EN ISO/IEC 17043

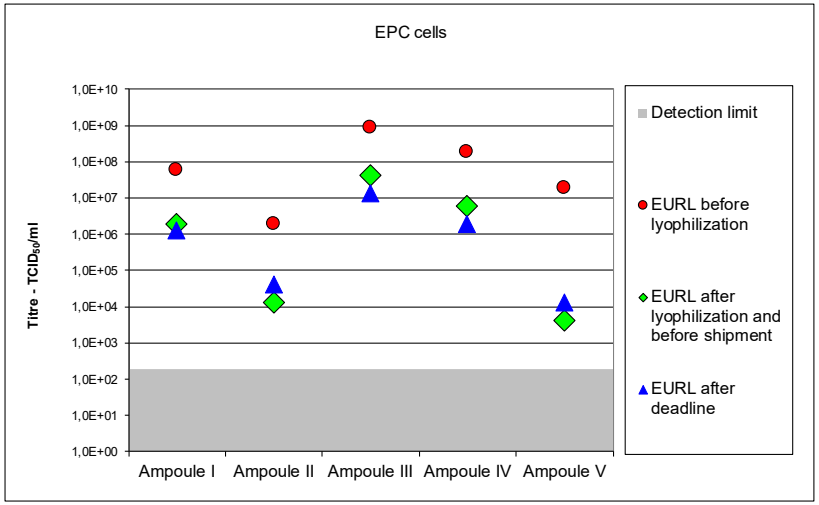
- The reductions of the titres after lyophilisation were between 1-3 log in the same cell line.
  Except for ampoule V (containing IPNV) which was 4 log in EPC and RTG-2 cells.

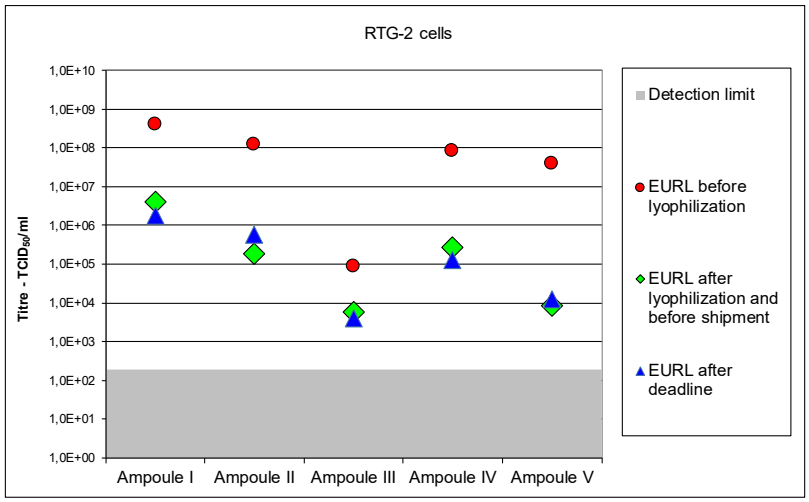
- All titres of the lyophilised viruses were above detection level, except for ECV (ampoule II) on FHM cells.

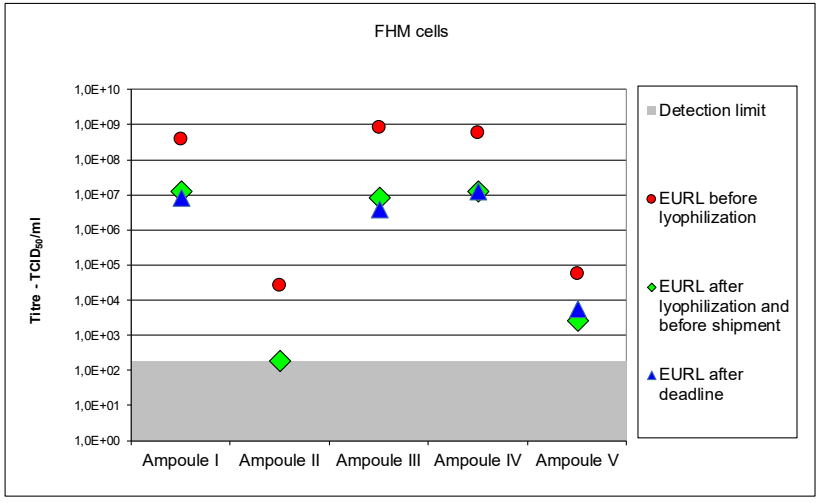
- **This highlights the importance of using two heterologous cell lines.**

Titers before and after lyophilization

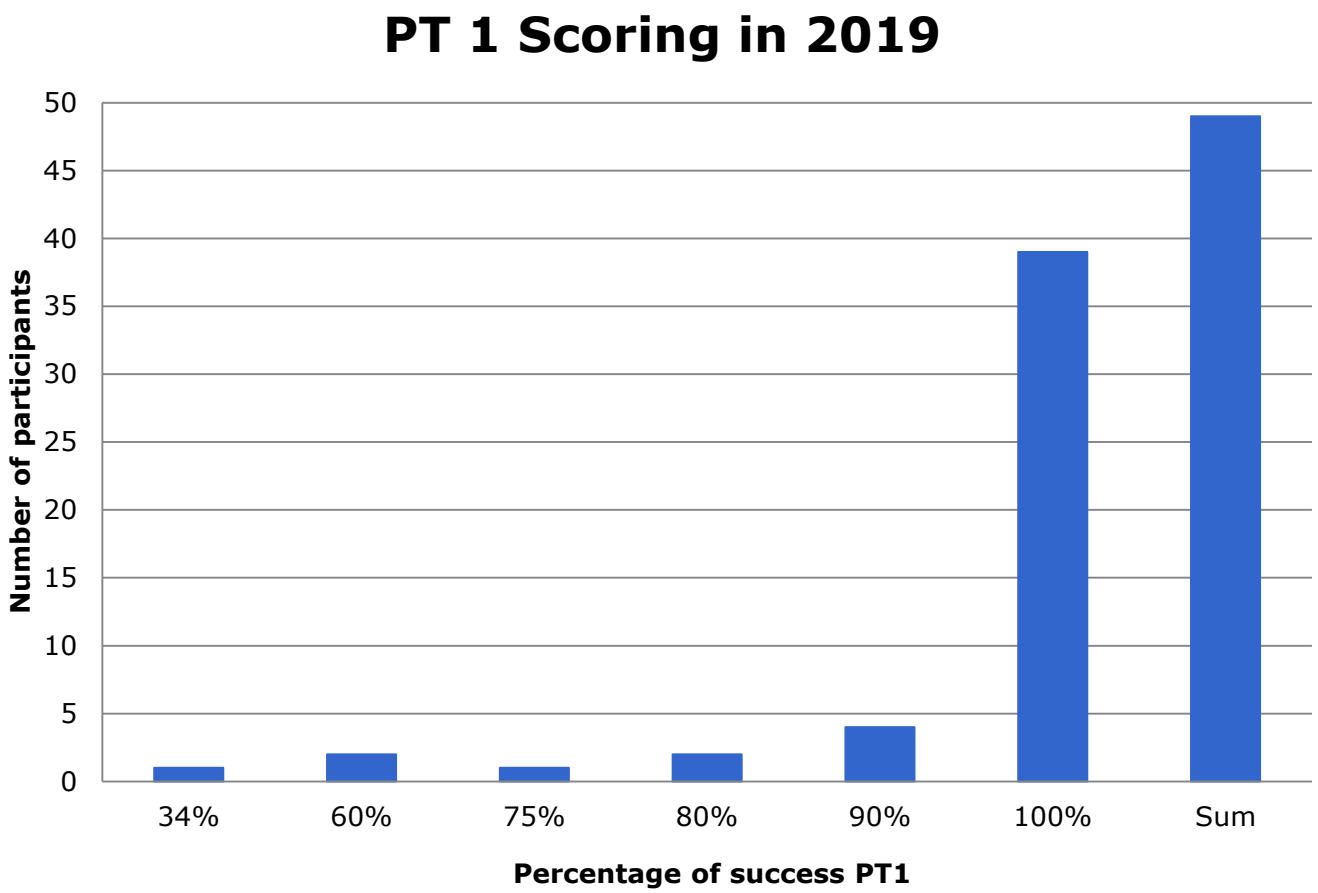








Laboratory scoring, PT1



Genotyping and sequencing – PT1

	Amp. I	Amp. II	Amp. III	Amp. IV	Amp. V
	VHSV, Ia IHNV, E	ECV	VHSV IIIa	SVCV Ia	IPNV Genogroup 5
No. of sequence	44		34	24	22
No. of correct genotypes	41		28	23	16
No. of correct sequences without genotype	2		1	1	6
No of incorrect genotype	1		5	0	0

Sequencing of Ranavirus is necessary to discriminate exotic EHNV from other ranavirus endemic in Europe

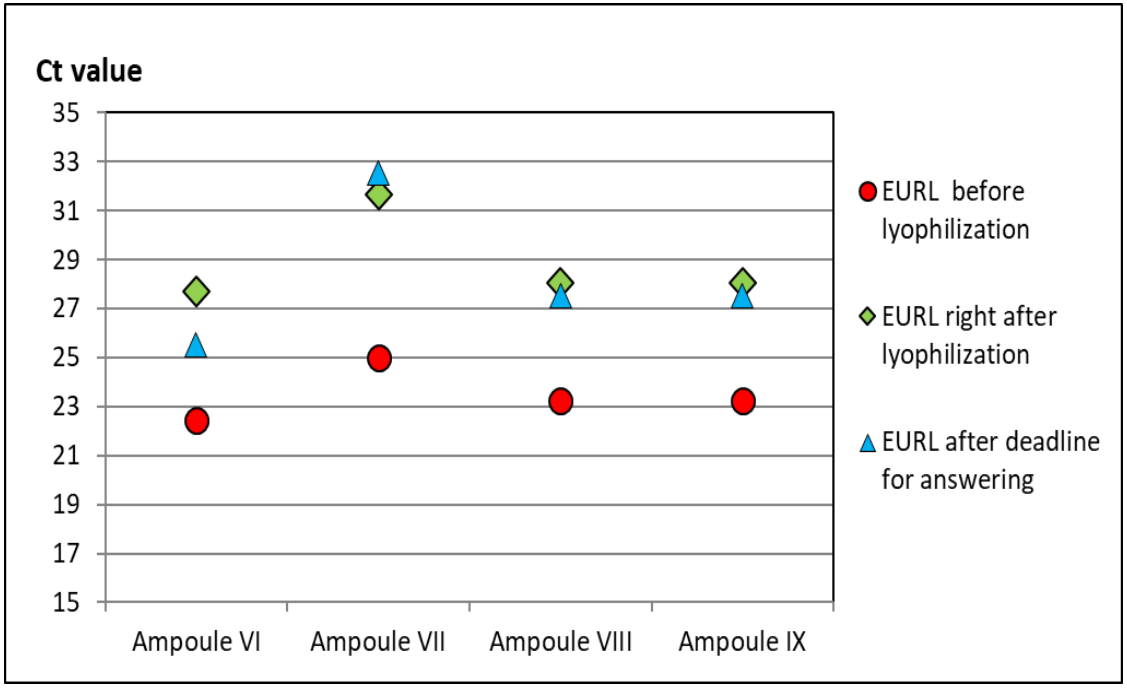
PT-2 Content of ampoules

Four ampoules containing pathogens / lyophilised tissue culture supernatant

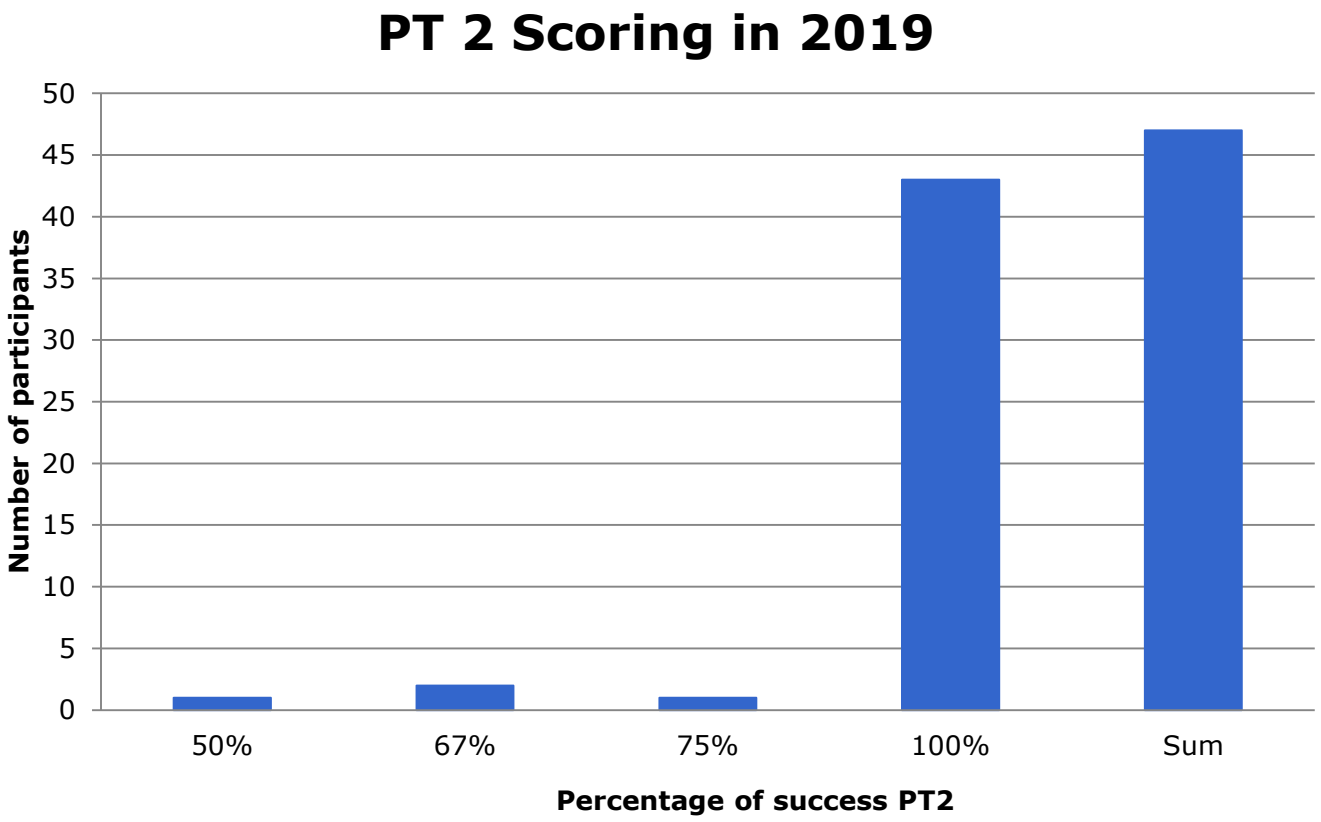
Code	Isolate
Ampoule VI:	**ISAV 390/98, HPRΔ**
Ampoule VII:	**Koi Herpesvirus, isolate NRIA 0301**
Ampoule VIII:	**Salmonid Alpha Virus (SAV) 2, Sleeping Disease Virus (SD) – MR-N1-2011**
Ampoule IX:	**ISAV 2016-70-1297_Vir4415, HPRΔ**

Testing PT2

- 5 ampoules were tested PCR.
  All the standard deviations were below 1 Ct value.
- The lyophilisation procedure caused a significant virus reduction at 4-7 Ct. values.
- Ct values are stable after lyophilisation



Laboratory scoring; PT2



Genotyping and sequencing – PT2

	Amp. VI	Amp. VII	Amp. VIII	
	ISAV HPRΔ	KHV CyHV-3	SAV Genotype 2	ISAV HPRΔ
No. of sequence	33	20	26	33
No. of correct genotypes	28	16	23	27
No. of correct sequences without genotype	4	4	0	2
No of incorrect genotype	1	0	3	4

”Underperfomance”

Due to:

- Answering Ranavirus without corroborating the finding with sequence analysis.
- Not identify the viral content of the ampoule.
- Identifying the wrong content of the ampoule.
- Contamination of the ampoule with an other virus.

The ampoules may contain high titered viruses, so appropriate procedures during handling and testing of the ampoules are important.

Feedback 2019

Only 47% of the participating laboratories completed the questionnaire with feedback.

Since the questionnaire will be included in the documentation for our quality assurance which is a demand from DANAK, I will kindly ask you to fill out the questionnaire with feedback in the future and of course return it ☺

A great thanks for support and contribution in the future.

Feedback from participants

- **Including Nodavirus sistematically**
- **Please label the plastic containers, so I can see which ampoule it contains.**
- **It wasn't simple (*to use the spreadsheet for submission of results*).**
- **There was an overlapping with the Crustacean PT test. Would prefer the two PT tests-Fish & Crustacean-to take place in different time periods since we lack lab personnel**
- **There was an discrepancy in the graph (figure 5 in the report) 'Ampul I BF-2 celler'**

Feedback - EURL

EURL COMMENTS

1) ISA isolate included shall be sequenced, distinguishing HPRΔ and HPR0 otherwise it will cause the loss of one point.

2) Rana isolate included shall be sequenced, distinguishing EHNV from the non listed Ranavirus otherwise it will cause the loss of one point.

3) Appropriate procedures during handling and testing of the ampoules are important to avoid contermination.

4) Nodavirus will not be included in the test OIE ref lab provide ringtest every second year.

InterLaboratory Proficiency test 2020

- 49 participants.
- Deadline December 4th
- Please have a look at instructions provided, from this year compile Ct values, and as last year provide score for genotyping

Correct completing of the Spreadsheets – Genotype

Ampoule number	Pathogen Identification	Amplicon sequenced (ref and primers)	Genotype	Sequence	Possible isolates:
Ampoule III	IHNV	Mid G gene Upstream Primer 5'-AGA-GAT-CCC-TAC-ACC-AGA-GAC-3'; Downstream Primer 5'-GGT-GGT-GTT-GTT-TCC-GTG-CAA-3'. Emmenegger E.J., Meyers T.R., Burton T.O & Kurath G. (2000). Genetic diversity and epidemiology of infectious	**U**	TTTTATTGGAGGAA AATGTACCAAATCA CCCTGCCAGACTC ATTGGTCCA ACGTAGTTTGGAT GGGTGATGCAGGG ATACCAGCCTGTG	DQ164100.1 Infectious hematopoietic necrosis virus isolate BLk94 glycoprotein (G) gene, 100% 645bps. Infectious hematopoietic necrosis virus gene for glycoprotein, complete cds, strain: ChAb76 643/645 99%
Ampoule VII	ISAV	HA gene Mjaaland et al (2002). Virology, 304:379-391 Klon1EGFP-F1 5'-GGGCTAGCATGGCACGATTCATAATT-3' Klon1EGFP-R1 5'-GGGGTACCGTAGCAACAGACAGGCTCGA	**HPR2**	CCAATGACTGCACT GACGGACCTACTG ACATGATCATCCCA ACTTCGATG ACACTGGACAACG CGGCAAGGGAGCT GTACCTGGGAGCA	ISAV4(90/09/400) (Genbank Accession DQ785248.1)
Only fill in the Genotype↑					
No suptype, serotype, etc.↓					
Ampoule III	IHNV	Emmenegger et al. (2000) Sequence (5´-> 3´) IHN-GF1 AGA GAT CCC TAC ACC AGA GAC IHN-GR1 GGT GGT GTT GTT TCC GTG CAA Enzmann et al. (2005)	**BLK94, genogroup U, subtype P**	GTGCAATCCGTGA AAGCCCTCCCACTC ATCCCCAAAGGGT CGTTCCCATTTCGT GAAGCTGGTAGCG CGATGGGCCCTGT ACGTCGTCCTGTCC	100% query cover and 665nt 100% identical with: DQ164100.1 - Infectious hematopoietic necrosis virus isolate BLk94 glycoprotein (G) gene, complete cds
Ampoule VII	ISAV (HPRdel)	HPR of segment 6 (HE gene); Markussen T, Jonassen CM, Numanovic S, Braaen S, Hjortaas M, Nilsen H, Mjaaland S. Evolutionary mechanisms involved in the virulence of infectious salmon anaemia virus (ISAV), a piscine orthomyxovirus. Virology. 2008 May	**HPR group 2/ EU-G2 group**	TGACCAGACAAGC TTAGGTAACACAG ACACACTTATCATG AGGGAGGTAGCAT TGCATAAGGAGAT GATCAGTAAACTTC AGAGGAACATCAC	ISAV4 90/09/400; DQ785248 Markussen,T., Jonassen,C.M., Numanovic,S., Braaen,S., Hjortaas,M., Nilsen,H. and Mjaaland,S. Evolutionary mechanisms involved in the virulence of infectious salmon anaemia virus (ISAV), a piscine orthomyxovirus. Virology 374 (2), 515-527 (2008) ISAV E72b/02: Isolate from Nova Scotia, belonging to the EU-E subgroup within the

Genotypes to be used

Virus	Genotype	Reference
VHSV	I (a-e), II, III, IV(a-d)	(Einer-Jensen et al., 2005) (Guðmundsdóttir et al., 2018)
IHNV	U,M,L,E,J	(Kurath et al.,2003) (Bellec et al., 2017)
IPNV	Genogroup 1,2,3,4,5 (Evt within genogroup 1 – genotype 1-4)	Blake et al.,2001 Ruane et al., 2015
SVCV	Genogroup 1 (a-d), 2,3,4	Sheppard et al., 2007
Ranavirus	EHNV- NOT EHNV	OIE Manual
ISAV	HPR Deleted / HPR0	Mjaaland et al 2002
KHV	CYHV 1-3	
SAV	1-6	Fringuelli et al.,2008

Correct completing of the Spreadsheets – Concluding Results

This goes for both PT1 and PT2

Ampoule no.	Isolate	ELISA	IFAT	Neutralisation	Conventional (RT-) PCR	Real-time (RT-) PCR	Sequencing: Fill in the information on the sheet regarding "Sequencing results"	Other	Concluding Result
Ampoule II	VHSV	-	-	N/A	N/A	-	N/A		**IPNV**
	IHNV	-	-	N/A	N/A	-	N/A		
	EHNV	N/A	-	N/A	-	N/A	N/A		
	Ranavirus	N/A	-	N/A	-	N/A	N/A		
	IPNV	+	+	N/A	N/A	+	+		
	SVCV	-	-	N/A	-	N/A	N/A		

Only fill in the virus name↑

No genotype, Isolate No., etc.↓

Ampoule II	VHSV	not performed	-	not performed	not performed	-			**100 % IPNV Genogroup 5 isolates 666/12; 470/07 and Sp**
	IHNV	not performed	-	not performed	not performed	-		Electron microscopy	
	EHNV	not performed	not performed	not performed	not performed	not performed		result: Birnavirus	
	Ranavirus	not performed	not performed	not performed	-	not performed			
	IPNV	not performed	+	not performed	+	+	+		
	SVCV	not performed	not performed	not performed	-	not performed			

Correct completing of the Spreadsheets - CPE

- Mark CPE with **X** - Notning else
- Mark if you have used the cell-line especially no CPE is obtained.

CELL LINES:		BF-2						EPC					
		BF-2 cells used?		Yes:	x	No:		EPC cells used?		Yes:	x	No:	
		1	2	3	4	5	6	7	8	9	10	11	12
10^{-0}	A		x				x	x	x	x	x	x	x
10^{-1}	B							x	x	x	x	x	x
10^{-2}	C								x				x
10^{-3}	D												
10^{-4}	E												
10^{-5}	F												
10^{-6}	G												
10^{-7}	H					C	C					C	C
No of:	X	2	$TCID_{50}$/ml:		2.7E+02			14	$TCID_{50}$/ml:		2.7E+04		

CELL LINES:		RTG-2						FHM					
		RTG-2 cells used?		Yes:	x	No:		FHM cells used?		Yes:		No:	x
		1	2	3	4	5	6	7	8	9	10	11	12
10^{-0}	A												
10^{-1}	B												
10^{-2}	C												
10^{-3}	D												
10^{-4}	E												
10^{-5}	F												
10^{-6}	G												
10^{-7}	H					C	C					C	C
No of:	X	0	$TCID_{50}$/ml:		< 1.9E+02			0	$TCID_{50}$/ml:		< 1.9E+02		

Proficiency test 2020 and 2021

- The test was send out in end of September 2020
- PT1: For identification of VHSV, IHNV and EHNV and in addition SVC, differentiating from other viruses as IPNV, Rana-viruses etc.
- PT2: Identification of ISAV, KHV and SAV
- From 2020 we will compare Ct.-values from the participating laboratories – there for please fill in your obtained Ct.values (cf. the updated 'Instruction for Spreadsheet 2020')

Acknowledgements

- Christina Flink Desler
- Argelia Cuenca
- Danny Darby
- Niccolò Vendramin

- **KHV-** Received from: Dr. Kei Yuasa, National Research Institute of Aquaculture, Japan
- **SAV- Received from**: Dr. Hilde Sindre, Norwegian Veterinary Institute, Norway
- **ISAV Received from:** Marine Scotland Science
- **ISAV – Received from:** Norwegian Veterinary Institute
- **ECV-Received from:** Dr. G. Bovo, ISZ-Ve, Padova, Italy