National Veterinary Institute

Technical University of Denmark

DTU

European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Copenhagen

Inter-Laboratory Proficiency Test 2017 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 2017

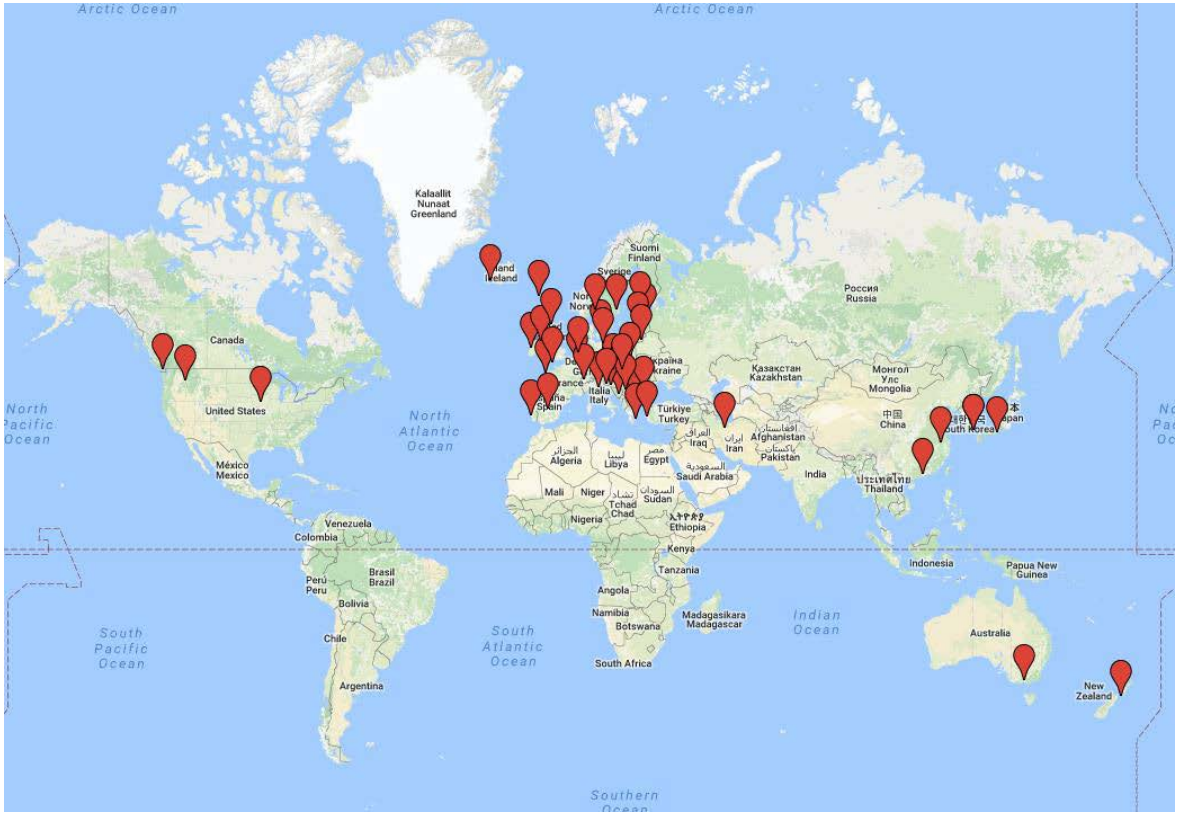


PT1 and PT2 was delivered to 45 laboratories

All NRL's for Fish Diseases in EU Member States

NRL's in:

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



In 2018 expected to participate Chile and Russia

National Veterinary Institute

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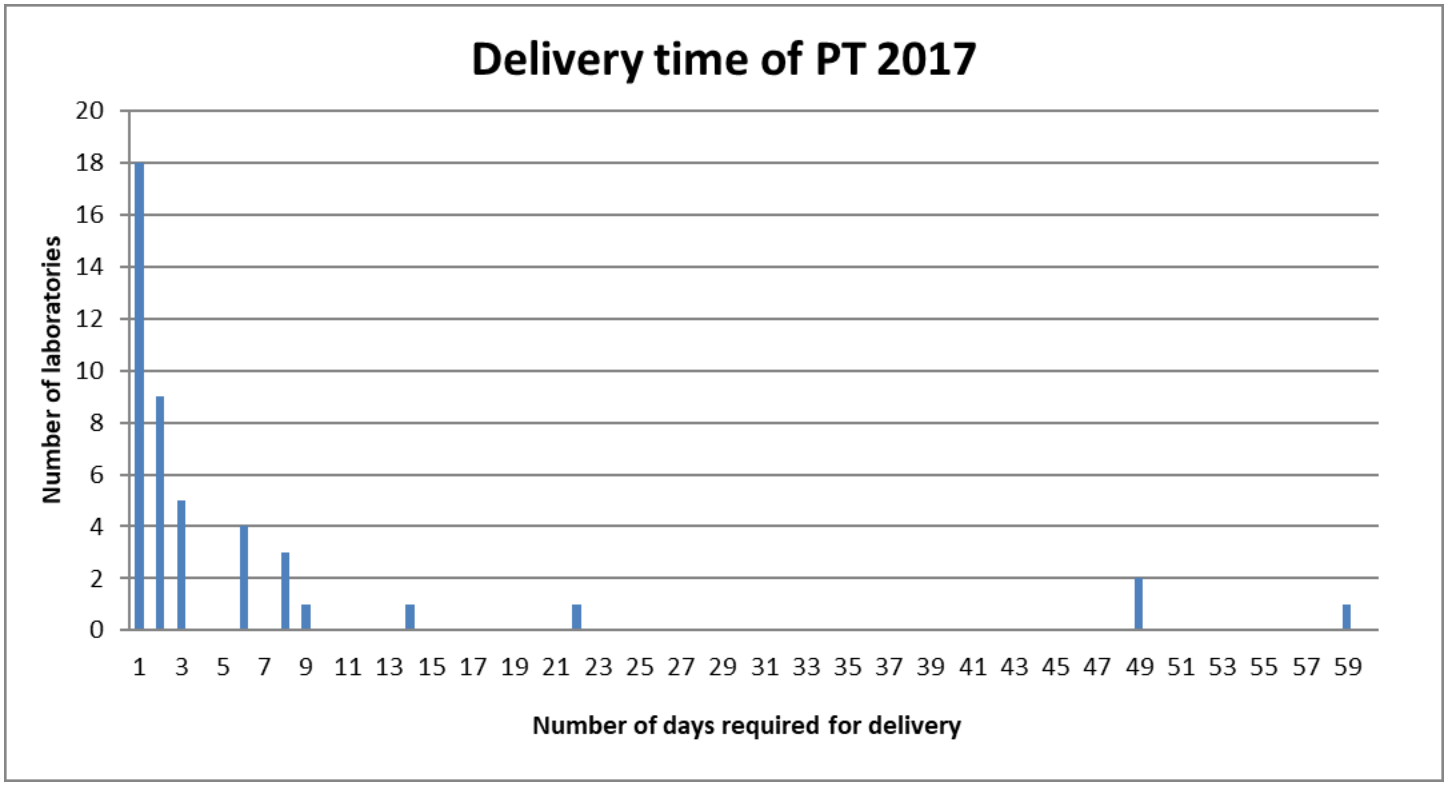
Distribution of PT1 and PT2



Within one day, the tests were delivered to 18 participants; 18 more tests were delivered within the first week; 5 more within the first two weeks; 1 further within four weeks; due to delivery problems in the receiving countries 3 tests were 7 – 9 weeks in transit. All the parcels were sent without cooling elements.

PT1: Content of ampoules

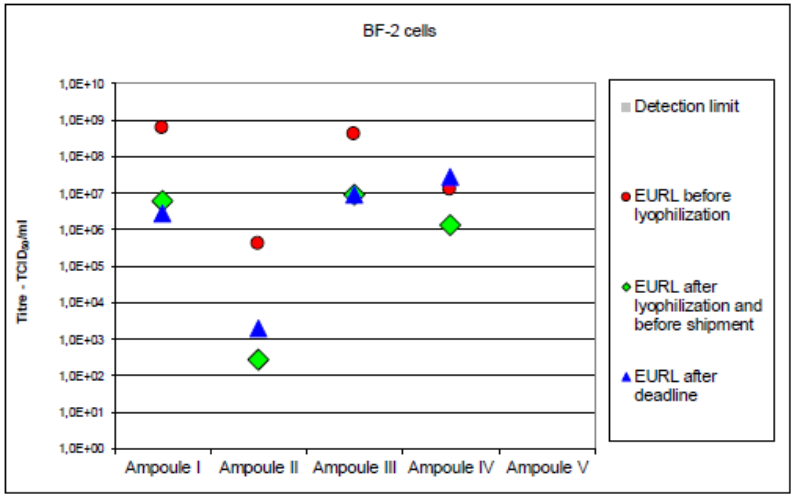
Five ampoules containing virus/ lyophilised tissue culture supernatant

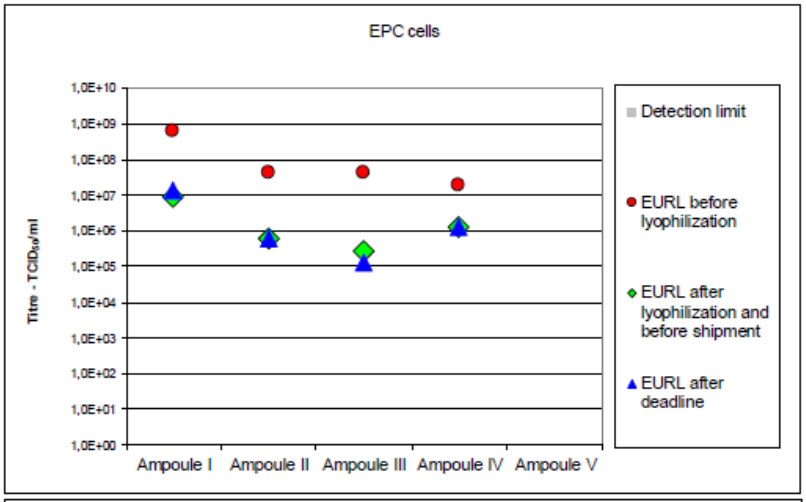
Code	Isolate
Ampoule I:	**VHSV strain DK-6137** (Ia)
Ampoule II:	**IHNV 217/A**
Ampoule III:	**VHSV - Isolate TR-WS13G (= TR-SW13G)** (Ie)
Ampoule IV:	**Ranavirus ECV: European catfish virus isolate 562/92.**
Ampoule V:	Sterile cell culture supernatant from BF-2 cells

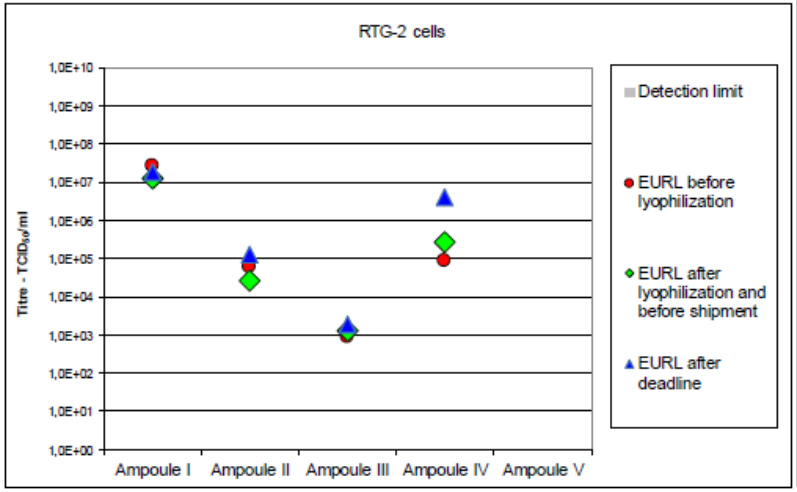
Testing PT1

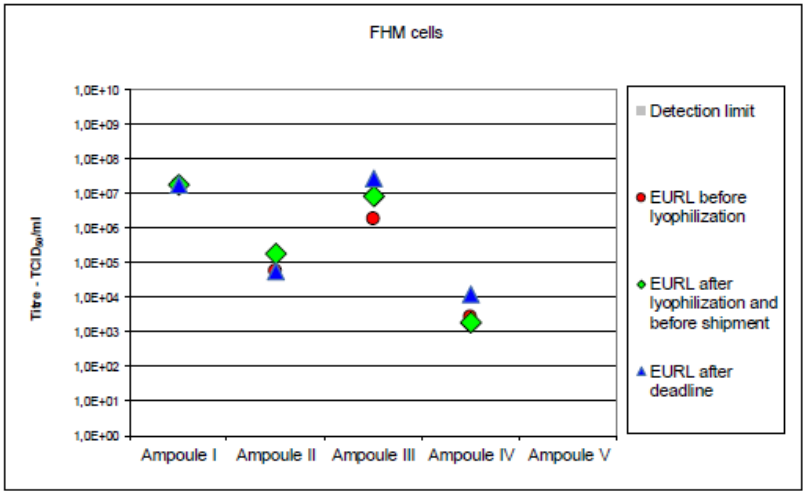
- The proficiency test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043
- The titre and homogeneity of the samples was tested prior to sending out the test by *titration of 5 ampoules of each virus preparation in 4 cell lines.*
- The identity of the virus in the 5 ampoules was checked by ELISA, IFAT, PCR and serum neutralisation.
- The lyophilisation procedure caused a significant titre reduction for IHNV with 1-2 log reduction, while for VHSV, IPNV, SVCV and EHNV almost no reduction was observed.
- All titres of the lyophilised viruses were above detection level, except for IHNV on BF-2 cells. As participants, however, are expected to use at least two different cell lines, IHNV would have been detected on the other cell line.

Titres before and after lyophilization









PT1

- Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in Commission Decision 2015-1554

The proficiency test was designed to primarily assess the ability of participating laboratories to **identify any of the fish viruses VHSV, IHNV and to be able to discriminate between the exotic listed EHNV from other ranaviruses(Council Directive 2006/88/EC Annex IV part II and Commission Implementing Directive 2014/22/EU of 13 February 2014).** Furthermore the interlaboratory proficiency test is also suitable for maintaining accreditation for identification of SVCV, and IPNV; participants have to consider that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses).

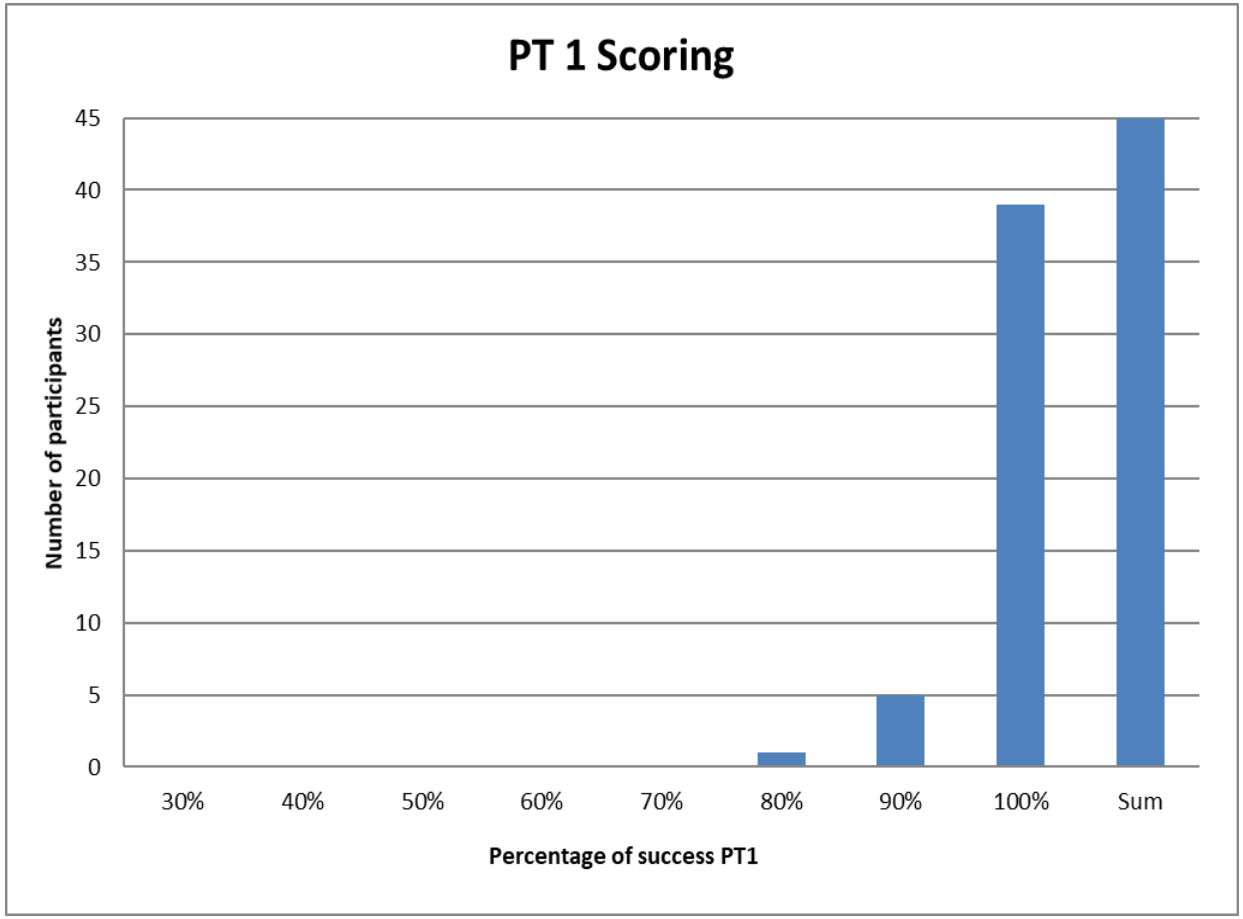
Genotyping and sequencing

Laboratory code number	Ampoule I VHSV DK-6137 Genotype Ia	Ampoule II IHNV 217/A Genotype M	Ampoule III VHSV TR-WS13G Genotype Ie	Ampoule IV ECV 562/92
1	1a	*	1e	*
2	No sequence provided	No sequence provided	No sequence provided	Do not test for Ranavirus
3	1a	E	1e	*
4	Ia	M	Ie	*
5	Ia	E	Ie	*
6	Europe Ia	Europe E	Europe Ie	*
7	No sequence provided	No sequence provided	No sequence provided	*
8	*		*	*
9	No sequence provided	BLAST analysis of the sequence obtained showed highest sequence identity with accession number: FJ265710.1	No sequence provided	BLAST analysis of the sequence obtained showed highest sequence identity with accession number: KT989884.1
10	No sequence provided	No sequence provided	No sequence provided	Do not test for Ranavirus
11	1e	M	1a	*
12	Ia1	E	Ie	ECV
13	Ia	*	Ie	ECV, ESV
14	*	*	*	*
15	*	*	*	*
16	1a	M	1e	Not EHNV
17	1a	M	3	ECV
18	Ia	E (M)	Ie	Cod iridovirus, ECV or ESV
19	*	*	*	*
20	VHSV:Ia SVCV:Ia	M	Ib	ECV
21	*	*	*	*
22	No sequence provided	No sequence provided	No sequence provided	*
23	No sequence provided	No sequence provided	No sequence provided	*
24	No sequence provided	No sequence provided	No sequence provided	No sequence provided
25	genotype Ia	genogroup M	genotype Ie	*

Genotyping and sequencing

Laboratory code number	Ampoule I VHSV DK-6137 Genotype Ia	Ampoule II IHNV 217/A Genotype M	Ampoule III VHSV TR-WS13G Genotype Ie	Ampoule IV ECV 562/92
26	*	*	*	*
27	Ia	European isolates (E–1–I–1)	Ie	*
28	Genotype Ia	Genotype E (Europe)	Genotype Ie	*
29	Ia genotype	M genogroup	Ie genotype	*
30	Genotype 1a	Genotype J	Genotype 1e	*
31	Ia	M	Ie	*
32	No sequence provided	No sequence provided	No sequence provided	*
33	No sequence provided	No sequence provided	No sequence provided	*
34	Ia	*	Genotype Ie	*
35	1a	M	1e	Not EHNV
36	No sequence provided	*	No sequence provided	European catfish virus (same virus as European sheatfish virus)
37	I	*	I	*
38	VHSV Genotype Ia	IHNV Genotype M	VHSV Genotype Ie	ECV/ESV
39	Ia	M	Ie	European, Italy, Hungary
40	No sequence provided	No sequence provided	No sequence provided	ECV/ESV
41	No sequence provided	No sequence provided	No sequence provided	Do not test for Ranavirus
42	No sequence provided	No sequence provided	No sequence provided	No sequence provided
43	Ia	E	Ie	*
44	*	*	Ie	*
45	I - Ia	M	I - Ie	*

Laboratory scoring, PT1



Any comments/questions to PT1?

PT-2 Content of ampoules

Four ampoules containing pathogens / lyophilised tissue culture supernatant

Code	Isolate
Ampoule VI:	**Salmonid alpha virus (SAV) 6, Pancreas Disease Virus (PD)** Ireland F104596
Ampoule VII:	**ISAV Glesvaer/2/90**
Ampoule VIII:	**BF-2 NON infected cell culture supernatant**
Ampoule IX:	**Cyprinid herpes virus 3 CyHV-3 – isolate KHV-TP 30 (syn: KHV-T (for Taiwan))**

PT2 Virus identification participating laboratories

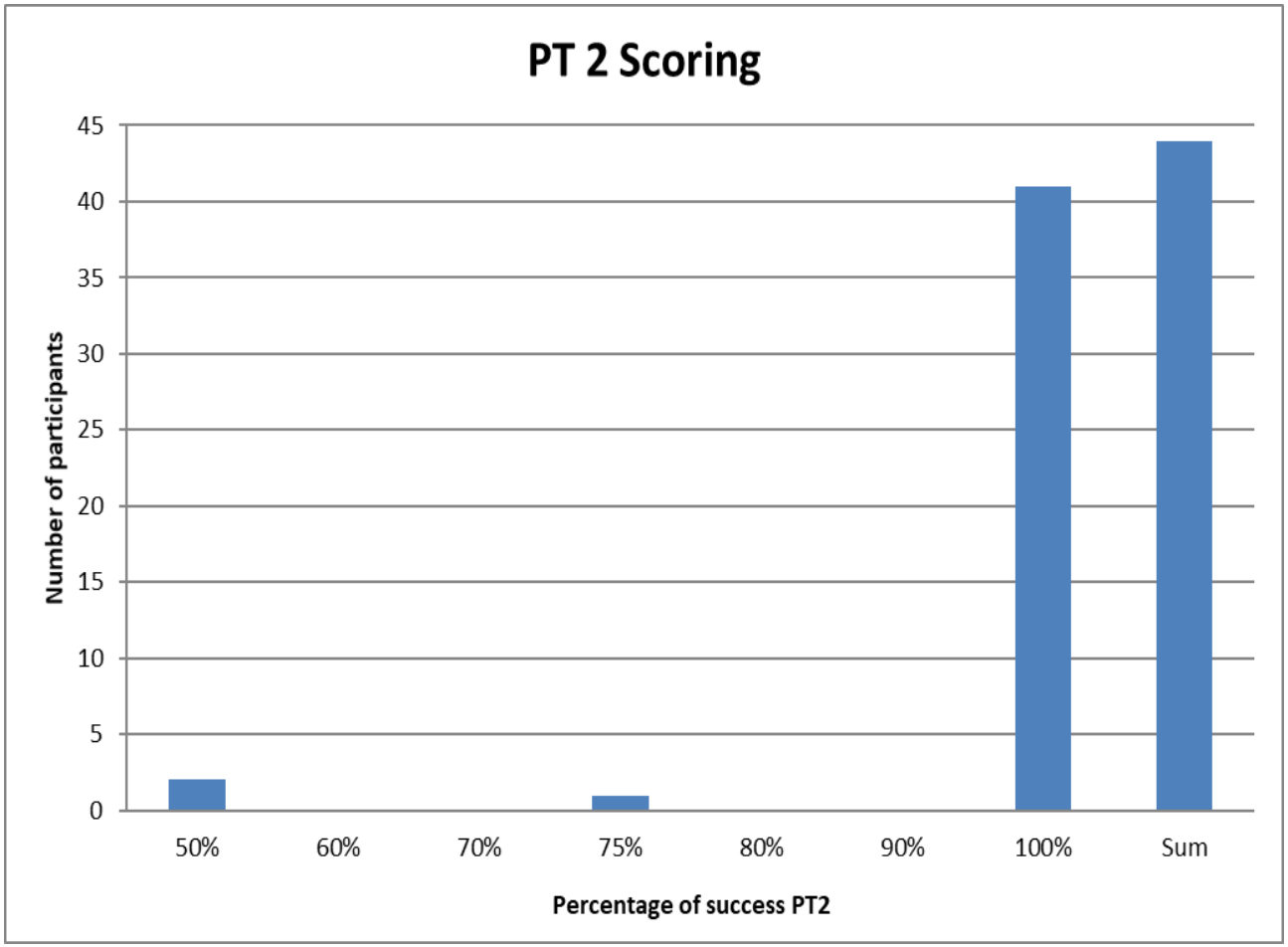
Genotyping and sequencing

Laboratory code number	Ampoule VI	Ampoule VII	Ampoule IX
	SAV SAV-6, PD	**ISAV Glesvaer/2/90 HPR Genotype: 2**	**KHV CyHV-3**
1	*	HPR2	*
2	Did not test for SAV	N/A	N/A
3	SAV6	EU-G2	N/A
4	*	HPR2	*
5	N/A	Genotype HPR deleted	N/A
6	SAV 2 FW (SD) (according to OIE)	2	*
7	N/A	N/A	N/A
8	N/A	N/A	N/A
9	N/A	N/A	Did not test for KHV
10	Did not test for SAV	Did not test for ISAV	N/A
11	SAV6	HPR deleted, genotype 2	CyHV-3
12	type VI	N/A	CyHV-3
13	VI	PR4	N/A
14	Did not test for SAV	*	*
15	Did not test for SAV	N/A	N/A
16	N/A	HPR Genotype 2	N/A
17	subtype 6	G2 (HPR/deleted)	N/A
18	Subtype 6	HPR deleted	CyHV3
19	*	*	*
20	*	HPR deleted	*
21	SAV6	*	N/A
22	Did not test for SAV	Genotype 2	N/A
23	N/A	N/A	N/A
24	N/A	N/A	N/A
25	SAV6	ISAV4	*

Genotyping and sequencing

Laboratory code number	Ampoule VI SAV SAV-6, PD	Ampoule VII ISAV Glesvaer/2/90 HPR Genotype: 2	Ampoule IX KHV CyHV-3
26	*	*	*
27	*	HPR-2	*
28	*	HPR deleted(HPR2)	*
29	SAV 6 (PD)	ISAV (HPR2)	CyHV 3
30	Subtype VI	HPRdeleted	N/A
31	*	*	N/A
32	N/A	N/A	N/A
33	N/A	N/A	N/A
34	*	*	KHV
35	Type 6	HPR Type 2	CyHV-3
36	N/A	European, HPR2	N/A
37	*	*	*
38	SAV Genotype VI	ISAV HPR2	KHV
39	SAV6	HPR Genotype: 2	Asian
40	N/A	N/A	N/A
41	Did not participate in PT2		
42	N/A	N/A	N/A
43	VI	HPR2	A1
44	Did not test for SAV	*	*
45	PD	*	*
No. of sequence preformed	26	31	22
No. of correct genotyping	16	20	-

Laboratory scoring; PT2



Any comments/questions to PT2?

Feedback 2017

Participant feedback form following the Inter-Laboratory Proficiency Test 2017 - PT1 & PT2			
In order to ensure a high quality of future inter-laboratory proficiency tests, we would like if you could provide us with feedback on the tests shipped in 2017, PT1 and PT2. Therefore, if you have any comments, please fill it in.			
Name of the National Reference Laboratory:			
Work area		**Specific points to be adressed**	**Reply**
Concerning the ampoules that you received:	1	Were they received safely and under proper conditions?	
	2	Were there enough time to perform the test?	
	3	Were instructions clear?	
	4	Were you able to use daily diagnostic procedures to analyse the content?	
	5	Any other comments?	
Concerning results and report?	6	Was it convenient for you to use the spreadsheet for submission of results?	
	7	Was the report straightforward to understand?	
	8	Was it easy to assess how you performed compared to other participants?	
If you have any other comments please fill in below:	9	Comments	

OBS. The questionnaire has been delivered 25.05.

Feedback 2017

OBS. The questionnaire has been delivered 25.05.

In 5 days 26 feedback (!) were received

2 years ago 23 were received ☺

A great thanks for support and contribution in very short time

Feedback- PARTICIPANTS COMMENTS

1) 2 comments require more time to run the whole test

2) 1 comment asked for including Nodavirus sistematically

Feedback- EURL COMMENTS

1) Sequencing scoring, on separate table. At **GENOTYPE** level, NOT at isolate level

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

3) New letter with instructions including remarks on sharing results before ampoule content is disclosed from EURL

”underperfomance”

It has been a concern that two laboratories has identified the correct virus but not in the right ampoule, meaning that some mistake in traceability of the ampoules during the working flow procedure has occurred.

A contamination in this step will be carried along the whole testing conducting to wrong answer for the ampoules involved.

Traceability of each samples and proper separation between different ampoules is necessary to achieve good performance in the test.

Ampoule may contain high titered viruses, so disinfection and appropriate procedures are necessary

Proficiency test 2018

- Aim: To send out the test in end of September 2018
- 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 (with option to opt in and out)

Acknowledgements

- Teena Vendel Klinge
- Christina Flink Desler
- Betina Lynnerup

- ISAV: OIE reference laboratory, Oslo, Norway, Birgit Dannevig

- KHV: Institute of Medical Biotechnology, Central Taiwan University of Science and Technology, Dr. Peiyu Lee and Friedrich-Loeffler-Institut (FLI), Sven M. Bergmann