Inter-Laboratory Proficiency Test 2020 for identification of VHSV, IHNV, EHNV SVCV and IPNV (PT1) and identification of CyHV-3 (KHV), ISAV and SAV (PT2)

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PT1 and PT2 was shipped to 45 laboratories in 2020

All NRL's for Fish Diseases in EU Member States

NRL's in:

Australia

Canada

Faroe Islands

Iceland

Japan

New Zealand

Norway

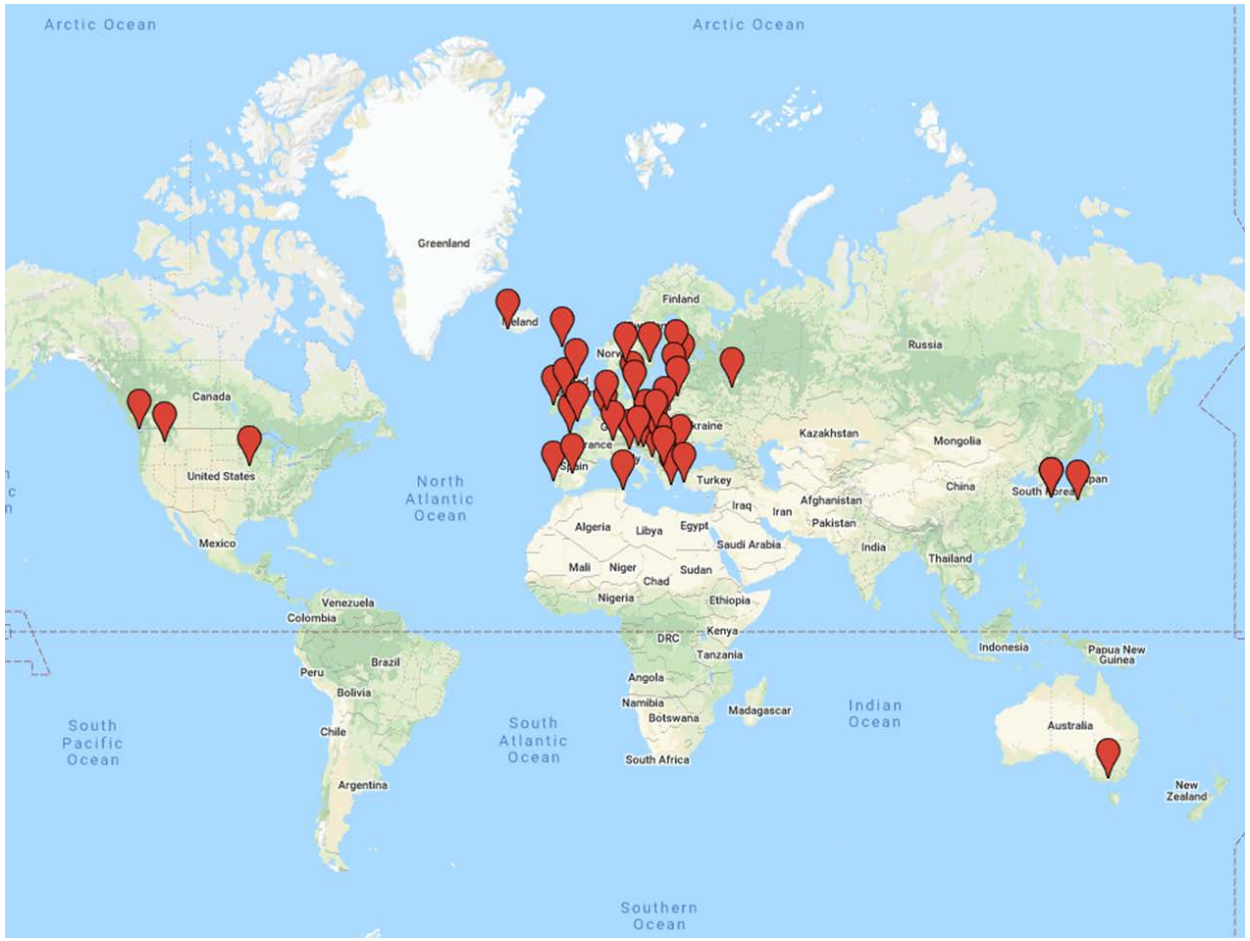
Republic of Korea (2)

Russia

Switzerland

Turkey

USA (2)



PT1: Content of ampoules

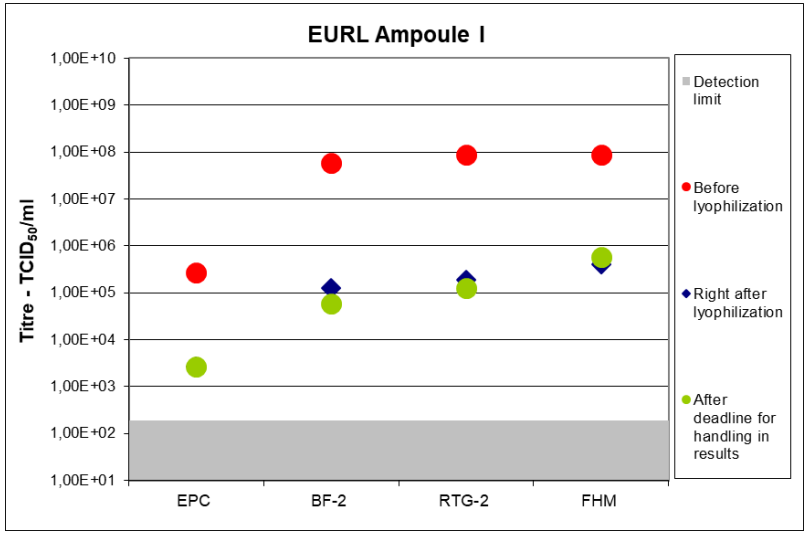
Five ampoules containing virus/ lyophilised tissue culture supernatant

Code	Isolate
Ampoule I:	**VHSV DK-9895174, Genotype Ia**
Ampoule II:	**IHNV 32/87, Genogroup E + IPN Sp, Genogroup 5**
Ampoule III:	**SVCV DK-203273, Genotype 1a**
Ampoule IV:	**Blank**
Ampoule V:	**EHNV 86/8774**

Testing PT1 after freeze drying
Approval of homogeneity

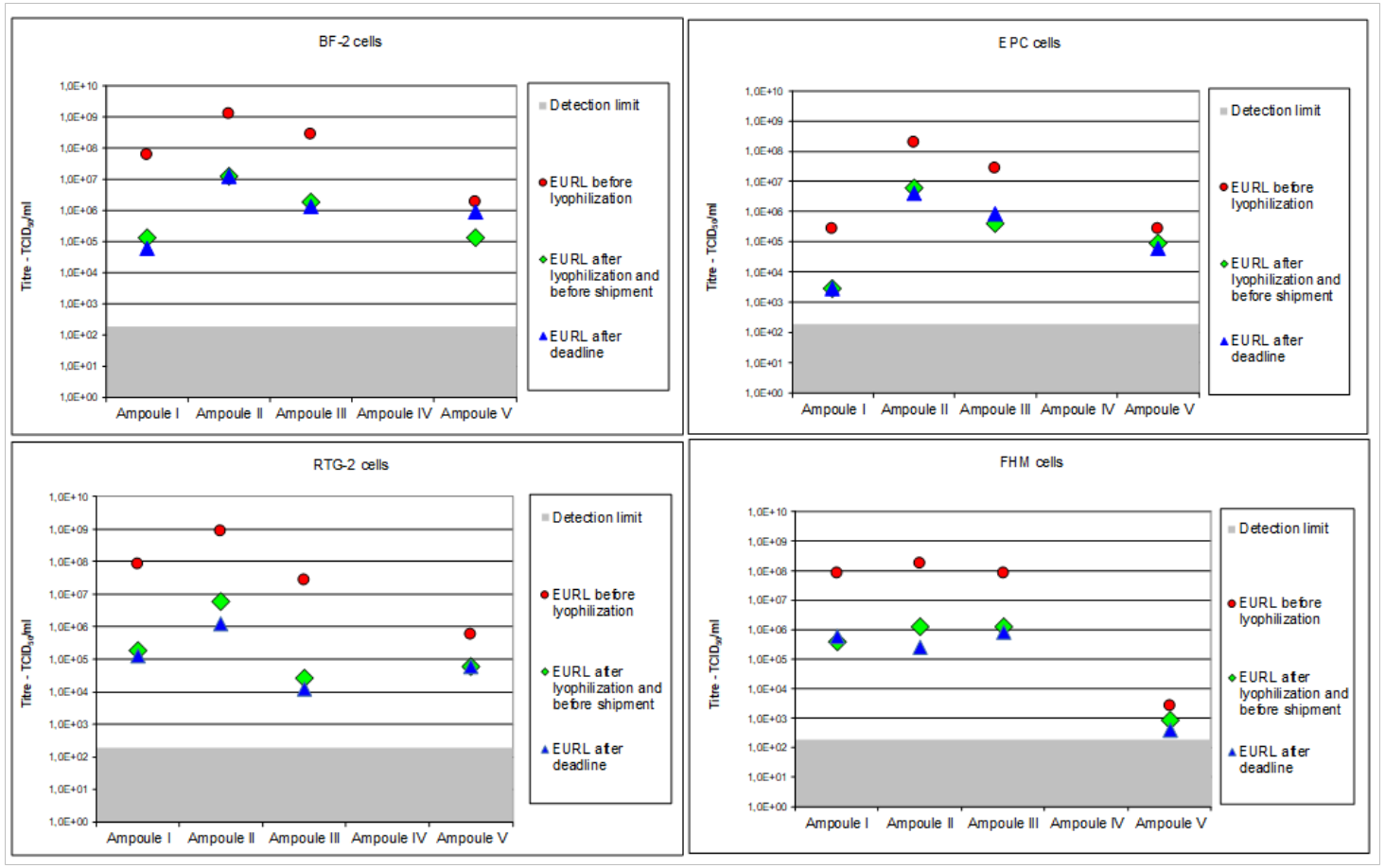
- The reduction of viral titre due to freeze-drying are very variable but only a variation within 1 log of viral titre across the 5 replicates is accepted.

Ampul I	Before lyophilization	Right after lyophilization	After deadline
Cell line			
EPC	2,73E+05	2,73E+03	2,73E+03
BF-2	5,87E+07	1,26E+05	5,87E+04
RTG-2	8,62E+07	1,86E+05	1,26E+05
FHM	8,62E+07	4,00E+05	5,87E+05

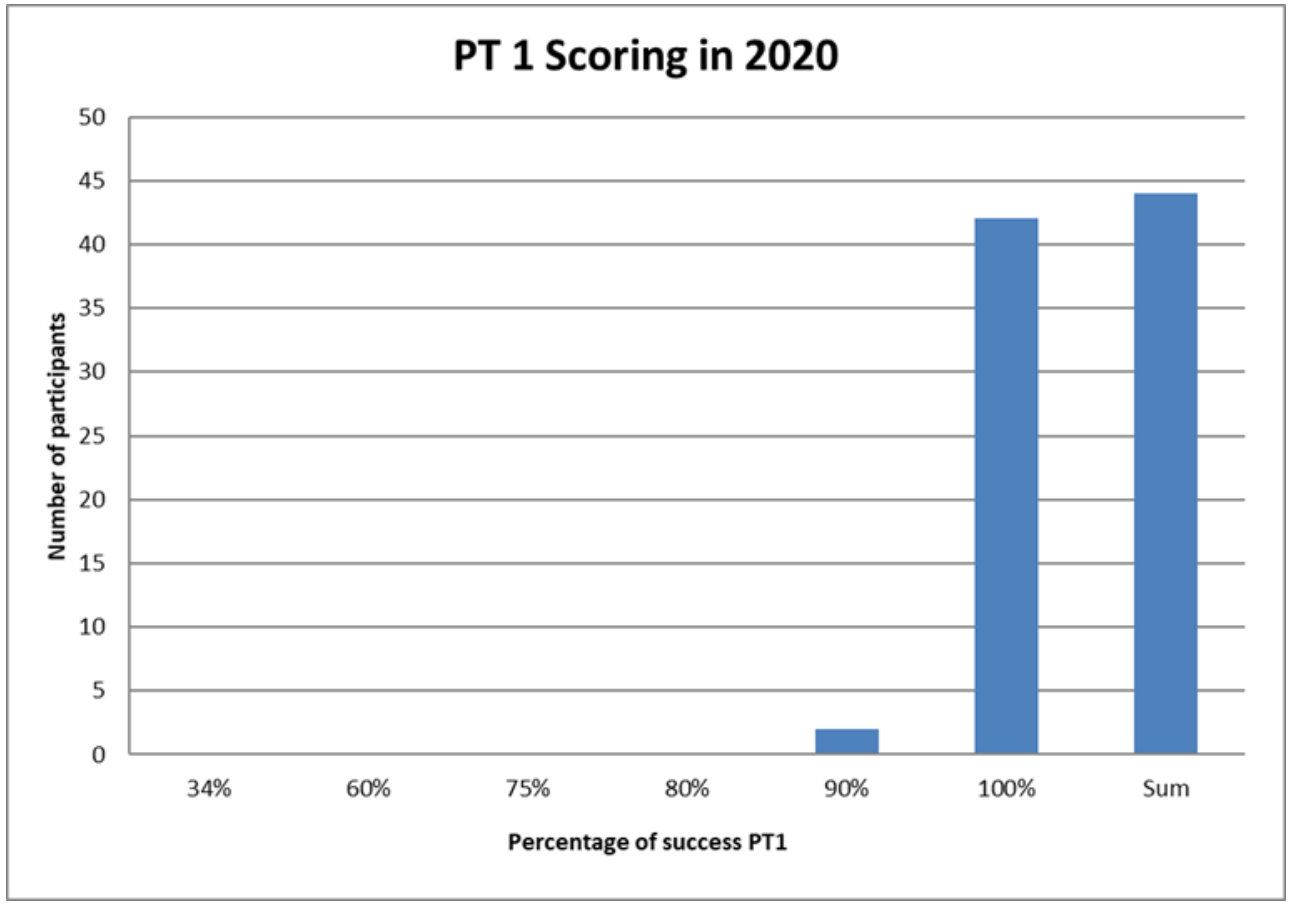


	Pathogen	VHSV
Cell line	Ampoule No. I	
EPC	a	1,3E+03
	b	5,9E+03
	c	2,7E+03
	d	2,7E+03
	e	1,9E+03
	Median	**2,7E+03**
BF-2	a	8,6E+04
	b	1,3E+05
	c	1,9E+05
	d	1,3E+05
	e	4,0E+05
	Median	**1,3E+05**
RTG-2	a	4,0E+05
	b	4,0E+05
	c	1,9E+05
	d	1,3E+05
	e	8,6E+04
	Median	**1,9E+05**
FHM	a	5,9E+05
	b	4,0E+05
	c	1,9E+05
	d	4,0E+05
	e	4,0E+05
	Median	**4,0E+05**

Titers before and after lyophilization



Laboratory scoring, PT1



PT-2 Content of ampoules

Four ampoules containing pathogens / lyophilised tissue culture supernatant

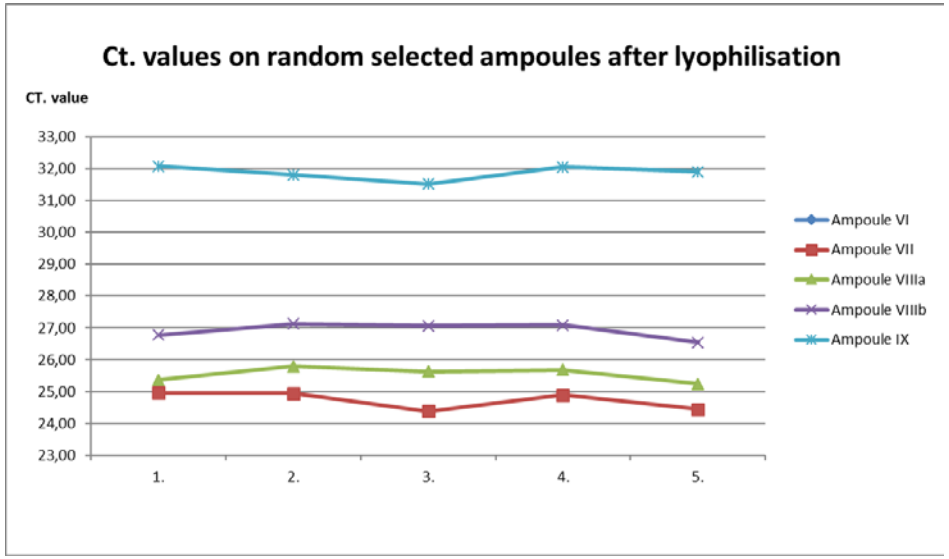
Code	Isolate
Ampoule VI:	Blank
Ampoule VII:	ISAV 390/98, HPRΔ
Ampoule VIII:	Salmonid Alpha Virus (SAV) 3, Pancreas Disease Virus (PD) – Norway-R-1_2007 + ISAV 2016-70-1297_Vir4415, HPRΔ
Ampoule IX:	Koi Herpesvirus, isolate NRIA 0301

Testing PT2 after freeze drying

Each of the 5 replicates are:

- Tested by qPCR/PCR directly from the freeze-dried material for all pathogens included in PT2
- Coefficient of variation (CV) below 0,05 on the 5 replicates is accepted.

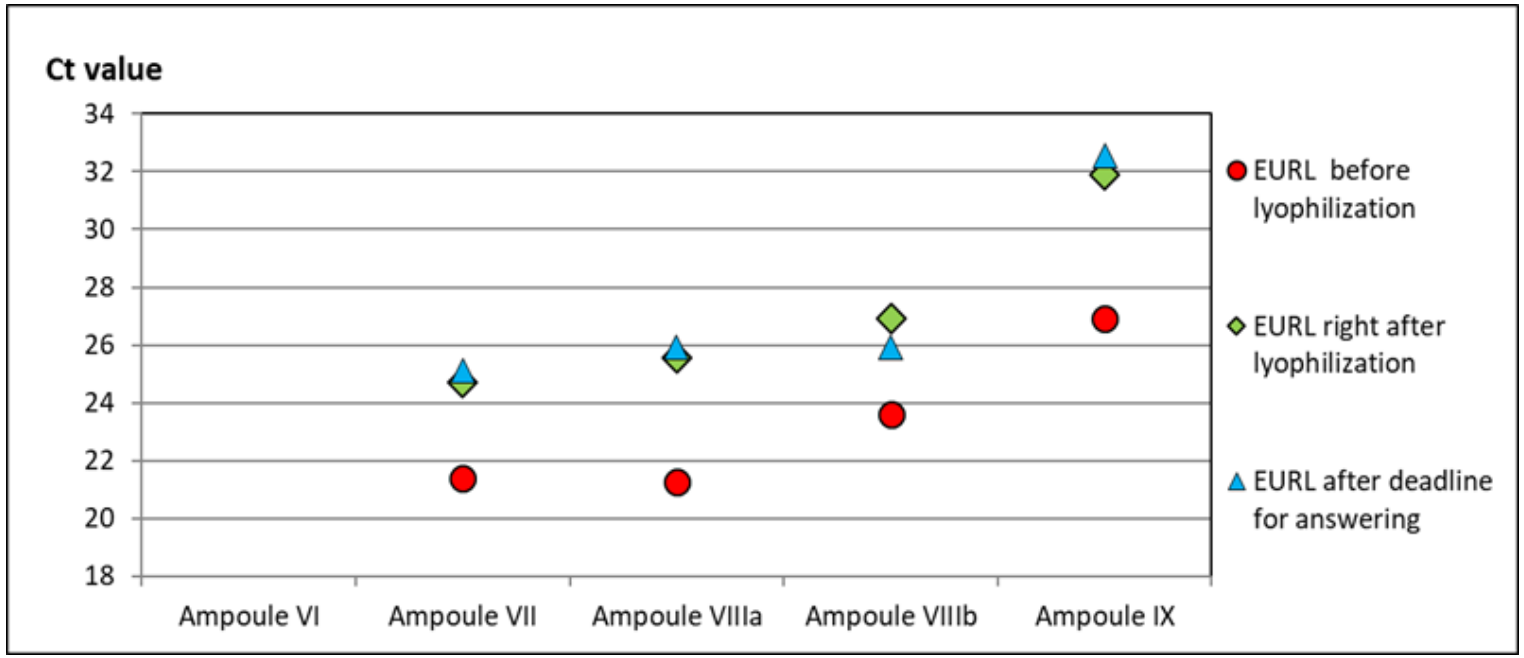
Pathogen	KHV
Ct. value	Amp. IX
a	32,08
b	31,80
c	31,52
d	32,05
e	31,90
Standard deviation (SD)	0,23
Avarages ($\overline{x}$)	31,87
Varritionskoffecient (CV)	0,01



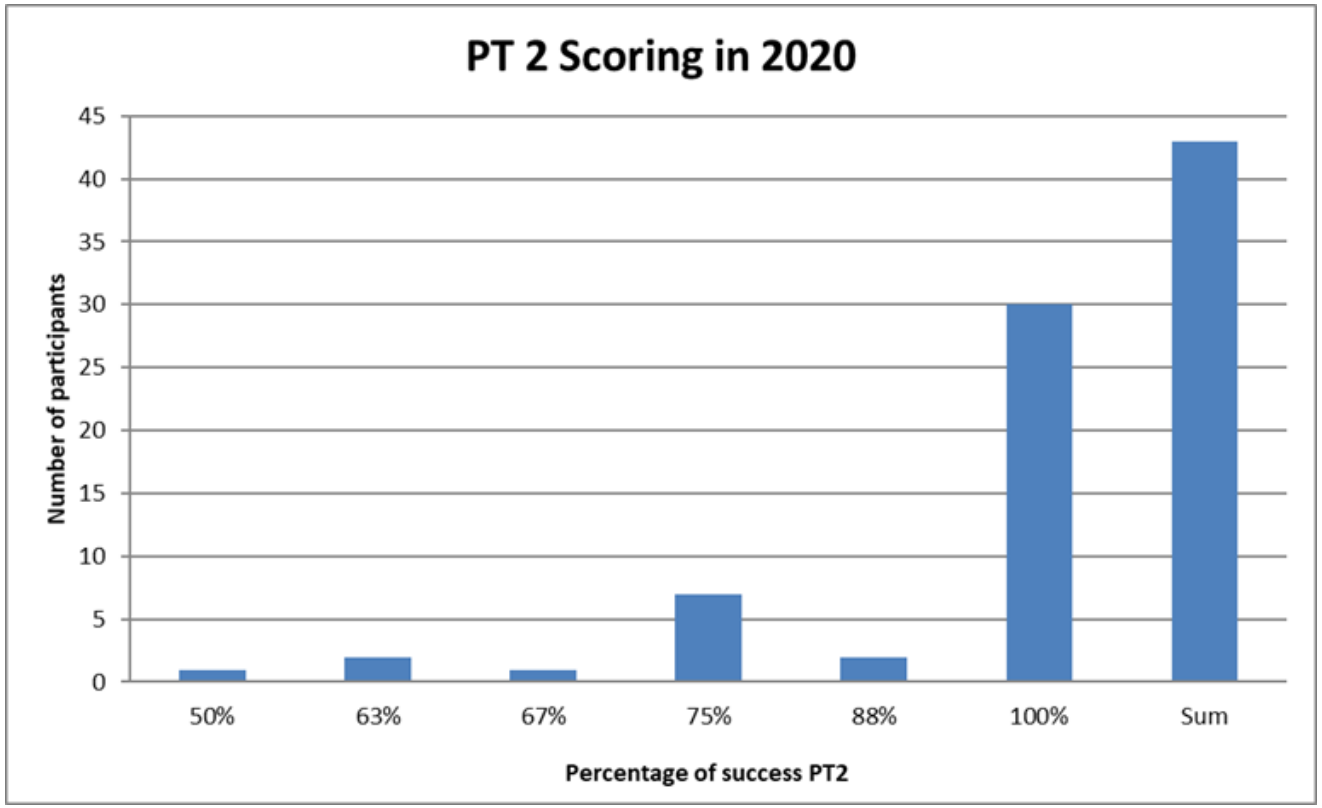
- Isolates are sequenced before freeze drying

Testing PT2

- The lyophilisation procedure can cause a significant virus reduction up to 7 Ct. values.
- Ct values are stable after lyophilisation



Laboratory scoring; PT2



Genotyping and sequencing – PT1

	Amp. I	Amp. II	Amp. III	Amp. IV	Amp. V
	VHSV, Ia	IHNV, E (M) + IPNV, 5	SVCV, Ia	Blank	EHNV
No. of participants performing sequencing	33	35	25		42
No. of participants getting full score	28	23	23		42
No. of correct sequences provided without genotype assigned or incomplete sequence	3	10	0		1
No. of incorrect genotype provided	2	2	2		0

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

Genotyping and sequencing – PT2

	Amp. VI	Amp. VII	Amp. VIII	Amp. IX
	Blank	ISAV, HPRΔ	SAV3 + ISAV, HPRΔ	KHV, CyHV-3
No. of sequences performed		34	33	24
No. of correct genotypes given		29	16	24
No. of correct sequences provided without genotype assigned		3	15	0
No. of incorrect genotype provided		2	2	0

”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.
- Identifying the wrong genotype of the virus

Feedback - EURL

EURL COMMENTS

- PT1- Rana isolate included shall be sequenced, distinguishing EHNV from the non listed Ranavirus otherwise it will cause the loss of one point.

- PT2- ISA isolate included shall be sequenced, distinguishing HPRΔ and HPR0 otherwise it will cause the loss of one point.

- FILLING THE SPREADSHEET IN STANDARDIZED WAY please use the instruction how to fill in the spreadsheet.

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.
- Use same set of cell lines across all ampoules

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		

Correct completing of the Spreadsheets – Concluding Results

Goes for both PT1 and PT2

Ampoule no.	Pathogen	ELISA	IFAT	Neutralisation	Conventional (RT-) PCR	Real-time (RT-) PCR Ct value	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	22.52	+		
	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	+	22.52	+		
	SVCV	not performed	not performed	not performed	-	not performed			

Correct completing of the Spreadsheets – Concluding Results

PT2

Ampoule No.		PCR	Real-time PCR	Ct. - value (Real-time PCR)	RT-PCR	Real-time RT-PCR	Ct. - value (Real-time RT-PCR)	Sequencing: Fill in the information on the sheet regarding "Sequencing results"	Other - specify:	Concluding Result
Ampoule VI	ISAV	N/A	N/A	N/A	+	+	23,70	+	N/A	ISAV HPR deleted
	KHV	-	-	-	N/A	N/A	N/A	-	N/A	
	SAV	N/A	N/A	N/A	N/A	-	-	-	N/A	

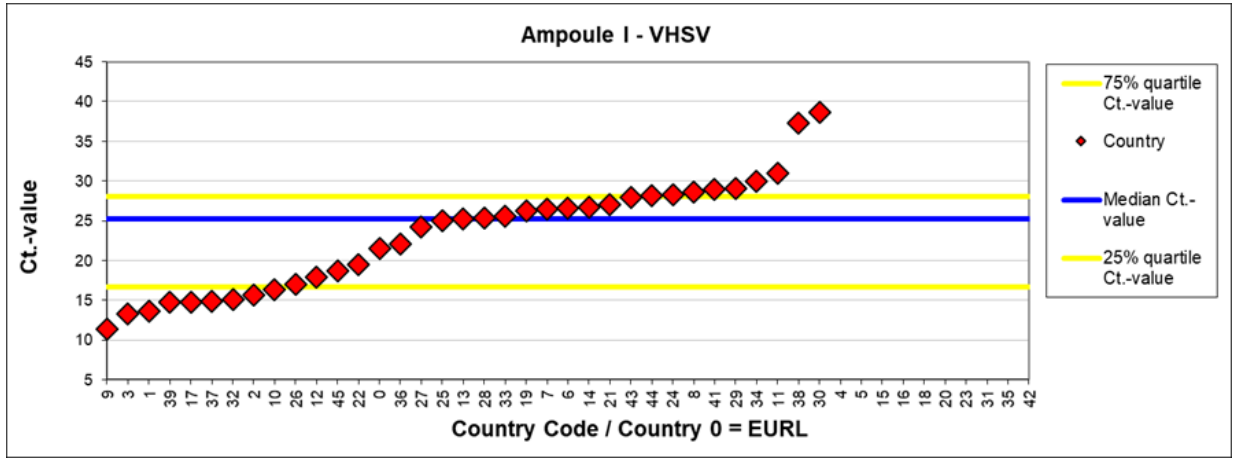
Fill in the correct ISA type ↑

Not only ↓

Ampoule No.		PCR	Real-time PCR	Ct. - value (Real-time PCR)	RT-PCR	Real-time RT-PCR	Ct. - value (Real-time RT-PCR)	Sequencing: Fill in the information on the sheet regarding "Sequencing results"	Other - specify:	Concluding Result
Ampoule VI	ISAV	N/A	N/A	N/A	+	+	23,70	+	N/A	ISAV
	KHV	-	-	-	N/A	N/A	N/A	-	N/A	
	SAV	N/A	N/A	N/A	N/A	-	-	-	N/A	

New in Proficiency test 2020

- In 2020 we have compare Ct.-values from the participating laboratories for both PT1 and PT2.
- The Ct values cannot be directly compered due to the use of different methods, reagents and equipment for nucleic acid extraction and (RT)-qPCR. Additionally, for PT1 some participants may have tested directly from the ampoule provided whereas others used cell supernatant from inoculation on cell culture.
- The difference stand out distinctly in PT1



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 CCCTGCCAGACTCA TTGGTCCA 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 HPR-deleted	HA gene Mjaaland et al (2002). Virology, 304:379-391 Klon1EGFP-F1 5'-GGGCTAGCATGGCACGATTCATAATT-3' Klon1EGFP-R1 5'-GGGGTACCGTAGCAACAGACAGGCTCGA	**HPR deleted**	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 F72b/02; Isolate from Nova Scotia, belonging to the EU-F subgroup within the EU-G2

Genotypes to be used

To be inserted in "Concluding results" for Ampoule I-IX	To be inserted in "Genotype" for Sequencing results.
VHSV	I (a-e), II, III, IV (a-d)
IHNV	U, M, L, E, J
EHNV	EHNV
Ranavirus – NOT EHNV	Not EHNV
SVCV	Genogroup 1 (a-d), 2, 3, 4
Birnavirus II (Telinavirus)	-
Perch Rhabdovirus	-
IPNV	Genogroup 1, 2, 3, 4, 5 Optionally: within genogroup 1 – genotype (1-4)
HPR-deleted ISAV or HPR0 ISAV	HPR-deleted / HPR0
KHV	CyHV (1-3)
SAV	1, 2, 3, 4, 5, 6

The freeze-drying procedure

- Looking into changing to ampoules pre-scored

Acknowledgements

- Christina Flink Desler
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- 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
- **EHNV-Received from:** Prof. Whittington, The OIE reference laboratory for EHN, University of Sidney, Australia
- **IHNV-Received from:** Pierre de Kinkelin - ANSES