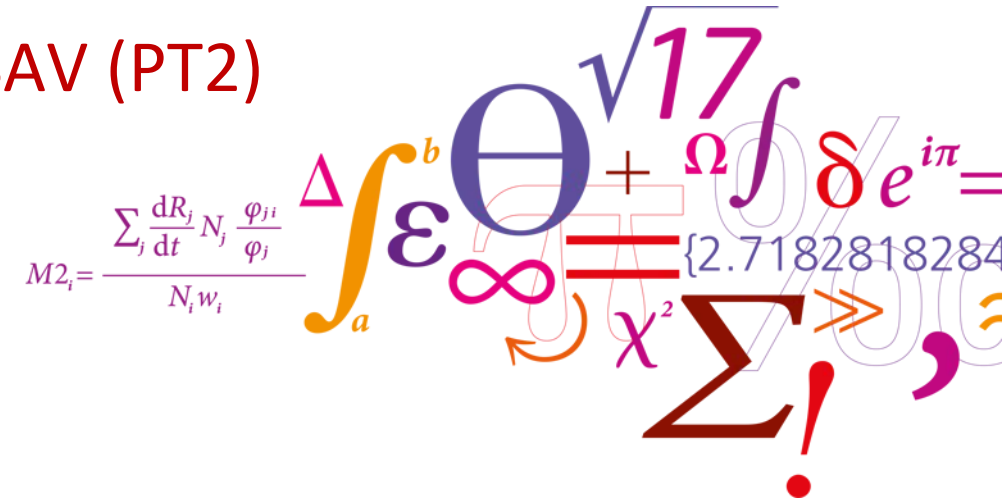
Inter-Laboratory Proficiency Test 2018 for identification of VHSV, IHNV, EHNV SVCV and IPNV (PT1) and identification of CyHV-3 (KHV), ISAV and SAV (PT2)



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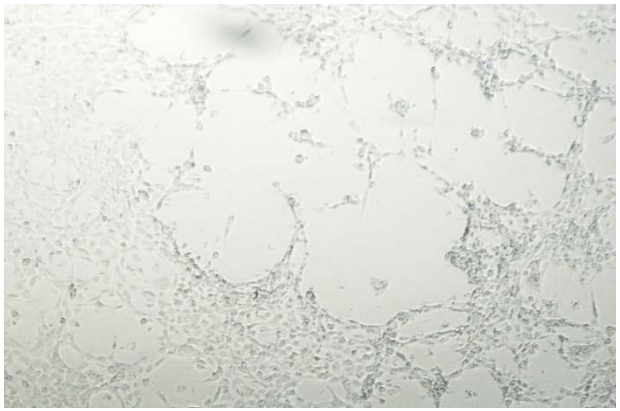
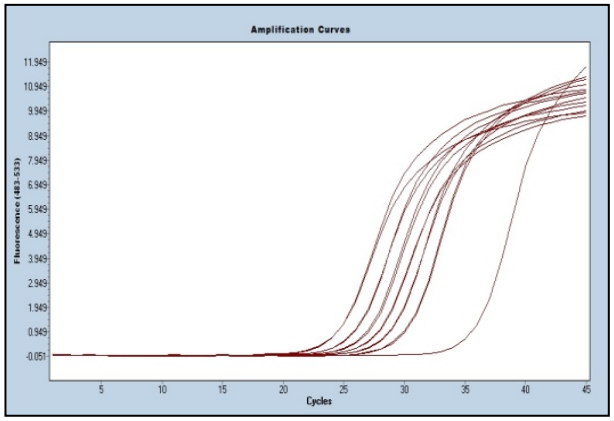
DANAK

PT Reg. no.: 515

Content

- Proficiency test 1, PT1
- Proficiency test 2, PT2
- Feedback from participants
- Proficiency test 2019





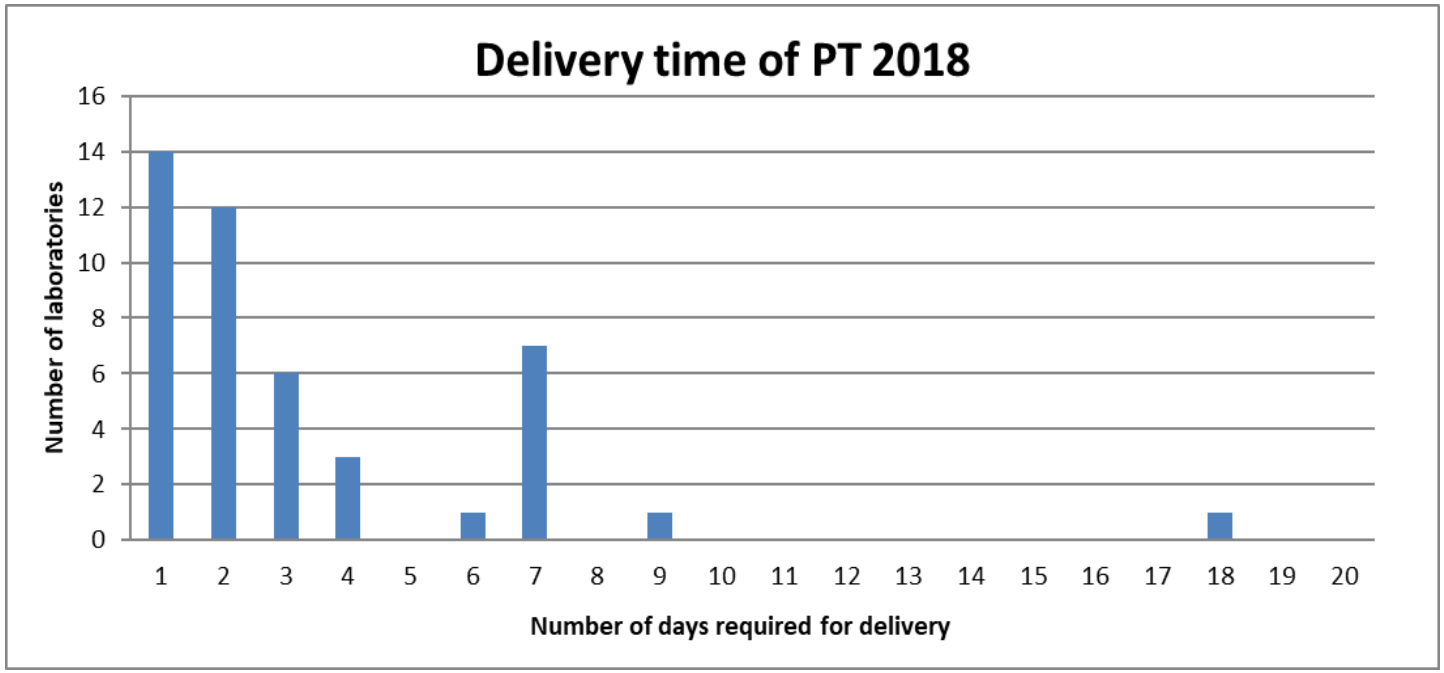
PT1 and PT2 was shipped to 47 laboratories

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



91% 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:	**SVCV strain 56/70 Genotype Id**
Ampoule II:	**IPNV strain Sp Genotype 5**
Ampoule III:	**IHNV - isolate BLK94 American Genotype U**
Ampoule IV:	**EHNV Isolate 86/8774**
Ampoule V:	**VHS virus, DK-3592B Genotype Ia**

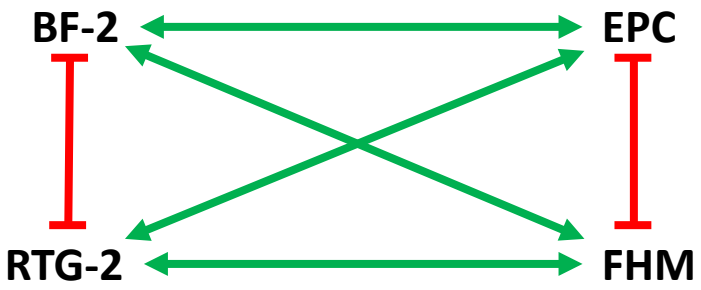
Testing PT1

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

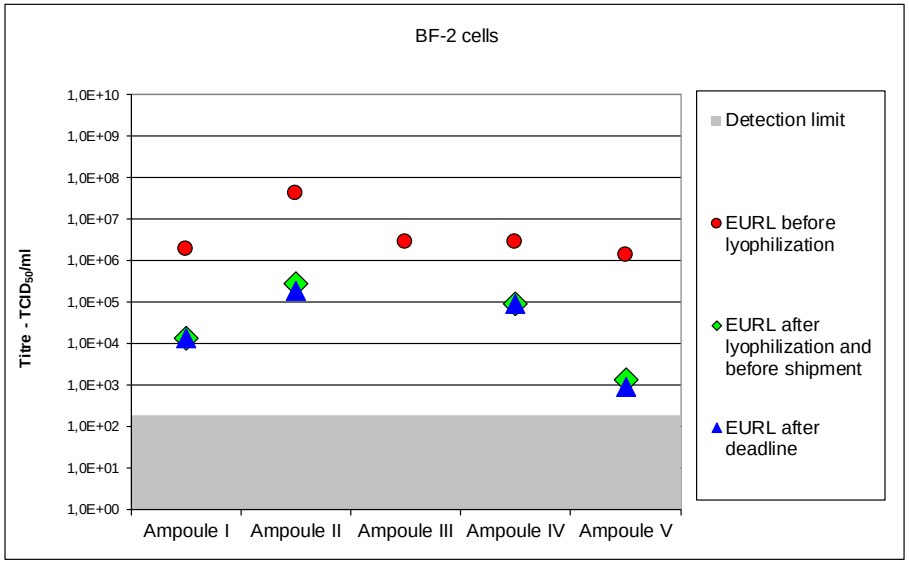
- The reductions of the titres after lyophilisation were between 1-3 log in the same cell line.
  Except for ampoule III (containing IHNV) which was more than 4 log in BF-2 cells

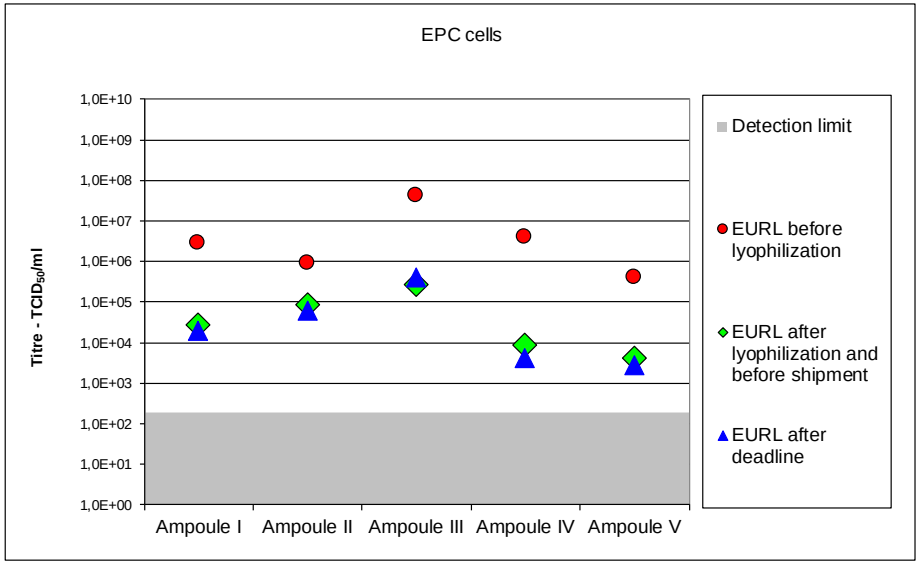
- All titres of the lyophilised viruses were above detection level, except for IHNV on BF-2 cells.

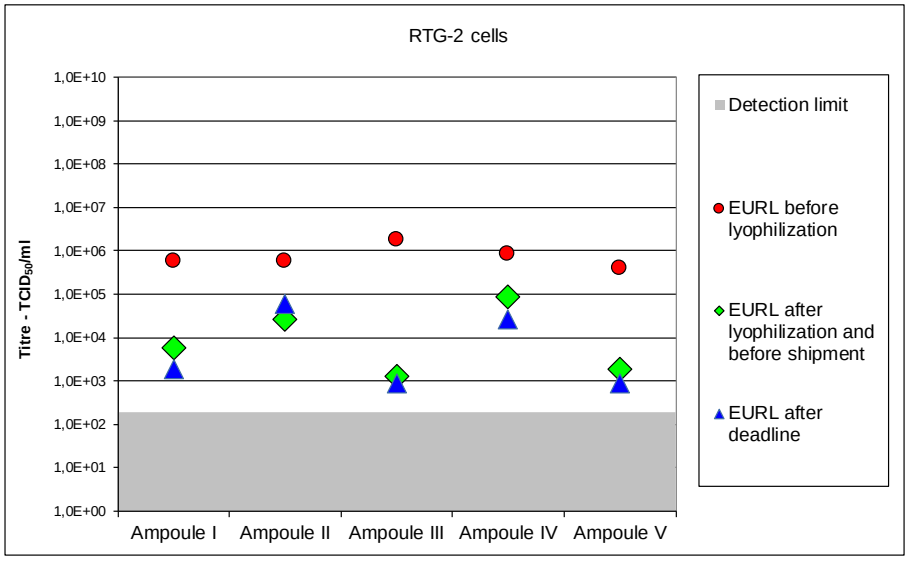
This highlights the importance of using two heterologous cell lines since IHNV would have been detected on one or the other.

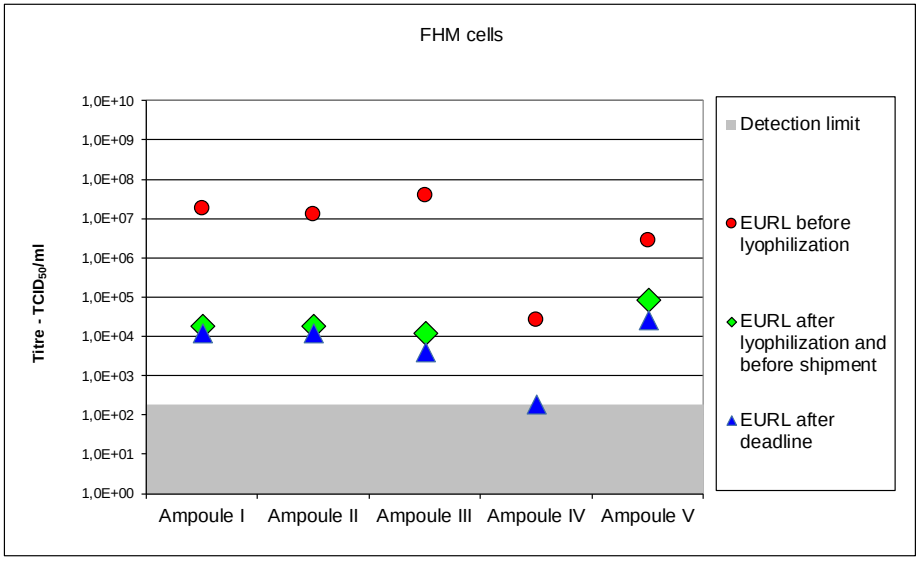


Titers before and after lyophilization

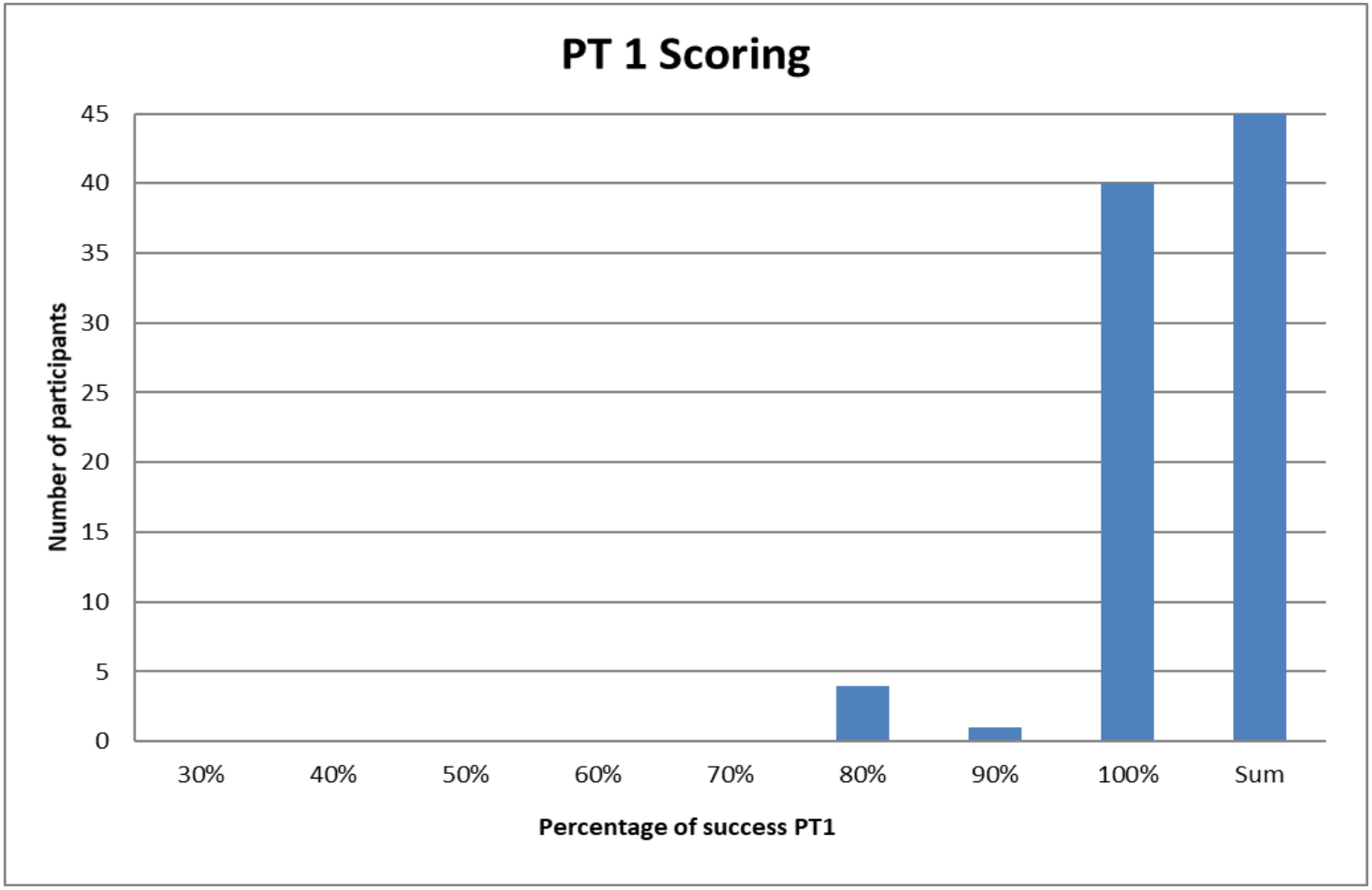








Laboratory scoring, PT1



Genotyping and sequencing – PT1

	Amp. I	Amp. II	Amp. III	Amp. IV	Amp. V
	SVCV Genotype: Id	IPNV Genotype: 5	IHNV Genotype: U	EHNV	VHSV Genotype: Ia
No. of sequence	24	22	32	44	30
No. of correct genotypes	10	12	27	14	26
No. of correct sequences without genotype	14	10	4	30	3
No of incorrect genotype	0	0	1	0	1

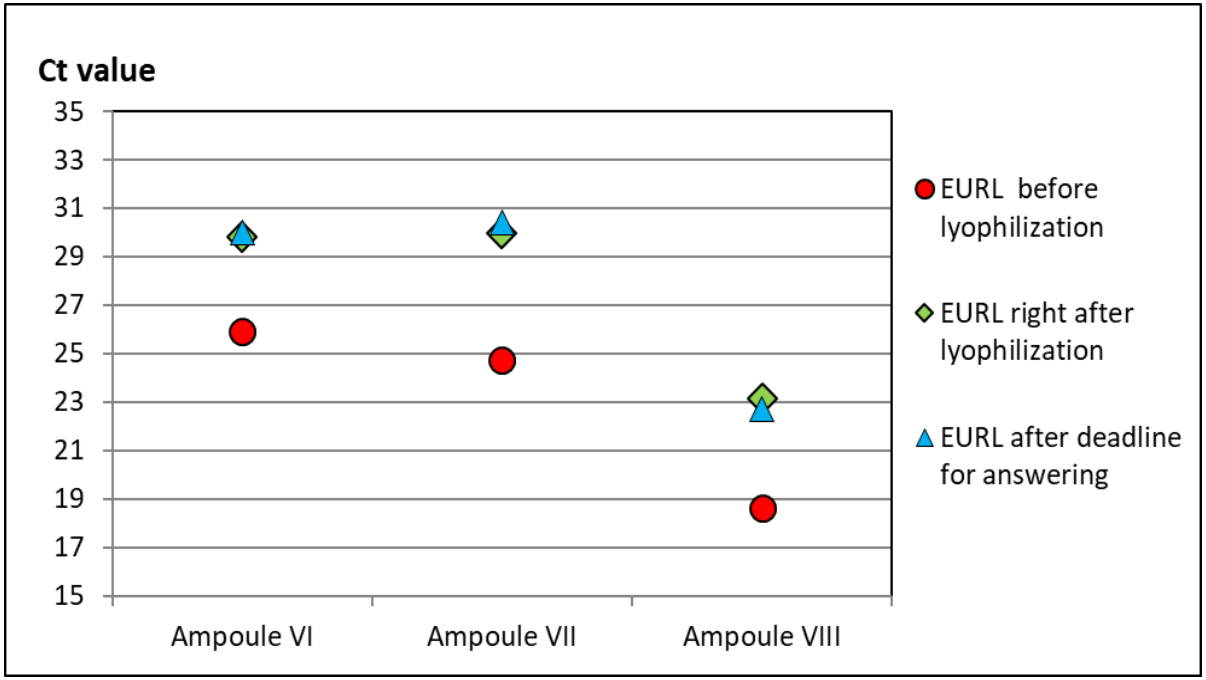
PT-2 Content of ampoules

Four ampoules containing pathogens / lyophilised tissue culture supernatant

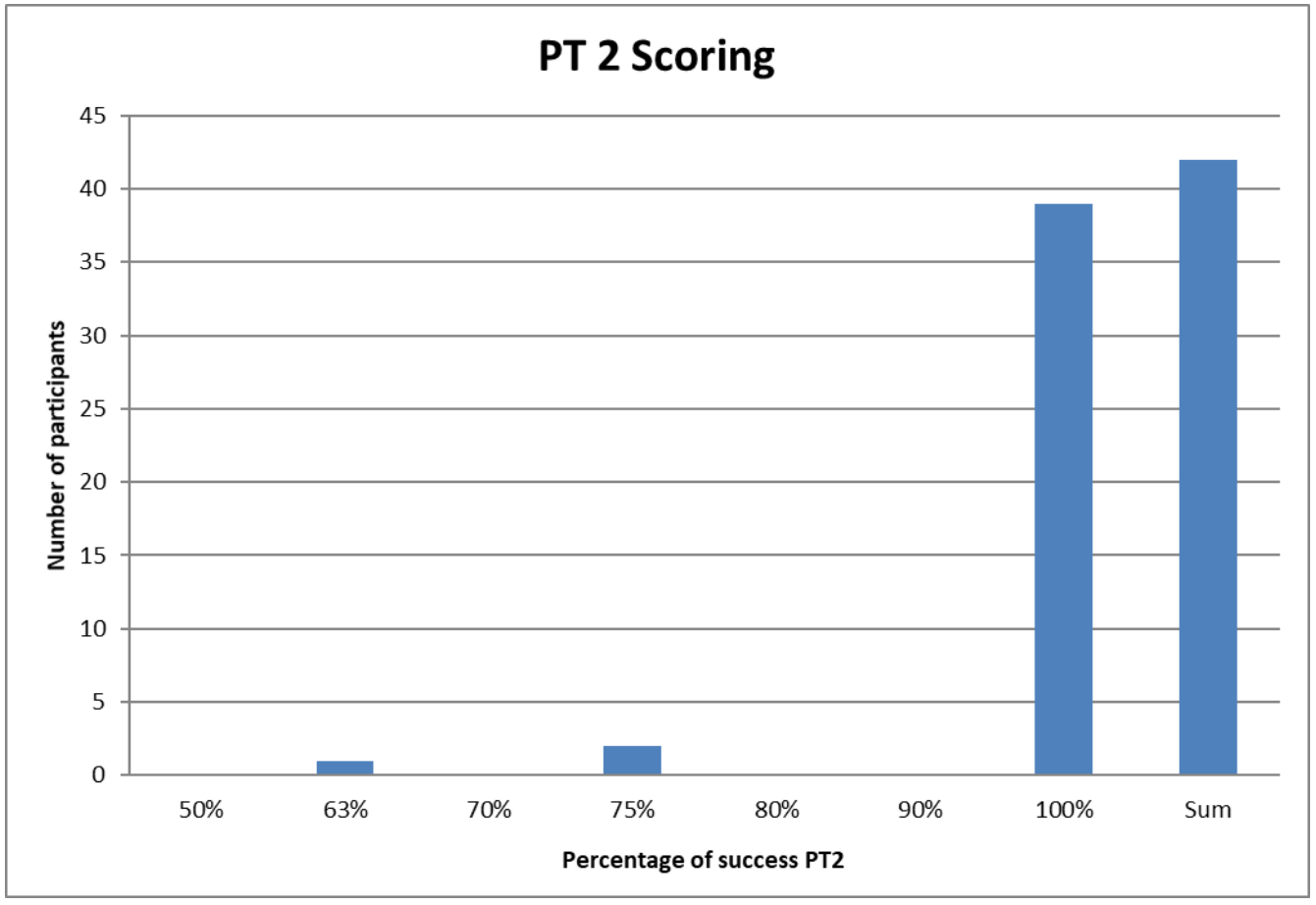
Code	Isolate
Ampoule VI:	**Koi Herpesvirus isolate KHV 1287**
Ampoule VII:	**ISAV Glesvaer/2/90. HPR Genotype: 2**
Ampoule VIII:	**Salmonid alpha virus (SAV) 3, Pancreas Disease Virus (PD)**
Ampoule IX:	**BF-2 NON Infected cell culture Supernatant**

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 4-5 Ct. values.
- Ct values are stable after lyophilisation



Laboratory scoring; PT2



Genotyping and sequencing – PT2

	Amp. VI	Amp. VII	Amp. VIII
	CyHV – KHV 1287 Genotype 3	ISAV HPRΔ HPR Genotype: 2	SAV Genotype 3
No. of sequence	21	32	25
No. of correct genotypes	9	19	19
No. of correct sequences without genotype	12	10	6
No of incorrect genotype	0	3	0

”Underperfomance”

Due to:

- Answering Ranavirus without corroborating the finding with sequence analysis.
- Not identify the viral 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 2018

30 completed questionnaire with feedback were received, they will be included in the documentation for our quality assurance.

A great thanks for support and contribution.

Feedback from participants

- **Including Nodavirus sistematically**

- **Reporting the Ct values recorded by the participating labs along with the assays used.**

- **Specify the corresponding values of titer and cq from the EU reference lab as standard.**

- **PT2 -The example for how to complete the table for tests not performed conflicts with the instructions. For PT2 perhaps not applicable tests could be greyed out or 'N/A' by default. e.g PCR for ISAV N/A as it is an RNA pathogen.**

- **The use of anti-IPNV serum is part of our routine diagnostic protocols when we suspect of notifiable diseases. We used it and we could not titrate nor amplify through qPCR the ampule containing IPNV.**

Feedback - EURL

EURL COMMENTS

1) ISA isolate included shall be sequenced, distinguishing HPRΔ and HPR0

2) Rana isolate included shall be sequenced, distinguishing EHNV from the non listed Ranavirus.

3) Sequencing scoring at Genotype level, NOT at isolate level

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 2019

- Aim: To send out the test in end of September 2019
- 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
- New participant expected (Macedonia)

Acknowledgements

- Christina Flink Desler
- Argelia Cuenca
- Bjørn Hørsvig
- 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:** Dr. B. Dannevig, OIE Reference Laboratory for ISA, Oslo, Norway
- **SVCV-** Received from**:** Prof. Fijan
- **IHNV-** Received from Gael Kurath USGS Western Fisheries Research Center
- **EHNV-Received from:** Prof. Whittington, The OIE reference laboratory for EHN, University of Sidney, Australia.