EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2018

for identification and titration of

VHSV, IHNV, EHNV, SVCV and IPNV (PT1)

and identification of

CyHV-3 (KHV), SAV and ISAV (PT2)

Organised by the
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DANAK
PT Reg. no.: 515

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Introduction

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
PT1 was designed to primarily assess the identification of the fish viruses causing notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and in addition other fish pathogenic viruses as pike fry rhabdovirus (PFR), spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) by cell culture based methods.
PT2 was designed for assessing the ability of participating laboratories to identify the fish viruses: infectious salmon anaemia virus (ISAV), salmonid alphavirus (SAV) and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by bio molecular methods (PCR based).
45 laboratories participated in PT1 while 42 participated in PT2. 2 laboratories were, due to internal clearance problems, not able to provide the answers before deadline.
Regarding PT1 and PT2, 40 and 36 laboratories respectively participated in identifying all viruses included.
The tests were sent from the EURL 1st of October 2018.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.
This report covers both the results of PT1 and PT2.

PT1 consisted of five coded ampoules (I-V). These ampoules contained SVCV, IPNV, IHNV, EHNV and VHSV (see table 1). 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) [1, 2]. Furthermore the inter-laboratory 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). In addition the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Implementing Decision (EU) 2015/1554 [3] and by using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.

If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals [4]. Laboratories were encouraged to identify VHSV and IHNV isolates by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al.* (2004) [5] for VHSV and either method as mentioned in the IHN chapter of the 2018 version of the Chapter 2.3.4 in the OIE Manual of Diagnostic Tests for Aquatic Animals (Emmenegger et al. 2000 or in Kurath et *al.* (2003)) [6–8] for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of four coded ampoules (VI-IX). One ampoule contained CyHV-3 (KHV), one contained ISAV, one contained SAV and one contained sterile cell culture supernatant from BF-2 cells, see table 9. The test was designed to primarily assess the ability of participating laboratories to identify

the notifiable fish pathogens ISAV and KHV (listed in Council Directive 2006/88/EC, Annex IV and Commission Implementing Directive 2014/22/EU) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test for SAV or not. The EURL team would then take care of calculating the score accordingly, overall 36 of 42 laboratories tested for SAV in 2018 and 6 of the 42 laboratories did not test for SAV in 2018 as occurred in 2017.
Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. Regarding SAV analysis, participants can refer to the Chapter 2.3.5. of the OIE Manual of Diagnostic Tests for Aquatic Animals [9]. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it was possible to replicate them in cell cultures.

During the preparation of the current report the EURL has acknowledged the big effort that many participants are putting in sequencing and genotyping the isolates of the PT panel. For this reason, the EURL proposes to provide a separate scoring system for the genotyping results, which will be attached to the annexes (Table 9 and 13) which display the genotyping results provided by all participants.

Each laboratory was given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. Furthermore, the EURL-team have included comments to the participants if relevant. An un-coded version of the report is sent to the European Commission.

Participants were asked to download an excel sheet from the EURL web site (http://www.eurl-fish.eu/) to be used for reporting results and to be submitted to the EURL electronically. Additionally, participants were requested to answer a questionnaire regarding the accreditation status of their laboratory. Collected accreditation data will not be presented in this report but will be presented at the 23th Annual Workshop of the NRLs for Fish Diseases week 22, 2019 in Kgs. Lyngby. Participants were asked to reply latest December 3rd 2018.

Distribution of the test

The test was sent out according to current international regulations for shipment of diagnostic specimens UN 3373, “Biological substance, Category B”. All proficiency tests parcels were delivered by courier and when possible participants were provided with a tracking number so they were able to follow the shipment.

Shipment and handling
Within one day, the tests were delivered to 14 participants; 29 more tests were delivered within the first week; 1 more within the first two weeks; 1 further within three weeks; due to clearance problems in the receiving country, 2 tests did not arrive in time for the laboratories to provide the results before deadline. (Figure 1). All the parcels were sent without cooling elements.
A relatively high stability was demonstrated to characterize the lyophilized pathogens in glass ampoules as describedproficiency test report.

Extra parcels were kept at 4°C in order to be able to provide fast substitutes in case of damage during transport.

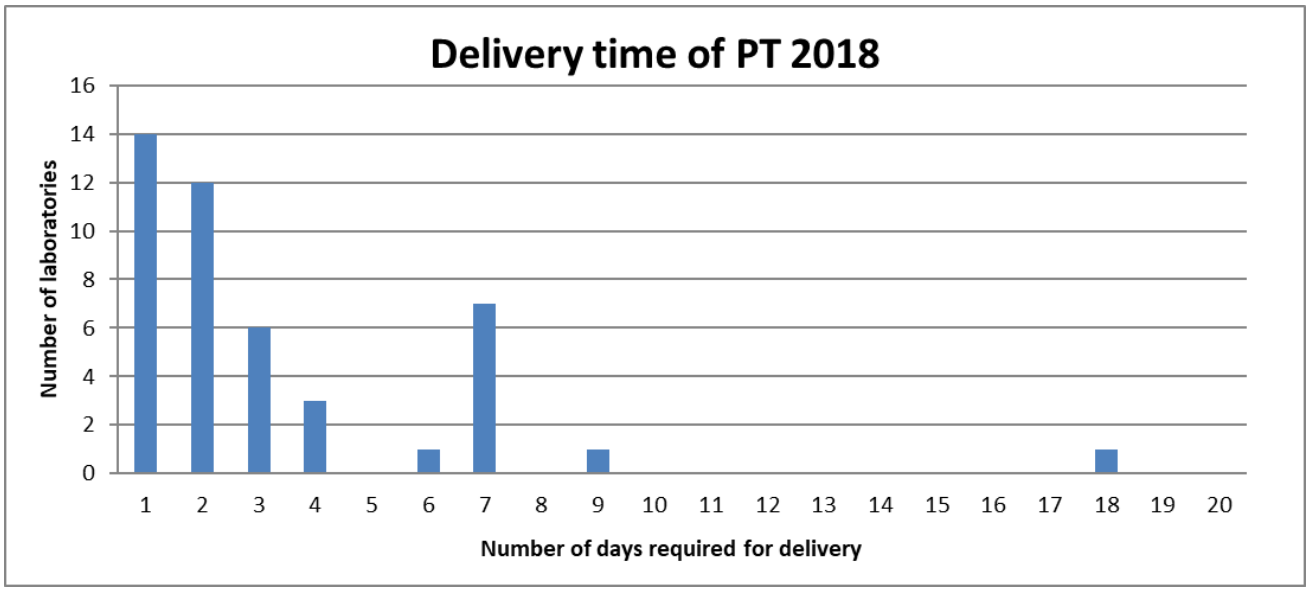


Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 45 laboratories received the annual proficiency test. 42 participants submitted the full spreadsheet within the deadline; 2 additional participants delivered the results for ampoule content within the deadline but were given an extended deadline for providing sequencing results; 1 laboratory submitted a spreadsheet from a former year, since this was discovered after the original deadline, the participant were allowed to submit the spreadsheet from 2018 after the original deadline. Figure 2 show how many laboratories that participated in the proficiency test from 1996 to 2018.

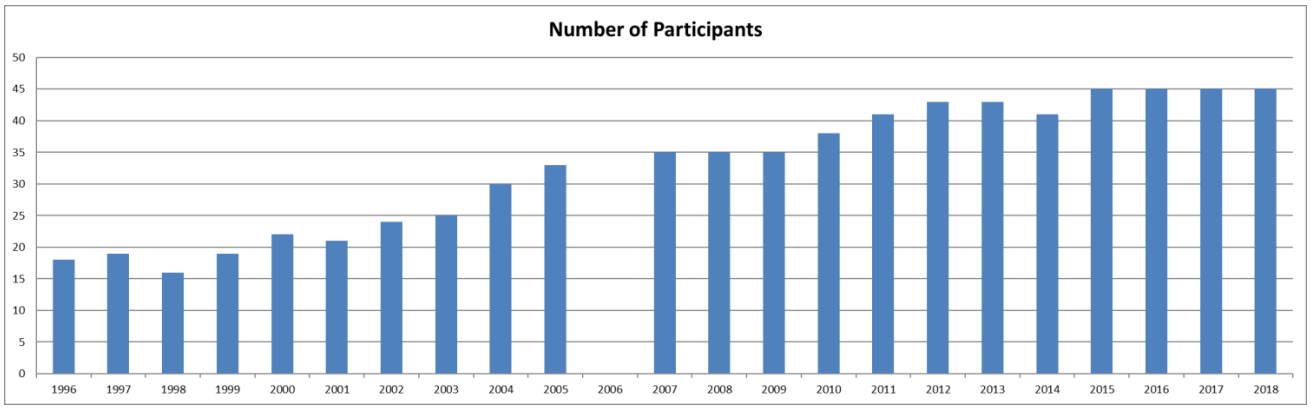


Figure 2. Participants in the EURL proficiency test over the years.

Proficiency test 1, PT1

Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States, including Denmark, and likewise to the NRLs in Australia, Bosnia and Herzegovina, Canada, Chile, Faroe Islands, Iceland, Iran, Japan, New Zealand, Norway, Russia, Serbia, Switzerland, Turkey, and to two laboratories in South Korea and USA, respectively. Due to local clearance problems the packages containing the PT2018 were never delivered to the two laboratories in P.R. China.

The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy, Cyprus and Malta for identification of all listed diseases. Figure 3 shows the worldwide distribution of the participating NRLs. This year there were two new participating countries; National Reference laboratorires of Chile and Russia.

Figure 3. Worldwide distribution of the participants in the EURL proficiency test 2018.

Content of ampoules

The viruses were propagated on each of their preferred cell line, and when total cytopathic effect (CPE) was observed, the supernatants were collected and filtrated through a 45 µm filter, mixed with equal volumes of 2% w/v lactalbumin hydrolysate solution and lyophilized in glass ampoules. The ampoules were sealed by melting. The details of the virus isolates used in the proficiency test are outlined in table 1.

Table 1. Content of each ampoule with reference to culture conditions and major publications of the included viruses.

Code	Specifications/References
Ampoule I: SVCV	**SVCV strain 56/70 Genotype Id** Spring viraemia of carp virus isolate from carp. The isolate is most likely identical to the S/30 isolate described in Fijan N, Petrinec Z, Sulimanovic D & Zwillenberg LO (1971). Isolation of the viral causative agent from the acute form of infectious dropsy of carp. *Veterinarski Archiv* 41, 125-138.[10] **Received from:** Prof. Fijan (January 1979 in a tube named Rhabdo virus carpio 56/70 and given as the reference strain of SVC virus). **Genotype:** Id **GenBank accession numbers:** Z37505.1 (Fijan), AJ538061.1 (S30) **Reference on sequence (S30) and genotype:** Stone DM, Ahne W, Denham KL, Dixon PF, Liu C-TY, Sheppard AM, Taylor GR & Way K (2003). Nucleotide sequence analysis of the glycoprotein gene of putative spring viraemia of carp virus and pike fry rhabdovirus isolates reveals four genogroups. *Diseases of Aquatic Organisms* 53, 203-210. [11]
Ampoule II: IPNV	**IPNV strain Sp Genotype 5** The Sp (Spjarup) reference strain of Infectious Pancreatic Necrosis (IPN) virus from farmed rainbow trout in Denmark, isolated in 1969 by Dr. Vestergaard Jørgensen. **Received from:** National Veterinary Institute, Technical University of Denmark. **GenBank accession numbers**: AM889221 **Reference on isolate:** Jørgensen PEV & Bregnballe F (1969) Infectious pancreatic necrosis in rainbow trout in Denmark. *Nordisk Veterinærmedicin* **21**, 142-148. [12] Jørgensen PEV & Grauballe PC (1971) Problems in the serological typing of IPN virus. *Acta Veterinaria Scandinavica* **12**, 145-147. [13] **References on sequences:** P. F. Dixon, G.-H. Ngoh, D. M. Stone, S. F. Chang, K. Way, S. L. F. Kueh (2008) Proposal for a fourth aquabirnavirus serogroup Archives of Virology 153:1937–1941 [14]
Ampoule III: IHNV	**IHNV - isolate BLK94** Isolated in 1994 from Sockeye salmon *Oncorhynchus nerka* smolt, in Washington USA. **Received from**: Gael Kurath **American Genotype** U Genogroup U G. Kurath, K. Garver, R. M. Troyer, E. J. Emmenegger, K. Einer-Jensen, E. Anderson Phylogeography of infectious haematopoietic necrosis virus in North America 2003, J. General Virology 84:803-814; [7] Mid G USD mG002U refers to Universal sequence designators (USD) defined for North American IHNV isolates as described in the MEAP-IHNV (Molecular Epidemiology of Aquatic Pathogens) database at http://gis.nacse.org/ihnv

Code	Specifications/References
Ampoule IV: EHNV	**EHNV Isolate 86/8774** Australian freshwater isolate of epizootic haematopoietic necrosis virus from rainbow trout from Adaminaby Trout Farm, NSW obtained in 1986 by Jeremy Langdon. **Received from:** Prof. Whittington, The OIE reference laboratory for EHN, University of Sidney, Australia. **GenBank accession numbers:** FJ433873, AY187045, AF157667 **Reference on isolate:** Langdon JS, Humphrey JD & Williams LM (1988). Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, *Salmo gairdneri* Richardson, in Australia. *Journal of Fish Diseases* 11, 93-96. [15] **References on sequences:** Hyatt AD, Gould AR, Zupanovic Z, Cunningham AA, Hengstberger S, Whittington RJ, Kattenbelt J & Coupar BEH (2000). Comparative studies of piscine and amphibian iridoviruses. *Archives of Virology* 145, 301-331. [16] Jancovich JK, Bremont M, Touchman JW & Jacobs BL (2010). Evidence for multiple recent host species shifts among the ranaviruses (family Iridoviridae). *Journal of Virology* 84, 2636-2647. [17] Marsh IB, Whittington RJ, O'Rourke B, Hyatt AD & Chisholm O (2002) Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Molecular and Cellular Probes* 16, 137-151. [18]
Ampoule V: VHSV	**VHS virus**, DK-3592B "**Voldbjerg strain**". Highly pathogenic Viral Haemorragic Septicaemia strain belonging to sero-pattern I isolated from Rainbow trout in 1989. Olesen NJ, Lorenzen N, Jørgensen PEV. Serological differences among isolates of viral haemorrhagic septicaemia virus detected by neutralizing monoclonal and polyclonal antibodies. Dis Aquat Org 1993;16:163–70 [19] **Genotype**: Ia **Reference on isolate:** Lorenzen N, Olesen NJ, Jørgensen PEV (1993) Antibody response to VHS virus proteins in rainbow trout. Fish Shellfish Immunol 3:461–473 [20] **References on sequences:** N gene MF594520 Nv gene DQ159198.1 Full Genome KC778774.1

Testing of the PT1 test

The PT1 test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043. Prior to distribution the EURL tested 5 ampoules of each virus preparation by titration in 4 cell lines (BF-2, EPC, RTG-2 and FHM), to ascertain a satisfactory titre in the preferred cell line and homogeneity of content of ampoules (Table 2 and Figure 4).

The lyophilisation procedure is known to determine some reduction especially for VHSV. Previous experience reported during the past Proficiency tests demonstrated a rather high stability for SVCV, EHNV and IPNV serotype Sp. We have previously shown that lyophilised virus kept in glass sealed ampoules is stable for more than half a year when kept at room temperature (Inter-Laboratory Proficiency Test report 2007).
We have furthermore shown that lyophilised virus in glass sealed ampoules is stable after exposure to 30°C for 24 hours (Inter-Laboratory Proficiency Test report 2010)
In 2011 we have shown that lyophilised virus in glass sealed ampoules is stable when temperature raised from 20-42°C over a period of 5 hours (Inter-Laboratory Proficiency Test 2011)

The identities of the viruses in all 5 ampoules were checked and confirmed before shipment by ELISA, IFAT, serum neutralisation tests (SNT), PCR and/or qPCR and RT-PCR and/or RT-qPCR. After shipment the stability of the content in the ampoules were assessed by titrating the virus on cell cultures, and identifying it by ELISA, furthermore PCR based tests were performed on the original content of all the ampoules. This year reductions of the titres after lyophilisation were observed. For the major part of the ampoules, the reduction of the titre was between 1-3 log in the same cell line. The reduction of the titre in ampoule III (containing IHNV) was more than 4 log in BF-2 cells. It is reported that BF-2 cell line is poorly susceptible to IHNV. Therefore the reduction of the titre, could have been caused by the 10-fold dilution when resuspending the ampoule and the subsequent lyophilisation of the ampoule contents. This highlights the importance of using two heterologous cell lines according to Commission Decision 2015-1554 [3] for conducting surveillance for listed viral disease in fish. No significant reductions were observed after long term storage (Table 2 and figure 4)

Ampoul No.	Cell line	Titre before Lyophilisation	Titre after Lyophilisation and before shipment	Titre after deadline for handling in results (storage 4°C in the dark)
		$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I: SVCV 56/70	BF-2	1,9E+06	1,3E+04	1,3E+04
	EPC	2,7E+06	2,7E+04	1,9E+04
	RTG-2	5,9E+05	5,9E+03	1,9E+03
	FHM	1,9E+07	1,9E+04	1,3E+04
Ampoule II: IPNV Sp	BF-2	4,0E+07	2,7E+05	1,9E+05
	EPC	8,6E+05	8,6E+04	5,9E+04
	RTG-2	5,9E+05	2,7E+04	5,9E+04
	FHM	1,3E+07	1,9E+04	1,3E+04
Ampoule III: IHNV BLK94	BF-2	2,7E+06	<1,9E+02	< 1,9E+02
	EPC	4,0E+07	2,7E+05	4,0E+05
	RTG-2	1,9E+06	1,3E+03	8,6E+02
	FHM	4,0E+07	1,3E+04	4,0E+03
Ampoule IV: EHNV 86/8774	BF-2	2,7E+06	8,6E+04	8,6E+04
	EPC	4,0E+06	8,6E+03	4,0E+03
	RTG-2	8,6E+05	8,6E+04	2,7E+04
	FHM	2,7E+04	<1,9E+02	1,9E+02
Ampoule V: VHSV DK-3592B	BF-2	1,3E+06	1,3E+03	8,6E+02
	EPC	4,0E+05	4,0E+03	2,7E+03
	RTG-2	4,0E+05	1,9E+03	8,6E+02
	FHM	2,7E+06	8,6E+04	2,7E+04

Table 2. PT1:

Titres in ampoules I to V stored in the dark tested on four cell lines at different time points:

- Before lyophilisation, (stored at -80°C).
- After lyophilisation and before shipment (median titre of 5 replicates), (stored at 4°C), the variation of the titre of the 5 replicates was within 1 log in the same cell line.
- After deadline for handling in results (1 ampoule), (stored at 4°C).

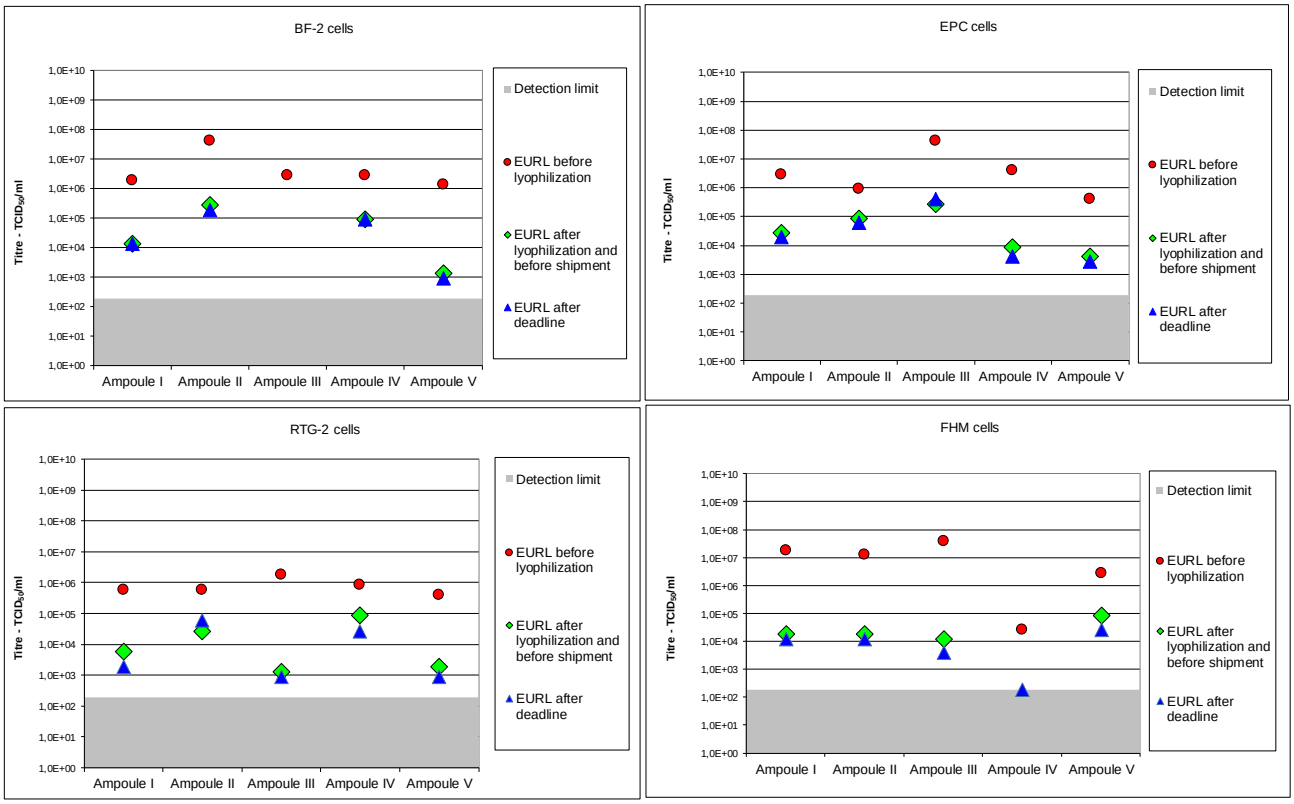


Figure 4. Virus titers in different cell lines:
Before lyophilisation, before shipment and after deadline for handling in results (storage 4°C in the dark).

Virus identification and titration

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 [3], i.e. by cell culture followed by ELISA, IFAT, neutralisation test and/or RT-PCR/RT-qPCR. The results of the content in the 5 ampoules as reported by the participating laboratories are summarised in table 3.

Participants were also asked to assess the viral load in the ampoules by conducting titrations. The titration procedures were described in the instructions enclosed with the test. All titres were calculated by the EURL based on the crude data submitted by each participant and given as Tissue Culture Infective Dose 50% per ml ($TCID_{50}$/ml). The titre of the re-dissolved virus was multiplied by a factor of 10 to compensate for the dilution of the original volume of virus in the ampoules (200 µl virus + 200 µl lactalbumin in vials re-dissolved in a total of 2.0 ml cell culture medium). The titration results obtained by the participating laboratories are summarised in tables 4 to 8. The titres obtained from each participating laboratory are represented graphically. In Figures 5-8, all titres submitted by the participants for each cell line and ampoule, respectively are compared to each other. On these figures, the median titre and the 25% and 75% inter-quartile range is displayed. In this way, the titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories. CHSE-214 cells are not displayed graphically or commented on in this report as only 9 laboratories used these cells.

Laboratories with the required facilities were strongly encouraged to identify the genotype of the virus isolates. It was not mandatory to perform these analyses for VHSV and IHNV. However, for ranaviruses it is mandatory to perform a sequence or restriction endonuclease analysis of the isolate in order to determine if the isolate is EHNV.

Table 3. Inter-Laboratory Proficiency Test, PT1, 2018 - Virus identification and score obtained by participants.

Laboratory code number	Score	Answer received at EURL	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
			SVCV 56/70	IPNV Sp	IHNV BLK94	EHNV 86/8774	VHSV DK-3592B
1	10/10	30-11-2018	svcv	IPNV	IHNV	Ranavirus (EHNV)	VHSV
2	10/10	29-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
3	10/10	29-11-2018	SVC	IPN	IHN	EHNV	VHS
4[1]	8/8	29-11-2018	SVCV	IPNV	IHNV	NO IHNV,VHSV,SVCV,IPNV	VHSV
5[2]	9/10	03-12-2018	SVCV	IPNV	IHNV	Ranavirus	VHSV
6	10/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	VHSV
7	8/10	03-12-2018	svcv	Negative sample	IHNV	EHNV	VHSV
8	10/10	29-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
9	10/10	29-11-2018	SVCV	IPNV	(IHNV)*	EHNV	(VHSV)*
10	10/10	03-12-2018	SVCV	IPNV	IHNV	Ranavirus, EHNV	VHSV
11[1]	8/8	15-02-2019	SVCV	IPNV	IHNV	No IPNV,IHNV, VHSV, SVCV	VHSV
12	10/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	VHSV
13	10/10	30-11-2018	SVCV	IPNV genogroup 5	IHNV genotype U	EHNV	VHSV genotype Ia
14	10/10	08-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
15	10/10	03-12-2018	100 % SVCV "Fijan"	100 % IPNV Genogroup 5 isolates 666/12; 470/07 and Sp	100 % IHNV BLK94 American Genogroup U	EHNV	100 % VHSV DK-3592B Genogroup Ia
16	10/10	30-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
17	10/10	29-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
18	10/10	29-11-2018	SVCV	IPNV	IHNV	Ranavirus	VHSV
19[1]	8/8	03-12-2018	SVC	IPN	IHN	NEG	VHS
20	10/10	29-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
21	10/10	29-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
22	10/10	19-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
23	10/10	26-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
24	8/8	03-12-2018	SVCV	IPNV	IHNV	not performed for EHNV and Ranavirus	VHSV
25	10/10	03-12-2018	SVCV	IPNV	IHNV	Ranavirus	VHSV
26	10/10	03-12-2018	SVCV viable virus	IPNV viable virus	IHNV viable virus	EHNV viable virus	VHSV viable virus
27	10/10	30-11-2018	SVCV	IPNV	IHNV	Ranavirus (EHNV)	VHSV
30	10/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	VHSV
31	10/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	VHSV
32	10/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	VHSV
33[3]	4/4	30-11-2018			IHNV		VHSV
34	8/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	
35	10/10	26-11-2018	SVCV	IPNV	IHNV	Ranavirus (EHNV)	VHSV
36	10/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	VHSV
37	8/10	30-11-2018	SVCV	Negative	IHNV	EHNV	VHSV
38	8/10	28-11-2018	Negative	IPNV	IHNV	EHNV	VHSV
39	10/10	26-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV

40	10/10	03-12-2018	SVCV	IPNV	IHNV	EHNV	VHSV
41	10/10	06-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
42	10/10	30-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
43	10/10	30-11-2018	SVCV	IPNV	IHN	Ranavirus was identified by conventional RT-PCR and than REA was applied as given by OIE manuel to identified EHNV	VHSV
44	10/10	29-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
45	10/10	23-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
46	10/10	27-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV
47	10/10	16-11-2018	SVCV	IPNV	IHNV	EHNV	VHSV

1) Do not test for Ranavirus

2) Did not corroborate the findings in ampoule IV by sequencing or REA

3) Do not test for Ranavirus, SVCV and IPNV

Table 4. Inter-Laboratory Proficiency Test, PT1, 2018 – Results of titration of **ampoule I**.

SVCV 56/70					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	svcv	8,6E+03	5,9E+03	1,3E+03	N/A
2	SVCV	8,6E+02	5,9E+03	N/A	N/A
3	SVC	4,0E+02	2,7E+04	< 1,9E+02	N/A
4	SVCV	5,9E+03	2,7E+03	N/A	N/A
5	SVCV	N/A	4,0E+05	N/A	N/A
6	SVCV	< 1,9E+02	5,9E+03	< 1,9E+02	4,0E+03
7	svcv	5,9E+02	1,3E+04	N/A	N/A
8	SVCV	8,6E+03	4,0E+03	N/A	N/A
9	SVCV	N/A	< 1,9E+02	< 1,9E+02	< 1,9E+02
10	SVCV	1,9E+04	8,6E+04	1,9E+04	4,0E+04
11	SVCV	2,7E+02	5,9E+05	N/A	N/A
12	SVCV	8,6E+02	5,9E+03	N/A	N/A
13	SVCV	2,7E+03	4,0E+03	N/A	N/A
14	SVCV	1,3E+03	2,7E+04	1,3E+03	N/A
15	SVCV	2,7E+02	2,7E+04	< 1,9E+02	N/A
16	SVCV	< 1,9E+02	5,9E+03	N/A	N/A
17	SVCV	1,3E+03	1,3E+04	N/A	N/A
18	SVCV	1,9E+03	4,0E+03	N/A	N/A
19	SVC	N/A	N/A	N/A	N/A
20	SVCV	4,0E+04	1,9E+04	N/A	N/A
21	SVCV	2,7E+04	5,9E+03	N/A	N/A
22	SVCV	< 1,9E+02	8,6E+02	< 1,9E+02	1,3E+03
23	SVCV	2,7E+02	1,3E+03	N/A	N/A
24	SVCV	N/A	1,3E+04	1,3E+03	N/A
25	SVCV	1,3E+04	1,3E+03	N/A	N/A
26	SVCV	< 1,9E+02	1,3E+03	N/A	8,6E+02
27	SVCV	4,0E+03	1,3E+04	N/A	N/A
30	SVCV	4,0E+03	1,9E+03	8,6E+03	1,3E+04
31	SVCV	4,0E+02	< 1,9E+02	N/A	N/A
32	SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
33		N/A	1,3E+04	1,3E+09	1,3E+05
34	SVCV	1,3E+06	5,9E+06	N/A	N/A
35	SVCV	1,3E+03	1,3E+03	1,3E+03	1,3E+03

36	SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
37	SVCV	< 1,9E+02	5,9E+02	< 1,9E+02	2,7E+02
38	Negative	N/A	< 1,9E+02	N/A	< 1,9E+02
39	SVCV	5,9E+03	4,0E+04	4,0E+03	8,6E+03
40	SVCV	1,3E+03	N/A	N/A	1,3E+03
41	SVCV	5,9E+03	1,3E+04	N/A	N/A
42	SVCV	< 1,9E+02	N/A	N/A	1,9E+02
43	SVCV	1,9E+03	1,3E+03	N/A	N/A
44	SVCV	5,9E+03	2,7E+05	N/A	N/A
45	SVCV	< 1,9E+02	N/A	N/A	1,9E+04
46	SVCV	2,7E+03	1,3E+03	N/A	4,0E+03
47	SVCV	4,0E+02	1,3E+03	1,3E+03	< 1,9E+02

N/A: Cell line not applied by the participating laboratory for titration of the virus

SVCV 56/70	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	39	41	15	16
Median titre	1,3E+03	5,9E+03	1,3E+03	1,3E+03
Maximum titre	1,3E+06	5,9E+06	1,3E+09	1,3E+05
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	2,7E+02	1,3E+03	<1,9E+02	2,5E+02
75% quartile titre	5,9E+03	1,3E+04	2,6E+03	9,6E+03

Table 5. Inter-Laboratory Proficiency Test, PT1, 2018 – Results of titration of **ampoule II**.

IPNV Sp					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	IPNV	2,7E+05	5,9E+04	2,7E+04	N/A
2	IPNV	4,0E+05	8,6E+03	N/A	N/A
3	IPN	5,9E+05	2,7E+05	1,3E+05	N/A
4	IPNV	1,26E+06	4,00E+04	N/A	N/A
5	IPNV	N/A	5,9E+07	N/A	N/A
6	IPNV	4,0E+04	5,9E+03	< 1,9E+02	< 1,9E+02
7	Negative sample	< 1,9E+02	< 1,9E+02	N/A	N/A
8	IPNV	4,0E+06	2,7E+04	N/A	N/A
9	IPNV	N/A	1,3E+06	1,3E+05	1,9E+04
10	IPNV	5,9E+05	8,6E+04	4,0E+05	8,6E+04
11	IPNV	2,7E+05	5,9E+04	N/A	N/A
12	IPNV	4,0E+05	2,7E+04	N/A	N/A
13	IPNV	1,3E+06	5,9E+05	N/A	N/A
14	IPNV	4,0E+05	1,3E+05	4,0E+05	N/A
15	IPNV	1,9E+06	1,3E+05	2,7E+05	N/A
16	IPNV	1,9E+05	1,3E+05	N/A	N/A
17	IPNV	8,6E+04	4,0E+04	N/A	N/A
18	IPNV	5,9E+05	1,3E+05	N/A	N/A
19	IPN	N/A	N/A	N/A	N/A
20	IPNV	5,9E+05	5,9E+04	N/A	N/A
21	IPNV	1,3E+05	4,0E+04	N/A	N/A
22	IPNV	5,9E+04	4,0E+04	4,0E+04	4,0E+03
23	IPNV	2,7E+05	1,9E+05	N/A	N/A
24	IPNV	N/A	2,7E+03	1,9E+05	N/A
25	IPNV	1,9E+05	1,3E+04	N/A	N/A
26	IPNV	2,7E+05	< 1,9E+02	N/A	< 1,9E+02
27	IPNV	1,9E+05	1,3E+05	N/A	N/A
30	IPNV	2,7E+05	1,9E+05	8,6E+05	4,0E+05
31	IPNV	2,7E+04	1,9E+03	N/A	N/A
32	IPNV	8,6E+04	8,6E+03	N/A	N/A
33		N/A	2,7E+08	1,3E+07	1,3E+07
34	IPNV	< 1,9E+02	< 1,9E+02	N/A	N/A
35	IPNV	1,9E+05	5,9E+05	1,3E+05	1,9E+05
36	IPNV	2,7E+06	1,9E+05	N/A	N/A
37	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02

38	IPNV	N/A	4,00E+04	N/A	8617,73876
39	IPNV	5,9E+05	8,6E+05	1,9E+06	5,9E+04
40	IPNV	1,9E+06	N/A	N/A	1,3E+05
41	IPNV	4,0E+05	1,9E+05	N/A	N/A
42	IPNV	1,9E+06	N/A	N/A	< 1,9E+02
43	IPNV	4,0E+05	1,9E+03	N/A	N/A
44	IPNV	1,9E+06	1,9E+05	N/A	N/A
45	IPNV	8,6E+05	N/A	N/A	< 1,9E+02
46	IPNV	8,6E+04	4,0E+04	N/A	1,9E+04
47	IPNV	4,0E+04	1,9E+03	4,0E+03	< 1,9E+02

N/A: Cell line not applied by the participating laboratory for titration of the virus

IPNV Sp Gen V	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	39	41	15	16
Median titre	2,7E+05	5,9E+04	1,3E+05	1,4E+04
Maximum titre	4,0E+06	2,7E+08	1,3E+07	1,3E+07
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	1,1E+05	8,6E+03	3,4E+04	<1,9E+02
75% quartile titre	5,9E+05	1,9E+05	4,0E+05	9,6E+04

Table 6. Inter-Laboratory Proficiency Test, PT1, 2018 – Results of titration of **ampoule III**.

IHNV BLK94					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	IHNV	8,6E+03	1,9E+06	8,6E+02	N/A
2	IHNV	< 1,9E+02	5,9E+04	N/A	N/A
3	IHN	< 1,9E+02	8,6E+05	< 1,9E+02	N/A
4	IHNV	1,26E+04	4,00E+05	N/A	N/A
5	IHNV	N/A	1,9E+08	N/A	N/A
6	IHNV	< 1,9E+02	2,7E+05	< 1,9E+02	2,7E+04
7	IHNV	2,7E+04	2,7E+05	N/A	N/A
8	IHNV	8,6E+03	4,0E+05	N/A	N/A
9	(IHNV)*	N/A	< 1,9E+02	< 1,9E+02	< 1,9E+02
10	IHNV	4,0E+03	1,3E+06	2,7E+03	4,0E+05
11	IHNV	2,7E+04	1,3E+06	N/A	N/A
12	IHNV	1,3E+03	5,9E+05	N/A	N/A
13	IHNV	1,3E+03	1,9E+03	N/A	N/A
14	IHNV	< 1,9E+02	5,9E+05	5,9E+02	N/A
15	IHNV	< 1,9E+02	1,9E+06	2,7E+02	N/A
16	IHNV	< 1,9E+02	1,3E+06	N/A	N/A
17	IHNV	4,0E+02	2,7E+05	N/A	N/A
18	IHNV	1,3E+03	8,6E+05	N/A	N/A
19	IHN	N/A	N/A	N/A	N/A
20	IHNV	5,9E+04	1,3E+06	N/A	N/A
21	IHNV	1,3E+04	2,7E+05	N/A	N/A
22	IHNV	< 1,9E+02	8,6E+04	4,0E+02	5,9E+05
23	IHNV	< 1,9E+02	5,9E+05	N/A	N/A
24	IHNV	N/A	2,7E+05	8,6E+04	N/A
25	IHNV	1,9E+04	1,3E+05	N/A	N/A
26	IHNV	< 1,9E+02	1,26E+05	N/A	2,73E+04
27	IHNV	4,0E+03	2,7E+05	N/A	N/A
30	IHNV	2,7E+03	2,7E+03	1,3E+04	1,9E+05
31	IHNV	< 1,9E+02	5,9E+02	N/A	N/A
32	IHNV	5,9E+03	1,3E+03	N/A	N/A
33	IHNV	N/A	1,3E+08	5,9E+08	1,3E+07
34	IHNV	1,3E+07	2,7E+08	N/A	N/A
35	IHNV	1,3E+03	1,3E+03	1,3E+03	1,3E+03

36	IHNV	< 1,9E+02	8,6E+06	N/A	N/A
37	IHNV	< 1,9E+02	2,73E+03	< 1,9E+02	2,73E+03
38	IHNV	N/A	2,73E+04	N/A	< 1,9E+02
39	IHNV	1,3E+03	1,3E+06	2,7E+04	1,3E+06
40	IHNV	1,9E+02	N/A	N/A	5,9E+04
41	IHNV	4,0E+04	2,7E+06	N/A	N/A
42	IHNV	< 1,9E+02	N/A	N/A	8,6E+03
43	IHN	1,3E+03	5,9E+04	N/A	N/A
44	IHNV	4,0E+03	2,7E+06	N/A	N/A
45	IHNV	< 1,9E+02	N/A	N/A	2,7E+05
46	IHNV	1,9E+06	4,0E+05	N/A	8,6E+05
47	IHNV	< 1,9E+02	4,0E+05	5,9E+05	< 1,9E+02

N/A: Cell line not applied by the participating laboratory for titration of the virus

IHNV BLK94	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	39	41	15	16
Median titre	1,3E+03	4,0E+05	8,6E+02	4,3E+04
Maximum titre	1,3E+07	2,7E+08	5,9E+08	1,3E+07
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	8,6E+04	1,4E+02	2,4E+03
75% quartile titre	8,6E+03	1,3E+06	2,0E+04	4,5E+05

Table 7. Inter-Laboratory Proficiency Test, PT1, 2018 – Results of titration of **ampoule IV**.

EHNV 86/8774					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	Ranavirus (EHNV)	2,7E+05	8,6E+04	1,3E+04	N/A
2	EHNV	5,9E+04	2,7E+04	N/A	N/A
3	EHNV	5,9E+05	2,7E+04	4,0E+04	N/A
4	NO IHNV,VHSV,SVCV,IPNV	1,3E+05	2,73E+04	N/A	N/A
5	Ranavirus	N/A	1,3E+05	N/A	N/A
6	EHNV	2,7E+05	8,6E+04	5,9E+02	< 1,9E+02
7	EHNV	4,0E+05	4,0E+04	N/A	N/A
8	EHNV	5,9E+06	5,9E+05	N/A	N/A
9	EHNV	N/A	1,3E+04	< 1,9E+02	< 1,9E+02
10	Ranavirus, EHNV	1,3E+06	2,7E+05	1,3E+06	5,9E+03
11	No IPNV,IHNV, VHSV, SVCV	4,0E+06	4,0E+04	N/A	N/A
12	EHNV	2,7E+05	2,7E+05	N/A	N/A
13	EHNV	8,6E+04	2,7E+05	N/A	N/A
14	EHNV	5,9E+05	8,6E+04	1,3E+06	N/A
15	EHNV	1,3E+04	1,3E+03	2,7E+02	N/A
16	EHNV	2,7E+05	2,7E+05	N/A	N/A
17	EHNV	1,3E+05	2,7E+05	N/A	N/A
18	Ranavirus	4,0E+04	8,6E+03	N/A	N/A
19	NEG	N/A	N/A	N/A	N/A
20	EHNV	5,9E+05	1,3E+04	N/A	N/A
21	EHNV	5,9E+05	8,6E+03	N/A	N/A
22	EHNV	1,3E+06	2,7E+04	1,9E+05	4,0E+04
23	EHNV	2,7E+04	5,9E+03	N/A	N/A
24	not performed for EHNV and Ranavirus	N/A	2,7E+03	2,7E+04	N/A
25	Ranavirus	5,9E+05	2,7E+03	N/A	N/A
26	EHNV viable virus	2,7E+05	< 1,9E+02	N/A	40000
27	Ranavirus (EHNV)	1,3E+06	4,0E+04	N/A	N/A
30	EHNV	2,7E+05	1,9E+04	4,0E+04	1,9E+04
31	EHNV	1,3E+05	< 1,9E+02	N/A	N/A
32	EHNV	1,9E+05	4,0E+03	N/A	N/A
33		N/A	2,7E+08	1,3E+09	2,7E+07
34	EHNV	1,3E+07	1,3E+08	N/A	N/A
35	Ranavirus (EHNV)	1,3E+05	5,9E+04	1,3E+05	1,3E+04

36	EHNV	5,9E+05	8,6E+04	N/A	N/A
37	EHNV	2,7E+05	1,86E+04	< 1,9E+02	1,9E+04
38	EHNV	N/A	5,87E+03	N/A	1,9E+03
39	EHNV	2,7E+05	1,3E+05	2,7E+05	8,6E+04
40	EHNV	1,3E+05	N/A	N/A	4,0E+02
41	EHNV	1,3E+06	1,9E+05	N/A	N/A
42	EHNV	8,6E+04	N/A	N/A	< 1,9E+02
43	EHNV	1,9E+05	1,9E+04	N/A	N/A
44	EHNV	5,9E+06	5,9E+06	N/A	N/A
45	EHNV	5,9E+05	N/A	N/A	1,9E+04
46	EHNV	8,6E+04	1,3E+05	N/A	5,9E+04
47	EHNV	1,3E+06	2,7E+05	2,7E+04	< 1,9E+02

N/A: Cell line not applied by the participating laboratory for titration of the virus

EHNV 86/8774	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	39	41	15	16
Median titre	2,7E+05	4,0E+04	4,0E+04	1,6E+04
Maximum titre	1,3E+07	2,7E+08	1,3E+09	2,7E+07
Minimum titre	1,3E+04	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	1,3E+05	1,3E+04	6,6E+03	3,0E+02
75% quartile titre	5,9E+05	1,9E+05	2,3E+05	4,0E+04

Table 8. Inter-Laboratory Proficiency Test, PT1, 2018 – Results of titration of **ampoule V**.

VHSV DK-3592B					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	VHSV	4,0E+03	1,9E+03	< 1,9E+02	N/A
2	VHSV	< 1,9E+02	< 1,9E+02	N/A	N/A
3	VHS	2,7E+04	2,7E+03	2,7E+03	N/A
4	VHSV	5,9E+03	1,3E+03	N/A	N/A
5	VHSV	N/A	2,7E+07	N/A	N/A
6	VHSV	1,3E+04	1,3E+03	1,9E+02	1,9E+02
7	VHSV	8,6E+02	5,9E+03	N/A	N/A
8	VHSV	1,3E+04	1,3E+04	N/A	N/A
9	(VHSV)*	N/A	< 1,9E+02	< 1,9E+02	< 1,9E+02
10	VHSV	2,7E+04	5,9E+03	2,7E+04	1,9E+05
11	VHSV	2,7E+05	1,9E+04	N/A	N/A
12	VHSV	4,0E+02	1,9E+03	N/A	N/A
13	VHSV	2,7E+03	4,0E+03	N/A	N/A
14	VHSV	8,6E+04	1,3E+03	1,3E+04	N/A
15	VHSV	8,6E+03	1,3E+04	8,6E+03	N/A
16	VHSV	< 1,9E+02	1,9E+03	N/A	N/A
17	VHSV	2,7E+03	1,9E+03	N/A	N/A
18	VHSV	1,3E+03	1,3E+03	N/A	N/A
19	VHS	N/A	N/A	N/A	N/A
20	VHSV	2,7E+04	2,7E+03	N/A	N/A
21	VHSV	4,0E+04	1,9E+03	N/A	N/A
22	VHSV	5,9E+02	1,3E+03	2,7E+03	1,9E+03
23	VHSV	2,7E+03	1,9E+03	N/A	N/A
24	VHSV	N/A	4,0E+04	4,0E+05	N/A
25	VHSV	8,6E+03	4,0E+02	N/A	N/A
26	VHSV	2,7E+02	< 1,9E+02	N/A	1,3E+03
27	VHSV	5,9E+04	1,3E+04	N/A	N/A
30	VHSV	8,6E+03	1,9E+03	1,9E+03	8,6E+03
31	VHSV	2,7E+02	1,9E+02	N/A	N/A
32	VHSV	1,9E+03	< 1,9E+02	N/A	N/A
33	VHSV	N/A	1,3E+09	1,3E+09	5,9E+08
34		< 1,9E+02	< 1,9E+02	N/A	N/A
35	VHSV	4,0E+03	5,9E+03	1,3E+03	5,9E+03
36	VHSV	1,9E+04	4,0E+03	N/A	N/A

37	VHSV	2,7E+03	2,7E+03	< 1,9E+02	1,3E+03
38	VHSV	< 1,9E+02	1,9E+04	< 1,9E+02	4,0E+03
39	VHSV	1,3E+03	2,7E+03	4,0E+03	2,7E+03
40	VHSV	2,7E+03	N/A	N/A	4,0E+04
41	VHSV	1,3E+04	4,0E+03	N/A	N/A
42	VHSV	8,6E+03	N/A	N/A	5,9E+04
43	VHSV	5,9E+04	2,7E+03	N/A	N/A
44	VHSV	8,6E+03	4,0E+03	N/A	N/A
45	VHSV	1,3E+03	N/A	N/A	1,9E+03
46	VHSV	1,9E+03	5,9E+03	N/A	1,3E+03
47	VHSV	1,9E+03	2,7E+03	< 1,9E+02	< 1,9E+02

N/A: Cell line not applied by the participating laboratory for titration of the virus

VHSV DK-3592B	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	40	41	16	16
Median titre	3,4E+03	2,7E+03	2,3E+03	2,3E+03
Maximum titre	2,7E+05	1,3E+09	1,3E+09	5,9E+08
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	1,3E+03	1,3E+03	<1,9E+02	1,3E+03
75% quartile titre	1,3E+04	5,9E+03	9,6E+03	1,6E+04

Figure 5. Virus titres obtained in BF-2 cells. The titre (red diamond) of each participating laboratory (country code) using BF-2 cells illustrated for ampoule I, II, III, IV and V. The detection level (grey shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line) are plotted on all graphs. For participants failing to obtain any titre no red diamond is shown.

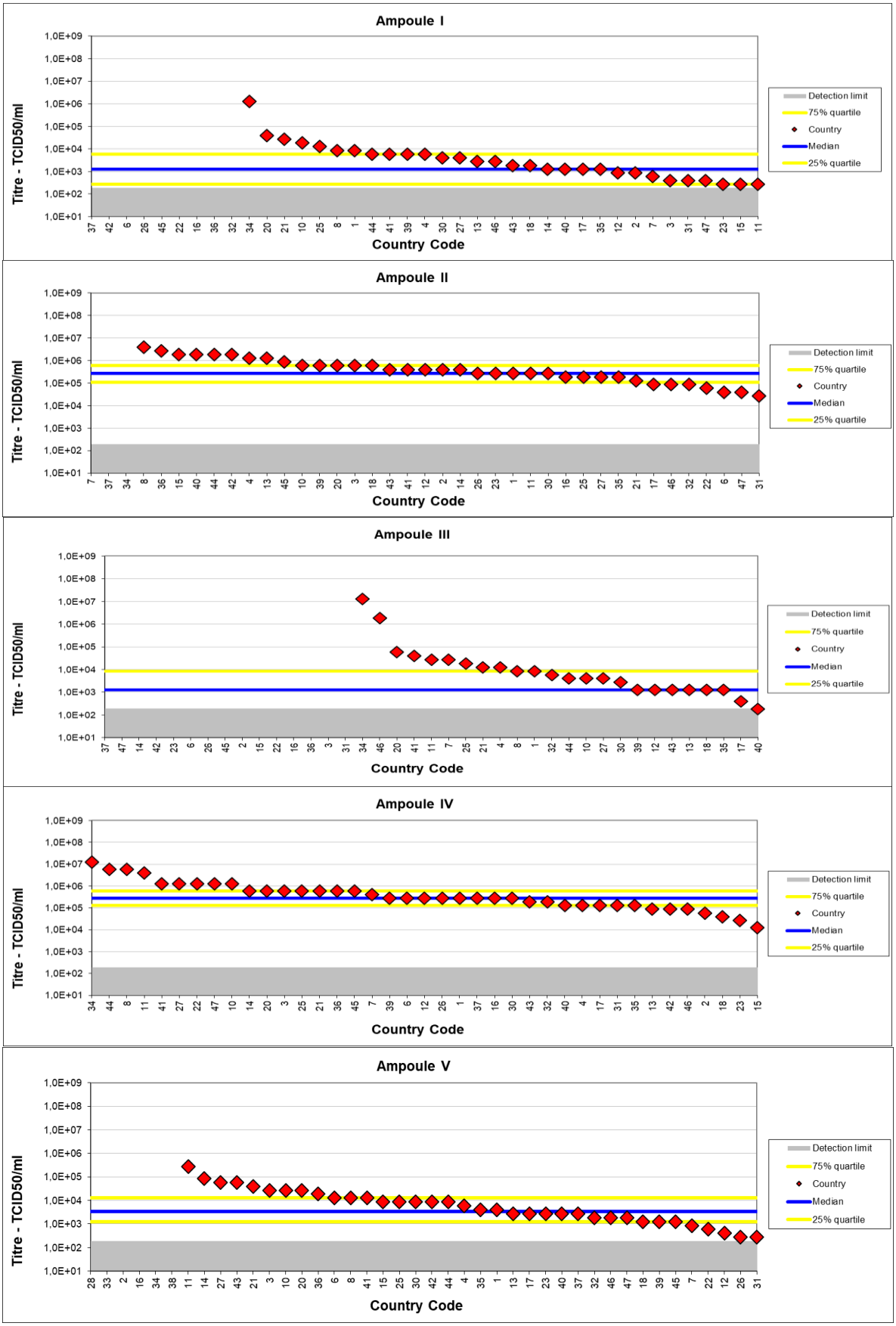


Figure 6. Virus titres obtained in EPC cells. For further details see description at Figure 5

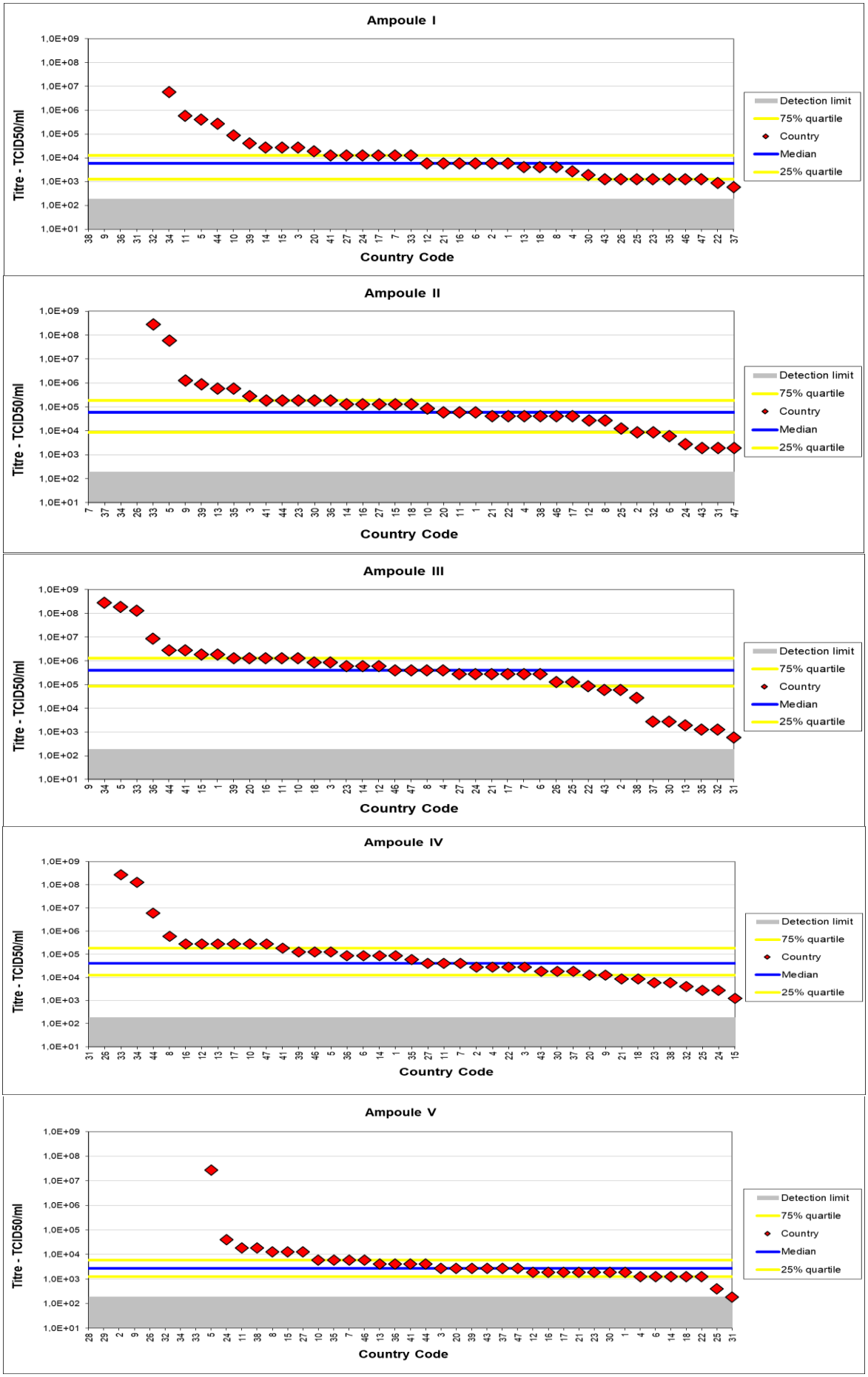


Figure 7. Virus titre obtained in RTG-2 cells. For further details see description at Figure 5

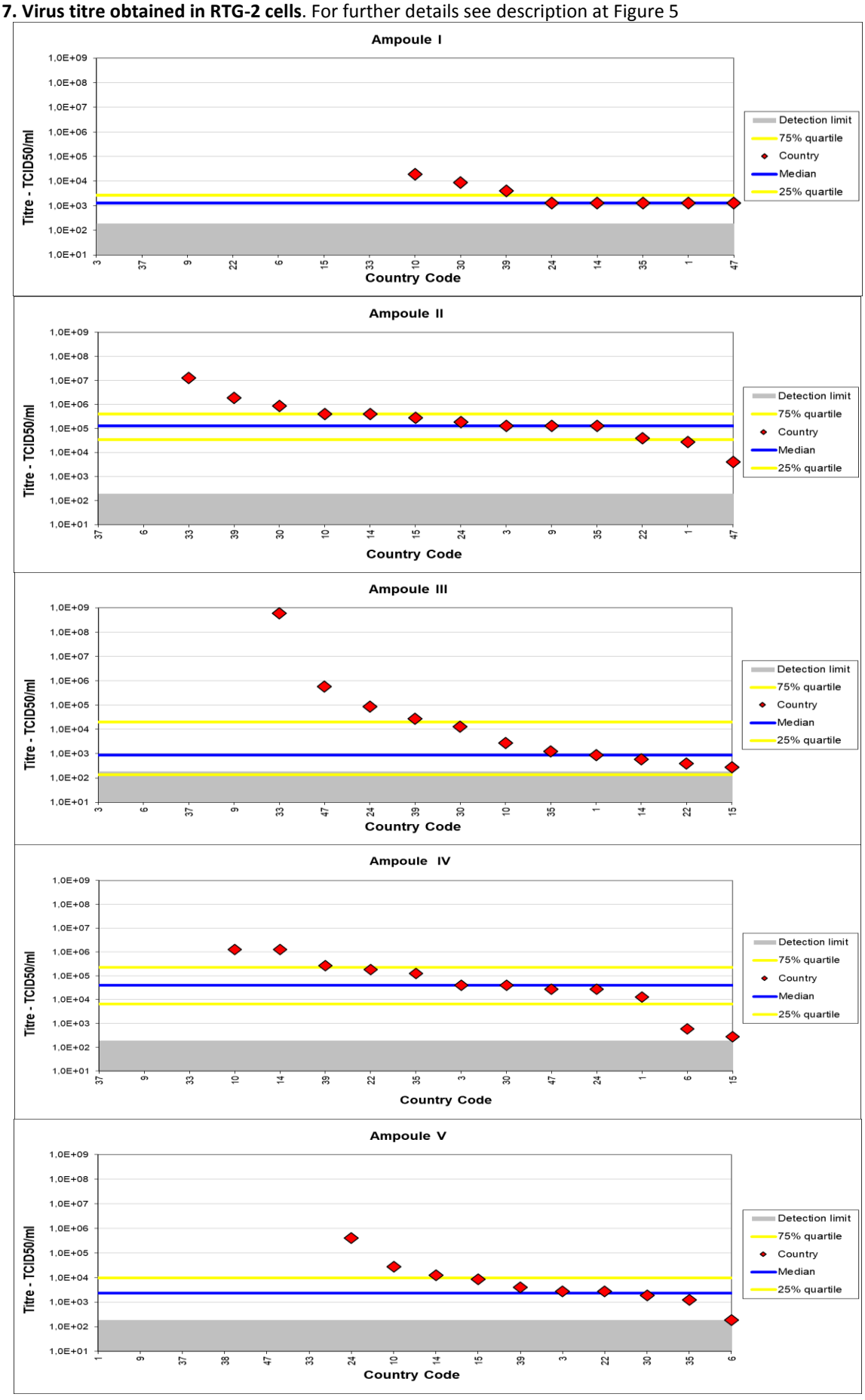
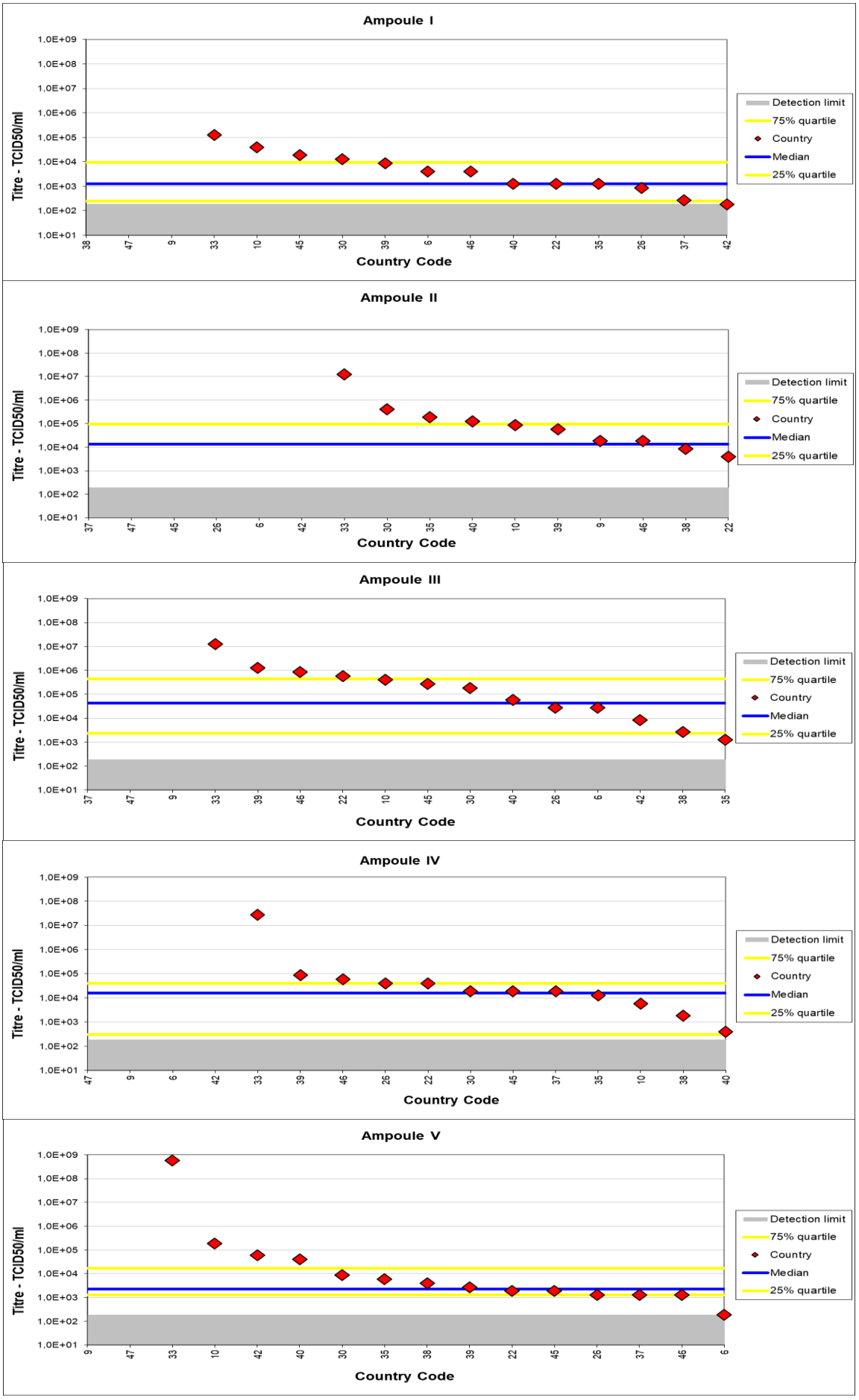


Figure 8. Virus titres obtained in FHM cells. For further details see description at Figure 5



Identification of content

- 40 laboratories analysed for all viruses; 35 of these laboratories correctly identified all viruses in all ampoules
- 42 participants submitted the spreadsheet within the deadline, 2 participants delivered the results for ampoule content within the deadline but needed a small extension to provide sequencing results. 1 participant submitted a spreadsheet from a former year by mistake and submitted the spreadsheet for 2018 after deadline, 2 participant did not delivered the results due to local delivery problems.

Ampoule I – SVCV (56/70)

- 43 laboratories correctly identified SVCV
- 1 laboratory answered 'Negative'
- 1 laboratory do not test for SVCV

Ampoule II – IPNV (Sp)

- 42 laboratories correctly identified IPNV
- 2 laboratories answered 'Negative'
- 1 laboratory do not test for IPNV

Ampoule III – IHNV (BLK94)

- All 45 laboratories correctly identified IHNV

Ampoule IV – EHNV (86/8774)

- 39 laboratories correctly identified the isolate as the listed EHNV by sequencing or REA (restriction enzyme analysis)
- 1 laboratory identified Ranavirus but did not specify if the isolate was the listed EHNV or not by sequencing or REA
- 5 laboratories do not test for Ranavirus.

Ampoule V – VHSV (DK-3592B)

- 44 laboratories correctly identified VHSV in ampoule V
- 1 laboratory did not identified VHSV

Scores

Starting with proficiency test 2003 we have provided a scoring system for the identification part of the proficiency tests. We have assigned a score of 2 for each correct answer/identification, giving the possibility for obtaining a maximum score of 10 (Table 3).

Ampoule I: SVCV identification was given the score 2. Incorrectly finding of "no virus" or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule II: IPNV identification was given the score 2. Incorrectly finding of "no virus" or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule III: IHNV identification was given the score 2. Incorrectly finding of "no virus" or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule IV: EHNV identification was given the score 2. The answer Ranavirus with no identification was given the score 1. Incorrectly finding of "no virus" or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule V: VHSV identification was given the score 2. Incorrectly finding of "no virus" or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Out of 45 laboratories participating in the PT 1 2018, 35 obtained score 10/10. The score 8/8 was assigned to 4 participants as they did not test Ranavirus; 1 participant obtained a score of 4/4 because did not test for SVCV, IPNV and Ranavirus. Finally 1 participant obtained 9/10 due to answering Ranavirus without corroborating the finding with sequence analysis and 4 obtained 8/10 because they did not identify correctly the viral content of one ampoule.

Cells applied for solving the test

Within the panel of cell lines available in the legislation the following ones were used by the participants:

- 40 laboratories used BF-2 cells
- 42 laboratories used EPC cells
- 15 laboratories used RTG-2 cells
- 17 laboratories used FHM cells
- 9 laboratories used CHSE-214 cells

- 3 laboratory used five cell lines: BF-2, EPC, RTG-2, FHM and CHSE-214

- 13 laboratories used four cell lines:
  - 8 laboratories used BF-2, EPC, RTG-2 and FHM
  - 3 laboratory used BF-2, EPC, RTG-2 and CHSE-214
  - 2 laboratory used BF-2, EPC, FHM and CHSE-214

- 9 laboratories used tree cell lines:
  - 4 laboratories used BF-2 cells in combination with EPC cells and RTG-2 cells
  - 3 laboratories used BF-2 cells in combination with EPC cells and FHM cells
  - 2 laboratory used RTG-2 cells in combination with EPC cells and FHM cells

- 27 laboratories used two cell lines:
  - 22 laboratories used BF-2 cells in combination with EPC cells
  - 3 laboratories used BF-2 cells in combination with FHM cells
  - 1 laboratories used RTG-2 cells in combination with EPC cells
  - 1 laboratory used EPC cells in combination with FHM cells

- 1 laboratory used only one cell line (EPC) and 1 laboratory did not titrate.

The combination of EPC and FHM cells or BF-2 and RTG 2 alone is not valid according to Commission Decision 2015-1554. The laboratories using these combinations are encouraged to include the use of BF-2 cells or RTG 2 cells and EPC cells or FHM cells.

The median titre calculated from the submitted results of each ampoule and in each cell line is illustrated in Figure 9.
It appears that:
Ampoule I (SVCV, 56/70) replicates equally on all four cell lines (EPC, BF-2, FHM and RTG-2).
Ampoule II (IPNV, sp) replicate on all four cell lines, however it grows best on BF-2 cells, a little less efficiently on RTG-2, EPC and FHM.
Ampoule III (IHNV, BLK94) replicates on all four cell lines, however it grows best on EPC cells and a little less efficiently on FHM and less on RTG-2.
Ampoule IV (EHNV, 86/8774) replicates well on BF-2 and a little less efficiently on EPC, FHM and RTG-2.
Ampoule V (VHSV, dk-3592B) replicates equally on all four cell lines (EPC, BF-2, FHM and RTG-2).
As it appears from Table 3-8 the variations in titres between laboratories was very high – with more than 7 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved and the ones with a high titre should ensure to follow the correct titration procedure.

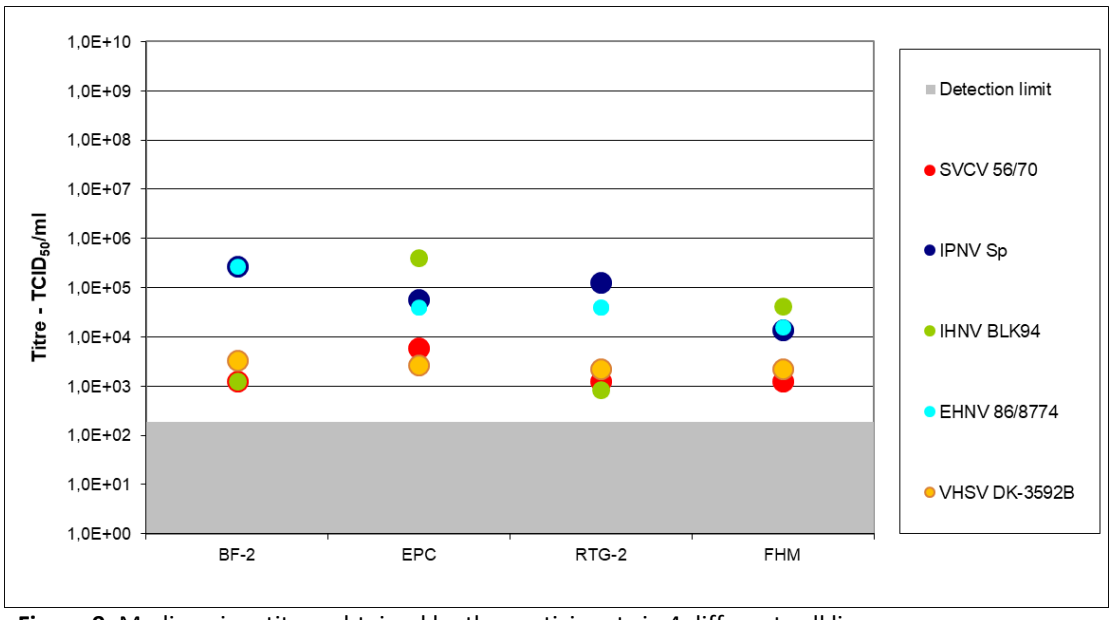


Figure 9. Median virus titres obtained by the participants in 4 different cell lines.

Genotyping and sequencing

In previous proficiency tests provided by the EURL, we have encouraged participants to genotype the identified viruses though it has not been a mandatory task. As ranaviruses are included in the test, it is mandatory to do sequence or REA analysis in order to discriminate EHNV from the non-listed ranaviruses. For IHNV and VHSV we still encouraged participants to genotype isolates according to the notification described in Einer-Jensen et *al.* (2004) [5] for VHSV and in Kurath et *al.* (2003) [7] and Emmenegger et *al.*, 2000 [8] for IHNV.

An Overview of the genotyping results obtained for PT1 by all participants is displayed in the following table 9

Table 9. The genotyping results obtained for PT1 by all 45 participants

Laboratory code number	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
	SVCV 56/70 Genotype: Id	**IPNV (Sp) Genotype: 5**	**IHNV BLK94 Genotype: U**	**EHNV 86/8774**	**VHSV DK-3592B Genotype: Ia**
1	Genogroup 1d	IPNV Genogroup 5	IHNV Genotype U	*	VHSV Genotype 1a
2	Id	sp	U	Australia	Ia
3	Nd	Nd	Nd	Epizootic haematopoietic necrosis virus	Nd
4	Nd	Nd	Nd	Nd	Nd
5	Nd	Nd	Nd	Nd	Nd
6	Id	V	U	*	Ia
7	Nd	Nd	Genogroup U	*	Genotype Ia
8	*	*	U	*	Ia1
9	*	Sp	U	*	Nd
10	Strain Fijan	Sp	BLK94, genogroup U, subtype P	*	DK-3592B, serotype I, genotype Ia
11	Nd	Nd	Nd	Nd	Nd
12	*	Genogroup 5	Genogroup U	*	Ia
13	*	5	U	*	Ia
14	European clade Genogroup Id	Genogroup 5	Genogroup U	EHNV	Genotype Ia
15	Sprivivirus	5	U	*	Ia
16	Nd	Nd	Nd	*	Nd
17	*	Nd	Nd	*	I a
18	Nd	Nd	Nd	Blast analysis of the sequence showed the highest sequence identity with accession nr. FJ433873.1	Nd
19	Nd	Nd	Nd	Nd	Nd
20	Genogroup Id (from Stone et al., 2003)	Genogroup 5 (Sp)	U	*	Ia
21	SVCV	Sp (VP1 genogroup 2; VP2 genogroup V)	U	EHN	Ia1
22	Id	V, (serotype: Sp)	U	*	Ia
23	Nd	Nd	*	*	*

24	Nd	Nd	Nd	Nd	Nd
25	*	IPNV	U	EHNV	1a
26	Nd	Nd	U	EHN	1a
27	Nd	Nd	U	*	1a
30	Nd	Nd	Genotype M	*	Genotype Ie
31	Nd	Nd	Nd	*	Nd
32	Nd	Nd	Nd	*	Nd
33	Nd	Nd	U	Nd	Nd
34	Nd	Nd	Nd	*	Nd
35	*	*	*	*	*
36	Nd	Nd	*	*	*
37	*	Nd	U	*	Ia
38	Nd	Genogroup 5	Genogroup U	*	Genotype Ia
39	*	Sp Serotype	U genogroup	*	Ia genotype
40	Nd	5	Genogroup U	*	1a
41	*	Nd	U	*	Ia
42	Nd	Nd	*	*	genotype I a/b
43	Nd	Nd	Nd	*	Nd
44	European Id	*	Upper genogroup	*	Genogroup Ia-1
45	1D	Genogroup 5 - SP A2	U	EHNV	IA
46	strain=Fijan	Serotype=Sp	genogroup U	Australian	Nd
47	Id	SP; Genogroup III	U clade	*	Ia
No. of sequence preformed	**24**	**22**	**32**	**44**	**30**
No. of correct genotypes given	**10**	**12**	**27**	**14**	**26**
No. of correct sequences provided without genotype assigned	**14**	**10**	**4**	**30**	**3**
No of incorrect genotype provided	**0**	**0**	**1**	**0**	**1**

*Sequence provided but no genotyping assigned under Genotype in the spreadsheet for Sequencing results – Proficiency Test 1
Nd: Not done - no sequence provided

AMPOULE I- SVCV strain 56/70 Genotype Id

24 laboratories sequenced the isolate in ampoule I.

10 laboratories correctly genotyped the isolate in ampoule I as genotype Id, 14 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype.

AMPOULE II- IPNV strain Sp Genotype 5

22 laboratories sequenced IPNV isolate in Ampoule II; 12 correctly identified the isolate as belonging to genotype 5, 10 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype.

AMPOULE III IHNV - isolate BLK94 American Genotype U

32 laboratories sequenced the IHNV isolate in ampoule III. Out of these 27 correctly identified the isolate as genotype U, 4 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype. 1 laboratory identified the isolate incorrectly as Genotye M

AMPOULE IV – EHNV Isolate 86/8774

44 laboratories sequenced EHNV in ampoule IV. 14 laboratories correctly identified the isolate as EHNV or Australian Genotype. 30 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype.

AMPOULE V – VHS virus, DK-3592B Genotype Ia.

30 laboratories sequenced the VHSV isolate in ampoule V. Out of these 26 correctly identified the isolate as genotype Ia, 3 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype. 1 laboratory identified the isolate incorrectly as Genotye Ie.

Résumé and concluding remarks PT1

30% of parcels were delivered by the shipping companies within 1 day after submission, 91% was delivered within 1 week and 94% was delivered within 2 week. One of the remaining three parcels took 18 days and 2 parcels were never delivered due to internal clearance problems.

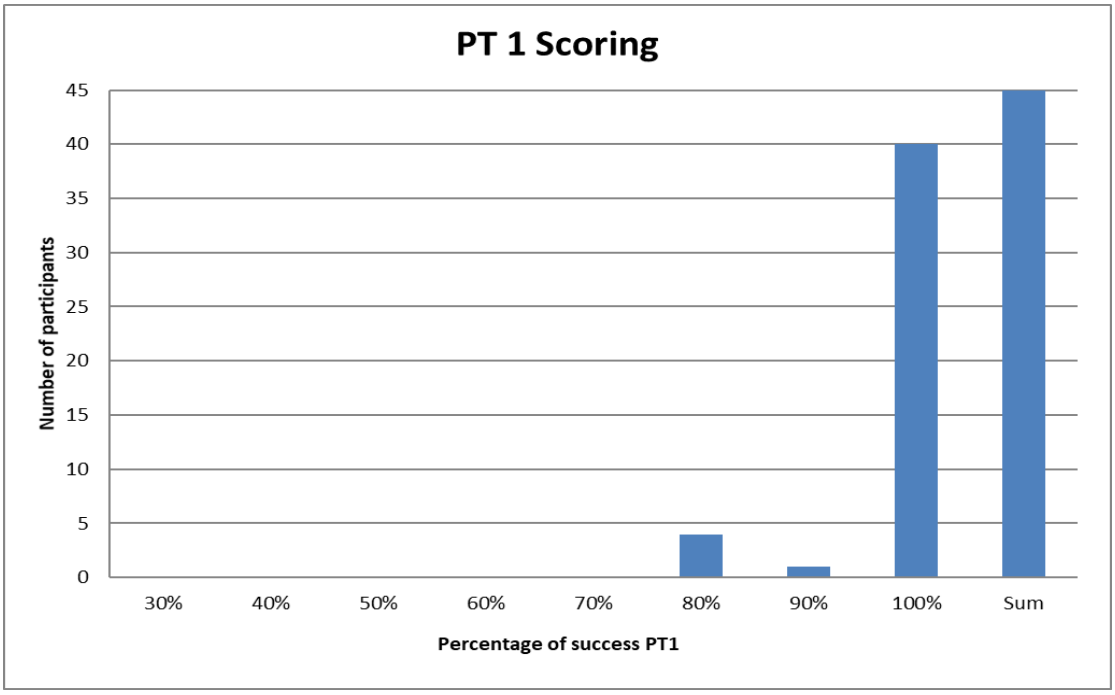
This year EHNV was included in the Proficiency test. 5 out of the 45 countries do not test for Ranavirus. 39 participants provided the correct identification, hereof one laboratory identified correctly the isolate as EHNV by sequencing but submitted the result as Ranavirus and one laboratory did not performed sequencing.

In this report (Figures 5-8), all the viral titres submitted by participants are compared to each other. In this way, the titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory is able to compare the sensitivity of its cell lines to the sensitivity of those used by the other participants. We recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose, especially as it appears that the variations in titres between laboratories was very high – with more than 7 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved and the laboratories scoring very high titres should ensure that the titration procedure is properly implemented.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We will also take the opportunity to provide a comment to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

Overall 40 out of 45 participants scored 100% success rate and 1 participant scored 90% due to sequencing of the content in ampoule IV (EHNV) and 3 participants scored 80% due to not finding one of the virus. These points will be assessed directly with the single participants that has underperformed.

The results presented in this report will be further presented and discussed at the 23rd Annual Workshop of National Reference Laboratories for Fish Diseases to be held 27th and 28th of May, 2019 in Kgs. Lyngby, Denmark.



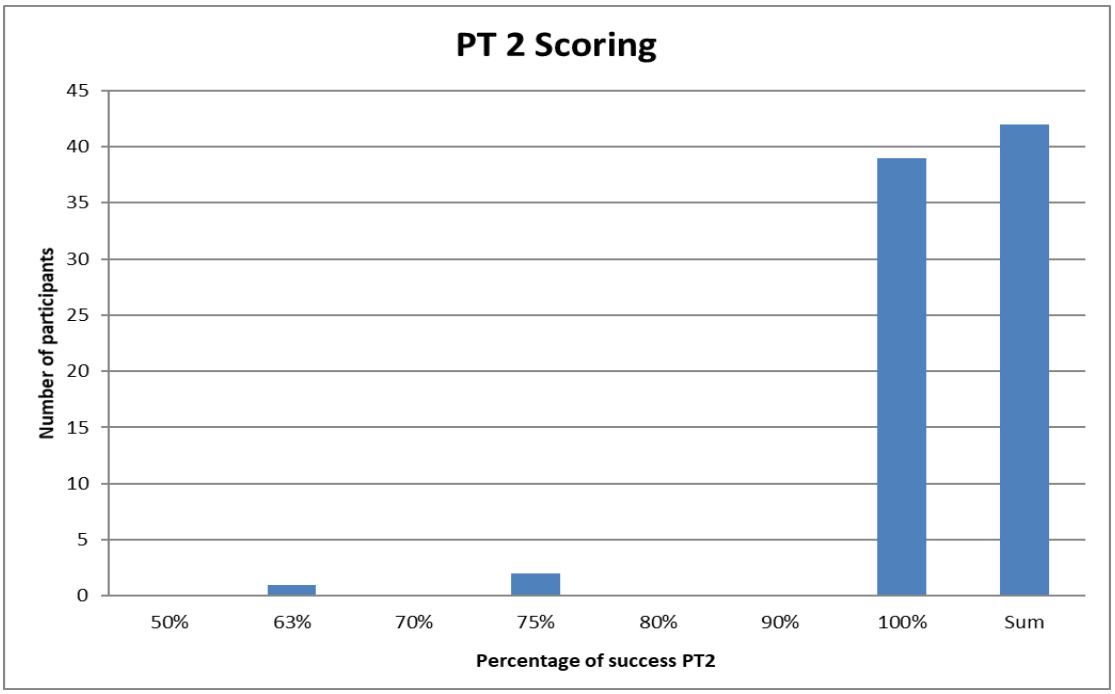


Figure 10 "a" and "b" Success-rate of participating laboratories 2018

Proficiency test 2, PT2

Four ampoules containing lyophilised cell culture supernatant were delivered to the same laboratories that participated in PT1 with the exception of three laboratories that participated only in PT1.

Content of ampoules

The viruses were propagated on each of their preferred cell line and when total cytopathic effect (CPE) was observed, the supernatants were collected and filtrated through a 45 µm filter, mixed with equal volumes of 2% w/v lactalbumin hydrolysate solution and lyophilized in glass ampoules. Before the ampoules were sealed by melting, the pathogen concentration was analysed by the KHV real-time PCR protocol described by Gilad et *al*. (2004) [21]and the conventional PCR protocol described by Bercovier et *al*. (2005) [22], the SAV real-time RT-PCR protocol described by Hodneland et al. (2006) [23], and the conventional PCR targeting segment E2 described by Fringuelli et al. (2008) [24] and the ISAV real-time RT-PCR protocol described by Snow et *al*. (2006) [25] and conventional RT-PCR protocol described by Mjaaland et *al*. (2002) [26].

The details of the virus isolates used in the proficiency test 2 are outlined in table 10.

Table 10. Content in each ampoule with reference to culture conditions and major publications of the included pathogens.

Code	Specifications/References
Ampoule VI: KHV	**Koi Herpesvirus isolate KHV 1287** Isolate from Common Carp (*Cyprinus Carpio*), from a river in the Okayama region, Japan in 2012. **Received from**: Dr. Kei Yuasa, National Research Institute of Aquaculture, Japan. **Passages no. in cell culture**: 4
Ampoule VII: ISAV	**ISAV Glesvaer/2/90** Virulent isolate of Infectious Salmon Anemia Virus, HPRΔ isolated from Atlantic salmon **HPR Genotype:** 2 **Received from:** Dr. B. Dannevig, OIE Reference Laboratory for ISA, Oslo, Norway **GenBank accession numbers:** HQ259676 or AF220607.1 or DQ785248.1 **References on isolate:** Dannevig BH, Falk K & Namork E (1995). Isolation of the causal virus of infectious salmon anaemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *Journal of General Virology* 76, 1353–1359. [27] Falk K, Namork E, Rimstad E, Mjaaland S & Dannevig BH (1997). Characterization of infectious salmon anemia virus, an orthomyxo-like virus isolated from Atlantic salmon (Salmo salar L.) *Journal of Virology* 71, 9016-9023. [28] **References on sequence:** Mérour E, LeBerre M, Lamoureux A, Bernard J, Brémont M & Biacchesi S (2011). Completion of the full-length genome sequence of the infectious salmon anemia virus, an aquatic orthomyxovirus-like, and characterization of mAbs. *Journal of General Virology* 92, 528-533. [29] **References on genotype:** Table 15. Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety 26.01.07. Which risk factors relating to spread of Infectious Salmon Anaemia (ISA) require development of management strategies? Dok.nr.06/804, 68 pages. [30]
Ampoule VIII: SAV	**Salmonid alpha virus (SAV) 3, Pancreas Disease Virus (PD)** Salmonid alphavirus strain Norway – R-1_2007, isolated from Atlantic salmon **Received from**: Dr. Hilde Sindre, Norwegian Veterinary Institute, Norway **Reference on isolate:** Taksdal T., Bang Jensen B., Bockerman I., McLoughlin M.F., Hjortaas M.J., Ramstad A. & Sindre H. (2015) Mortality and weight loss of Atlantic salmon, Salmon salar L., experimentally infected with salmonid alphavirus subtype 2 and subtype 3 isolates from Norway. Journal of Fish Diseases 38, 1047–1061. [31] **Gene Bank Ref**. **E2 gene**: LT630447 **References on the sequences**: Hjortaas M.J., Bang Jensen B., Taksdal T., Olsen a B., Lillehaug a, Trettenes E. & Sindre H. (2016) Genetic characterization of salmonid alphavirus in Norway. *Journal of Fish Diseases* **39**, 249–257. [32]
Ampoule IX : BLANK	BF-2 NON Infected Supernatant

Testing of the test

The PT2 test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043. Prior to the distribution we tested 5 ampoules of each virus preparation, by real-time PCR (Gilad et *al*. (2004))[21] for KHV, by real-time RT-PCR (Snow et *al*. (2006)) [25]for ISAV and by real-time RT PCR (Hodneland et al. (2006))[23] for SAV, to ascertain identity and homogeneity of the content in the ampoules (Figure 11). As a result all the standard deviations were below 1 Ct. value. Furthermore, after deadline for handling in results and minimum 3 months after lyophilisation and storage in the dark at 4°C, the content of the ampoules were tested to assess their stability (Table 11 and Figure 12).
Conventional PCR/RT-PCR fragments were sequenced and so was the HPR region in segment 6 of the ISAV isolate.

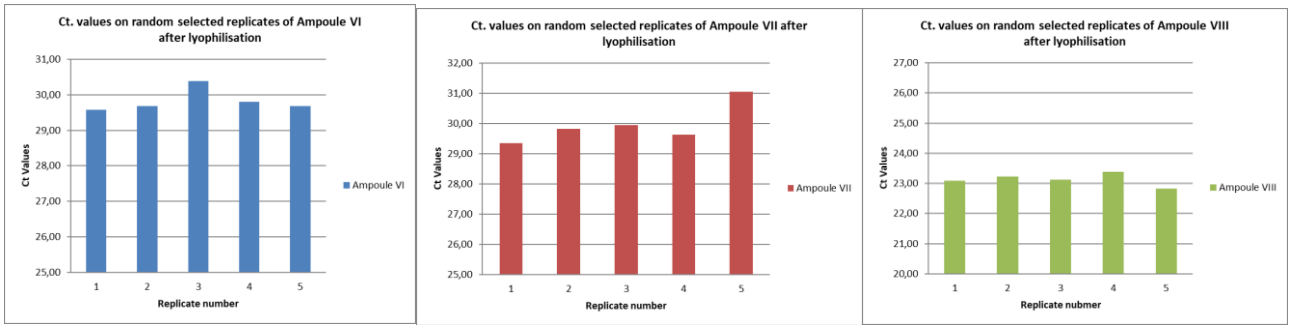


Figure 11, Ampule VI (KHV), VII (ISAV) and VIII (SAV) tested shortly after lyophilisation to assess homogeneity of the content.

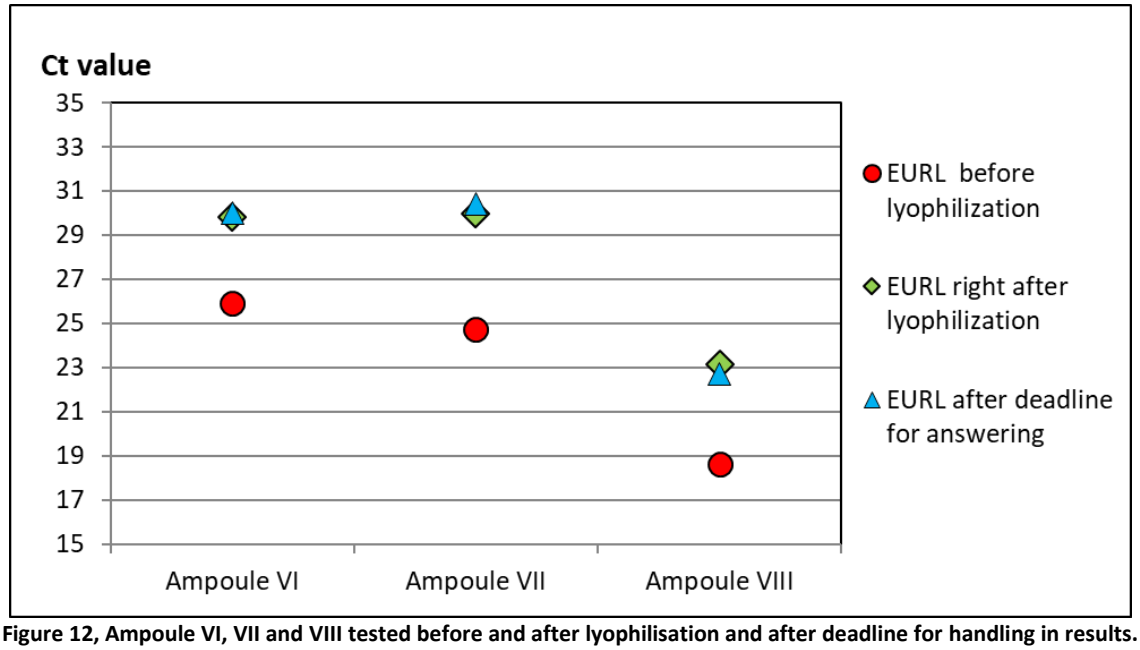


Figure 12, Ampoule VI, VII and VIII tested before and after lyophilisation and after deadline for handling in results.

Table 11, Ct-value of ampoules VI to IX tested before and immediately after lyophilisation and after deadline for handling in results.

Ampoule	Content	Cell line	EURL before lyophilization	EURL right after lyophilization	EURL after deadline for answering
Ampoule VI	KHV	a	25,92	29,58	29,98
		b		29,68	
		c		30,38	
		d		29,80	
		e		29,68	
	Median Value		**25,92**	**29,82**	**29,98**
Ampoule VII	ISAV	a	24,75	29,35	30,42
		b		29,83	
		c		29,94	
		d		29,63	
		e		31,06	
	Median Value		**24,75**	**29,96**	**30,42**
Ampoule VIII	SAV	a	18,61	23,08	22,74
		b		23,23	
		c		23,12	
		d		23,39	
		e		22,82	
	Median Value		**18,61**	**23,13**	**22,74**
Ampoule IX	BF-2 cells*	a	No Ct	No Ct.	No Ct
		b		No Ct.	
		c		No Ct.	
		d		No Ct.	
		e		No Ct.	
	Median Value		**No Ct.**	**No Ct.**	**No Ct.**

*Tested for KHV, ISAV and SAV

The lyophilisation procedure caused a significant virus reduction (mainly in ampule VII with ISAV and VIII with SAV) as detected by real-time PCR or real-time RT-PCR.

For each ampoule no other pathogens than the expected were detected.

Pathogen identification

In PT2, participants were asked to identify any of the fish viruses ISAV and KHV (both listed in Council Directive 2006/88/EC) [1] according to diagnostic procedures described in Council implementing directive 2015-1554 [3]. Bearing in mind that the test ampoules also could contain other viruses. Participants were expected to use their normal PCR or real-time PCR methods for detection of KHV and their normal RT-PCR or real-time RT-PCR methods for detection of ISAV.
It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses had not been inactivated and should thus be viable.
Also this year, the panel of pathogens to be investigated included SAV – salmonid alpha virus. Since this is not a listed disease in the European legislation the participation was voluntary and therefore the participants were asked to declare if the ampoules were tested for SAV or not. Regarding methods for detection of SAV the participants were notified that they could refer to the chapter 2.3.5 – Infection with salmonid alpha virus OIE Manual of Diagnostic Tests for Aquatic Animals.
In order to obtain uniform answers, participants were requested to download a spreadsheet available from the http://www.eurl-fish.eu, insert results in this and return by email.
The results from participating laboratories are shown in table 12.

Table 12. Inter-Laboratory Proficiency Test, PT2, 2017 - Virus identification.

Laboratory code number	Score	Answer received at EURL	Ampoule VI KHV (CyHV-3) 1287	Ampoule VII ISAV Glesvaer/2/90	Ampoule VIII SAV 3 PD	Ampoule IX Blank Non infected BF-2 cell culture
1	8/8	30-11-2018	KHV	ISAV	SAV	Negative
2	8/8	29-11-2018	KHV	ISA	SAV	No virus detected
3	5/8	29-11-2018	ISAV KHV	no pathogen found	SAV	no pathogen found
4[2]		29-11-2018	0	0	0	0
5	6/8	03-12-2018	KHV	ISAV	SAV	KHV
6	8/8	03-12-2018	KHV	ISAV	SAV	Neg
7[2]		03-12-2018	0	0	0	0
8	8/8	29-11-2018	KHV	ISAV	SAV	Neg for KHV, ISAV and SAV
9	6/8	29-11-2018	KHV	ISAV	*	0
10	8/8	03-12-2018	KHV	ISAV	SAV	Not ISAV, not KHV and not SAV
11[1]	6/6	15-02-2019	KHV	ISAV	No KHV and no ISAV	No KHV and no ISAV
12	8/8	03-12-2018	KHV	ISAV	SAV	No virus detected
13	8/8	30-11-2018	KHV	ISAV HPR2	SAV	No virus
14	8/8	08-11-2018	KHV Japanese lineage	ISAV HPR deleted	SAV 3	negative for ISAV, KHV and SAV
15	8/8	03-12-2018	KHV	ISAV (HPRdel)	SAV	negative
16	8/8	30-11-2018	KHV	ISAV	SAV	NO KHV- NO ISA NO SAV
17	8/8	29-11-2018	KHV	ISAV	SAV	-
18[3]	6/6	29-11-2018	No virus detected	ISAV	SAV	No virus detected
19[4]	4/4	03-12-2018	KHV	NEG	NEG	NEG
20	8/8	29-11-2018	KHV	ISAV	SAV	Blank
21	8/8	29-11-2018	KHV	ISAV	SAV	Negative
22	8/8	19-11-2018	KHV	ISAV	SAV	-
23[1]	6/6	26-11-2018	KHV	ISAV HPR2	Not KHV, not ISAV	Not KHV, not ISAV
24[1]	6/6	03-12-2018	KHV	ISAV	0	0
25	8/8	03-12-2018	KHV	ISAV	SAV	No virus was detected in this ampoule
26	8/8	03-12-2018	KHV	ISAV	SAV viable virus	Negative
27	8/8	30-11-2018	KHV	ISAV	SAV	No virus detected
30	8/8	03-12-2018	KHV	ISAV	SAV	-
31[1]	6/6	03-12-2018	KHV	ISAV	Not ISAV Not KHV	Not ISAV Not KHV
32	8/8	03-12-2018	KHV	ISAV	SAV	Negative

33[2]		30-11-2018	0	0	0	0
34	8/8	03-12-2018	KHV	ISAV	SAV	0
35	8/8	26-11-2018	KHV	ISAV	SAV	-
36	8/8	03-12-2018	KHV	ISAV	SAV	0
37	8/8	30-11-2018	KHV	ISAV	SAV	NEGATIVE
38	8/8	28-11-2018	KHV	ISAV	SAV	Negative
39	8/8	26-11-2018	KHV	ISAV	SAV	NO VIRUS
40	8/8	03-12-2018	KHV	ISAV	SAV	Negativ (no virus)
41	8/8	06-11-2018	KHV	ISA	SAV	no KHV, ISAV, SAV
42	8/8	30-11-2018	KHV	ISAV	SAV	Negative: No KHV, ISAV or SAV
43	8/8	30-11-2018	KHV	ISAV	SAV	Virus was not detected.
44	8/8	29-11-2018	KHV	ISAV	SAV	NEG
45	8/8	23-11-2018	CyHV-3	ISAV	SAV	Negative
46	8/8	27-11-2018	KHV	ISAV	SAV	Negative
47	8/8	16-11-2018	KHV	ISAV	SAV	not ISAV, KHV, or SAV

[1)] Did not test for SAV, [2)] Did not participate in PT2, [3)] Did not test for KHV, [4)] Did only test for KHV

All laboratories are asked to sequence the HPR region of ISAV isolates to distinghish from the pathogenic HPRΔ variant from ISAV HPR0 which has been delisted in Council Directive 2006/88/EC Annex IV .

It was requested that the pathogens in the samples were not forwarded to third parts without having contacted the EURL for permission in advance.

Identification of content

- 42 laboratories submitted results
- 33 laboratories correctly identified all four ampoules (KHV, ISAV, SAV, Blank)
- 40 laboratories tested for the two listed pathogens
- 41 laboratories tested for ISAV
- 41 laboratories tested for KHV
- 37 laboratories tested for SAV
- 3 laboratories that did participate in PT 1 did not participate in PT2

Ampoule VI – KHV

- 40 laboratories correctly identified KHV
- 1 laboratory did not participate for KHV and answered 'No virus detected'
- 1 laboratory correctly identified KHV but contaminated the ampoule with an ISAV which was in ampoule VII.

Ampoule VII – ISAV

- 40 laboratories correctly identified ISAV
- 1 laboratory answered 'No pathogen found''
- 1 laboratory did not participate for ISAV and answered 'NEG'

Ampoule VIII – SAV

- 36 laboratories correctly identified SAV
- 5 laboratories did not participated in identifying SAV and 3 correctly ruled out the other 2 listed pathogens (KHV and ISAV) in this ampoule. 1 Laboratory answered 'NEG' and 1 did not fill in an answer.
- 1 laboratory answered '*In ampoule VIII we received strong band in SAV PCR, but its size was much lower and sequencing was unsuccessful'

Ampoule IX – BLANK

- 41 laboratories ruled out the presence of pathogens they were testing for, the answers varied from 'Neg for KHV, ISAV and SAV' to leaving the field empty.
- 1 laboratory incorrectly identified KHV in ampoule IX

Scores

We have assigned a score of 2 for each correct answer (Table 12), giving the possibility for obtaining a maximum score of 8.
Not finding of the pathogen present in the ampoule gives the score 0.
Incorrectly finding of pathogens not present in the ampoules gives the score 0.
Finding of correct pathogen present in the ampoule but with contamination of another pathogen gives the score 1.

Of the 42 laboratories submitting results 39 laboratories obtained maximum score. The maximum score was calculated according to the number of pathogen tested by the laboratory.
A laboratory could obtain a maximum score of 8 if tested for all three pathogens included (ISAV, KHV and SAV) or the two listed pathogens (ISAV and KHV) .
If a laboratory did not test for KHV or ISAV but tested for SAV the maximum score was 6 points.
1 laboratory did neither test for ISAV or SAV therefor the maximum score was 4 points.

Methods applied
The following methods were used by the participants:

KHV detection
- 26 laboratories used Real Time PCR protocols for KHV detection.
- 31 laboratories used KHV PCR.

ISAV detection
- 27 laboratories used ISAV real-time RT-PCR.
- 29 laboratories used conventional RT-PCR.

SAV detection
- 23 laboratories used SAV real-time RT-PCR.
- 27 laboratories used SAV RT-PCR.

Genotypning and sequencing
Participants were asked to sequence the HPR region of possible ISAV isolates

An Overview of the genotyping results obtained for PT2 by all participants is displayed in the following table 13

Tabel 13. The genotyping results obtained for PT2 by all 42 participants

Laboratory code number	Ampoule VI	Ampoule VII	Ampoule VIII
	CyHV – Genotype 3 KHV 1287	ISAV HPRΔ Glesvaer/2/90 HPR Genotype: 2	SAV – Genotype 3 NO-R-1_2007
1	KHV	ISAV HPR2	SAV Genotype 3
2	Genotype 3 (Asian)	HPR Genotype: 2	Subtype 3 (SAV 3)
3	Nd	Nd	Nd
4	Did not participate in PT2		
5	Nd	Nd	Nd
6	*	HPR2	subtype 3
7	Did not participate in PT2		
8	*	*	*
9		*	N/A
10	*	HPR2	Subtype 3
11	Nd	Nd	Nd
12	*	EU-G2	SAV3
13	*	HPR2	*
14	Nd	Genotype HPR deleted	SAV3
15	Nd	HPR group 2/ EU-G2 group	SAV subtype 3
16	Nd	*	Nd
17	Nd	ISAV 4	Nd
18	Nd	Nd	Nd
19	Nd	Nd	Nd
20	CyHV-3	HPR2	SAV3
21	CyHV-3	Nd	IIII (SAV 3)
22	CyHv-3	HPR2	III
23	*	HPR2	Nd
24	Nd	Nd	Nd
25	Nd	HPR2	E2 (III)
26	Nd	G2 (HPR/deleted)	subtype 3
27	CyHV3	HPR deleted	SAV3
30	Nd	HPR genotype: 2	Nd
31	Nd	G2	Nd
32	Nd	*	Nd
33	Did not participate in PT2		
34	Nd	Nd	Nd

35	*	*	*
36	*	*	*
37	*	HPR-4	*
38	*	HPR deleted(HPR2)	*
39	CyHV 3	ISAV (HPR2)	SAV 3 (PD)
40	*	HPR deleted	3A
41	Nd	HPR0	SAV3
42	Nd	Nd	Nd
43	Nd	Nd	Nd
44	Wildtype KHV	HPR2	SAV-3
45	CyHV-3	HPR2	SAV 3
46	Nd	HPR2 , genogroup EU-G2	Nd
47	*	European- deleted	Subtype 3
No. of sequences performed	**21**	**32**	**25**
No. of correct genotypes given	**9**	**19**	**19**
No. of correct sequences provided without genotype assigned	**12**	**10**	**6**
No of incorrect genotype provided	**0**	**3**	**0**

*Sequence provided but no genotyping assigned under Genotype in the spreadsheet for Sequencing results – Proficiency Test 1
Nd: Not done - no sequence provided

AMPOULE VI KHV CyHV-3:
21 laboratories sequenced the KHV isolate included in ampoule VI.
9 laboratories correctly genotyped the isolate in ampoule VI as CyHV-3 or KHV; 13 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype.

AMPOULE VII ISAV Glesvaer/2/90 HPR Genotype: 2:
32 laboratories sequenced the ISAV isolate included in ampoule VII.
19 laboratories correctly genotyped the isolate in ampoule VII as ISAV HPRΔ HPR 2; 10 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype.
Finally 3 laboratories provided a sequence but assigned the incorrect genotype; 1 laboratory described the isolate as HPR0 and 2 laboratories characterized the isolate as HPR4.

AMPOULE VIII SAV – Gen.3 NO-R-1_2007:
25 laboratories sequenced the SAV isolate included in ampoule VIII.
19 laboratories correctly genotyped the isolate in ampoule VIII as SAV-3; 6 laboratories provided the sequence but did not assign a genotype or provided an incomplete genotype.

Concluding remarks PT2

The EURL have decided to include SAV in the panel of viruses included in PT2 since this was regarded as a proper initiative that strengthen the diagnostic capacities of the NRLs in detecting emerging pathogens.
42 laboratories participated in PT2.
37 laboratories tested for SAV and 36 correctly identified the virus in Ampoule VIII, 1 laboratory experienced some difficulties due to a non-specific PCR amplification.
40 out of the 42 laboratories correctly identified the ISA virus in ampoule VII, 1 did not test for ISAV and 1 laboratory answered 'No pathogen found'.
40 laboratories correctly identified the KHV in ampoule VI, 1 did not test for KHV and 1 laboratory correctly identified KHV but detected also another virus in this ampoule.

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. These points will be assessed directly with the single participants that has underperformed.

It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis, as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains, especially after the delisting of ISAV HPR0 (Commission Implementing Directive 2014/22/EU)[2].

It was proposed in 2017 to include a scoring system for the sequencing results provided by the participants. All sequencing results have been compiled in Table 9 and 13. It is possible to observe that results from participants are very heterogeneous and include participants that sequenced and genotyped all viruses in the PT, others that sequence one or two isolates and some that do not

perform any sequence. When analysing the results "per ampoule" there is also significant heterogeneity on how the genotype is provided from each participants.
For the aforementioned reasons, a comment on the sequencing performance is provided to each participant in the certificate provided from the EURL.
Notably the sequence analysis were highly satisfactory, incorrect identification of the genotype has only occurred in 2 cases in PT1 and 3 cases in PT2.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they fare in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We take the opportunity to provide comments to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

The results given in this report will be further presented and discussed at the 23rd Annual Workshop of National Reference Laboratories for Fish Diseases to be held 27th-28th of May 2019 in Kgs. Lyngby, Denmark.

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European Union Reference laboratory for Fish diseases
National Institute of Aquatic Resources, Technical University of Denmark, March 2019.

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