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 2016

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

Niels Jørgen Olesen, Niccolò Vendramin and Teena Vendel Klinge

DANAK

PT Reg. no.: 515

Content

- Proficiency test 1, PT1
- Proficiency test 2, PT2
- Feedback from participants
- Proficiency test 2016

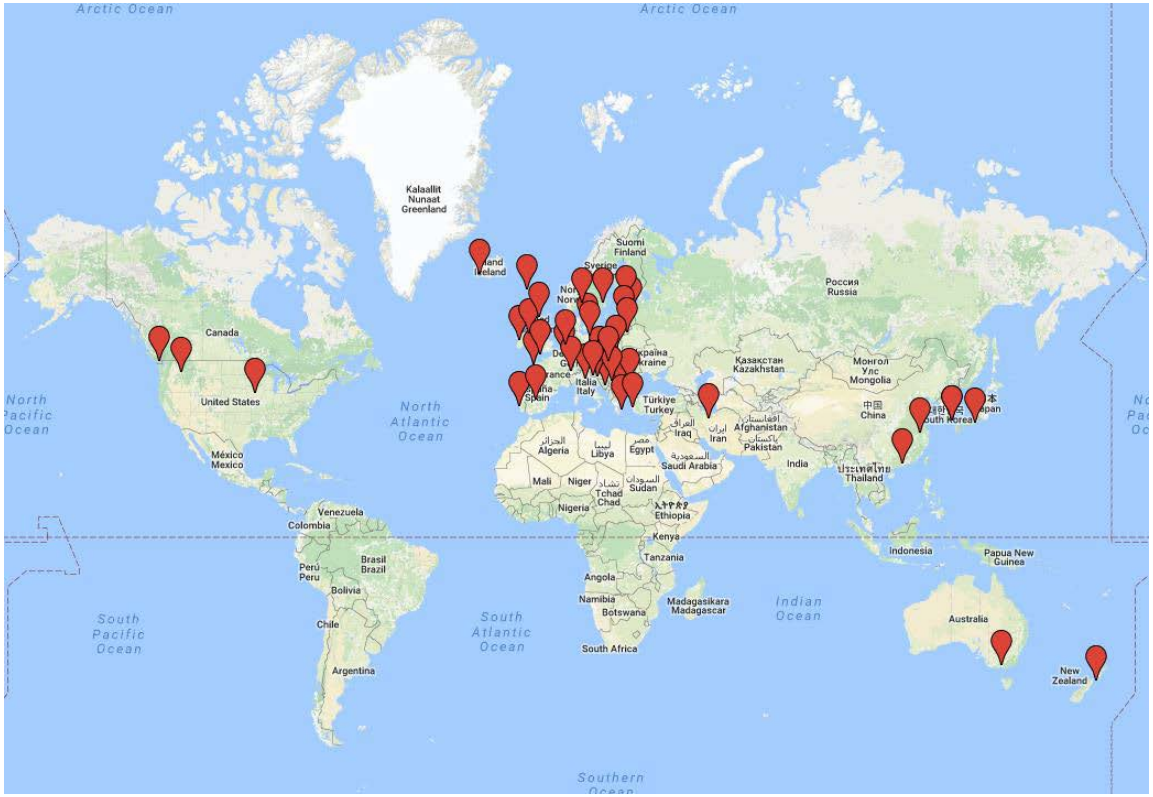


PT1 and PT2 was delivered to 45 laboratories

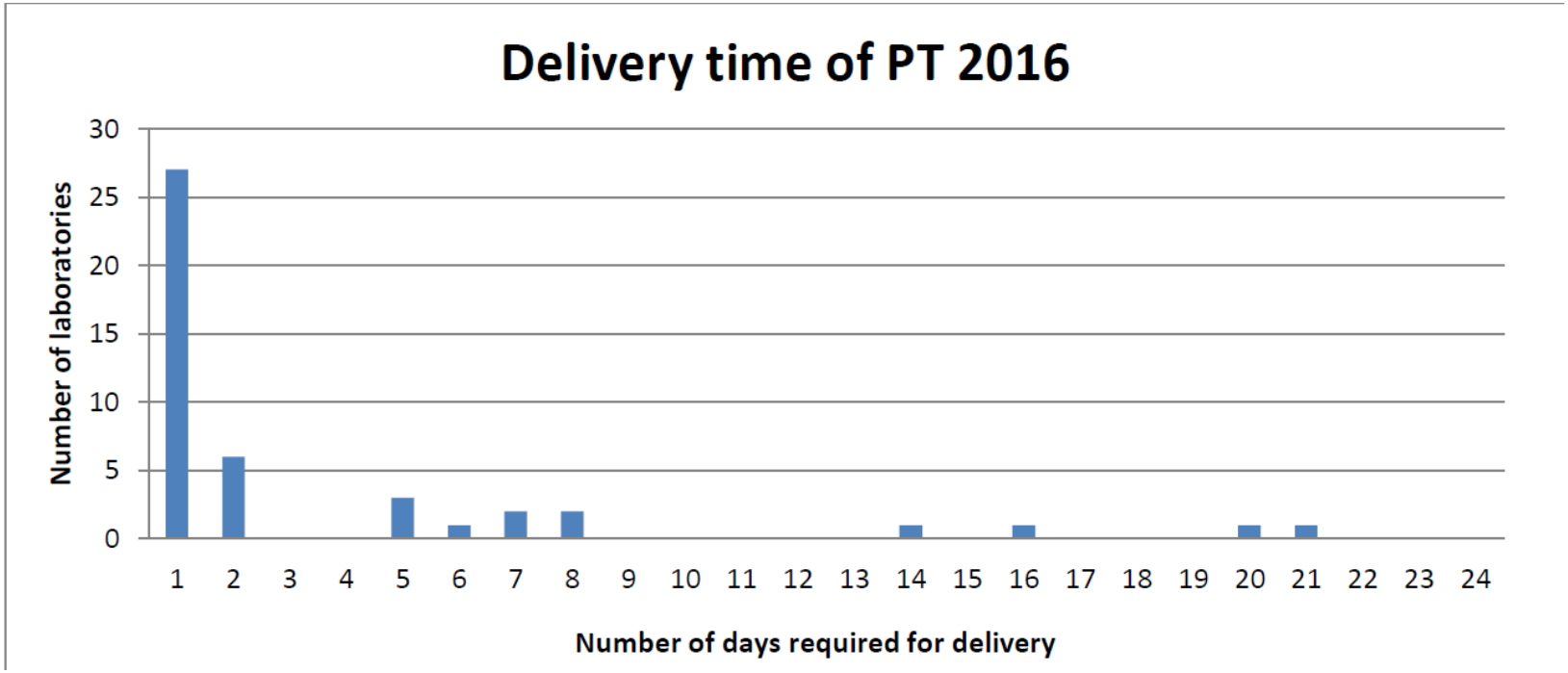
All NRL's for Fish Diseases in EU Member States

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)



Distribution of PT1 and PT2



Within one day, the tests were delivered to 27 participants; 12 more tests were delivered within the first week; 3 more within the first two weeks; 3 further within three weeks (Figure 1). All the parcels were sent without cooling elements.

PT1: Content of ampoules

Five ampoules containing virus/ lyophilised tissue culture supernatant

Code	Isolate
Ampoule I:	PFR - Pike Fry Rhabdovirus. Reference strain received from Dr. P.de Kinkelin, INRA, 1987
Ampoule II:	IHNV - isolate BLK94 07699 24:05
Ampoule III:	VHSV - Isolate TR-WS13G (= TR-SW13G)
Ampoule IV:	Ranavirus ECV: European catfish virus isolate 562/92.
Ampoule V:	IPNV strain Sp

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

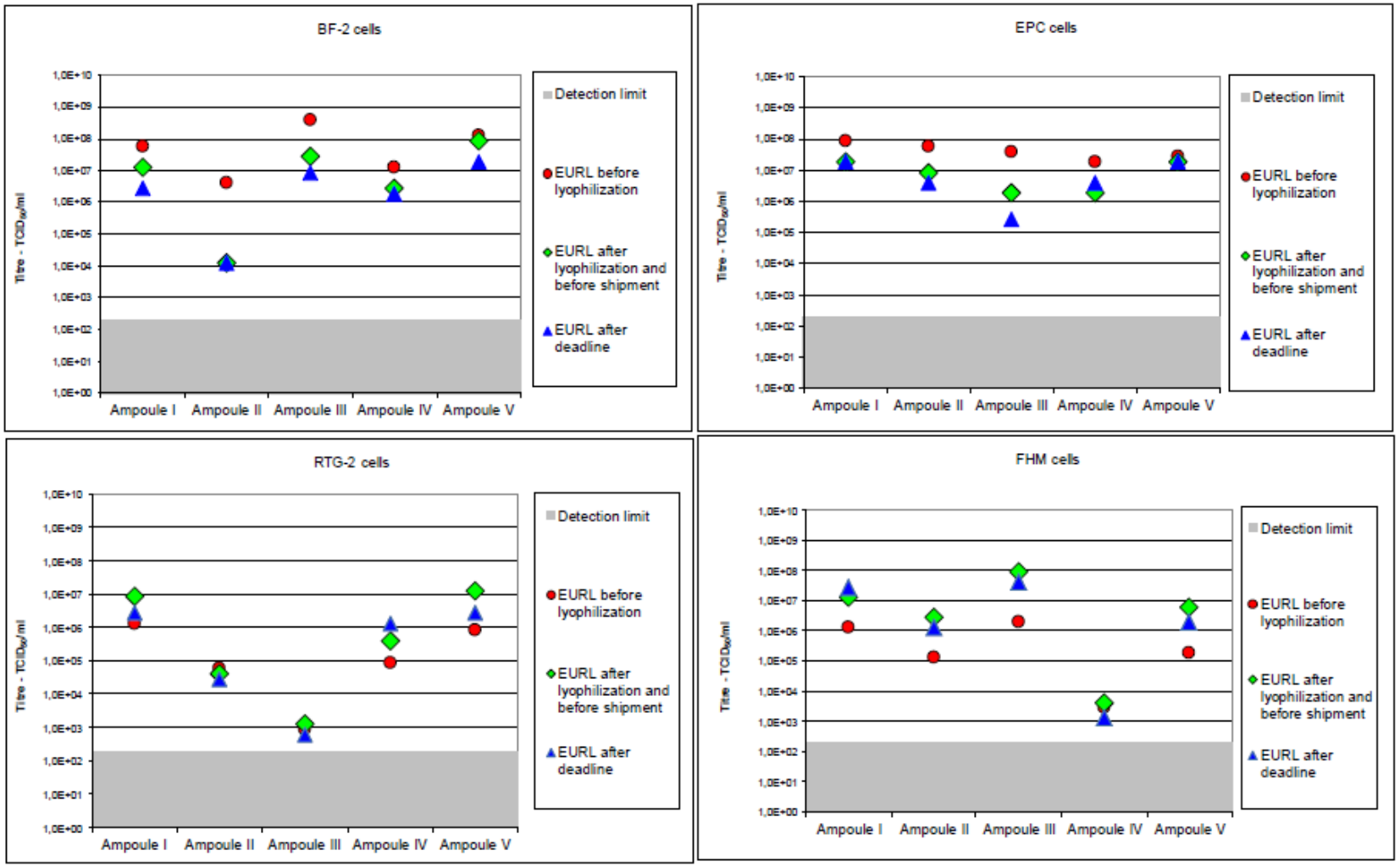


Figure 4. Virus titers in different cell lines:
Before lyophilisation, After lyophilisation-before shipment and After minimum 3 months after lyophilisation (storage 4°C in the dark) (1 ampoule).

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).

AMPOULE I PFRV

Ampoule I: PFR identification was given the score 2. Virus isolated but not identified providing that the ampoule content was NOT VHSV; IHNV; IPNV; SVCV or RANAvirus 1 point, but reducing the maximum score achievable to 9. In this way if a participant has not identified PFR but has ruled out all other pathogen will get a success rate of 100%. Identification as SVCV 1 point on a total maximum score for PT 1 of 10. Other incorrect findings or “no virus” or additional types of viruses than those included in the ampoule scored 0.

This year pike fry rhabdovirus was included in ampoule I. This virus has generated some challenges to the participants due to its antigenic similarity with SVCV, however the increase implementation of biomolecular techniques has allowed 17 laboratories to identify it correctly and other 17 were able to rule out the presence of VHSV,IHNV,IPNV,SVCV and ranavirus. The scoring system has been adjusted accordingly.

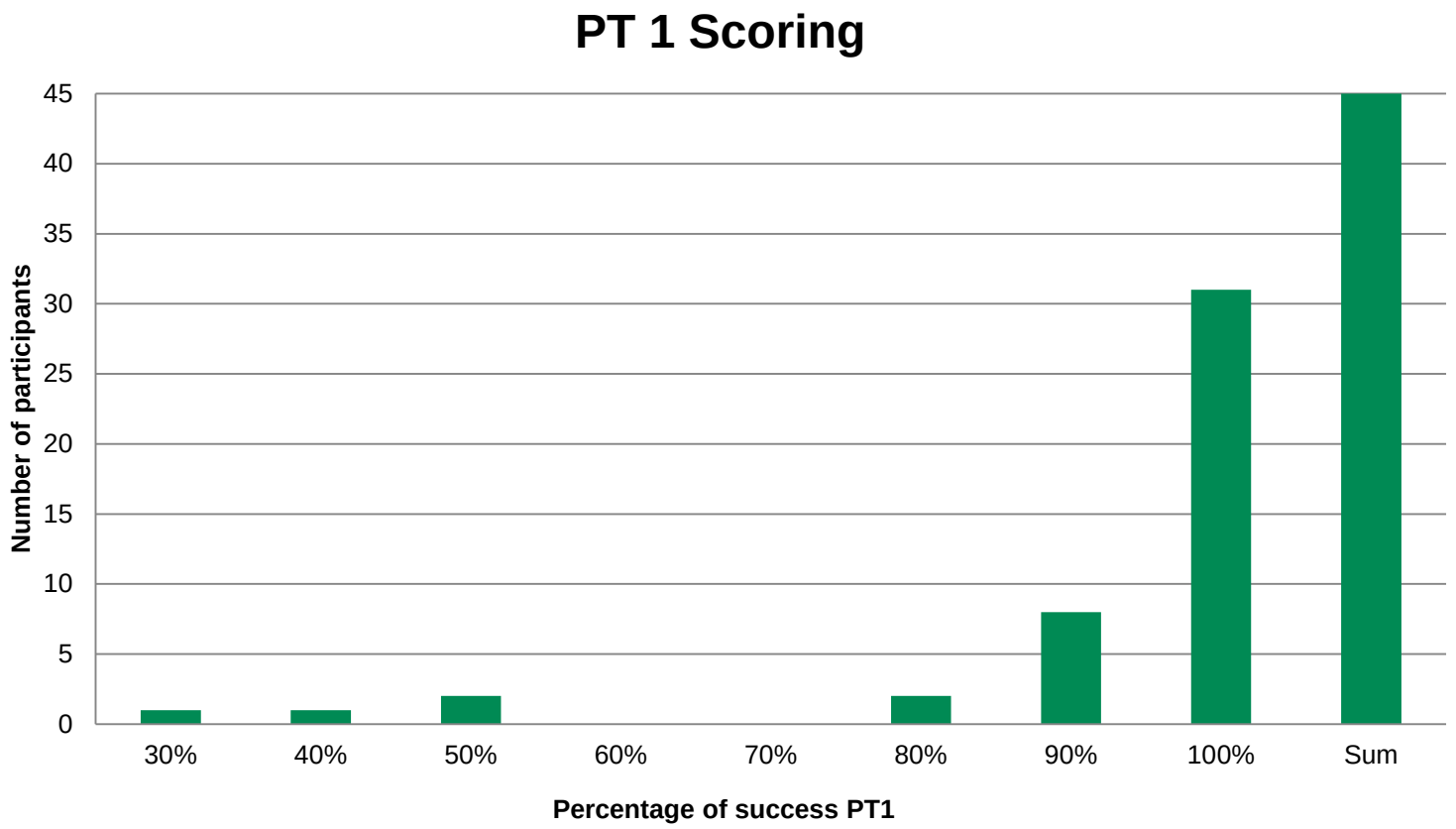
Genotyping and sequencing

Laboratory code number	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
	PFR	IHNV BLK94	VHSV TR-WS13G	ECV 562/92	IPNV Sp
1	Vesiculovirus Genogroup III	IHNV Genogroup U	VHSV Genogroup Ie	ECV/ESV	IPNV Genogroup 5
2	France "1972", originally from The Netherlands	USA, genotype U	Ie, Georgia "1981"	Hungary, strain 13051/2012	IPN: Sp/ EHNV: Hungary, strain 13051/2012
3				ECV/ESV	
4					
5					
6		U	Ie		
7		L	Ie		
8		U	I		Sp
9			III		Sp
10					
11		Genogroup U	Genogroup I		Genogroup V
12		U	Ie		5
13		Genotype U	Genotype Ie		
14		Asia	Ie		Sp
15					
16			1 E		
17				KT989884.1	
18					
19	Genogroup III (from Stone et al., 2003)	U	Genotype Ie		Genogroup 5 (Sp)
20		U	Ie	ECV/ESV	genotype 5 serotype Sp
21	Genogroup III	U	Ie	ECV, ESV	
22					
23		IHNV L genogroup			

Genotyping and sequencing

24		U	1e	Not EHNV	N/a
25		Genogroup U	Genogroup 1b		-
26		IHNV U	VHSV Ie and IHNV U	ECV or ESV	VHSV Ie
27					
28		U	Ib	ECV	
29			Ib		
30					
31					
32					
33		Genogroup U	Genotype Ie		Sp
34					
35			Ie		
36		Genotype U (North American)	Genotype Ie		
37		U genogroup	Ie genotype		Sp Serotype
38		Genogroup U	Genotyp 1e		Genogroup 5
39		IM2Ws+ID3a-Primer: M, E for X89213 IHN-OIE Primer: U	Depending on primers different subtypes of gentype 1 were found: With GA-Primers Ia, Id & Ie; with the EURL-primer pair Ib and Ie; with both primer pairs isolate GE-1.2 under different accession numbers		
40					
41					
42		Upper genogroup	Genotype Ie	N/A	Genogroup V, Serotype A2
43		U	1e	Not EHNV	Genogroup 5
44					
45		IHNV-U	I-b		SP; Genogroup III

Laboratory scoring, PT1



”underperfomance”

The great majority of the underperformance, to our understanding, is dued to contamination of the samples when opening the ampoules, resuspending the content.

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

The ”saw” has been suggested as possible vector of contamination, in our experience this is not likely to be the case. The saw will not get in touch with the content of the ampoule and there should be absolutely no virus on the external side of the ampoule.

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) 2, Sleeping disease virus (SD) received from Dr. J. Castric, ANSES, France in 1998 as isolate s49p
Ampoule VII:	ISAV Glesvaer/2/90
Ampoule VIII:	Cyprinid herpes virus 3 CyHV-3 – isolate KHV-TP 30 (syn: KHV-T (for Taiwan))
Ampoule IX:	Blank

PT2 Virus identification participating laboratories

Genotyping and sequencing

Laboratory code number	Ampoule VI	Ampoule VII	Ampoule VIII
	SAV 2, Sleeping disease virus (SD)	ISAV ISAV Glesvaer/2/90	KHV- CyHV-3 – isolate KHV-TP 30
1	SAV Subtype II	ISAV HPR2	KHV
2	France, isolate S49P	Faroe Islands, isolate F72b/02	Indonesia, isolate PP3_070411
3	0	0	0
4[2]	0	0	0
5	0	0	0
6	II	HPR2	3
7[1]	0	0	0
8	SAV 2	0	0
9	0	0	0
10[1]	0	0	0
11	Subtype II	EU-H1	0
12	0	HPR2	0
13	0	Genotype HPR deleted	0
14	SAV 2 FW (SD) (according to OIE)	2	0
15	0	0	0
16	0	0	0
17[3]	0	0	0
18[2]	0	0	0
19	SAV2	HPR deleted	0
20	type 2	0	CyHV-3
21	subtype II	PR4	0
22[1]	0	0	0
23	0	0	0

Natior

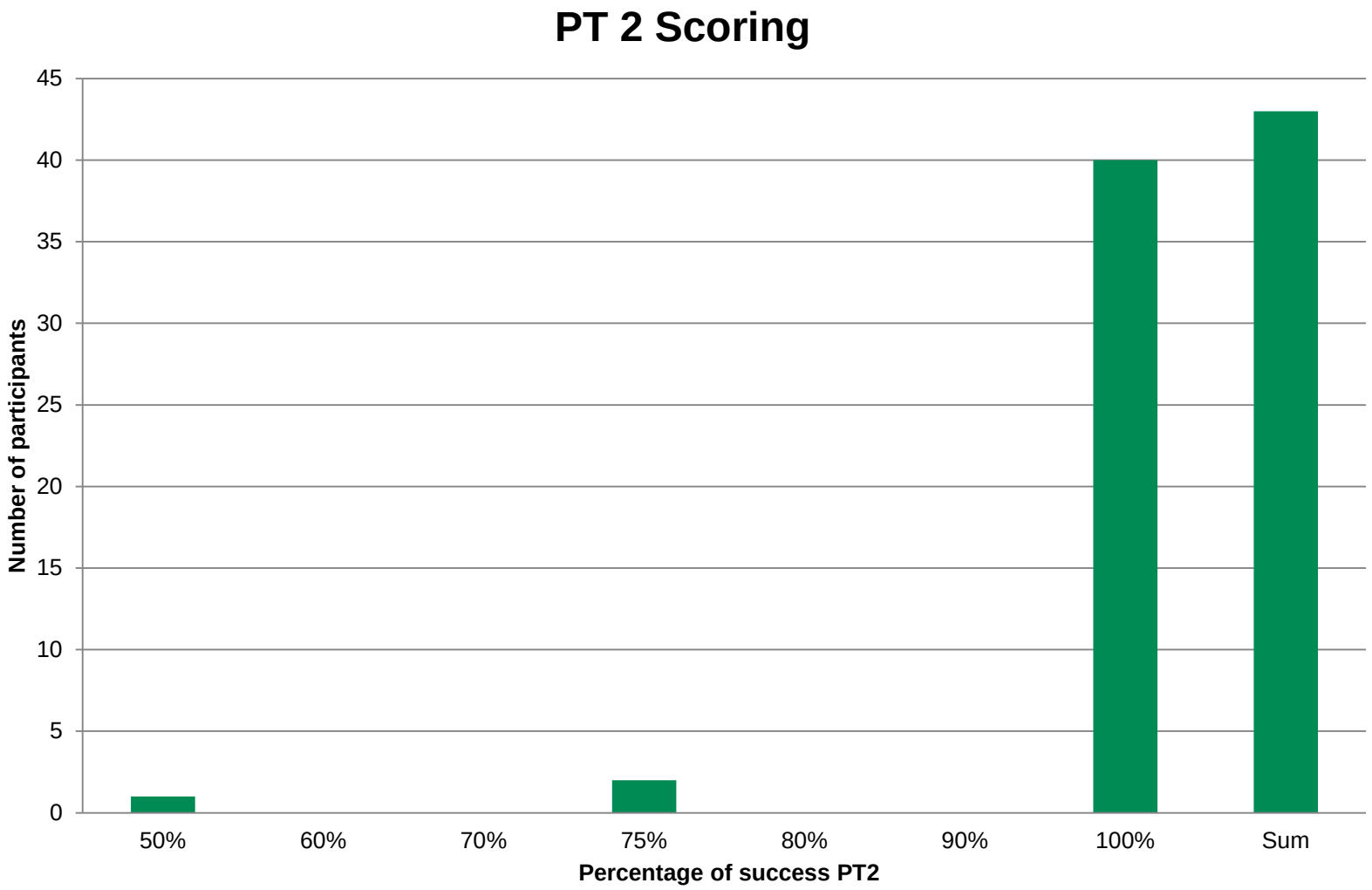
DTU

Genotyping and sequencing

Laboratory code number	Ampoule VI	Ampoule VII	Ampoule VIII
	SAV 2, Sleeping disease virus (SD)	ISAV ISAV Glesvaer/2/90	KHV- CyHV-3 – isolate KHV-TP 30
24	N/a	N/a	HPR Genotype 2
25	Subtype 2	G2 (HPR/deleted)	-
26	SAV2	ISAV HPR-deleted	CyHV3 (KHV)
27	0	0	0
28	SAV2	HPR Deleted	E
29	0	0	0
30	0	EU-G2	0
31[1]	0	0	0
32	0	0	0
33	0	0	0
34	0	0	0
35	-	HPR-4	-
36	-	HPR deleted(HPR4)	-
37	SAV 2 FW (SD)	ISAV (HPR2)	CyHV 3
38	Genogroup 2	HPR deleted variant	0
39	0	0	0
40	0	0	0
41	0	0	0
42	Subtype 2	HPR2	Wild type KHV
43	TYPE II	HPR2	CyHv-3
44	0	North American	0
45	0	European; HPR-deleted	0

DTU

Laboratory scoring; PT2



Any comments/questions to PT2?

Feedback 2016

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 your 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	

Feedback- Comments

I think it would be interesting to be able to compare the different Ct values of the participants.

2016 PTs was the first PT for us.We couldn,t do PT2,so we hope for the Next

We welcome to use the same spread sheet every year.

The test has well established and the tasks are clear. Nevertheless, the workload for a small laboratory to perform all tasks is considerable

Please keep including IPNV and SVCV, but does not have to be every year.

one mistake in the name of SAV isolate (ampoule VI) : it is S49p and not sp49.

When the sequence alignment result of ampoule I tuned out to be Pike fry-like rhabdovirus which we thought was not inclued in the instruction, we were confused how to fill the spread sheet.The description of the relevant contents should be emphasized.

Proficiency test 2016

- Aim: To send out the test in end of September 2017
- 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