EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2015

for identification and titration of

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

and identification of

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

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PT Reg. no.: 515

DANAK

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Introduction

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish 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 the 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 the fish pathogenic viruses: spring viremia 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 pathogens: infectious salmon anaemia virus (ISAV), salmon alphavirus (SAV) and Cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by biomolecular methods (PCR based). 45 laboratories participated in PT1 while 44 participated in PT2 of which 34 participated in identifying SAV.
The tests were sent from the EURL in mid-September 2015.
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 IHNV, VHSV alone and in a co-infection setting, ECV and sterile cell culture medium (Eagles MEM with 10%FBS) (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, EHNV, SVCV, and IPNV (Council Directive 2006/88/EC Annex IV part II and Commission Implementing Directive 2014/22/EU of 13 February 2014); bearing in mind 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 Decision 2015-1554 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 2015. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible 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) for VHSV and either method as mentioned in the IHN chapter of the 2013 version of the OIE manual on Aquatic Animal Diseases (Emmenegger et al. (2000)) or in Kurath et *al*. (2003) 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 SAV, one contained ISAV and one contained sterile cell culture medium (Eagles MEM with 10%FBS), 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. For 2015 the EURL decided that the panel of pathogens to be investigated should be expanded to include Salmonid Alphavirus (SAV)-. 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 or not. The EURL would then take care of calculating the score accordingly.
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 OIE manual Chapter 2.3.5b. — Infection with salmonid alphavirus . It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might had been possible to replicate them in cell cultures.

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 providers of the proficiency test 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 20th Annual Workshop of the NRLs for Fish Diseases week 22, 2016 in Copenhagen. Participants were asked to reply latest November 13th 2015.

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 follow the shipment.

Shipment and handling
Within one day, the tests were delivered to 22 participants; 14 more tests were delivered within the first week; 3 more within the first two weeks; 5 further within three weeks and the last test was delivered within 35 days (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 described in the PT 2012 report.

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

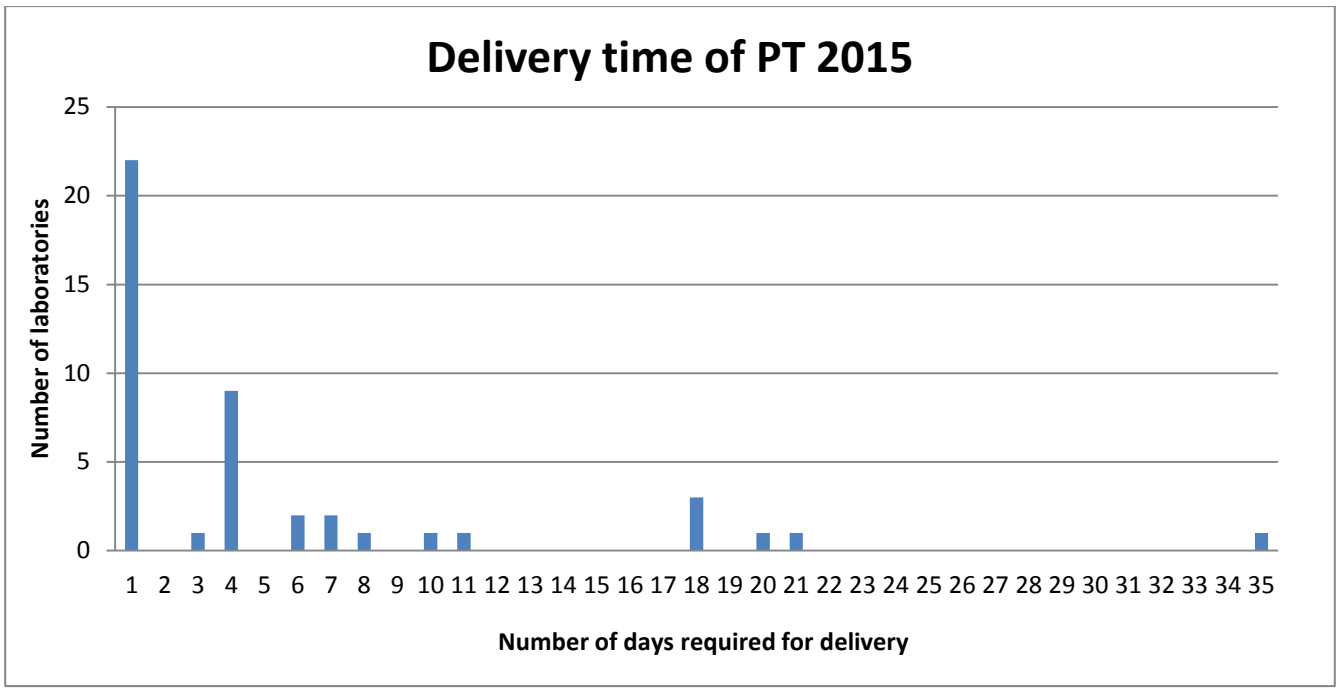


Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 45 laboratories received the annual proficiency test. 43 of the participants submitted results within the deadline, 2 participants got the deadline extended due to delivery problems or technical problems in the laboratory. Figure 2 show how many laboratories that participated in the proficiency test from 1996 to 2015.

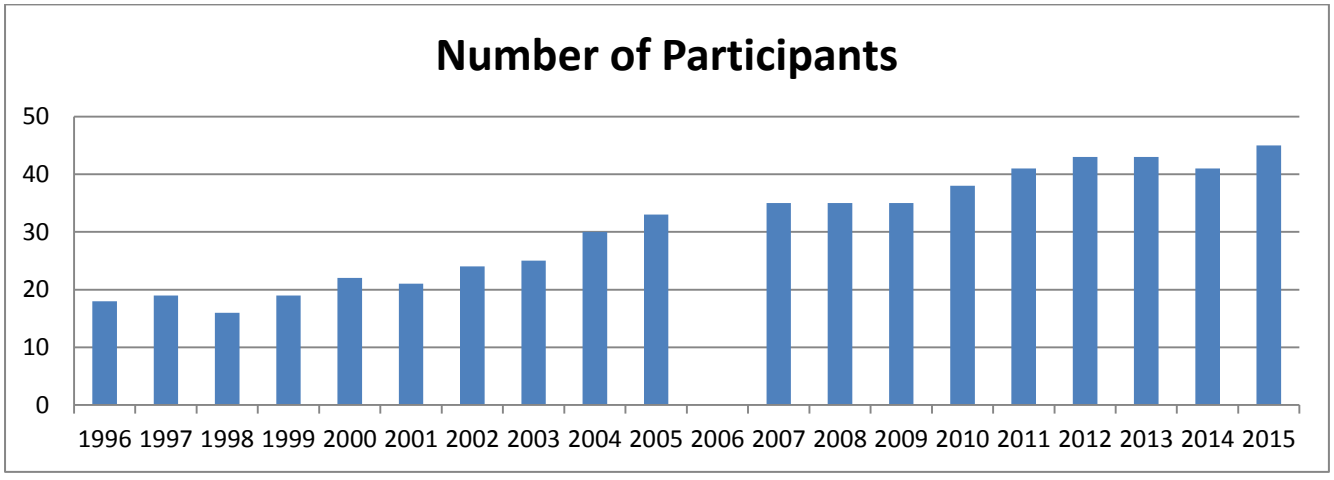


Figure 2. Participants in the EURL proficiency test over the years.

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, Faroe Islands, Iceland, Japan, New Zealand, Norway, Serbia, Switzerland, Turkey, Singapore and 2 from: P.R. China, South Korea and USA.

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.

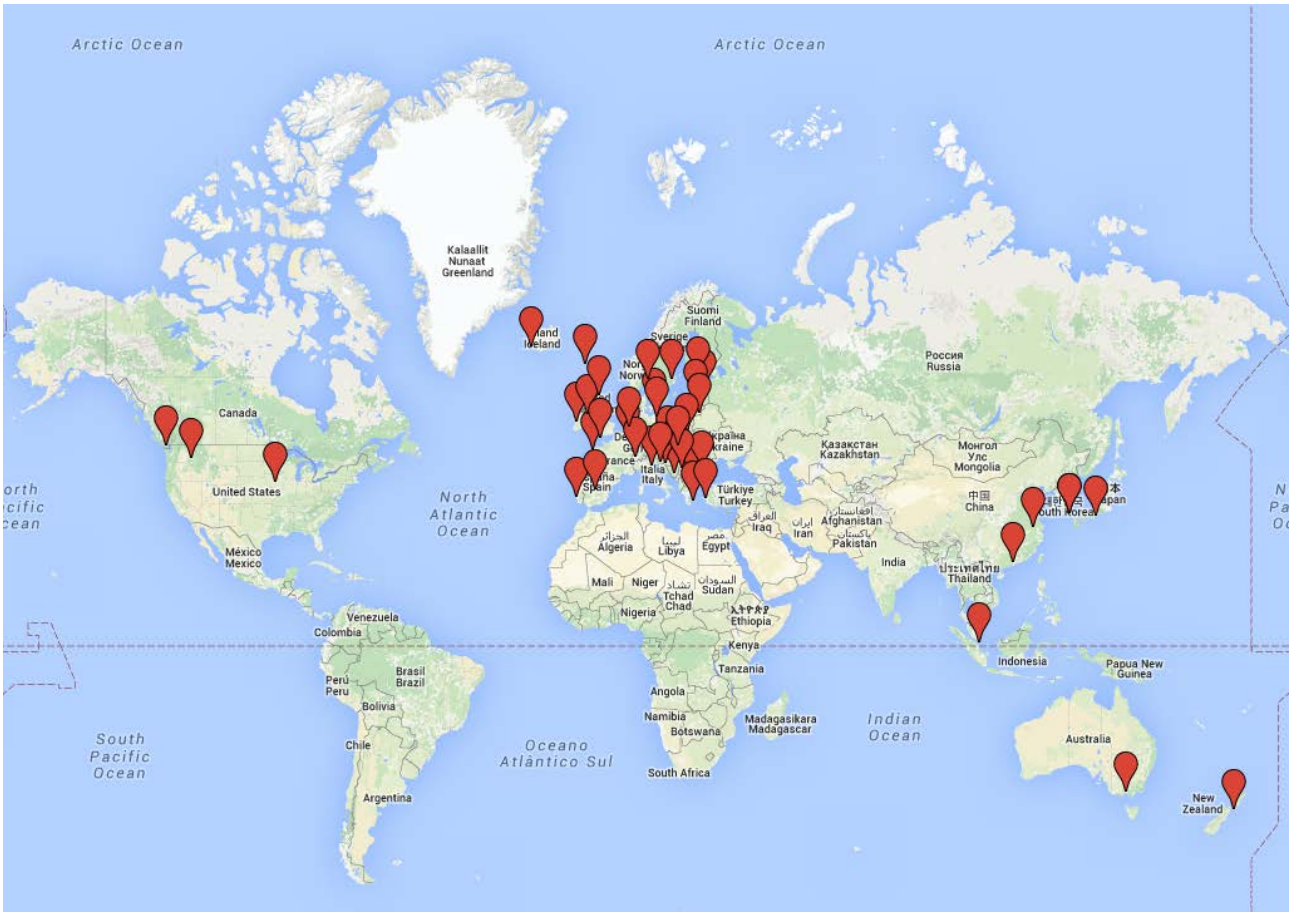


Figure 3. Worldwide distribution of the participants in the EURL proficiency test 2015.

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:	**IHNV 32/87** **Received from:** First French isolate (April 1987) from rainbow trout. **Cell culture passage number: 9 passages in EPC.** **GenBank accession numbers:** J265717 AY524121 (G-gene), FJ265711 (N gene).. **Reference on isolate:** *Hattenberger-Baudouy et al.* **1989** *Baudin Laurencin F* **(1987)**
Ampoule II:	**VHSV strain 1P8:** Marine isolate (1996) from herring (*Clupea harengus*) caught in the Baltic Sea. (Mortensen et al. 1999). Cell culture passage number: 7 Genotype Ib. GenBank accession numbers: AY546573 (G-gene) and GQ325430, AY356652 (N-gene) www.fishpathogens.eu ID number: 2251 **Reference on isolate:** (Mortensen et al. 1999). **References on sequences:** Campbell et al. (2009) Einer-Jensen et al. 2004. Snow et al. (2004).
Ampoule III:	**European Catfish virus (ECV), Isolate 562/92**Italian isolate from catfish suffering high mortality. Received from Dr. G. Bovo, ISZ-Ve, Padova, Italy. Cell culture passage number: 7 GenBank accession number: FJ358608 Reference on isolate: Bovo et al. (1993). Reference on sequence: Holopainen et al. (2009) .
Ampoule IV:	**BLANK** Supernatant from NON infected BF-2 cells

Code	Specifications/References
Ampoule V[1]:	**VHSV strain, DK-5151 + IHNV 32/87** **VHSV DK-5151 (Rindsholm, 1992)** Danish freshwater VHSV isolate from rainbow trout. Cell culture passage number: 4 in BF-2 and 6 in EPC. . Genotype Ia (Ejner-Jensen et al. 2004). **References:** Olesen et al. 1993 Einer-Jensen et al 2004 GenBank accession number: AF345859.1 **IHNV 32/87** **Received from:** First French isolate (April 1987) from rainbow trout. **Accept for using the isolate:** ☒ **Cell culture passage number: 9 passages in EPC.** **GenBank accession numbers:** J265717 AY524121 (G-gene), FJ265711 (N gene).. **Reference on isolate:** *Hattenberger-Baudouy et al.* **1989,** *Baudin Laurencin F* **(1987)**

[1] **A specific description of ampoule V content is provided further in the text**

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), RT-PCR/Q-PCR. 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.

During results receipt 24 of 44 participating laboratories reported the detection of an additional rhabdovirus in ampoule V, being a SVCV or a SVCV-like isolate.
Different analyses were therefore initiated by the EURL to assess and corroborate the presence of this virus in ampoule V.
Direct RT-PCR on re-suspended content of 4 ampoule V replicates both using diagnostic and sequencing primer sets as described by Koutná et al. (2003) and Stone et al., (2003) all tested negative.
Re-suspended content in ampoule V was following inoculated on BF-2; EPC, RTG-2 and FHM cell lines. Harvested supernatant tested by SVCV-ELISA and both SVCV RT- PCR protocols tested negative. However an IFAT analysis performed using polyclonal antibody K42 raised against pike fry rhabdovirus (Jørgensen et al. 1989) provided a positive staining.
Further examinations were then initiated as re-suspended content of ampoule V was inoculated on BF-2-; EPC-, RTG-2- and FHM cell lines, respectively with polyclonal neutralizing antisera against VHSV and IHNV and cells were incubated at 24°C, a temperature considered not permissive to the growth of VHSV and IHNV.
An isolate was finally obtained and tested with the two PCR protocols mentioned above, where only the more generic test performed with sequencing primers tested positive.
The amplicon was sequenced and the sequence analysis blasted against the ones retrieved from the other participants.
Sequence analysis finally confirmed that the additional isolate from Ampoule V obtained from cell culture at non permissive temperature for the growth of VHSV and IHNV, was 99% identical to the tench rhabdovirus S64 (Jørgensen et al. 1989).
The VHSV isolate DK-5151 in Ampoule V was retrieved from a large virus panel produced in 2009 which included a number of various fish pathogenic viruses and among these the S64 isolate. Our theory is that the DK-5151 vial was contaminated with S64 during this production, but as this happened in our previous facilities in Aarhus we have not been able to exactly trace the procedures followed at that time.

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 4°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 and five months after lyophilisation, (1 ampoule), (stored at 4°C).

- Ampoul No.	Cell line	Titre before Lyophilisation	Titre after Lyophilisation and before shipment	Titre after deadline for handling in results (and five months after lyophilisation) (storage 4°C in the dark)
		$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I: IHNV, 32/87	BF-2	1,3E+03	1,9E+02	<1,9E+02
	EPC	8,6E+06	4,0E+05	2,7E+05
	RTG-2	8,6E+05	<1,9E+02	8,6E+03
	FHM	1,9E+06	8,6E+04	2,7E+04
Ampoule II: VHSV, 1p8	BF-2	1,9E+06	2,7E+04	2,7E+03
	EPC	< 1,9E+02	<1,9E+02	<1,9E+02
	RTG-2	< 1,9E+02	<1,9E+02	<1,9E+02
	FHM	2,7E+06	5,9E+04	2,7E+04
Ampoule III: ECV, 562/92	BF-2	1,9E+07	1,3E+06	1,3E+05
	EPC	1,3E+06	1,3E+05	1,3E+03
	RTG-2	1,3E+03	8,6E+02	2,7E+03
	FHM	2,7E+03	<1,9E+02	<1,9E+02
Ampoule IV: Blank, BF2 cell supernatant	BF-2	< 1,9E+02	<1,9E+02	<1,9E+02
	EPC	< 1,9E+02	<1,9E+02	<1,9E+02
	RTG-2	< 1,9E+02	<1,9E+02	<1,9E+02
	FHM	< 1,9E+02	<1,9E+02	<1,9E+02
Ampoule V: VHSV, DK-5151 + IHNV, 32/87	BF-2	2,7E+08	8,6E+06	1,9E+06
	EPC	1,9E+08	8,6E+06	4,0E+06
	RTG-2	1,3E+08	8,6E+06	2,7E+06
	FHM	1,3E+08	4,0E+06	4,0E+06

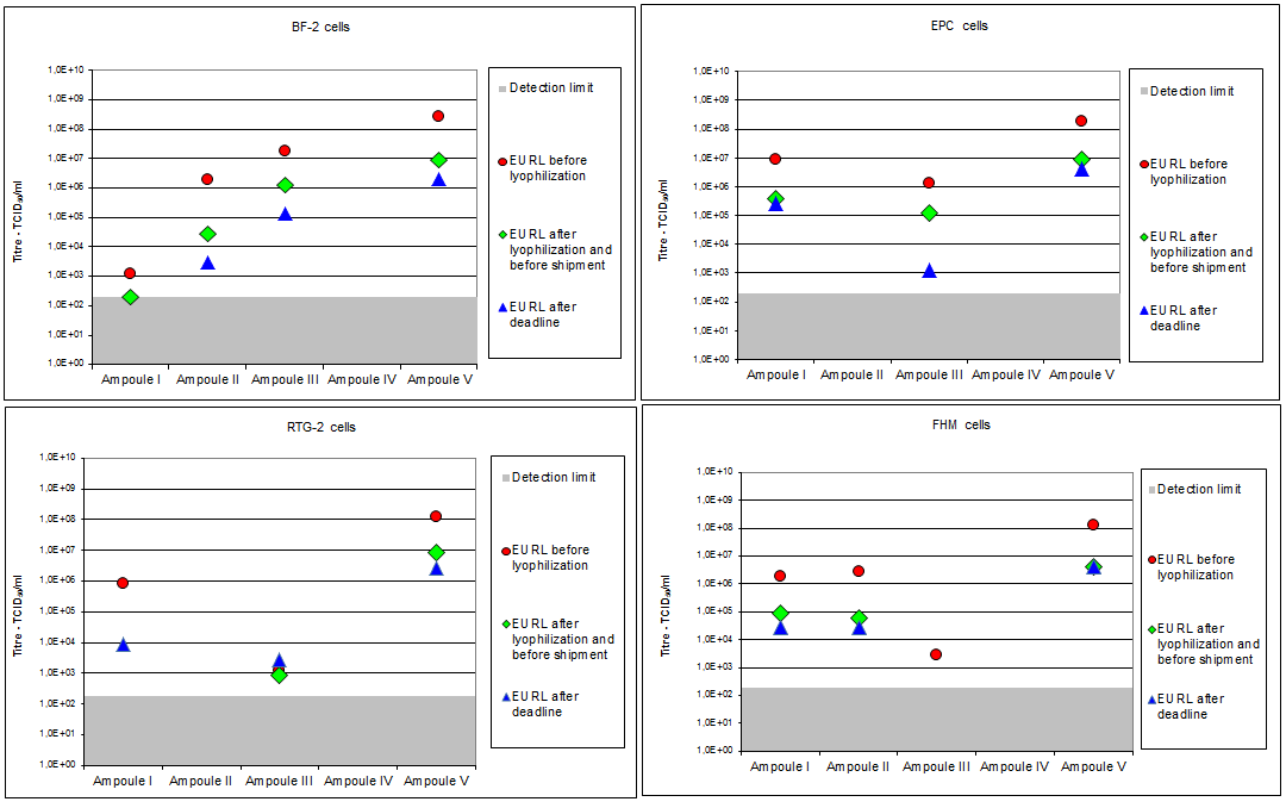


Figure 4. Virus titers in different cell lines:
Before lyophilisation, After lyophilisation-before shipment and After minimum 3 months after lyophilisation (storage 4°C in the dark) (1 ampoule).

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, i.e. by cell culture followed by ELISA, IFAT, neutralisation test and/or RT-PCR/Q-PCR. Identification results of the content of the 5 ampoules at 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). Viruses titration results obtained in 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 6 laboratories used these cells. Laboratories with the required facilities were encouraged to examine and 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, 2015 - Virus identification and score obtained by participants.

Laboratory code number	Score 10/10	Answer received at EURL	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
			IHNV 32/87	VHSV 1P8	ECV 562/92	Blank BF-2 cell supernatant	VHSV DK-5151 + IHNV 32/87
46	10/10	09.11.15	IHNV	VHSV	ESV	Negative	VHSV, IHNV, TenRV
45	10/10	06.11.15	IHNV	VHSV	ESV	no virus detected	VHSV & IHNV
44	8/10	12.11.15	IHNV	VHSV	RANA (ESV/ECV)	No virus growth	VHSV SVCV RANA (ECV/ESV)
43	8/8	30.11.15	IHNV	VHSV	no IHNV, no VHSV, no SVCV, no IPNV[4]	no CPE	IHNV, VHSV, SVCV
42	9/10	13.11.15	IHNV	VHSV	Rana V[2]	No virus	VHSV IHNV SVCV
41	10/10	11.11.15	IHNV	VHSV	ESV	Negative	IHNV + VHSV
40	10/10	13.11.15	IHNV	VHSV	European sheatfish ranavirus	not IHNV,VHSV, EHNV, Ranavirus,IPNV, SVCV	VHSV and IHNV
39	10/10	11.11.15	IHNV	VHSV	ESV		VHSV and IHNV
38	10/10	13.11.15	IHNV	VHSV	Ranavirus not EHNV [1]	Not VHSV, not IHNV and not Ranavirus (see comment)	VHSV and IHNV
37	8/10	12.11.15	IHNV	VHSV	Not VHSV,IHNV, IPNV,SVCV[4]	Virus not found	VHSV, IHNV
36	10/10	13.11.15	IHNV	VHSV	Ranavirus[1]	No virus detected	IHNV, VHSV & PFRV
35	10/10	12.11.15	IHNV genogroup M/Eur1	VHSV genotype Ib	European catfish virus ECV	no virus	VHSV genotype Ia, IHNV genogroup M/Eur1, Pikefry-like rhabdovirus
34	10/10	26.10.15	IHNV	VHSV	ECV / ESV	negative for all viruses tested	IHNV + VHSV +PFR-like virus
33	9/10	12.11.15	rhabdovirus, IHNV France 1987: IHNV 32/87, genogroup Europe	rhabdovirus, VHSV Denmark 1996: DK/1e62, DK/1p8, DK/1p12, sub/genogroup Ib	ranavirus, ECV/ ESV		rhabdovirus, VHSV Denmark DK/5151 Rindsholm, sub/genogroup Ia
32	10/10	13.11.15	IHNV	VHSV	ECV or ESV	NO VHSV NO IHNV NO EHNV	VHSV & IHNV
31	9/10	13.11.15	IHNV	VHSV	ESV	Negativ	VHSV+SVCV
30	9/10	11.11.15	IHNV	VHSV	ECV	No virus detected	VHSV and SVCV
28	9/10	13.11.15	IHNV	VHSV	Ranavirus not EHNV (ECV)	Negative	VHSV + Sprivivirus (TenRV)

Laboratory code number	Score 10/10	Answer received at EURL	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
			IHNV 32/87	VHSV 1P8	ECV 562/92	Blank BF-2 cellsupernantant	VHSV DK-5151 + IHNV 32/87
27	10/10	16.11.15	IHNV	VHSV	ESV	Negative	VHSV+IHNV+SVCV
26	10/10	13.11.15	IHNV	VHSV	Ranavirus[1]		VHSV, IHNV, Pike fry-like rhabdovirus
25	9/10	12.11.15	IHNV	VHSV	EHNV[3]	no VHSV/ no IHNV/no EHNV/ no IPNV/ no SVCV	VHSV/IHNV/SVCV
24	10/10	12.11.15	IHNV	VHSV	RANAVIRUS[1]	NEGATIVE	VHSV, IHNV
23	10/10	13.11.15	IHNV	VHSV	Ranavirus[1]	No IHNV, VHSV, Ranavirus, SVCV or IPNV detected	VHSV, IHNV & SVCV
22	9/10	13.11.15	IHNV viable virus detected	VHSV viable virus detected	EHNV viable virus detected[3]	No viruses detected	IHNV and VHSV viable virus detected
21	10/10	10.11.15	IHNV	VHSV	Ranavirus (ECV or ESV)	No virus detected	IHNV and VHSV
20	10/10	13.11.15	IHNV	VHSV	Ranavirus[1]	0	VHSV/SVCV/IHNV
19	9/10	13.11.15	IHNV	VHSV	EHNV[3]	No Virus	IHNV、VHSV、PFRV
18	10/10	13.11.15	IHNV	VHSV	ECV	Negative for VHSV, IHNV, IPNV, EHNV, ranavirus, SVCV	VHSV, IHNV
17	10/10	13.11.15	IHNV	VHSV	Ranavirus[1]	Negative	VHSV, IHNV, SVCV
16	10/10	13.11.15	IHNV	VHSV	ESV/ECV	Negative	VHSV IHNV Rhabdovirus
15	8/10	10.11.15	IHNV	VHSV	EHNV[2]	Negative	VHSV
14	9/10	13.11.15	IHNV	VHSV	EHNV	No Viruses isolated by BF2/EPC cell culture	IHNV/VHSV
13	10/10	13.11.15	IHNV	VHSV	Ranavirus[1]	/	VHSV, IHNV
12	10/10	13.11.15	IHNV	VHSV	ESV (very closely related to EHNV)		IHNV, VHSV
11	10/10	12.11.15	IHNV	VHSV	Ranavirus[1]	negative	VHSV, IHNV & Tench rhbdovirus(or Pike fry/like rhabdovirus)
10	9/10	12.11.15	IHNV	VHS	EHN	Negative	IHN, VHS
9	10/10	12.11.15	IHNV	VHSV	Ranavirus ESV	No virus	IHNV, VHSV, SVCV/like*
8	9/10	13.11.15	IHNV	VHSV	Ranavirus[2]	Negativ	VHSV, IHNV
7	10/10	10.11.15	IHNV	VHSV	EHNV[1]	no virus found	VHSV, IHNV, SVCV

Laboratory code number	Score 10/10	Answer received at EURL	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
			IHNV 32/87	VHSV 1P8	ECV 562/92	Blank BF-2 cellsupernantant	VHSV DK-5151 + IHNV 32/87
6	8/10	12.11.15	IHNV	Sterile	ECV	Sterile	IHNV & VHSV
5	8/10	13.11.15	IHNV	VHSV	Ranavirus was identified by conventional RT/PCR and than REA was applied as given by OIE manuel to identified ECV	IHNV	VHSV,IHNV,SVCV
4	10/10	13.11.15	IHNV	VHSV	European sheatfish virus	NEGATIVE	VHSV and IHNV
3	9/10	06.11.15	IHNV	VHSV	Ranavirus, not EHNV[1]	Negative	VHSV & TenRV
2	9/10	13.11.15	IHNV	VHSV	Ranavirus[1] (Not EHNV, see sequencing result)	negative	VHSV
1	10/10	12.11.15	IHNV/M	VHSV I/b	European sheatfish virus	no virus detected	mixed infection: tench rhabdovirus; IHNV; and VHSV

1: Correct sequence and answered ESV in sequences sheet.
2: Genomic analysis not performed
3: Correct sequence, wrong answer after blast.
4: Did not perform test for Ranavirus

Table 4. Inter-Laboratory Proficiency Test, PT1, 2015 – Results of titration of ampoule I.

Ampoule I - IHNV 32/87					
Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
46	IHNV	< 1,9E+02	1,9E+05	2,7E+03	N/A
45	IHNV	< 1,9E+02	8,6E+04	< 1,9E+02	N/A
44	IHNV	< 1,9E+02	1,9E+04	5,9E+02	N/A
43	IHNV	< 1,9E+02	2,7E+04	N/A	N/A
42	IHNV	1,3E+04	1,3E+03	N/A	N/A
41	IHNv	< 1,9E+02	4,0E+05	5,9E+03	5,9E+05
40	IHNV	2,7E+03	8,6E+04	N/A	N/A
39	IHNV	N/A	2,7E+03	8,6E+02	2,7E+02
38	IHNV	< 1,9E+02	2,7E+05	2,7E+05	4,0E+05
37	IHNV	1,3E+03	4,0E+04	N/A	N/A
36	IHNV	< 1,9E+02	5,9E+04	N/A	N/A
35	IHNV genogroup M-Eur1	1,3E+03	5,9E+05	N/A	N/A
34	IHNV	< 1,9E+02	1,3E+06	5,9E+04	N/A
33	rhabdovirus, IHNV France 1987: IHNV 32/87, genogroup Europe	< 1,9E+02	1,0E+04	< 1,9E+02	< 1,9E+02
32	IHNV	< 1,9E+02	5,9E+04	N/A	N/A
31	IHNV	< 1,9E+02	8,6E+05	N/A	N/A
30	IHNV	< 1,9E+02	2,7E+06	N/A	N/A
28	IHNV	2,7E+02	2,7E+05	N/A	N/A
27	IHNV	8,6E+02	4,0E+05	N/A	N/A
26	IHNV	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
25	IHNV	< 1,9E+02	1,9E+05	N/A	N/A
24	IHNV	N/A	5,9E+05	2,7E+04	N/A
23	IHNV	< 1,9E+02	2,7E+04	N/A	N/A
22	IHNV viable virus detected	N/A	4,0E+04	N/A	4,0E+05
21	IHNV	5,9E+02	8,6E+03	N/A	N/A
20	IHNV	5,9E+02	1,9E+03	N/A	N/A
19	IHNV	N/A	2,7E+04	< 1,9E+02	1,3E+04
18	IHNV	2,7E+02	1,9E+05	4,0E+02	5,9E+05
17	IHNV	N/A	4,0E+04	8,6E+03	N/A
16	IHNV	1,3E+03	4,0E+02	N/A	N/A
15	IHNV	2,7E+04	1,9E+05	N/A	N/A

Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
14	IHNV	< 1,9E+02	< 1,9E+02	N/A	N/A
13	IHNV	4,0E+03	1,3E+03	1,3E+03	1,3E+03
12	IHNV	< 1,9E+02	8,6E+03	N/A	N/A
11	IHNV	< 1,9E+02	1,9E+03	< 1,9E+02	< 1,9E+02
10	IHNV	< 1,9E+02	1,3E+03	< 1,9E+02	< 1,9E+02
9	IHNV	< 1,9E+02	8,6E+05	< 1,9E+02	8,6E+05
8	IHNV	< 1,9E+02	N/A	N/A	5,9E+04
7	IHNV	1,9E+02	1,3E+06	N/A	N/A
6	IHNV	N/A	1,3E+05	N/A	4,0E+05
5	IHNV	< 1,9E+02	1,3E+04	N/A	N/A
4	IHNV	1,3E+03	1,3E+05	N/A	N/A
3	IHNV	4,0E+03	N/A	N/A	1,3E+05
2	IHNV	< 1,9E+02	1,3E+04	N/A	4,0E+04
1	IHNV-M	1,9E+04	1,3E+04	1,3E+04	8,6E+03

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

	BF-2	EPC	RTG-2	FHM
Median titre	<1,9E+02	4,1E+04	7,2E+02	4,1E+04
Maximum titre	2,7E+04	2,7E+06	2,7E+05	8,6E+05
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	9,9E+03	<1,9E+02	2,7E+02
75% quartile titre	1,1E+03	2,3E+05	7,9E+03	4,1E+05

Table 5. Inter-Laboratory Proficiency Test, PT1, 2015 – Results of titration of ampoule II.

Ampoule II - VHSV 1P8					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
46	VHSV	2,7E+03	4,0E+02	< 1,9E+02	N/A
45	VHSV	4,0E+03	< 1,9E+02	1,9E+03	N/A
44	VHSV	1,9E+04	1,3E+03	8,6E+03	N/A
43	VHSV	2,73E+03	< 1,9E+02	N/A	N/A
42	VHSV	8,6E+03	5,9E+02	N/A	N/A
41	VHSv	1,3E+04	< 1,9E+02	< 1,9E+02	2,7E+04
40	VHSV	1,3E+04	5,9E+03	N/A	N/A
39	VHSV	N/A	1,9E+02	1,3E+03	1,9E+03
38	VHSV	1,3E+04	< 1,9E+02	< 1,9E+02	2,7E+04
37	VHSV	2,7E+03	1,3E+03	N/A	N/A
36	VHSV	2,7E+03	4,0E+02	N/A	N/A
35	VHSV genotype Ib	8,6E+03	4,0E+02	N/A	N/A
34	VHSV	5,9E+04	< 1,9E+02	< 1,9E+02	N/A
33	rhabdovirus, VHSV Denmark 1996: DK-1e62, DK-1p8, DK-1p12, sub-genogroup Ib	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
32	VHSV	1,3E+03	< 1,9E+02	N/A	N/A
31	VHSV	4,0E+03	1,3E+04	N/A	N/A
30	VHSV	8,6E+03	1,9E+02	N/A	N/A
28	VHSV	1,9E+04	1,9E+02	N/A	N/A
27	VHSV	8,6E+03	< 1,9E+02	N/A	N/A
26	VHSV	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
25	VHSV	4,0E+05	< 1,9E+02	N/A	N/A
24	VHSV	N/A	1,9E+04	5,9E+03	N/A
23	VHSV	8,6E+03	< 1,9E+02	N/A	N/A
22	VHSV viable virus detected	4,0E+04	5,9E+03	N/A	8,6E+03
21	VHSV	4,0E+03	< 1,9E+02	N/A	N/A
20	VHSV	1,9E+02	1,9E+02	N/A	N/A
19	VHSV	N/A	4,0E+02	< 1,9E+02	< 1,9E+02
18	VHSV	4,0E+04	1,9E+03	5,9E+03	2,7E+02
17	VHSV	N/A	8,6E+05	4,0E+04	N/A
16	VHSV	2,7E+03	< 1,9E+02	N/A	N/A

Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
15	VHSV	2,7E+04	1,3E+04	N/A	N/A
14	VHSV	2,73E+02	< 1,9E+02	N/A	N/A
13	VHSV	1,3E+04	1,3E+04	1,3E+04	1,3E+04
12	VHSV	4,0E+04	5,9E+02	N/A	N/A
11	VHSV	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
10	VHS	< 1,9E+02	1,26E+03	< 1,9E+02	< 1,9E+02
9	VHSV	1,9E+04	8,6E+02	1,3E+04	< 1,9E+02
8	VHSV	2,7E+03	N/A	N/A	1,3E+04
7	VHSV	2,7E+04	< 1,9E+02	N/A	N/A
6	Sterile	N/A	1,9E+02	N/A	1,9E+02
5	VHSV	1,9E+03	8,6E+02	N/A	N/A
4	VHSV	1,3E+04	5,9E+02	N/A	N/A
3	VHSV	2,7E+04	N/A	N/A	4,0E+02
2	VHSV	2,7E+03	< 1,9E+02	N/A	< 1,9E+02
1	VHSV I-b	1,9E+03	2,7E+02	< 1,9E+02	< 1,9E+02

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

	BF-2	EPC	RTG-2	FHM
Median titre	8,6E+03	1,9E+02	<1,9E+02	1,9E+02
Maximum titre	4,0E+05	8,6E+05	4,0E+04	2,7E+04
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	2,7E+03	<1,9E+02	<1,9E+02	<1,9E+02
75% quartile titre	1,9E+04	1,1E+03	5,9E+03	8,6E+03

Table 6. Inter-Laboratory Proficiency Test, PT1, 2015 – Results of titration of ampoule III.

Ampoule III - ECV 562/92					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
46	ESV	4,0E+05	4,0E+05	< 1,9E+02	N/A
45	ESV	4,0E+05	4,0E+04	1,3E+05	N/A
44	RANA (ESV/ECV)	1,9E+04	4,0E+03	1,9E+04	N/A
43	no IHNV, no VHSV, no SVCV, no IPNV	5,87E+04	2,73E+04	N/A	N/A
42	Rana V	1,3E+05	4,0E+03	N/A	N/A
41	ESV	1,9E+05	1,9E+03	< 1,9E+02	1,3E+03
40	European sheatfish ranavirus	4,0E+06	4,0E+05	N/A	N/A
39	ESV	N/A	1,3E+05	1,9E+04	4,0E+03
38	Ranavirus not EHNV	4,0E+05	4,0E+04	4,0E+04	2,7E+02
37	Not VHSV,IHNV, IPNV,SVCV	8,6E+05	2,7E+04	N/A	N/A
36	Ranavirus	5,9E+03	5,9E+03	N/A	N/A
35	European catfish virus ECV	1,3E+05	1,3E+05	N/A	N/A
34	ECV / ESV	2,7E+05	5,9E+04	1,9E+02	N/A
33	ranavirus, ECV/ ESV	6,8E+03	< 1,9E+02	< 1,9E+02	< 1,9E+02
32	ECV or ESV	1,3E+05	4,0E+04	N/A	N/A
31	ESV	2,7E+05	4,0E+04	N/A	N/A
30	ECV	2,7E+03	4,0E+02	N/A	N/A
28	Ranavirus not EHNV (ECV)	4,0E+05	8,6E+03	N/A	N/A
27	ESV	1,9E+06	8,6E+05	N/A	N/A
26	Ranavirus	2,7E+05	5,9E+05	8,6E+05	< 1,9E+02
25	EHNV	2,7E+04	1,9E+04	N/A	N/A
24	RANAVIRUS	N/A	8,6E+02	5,9E+02	N/A
23	Ranavirus	8,6E+05	< 1,9E+02	N/A	N/A
22	EHNV viable virus detected	4,00E+05	N/A	N/A	N/A
21	Ranavirus (ECV or ESV)	1,9E+04	5,9E+03	N/A	N/A
20	Ranavirus	4,0E+04	1,3E+03	N/A	N/A
19	EHNV	N/A	1,9E+04	< 1,9E+02	5,9E+03
18	ECV	1,3E+05	2,7E+03	4,0E+06	1,9E+05
17	Ranavirus	N/A	5,9E+03	< 1,9E+02	N/A
16	ESV/ECV	1,9E+05	2,7E+02	N/A	N/A
15	EHNV	1,9E+05	5,9E+04	N/A	N/A

Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
14	EHNV	126491,1064	1264,911064	N/A	N/A
13	Ranavirus	2,7E+05	1,3E+05	2,7E+05	1,3E+05
12	ESV (very closely related to EHNV)	2,7E+05	8,6E+05	N/A	N/A
11	Ranavirus	1,86E+03	1,86E+04	< 1,9E+02	1,26E+03
10	EHN	1,26E+03	4,00E+03	< 1,9E+02	2,73E+03
9	Ranavirus ESV	2,7E+05	2,7E+05	1,3E+05	2,7E+05
8	Ranavirus	8,6E+04	N/A	N/A	< 1,9E+02
7	EHNV	8,6E+06	5,9E+05	N/A	N/A
6	ECV	N/A	5,9E+05	N/A	1,9E+04
5	Ranavirus was identified by conventional RT-PCR and than REA was applied as given by OIE manuel to identified ECV	2,7E+04	2,7E+04	N/A	N/A
4	European sheatfish virus	2,7E+05	8,6E+05	N/A	N/A
3	Ranavirus, not EHNV	2,7E+04	N/A	N/A	1,9E+03
2	Ranavirus (Not EHNV, see sequencing result)	1,3E+05	1,3E+04	N/A	8,6E+03
1	European sheatfish virus	2,7E+05	8,6E+04	5,9E+03	1,9E+03

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

	BF-2	EPC	RTG-2	FHM
Median titre	1,9E+05	2,7E+04	3,2E+03	2,3E+03
Maximum titre	8,6E+06	8,6E+05	4,0E+06	2,7E+05
Minimum titre	1,3E+03	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	3,7E+04	4,0E+03	<1,9E+02	1,0E+03
75% quartile titre	3,0E+05	1,3E+05	1,0E+05	1,1E+04

Table 7. Inter-Laboratory Proficiency Test, PT1, 2015 – Results of titration of ampoule IV.

Ampoule IV - Blank, BF-2 cellesupernantant					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
46	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
45	no virus detected	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
44	No virus growth	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
43	no CPE	< 1,9E+02	< 1,9E+02	N/A	N/A
42	No virus	< 1,9E+02	< 1,9E+02	N/A	N/A
41	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
40	not IHNV,VHSV, EHNV, Ranavirus,IPNV, SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
39		N/A	< 1,9E+02	< 1,9E+02	< 1,9E+02
38	Not VHSV, not IHNV and not Ranavirus (see comment)	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
37	Virus not found	< 1,9E+02	< 1,9E+02	N/A	N/A
36	No virus detected	< 1,9E+02	< 1,9E+02	N/A	N/A
35	no virus	< 1,9E+02	< 1,9E+02	N/A	N/A
34	negative for all viruses tested	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
33		< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
32	NO VHSV NO IHNV NO EHNV	< 1,9E+02	< 1,9E+02	N/A	N/A
31	Negativ	< 1,9E+02	< 1,9E+02	N/A	N/A
30	No virus detected	< 1,9E+02	< 1,9E+02	N/A	N/A
28	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
27	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
26		< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
25	no VHSV/ no IHNV/no EHNV/ no IPNV/ no SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
24	NEGATIVE	N/A	< 1,9E+02	< 1,9E+02	N/A
23	No IHNV, VHSV, Ranavirus, SVCV or IPNV detected	< 1,9E+02	< 1,9E+02	N/A	N/A
22	No viruses detected	< 1,9E+02	< 1,9E+02	N/A	< 1,9E+02
21	No virus detected	< 1,9E+02	< 1,9E+02	N/A	N/A
20	0	4,0E+02	< 1,9E+02	N/A	N/A
19	No Virus	N/A	< 1,9E+02	< 1,9E+02	< 1,9E+02
18	Negative for VHSV, IHNV, IPNV, EHNV, ranavirus, SVCV	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
17	Negative	N/A	< 1,9E+02	< 1,9E+02	N/A
16	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
15	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A

Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
14	No Viruses isolated by BF2/EPC cell culture	< 1,9E+02	< 1,9E+02	N/A	N/A
13	-	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
12		< 1,9E+02	< 1,9E+02	N/A	N/A
11	negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
10	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
9	No virus	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
8	Negativ	< 1,9E+02	N/A	N/A	< 1,9E+02
7	no virus found	< 1,9E+02	< 1,9E+02	N/A	N/A
6	Sterile	N/A	< 1,9E+02	N/A	< 1,9E+02
5	IHNV	< 1,9E+02	< 1,9E+02	N/A	N/A
4	NEGATIVE	< 1,9E+02	< 1,9E+02	N/A	N/A
3	Negative	< 1,9E+02	N/A	N/A	< 1,9E+02
2	negative	< 1,9E+02	< 1,9E+02	N/A	< 1,9E+02
1	no virus detected	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02

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

	BF-2	EPC	RTG-2	FHM
Median titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
Maximum titre	4,0E+02	<1,9E+02	<1,9E+02	<1,9E+02
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
75% quartile titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02

Table 8. Inter-Laboratory Proficiency Test, PT1, 2015 – Results of titration of ampoule V.

Ampoule V - VHSV/IHN					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
46	VHSV, IHNV, TenRV	2,7E+05	5,9E+06	4,0E+05	N/A
45	VHSV & IHNV	1,9E+06	1,9E+06	4,0E+06	N/A
44	VHSV SVCV RANA (ECV/ESV)	2,7E+06	2,7E+06	1,3E+06	N/A
43	IHNV, VHSV, SVCV	5,87E+05	8,62E+05	N/A	N/A
42	VHSV IHNV SVCV	5,9E+04	5,9E+03	N/A	N/A
41	IHNv + VHSv	4,0E+06	8,6E+06	8,6E+05	8,6E+06
40	VHSV and IHNV	2,7E+06	1,9E+07	N/A	N/A
39	VHSV and IHNV	N/A	1,9E+06	1,3E+05	1,9E+05
38	VHSV and IHNV	5,9E+06	5,9E+06	4,0E+06	5,9E+06
37	VHSV , IHNV	1,9E+05	4,0E+05	N/A	N/A
36	IHNV, VHSV & PFRV	1,9E+06	4,0E+06	N/A	N/A
35	VHSV genotype Ia, IHNV genogroup M-Eur1, Pike-fry-like rhabdovirus	8,6E+05	1,3E+07	N/A	N/A
34	IHNV+VHSV+PFR-like virus	2,7E+07	5,9E+07	2,7E+06	N/A
33	rhabdovirus, VHSV Denmark DK-5151 Rindsholm, sub-genogroup Ia	1,5E+04	4,6E+04	6,8E+03	4,6E+03
32	VHSV & IHNV	1,3E+06	5,9E+05	N/A	N/A
31	VHSV+SVCV	8,6E+05	2,7E+06	N/A	N/A
30	VHSV and SVCV	8,6E+06	1,3E+07	N/A	N/A
28	VHSV + Sprivivirus (TenRV)	2,7E+06	1,9E+06	N/A	N/A
27	VHSV+IHNV+SVCV	5,9E+06	2,7E+06	N/A	N/A
26	VHSV, IHNV, Pike fry-like rhabdovirus	1,9E+05	1,9E+05	2,7E+05	4,0E+05
25	VHSV/IHNV/SVCV	2,7E+04	5,9E+04	N/A	N/A
24	VHSV, IHNV	N/A	1,3E+07	5,9E+06	N/A
23	VHSV, IHNV & SVCV	5,9E+06	4,0E+06	N/A	N/A
22	IHNV and VHSV viable virus detected	4,0E+06	4,0E+06	N/A	4,0E+07
21	IHNV and VHSV	2,7E+05	2,7E+05	N/A	N/A
20	VHSV/SVCV/IHNV	1,3E+04	1,9E+04	N/A	N/A
19	IHNV、VHSV、PFRV	N/A	4,0E+05	2,7E+05	1,3E+06
18	VHSV, IHNV	2,7E+06	4,0E+06	8,6E+06	1,3E+07
17	VHSV, IHNV, SVCV	N/A	5,9E+06	4,0E+04	N/A

Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
16	VHSV IHNV Rhabdovirus	1,3E+05	5,9E+03	N/A	N/A
15	VHSV	5,9E+05	1,9E+06	N/A	N/A
14	IHNV/VHSV	1,9E+05	400	N/A	N/A
13	VHSV, IHNV	5,9E+05	1,3E+06	1,3E+05	1,3E+05
12	IHNV, VHSV	8,6E+04	5,9E+06	N/A	N/A
11	VHSV, IHNV & Tench rhbdovirus(or Pike fry-like rhabdovirus)	1,86E+06	2,73E+05	5,87E+04	1,26E+05
10	IHN, VHS	1,26E+04	2,73E+04	< 1,9E+02	< 1,9E+02
9	IHNV, VHSV, SVCV-like*	2,7E+06	1,3E+06	1,9E+06	8,6E+05
8	VHSV, IHNV	1,9E+06	N/A	N/A	2,7E+06
7	VHSV, IHNV, SVCV	8,6E+06	8,6E+06	N/A	N/A
6	IHNV & VHSV	N/A	1,3E+07	N/A	4,0E+07
5	VHSV,IHNV,SVCV	5,9E+05	8,6E+07	N/A	N/A
4	VHSV and IHNV	2,7E+06	1,3E+07	N/A	N/A
3	VHSV & TenRV	5,9E+06	N/A	N/A	8,6E+06
2	VHSV	5,9E+05	8,6E+05	N/A	1,3E+06
1	mixed infection: tench rhabdovirus; IHNV; and VHSV	8,6E+05	4,0E+06	1,3E+05	8,6E+05

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

	BF-2	EPC	RTG-2	FHM
Median titre	1,1E+06	2,7E+06	3,4E+05	1,3E+06
Maximum titre	2,7E+07	8,6E+07	8,6E+06	4,0E+07
Minimum titre	1,3E+04	4,0E+02	<1,9E+02	<1,9E+02
25% quartile titre	2,5E+05	4,0E+05	1,3E+05	1,9E+05
75% quartile titre	2,7E+06	5,9E+06	2,5E+06	8,6E+06

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.

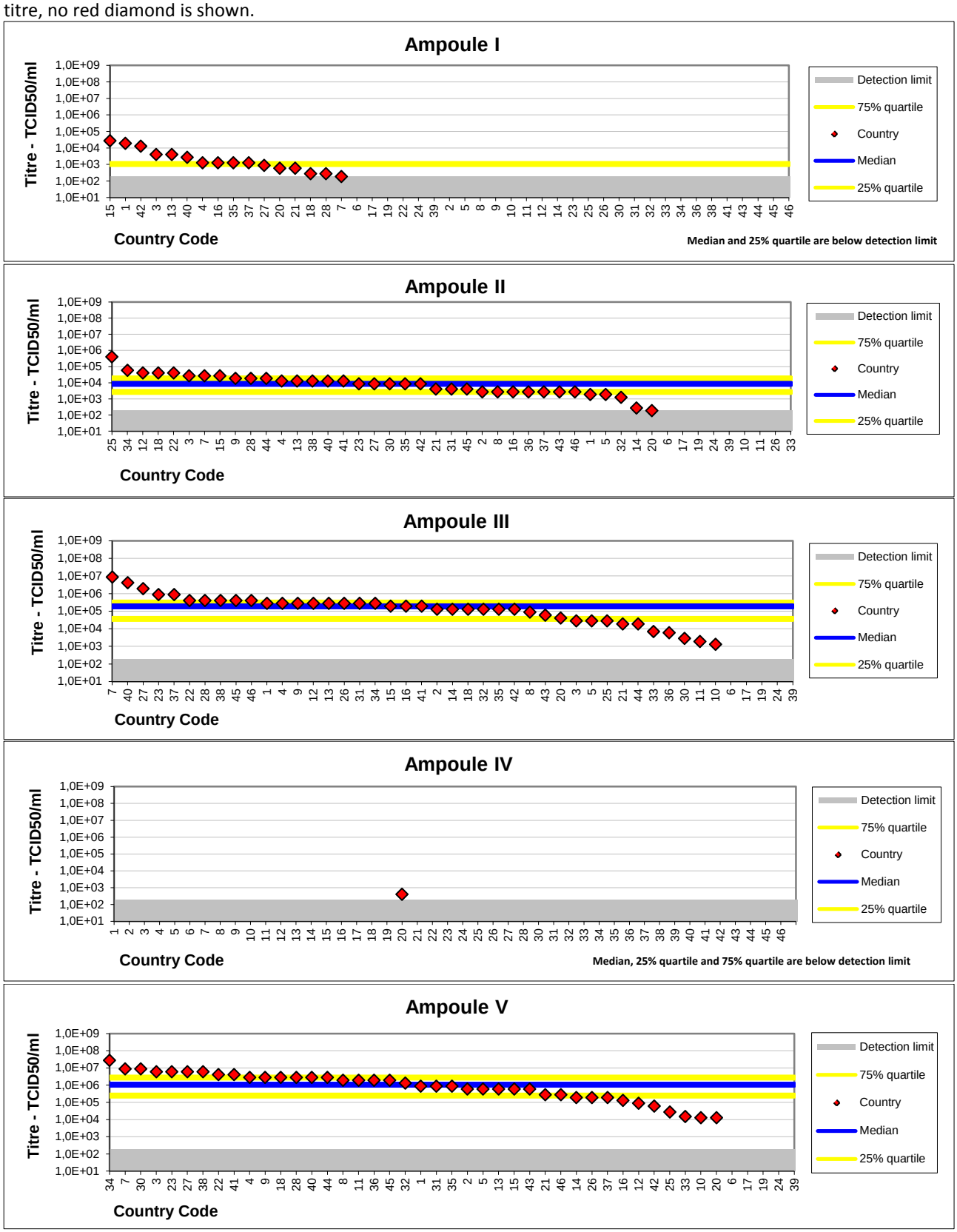


Figure 6. Virus titres obtained in EPC cells. For further details see Figure 5

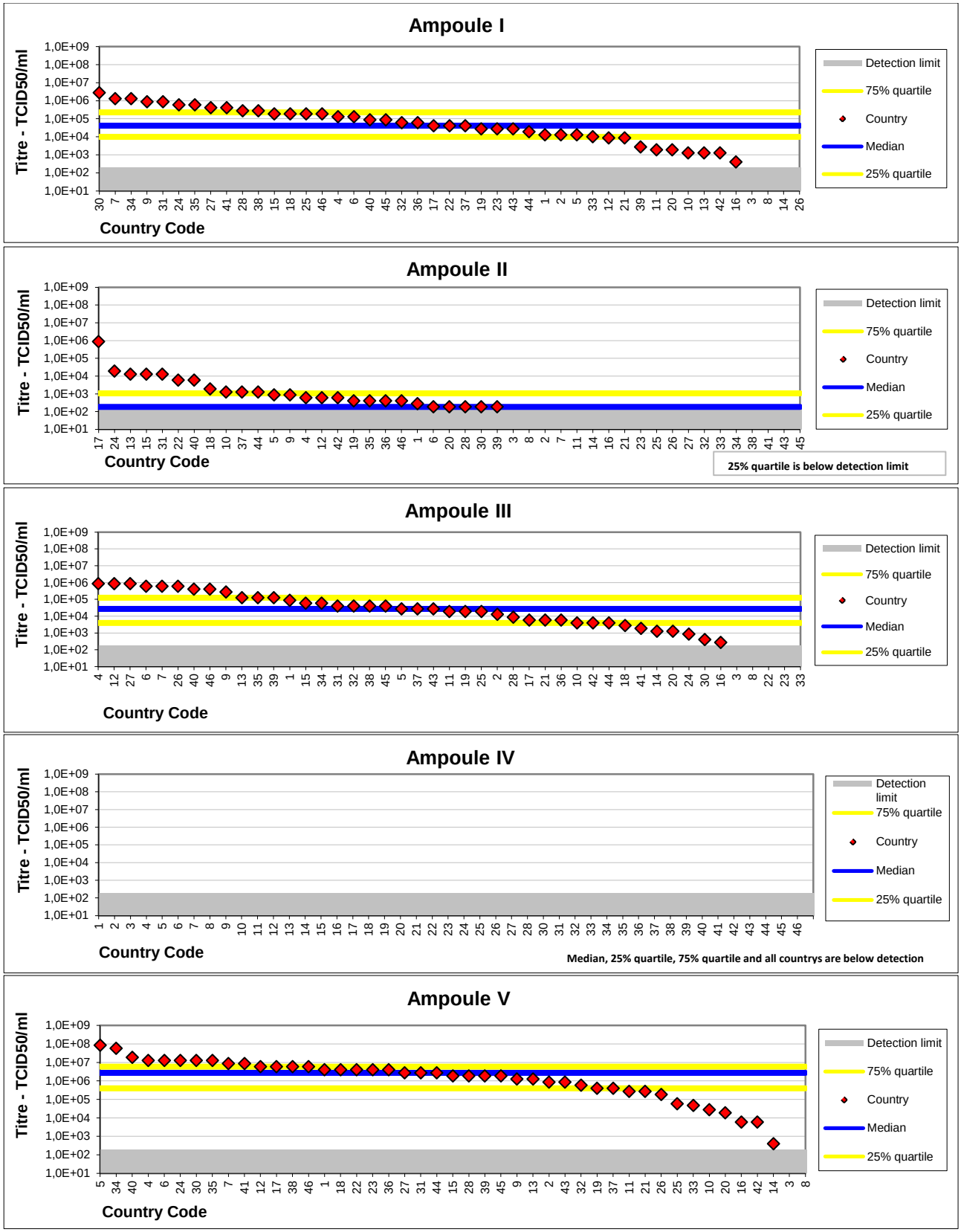


Figure 7. Virus titre obtained in RTG-2 cells. For further details see Figure 5

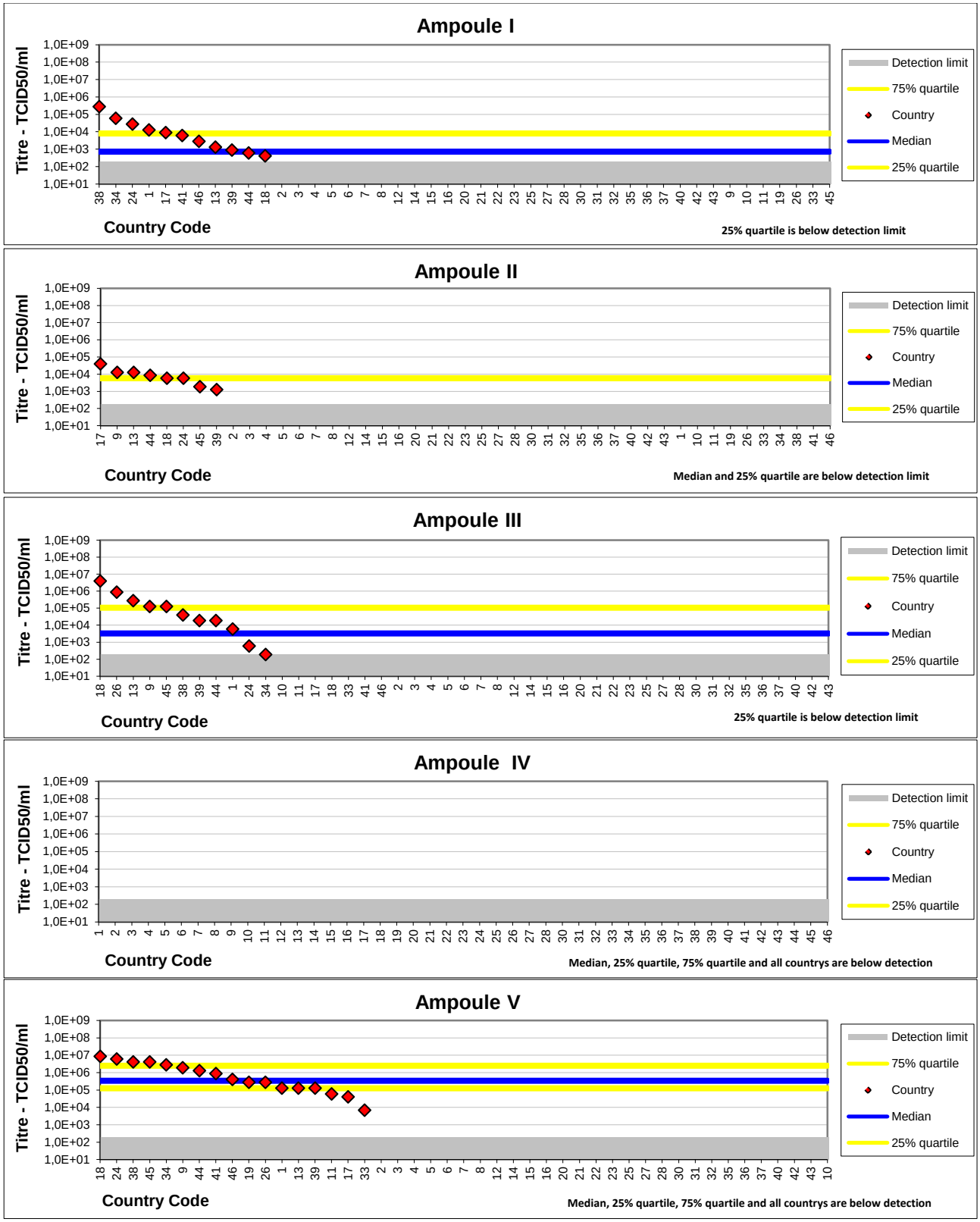
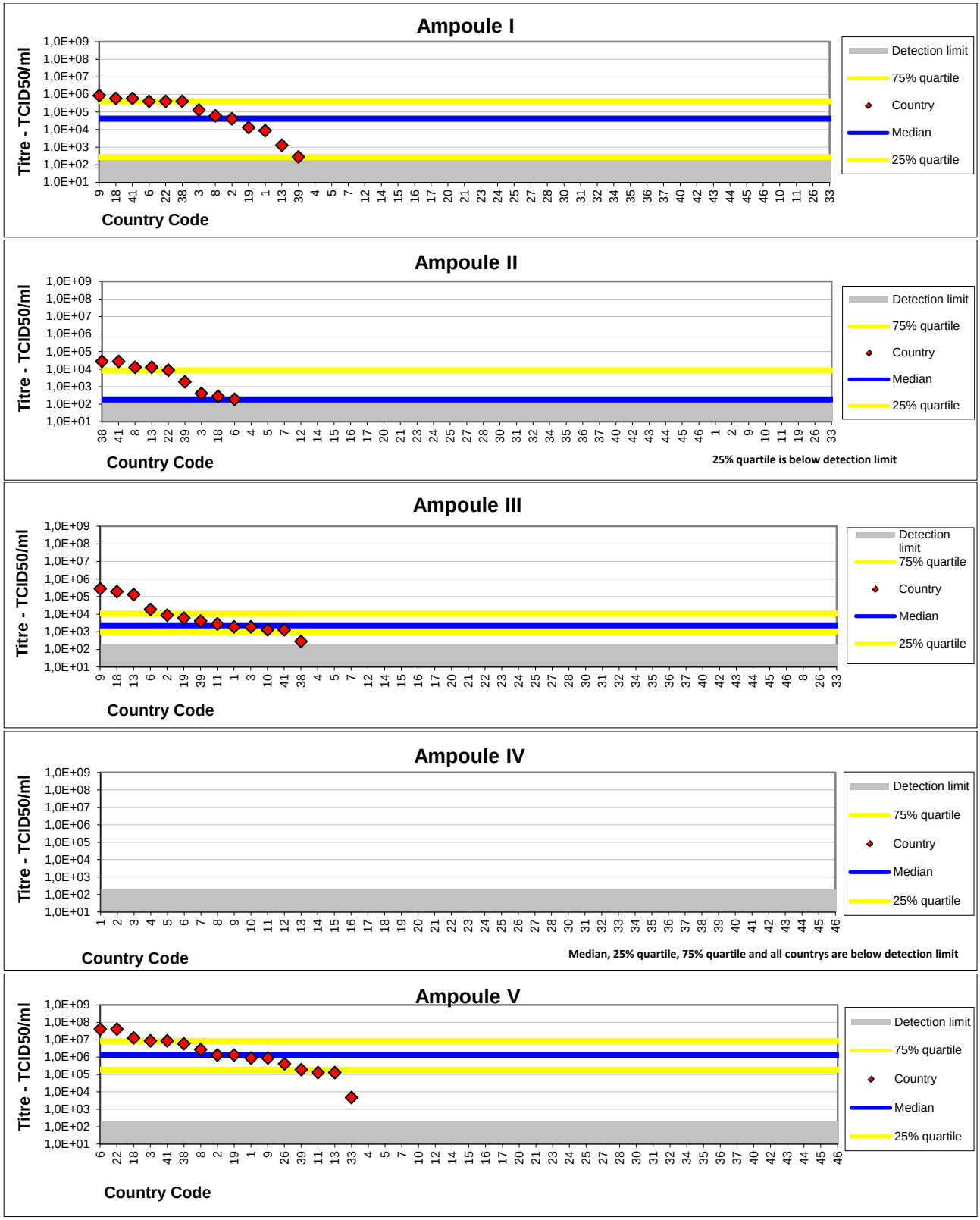


Figure 8. Virus titres obtained in FHM cells. For further details see Figure 5



Identification of content

- 29 laboratories correctly identified all viruses in all ampoules
- 43 of the participants submitted the spreadsheet within the deadline, 2 participants got the deadline extended due to delivery problems or technical problems in the laboratory.

Ampoule I – IHNV

- All 45 laboratories correctly isolated and identified IHNV

Ampoule II - VHSV

- 44 laboratories correctly identified VHSV
- 1 laboratory did not isolate any viruses.

Ampoule III – ECV

- 35 laboratories correctly isolated and identified ECV
- 3 laboratories correctly isolated Rana-virus but did not identify it – of which 1 by agreement does not have to sequence.
- 5 laboratories isolated Rana-virus but identified it as EHNV.
- 2 laboratories does not perform test for Ranaviruses.

Ampoule IV – BLANK

- 44 laboratories correctly identified 'not IHNV, not VHSV, not IPNV, not SVCV, not EHNV'
- 1 Laboratory isolated and identified IHNV

Ampoule V – VHSV + IHNV (+SVC-like)

- 37 laboratories correctly isolated and identified both VHSV and IHNV
- 7 laboratories isolated and identified only VHSV
- 1 laboratory isolated and identified VHSV and Rana-virus
- 24 laboratories isolated and identified SVC-like virus

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: IHNV identification was given the score 2. IHNV not identified was given the score 0. Incorrectly finding of "no virus" or additional types of viruses than those included in the ampoule scored 0, even though the included virus (IHNV) was amongst the identified viruses.

Ampoule II: VHSV identification was given the score 2. VHSV not identified was given the score 0. Incorrectly finding of "no virus" or additional types of viruses than those included in the ampoule scored 0, even though the included virus (VHSV) was amongst the identified viruses.

Ampoule III: ECV/ESV identification backed up by genomic analysis was given the score 2.
Ranavirus answer for this ampoule with a genomic analysis showing ECV/ESV was given the score 2.
Ranavirus/iridovirus as the only answer (without genomic analysis) was given the score 1.
EHNV identification (with or without genomic analysis) was given the score 1.

Ampoule IV: Identification "no virus", "Blank", "not IHNV, not VHSV, not IPNV, not SVCV, not EHNV" or similar answer was given the score 2. Incorrectly finding of virus in the ampoule was given the scored 0.

Ampoule V: Identification of both VHSV and IHNV was given the score 2. Identification of VHSV, IHNV and SVCV or SVCV/PFR-like was given the score 2. VHSV identification only was given the score 1. VHSV and SVCV identification only was given the score 1.
Incorrectly finding of "no virus" or additional types of viruses other than VHSV, IHNV anda SVC/PFR-like virus scored 0 even though VHSV and IHNV was amongst the identified viruses.

Out of 45 laboratories participating in the PT 1 2015, 27 obtained maximum score.
Serotyping and genotyping of VHSV and IHNV and submission of sequencing results are not a mandatory part of the test and is not included in the score of participants.

Cells applied for solving the test

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

- 39 laboratories used BF-2 cells
- 43 laboratories used EPC cells
- 18 laboratories used RTG-2 cells
- 17 laboratories used FHM cells
- 6 laboratories used CHSE-214 cells

- 10 laboratories used four cell lines (BF-2, EPC, RTG-2 and FHM)

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

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

The combination of EPC and FHM cells or BF-2 and RTG 2 as well is not valid according to Commission Decision 2015-1554. The laboratories using these combinations are encouraged to include the use of BF-2 cells and EPC or FHM.
The median titre calculated from the submitted results of each ampoule and in each cell line is illustrated in Figure 11.
It appears that:
Ampoule I (IHNV) replicates equally well on EPC and FHM cells, less efficiently on RTG-2 and not at all on BF-2 cells.
Ampoule II (VHSV Ib) replicate well on BF-2 cells, whereas its viral content was barely detectable on EPC and FHM cells, finally did not grow at all on RTG-2.
Ampoule III (ECV) replicates well on all four cell lines, however it grows best on BF-2 cells.
Ampoule V (VHSV + IHNV) replicates equally well on all four cell lines, which probably have to due to the fact that the two viruses replicates best on different cell lines.

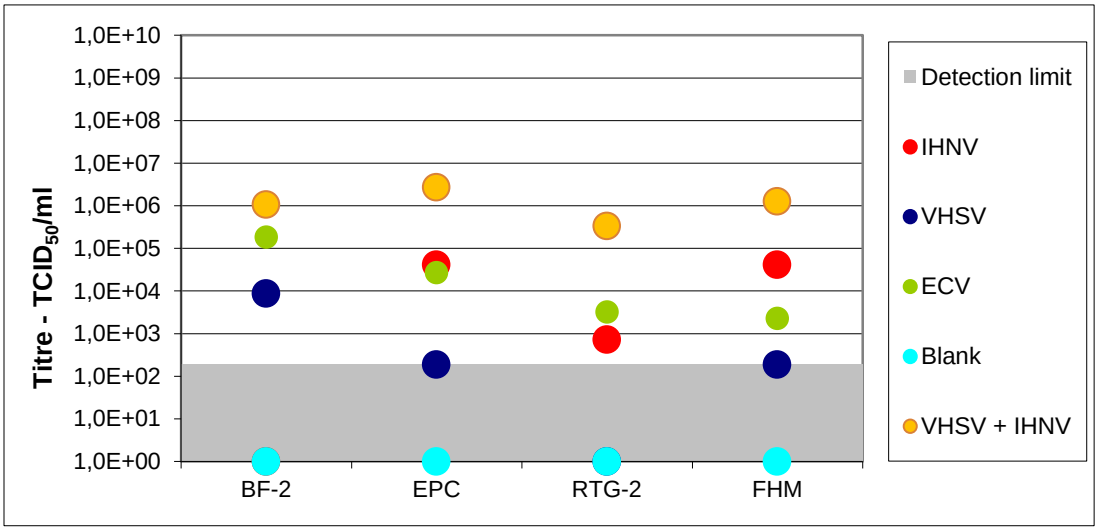


Figure 9. Median virus titres obtained by the participants in 4 different cell lines.

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) for VHSV and in Kurath et *al*. (2003) and Emmenegger et *al*., 2000 for IHNV but this was not an obligatory task.

AMPOULE I - IHNV

27 laboratories sequenced correctly IHNV in ampoule 1

- 18 laboratories partially sequenced the Glycoprotein Gene according to protocol from Emmeneger et al., 2000
- 1 Laboratory sequenced the full G Gene with in house developed primer sets
- 1 laboratory targeted the G gene according to the protocol provided by Miller et al., 1998
- 2 laboratories targeted the G gene according to the protocol provided by Kolodzejek et al., 2008
- 1 laboratory targeted the G gene according to Williams K. et al., 1999
- 1 laboratory reported to sequence the G protein without providing a protocol
- 1 laboratory targeted the Nucleocapsid protein according to protocol by Bergmann et al 2000
- 1 laboratory targeted the Nucleocapsid protein according to the protocol provided in the OIE manual 2006
- 1 laboratory referred to OIE Manual of 2006 without providing specifics for targeted region and primer sets
- 1 laboratory sequenced the NV region

It has to be specified that some participants used more than one protocol for sequencing the isolate.

AMPOULE II- VHSV

25 laboratories sequenced correctly VHSV in ampoule 4

- 6 laboratories targeted the G gene according to the protocol from Einer-Jensen et al., 2004
- 10 laboratories targeted the Nucleocapsid protein according to protocol from Snow et al.,2004
- 1 laboratory targeted the Nucleoprotein gene according to protocol form Bergmann et al.,2000
- 2 laboratory targeted the G gene according to protocol form Hedrick et al.,2003
- 1 laboratory targeted the G gene according to Miller et al. 1998
- 1 laboratory targeted the G gene according to Williams et al., 1999.
- 1 laboratory targeted the G gene according to OIE manual 2009
- 1 laboratory referred to OIE manual (non-specified year)
- 2 laboratories used unpublished results targeting the G gene

It has to be specified that some participants used more than one protocol for sequencing the isolate.

AMPOULE III - EHNV

35 laboratories sequenced ECV in ampoule III,

- 11 laboratories targeted the Major Capsid Protein MCP according to the protocol provided by Hyatt et al., 2000.
- 1 laboratory targeted the Major Capsid Protein MCP according to the protocol provided by Ohlemeyer et al., 2011
- 2 laboratories targeted the Major Capsid Protein MCP according to the protocol provided by OIE Manual 2011
- 1 laboratory targeted the Major Capsid Protein MCP according to protocol provided by Marsh et al.,2002
- 19 laboratories targeted the Major Capsid Protein MCP according to protocol provided by Holopainen et al, 2009
- 1 laboratory targeted the Polymerase according to the protocol provided by Holopainen et al, 2009
- 1 laboratory targeted the Major Capsid Protein MCP according to the protocol provided by Bigarre et al. 2008
- 1 laboratory targeted the Major Capsid Protein MCP without providing a protocol of reference
- 2 laboratories referred to OIE manual without providing details on protocol

It has to be specified that some participants used more than one protocol for sequencing the isolate.

AMPOULE V

Ampoule V – IHNV

24 laboratories sequenced IHNV isolate in Ampoule V

- 9 laboratories partially sequenced the Glycoprotein Gene according to protocol from Emmeneger et al., 2000
- 1 Laboratory sequenced the full G Gene with in house developed primer sets
- 1 laboratory targeted the Nucleocapsidprotein according to protocol by Bergmann et al 2000
- 3 laboratories targeted the G gene according to the protocol provided by Kolodzejek et al., 2008
- 5 laboratories targeted the G gene according to OIE manual
- 2 laboratories targeted the N gene according to OIE manual
- 1 laboratory targeted the G gene according to Williams et al., 1999
- 1 laboratory targeted the G gene without providing protocol of reference
- 1 laboratory provided the primersets without referring to a protocol
- 1 laboratory did not provide a protocol

It has to be specified that some participants used more than one protocol for sequencing the isolate.

Ampoule V – VHSV

25 laboratories sequenced the VHSV isolate included in Ampoule V

- 6 laboratories targeted the G gene according to the protocol from Einer-Jensen et al., 2004
- 7 laboratories targeted the Nucleocapsid protein according to protocol from Snow et al.,2004
- 1 laboratory targeted the Nucleocapsidprotein according to protocol by Bergmann et al 2000
- 1 laboratory targeted the G gene according to the protocol provided by Miller et al., 1998
- 1 laboratory targeted the G gene according to Williams et al.,1999
- 1 laboratory targeted the G gene according to Hedrick et al.,2003
- 1 laboratory targeted the G gene according to Raja-Halli et al., 2006
- 1 laboratory sequenced both N and G gene according to OIE manual
- 2 laboratories provided primer sets without reference and target region
- 2 laboratory targeted the g gene region with an “in house” developed protocol
- 1 laboratory sequenced the product obtained by qPCR analysis from Jonstrup et al., 2012.

It has to be specified that some participants used more than one protocol for sequencing the isolate.

Ampoule V – Tench rhabdovirus

9 Laboratories sequenced a rhabdovirus identified as tench rhabdovirus using protocols designed by Stone et al., 2003. This virus was not expected to be included in Ampoule V of the Interlaboratory Proficiency test 2015, a specific paragraph address this issue in the report.

Résumé and concluding remarks PT1

. 49% of parcels were delivered by the shipping companies within 1 day after submission and 80% was delivered within 1 week. It was, however, unfortunate that six parcels were more than 2 weeks on the way and one of these was 35 days on the way before delivered to the laboratory primarily due to border controls.

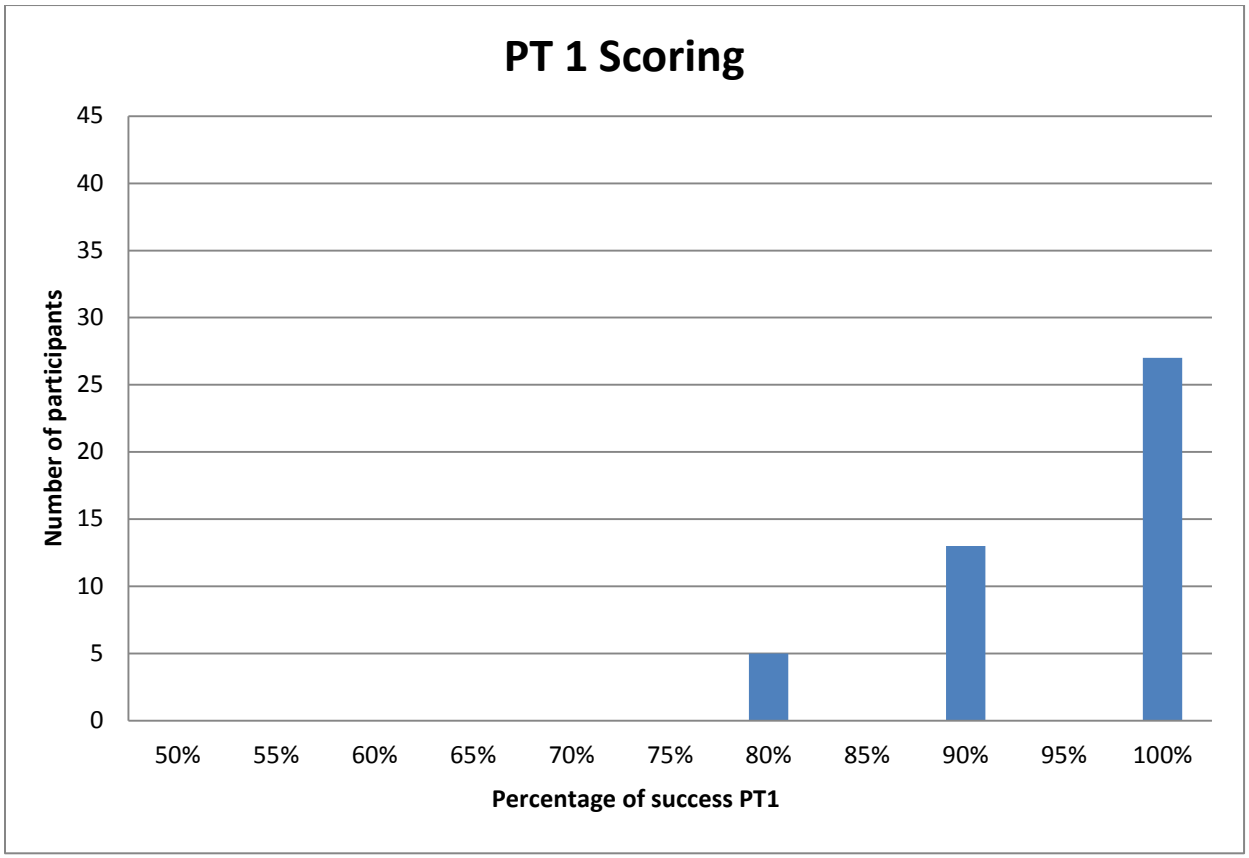
This year ECV was included. 43 participants were able to identify Ranavirus of these laboratories 38 correctly identified 'Ranavirus' or 'not EHNV'. 40 laboratories performed sequencing and among these 35 identified 'ESV/ECV' correctly.

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.

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.

It was unexpected and quite unfortunate that the PFR/ tenchRV like virus – S64- showed up in Ampoule V.
The presence of this rhabdovirus was confirmed in the ampoules V in addition to the expected VHSV strain DK-5151 + IHNV strain 32/87. The virus identified was Tench Rhabdovirus S64. The scoring system has been adjusted on the background of the finding from the participants and the final confirmation conducted at the EURL. This issue has been taken seriously into consideration by the EURL and managed both with the participants and DANAK the accreditation body that audit the QA system at DTU.

The results presented in this report will be further presented and discussed at the 20th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 31th of May - 1st of June 2016 in Copenhagen, Denmark.



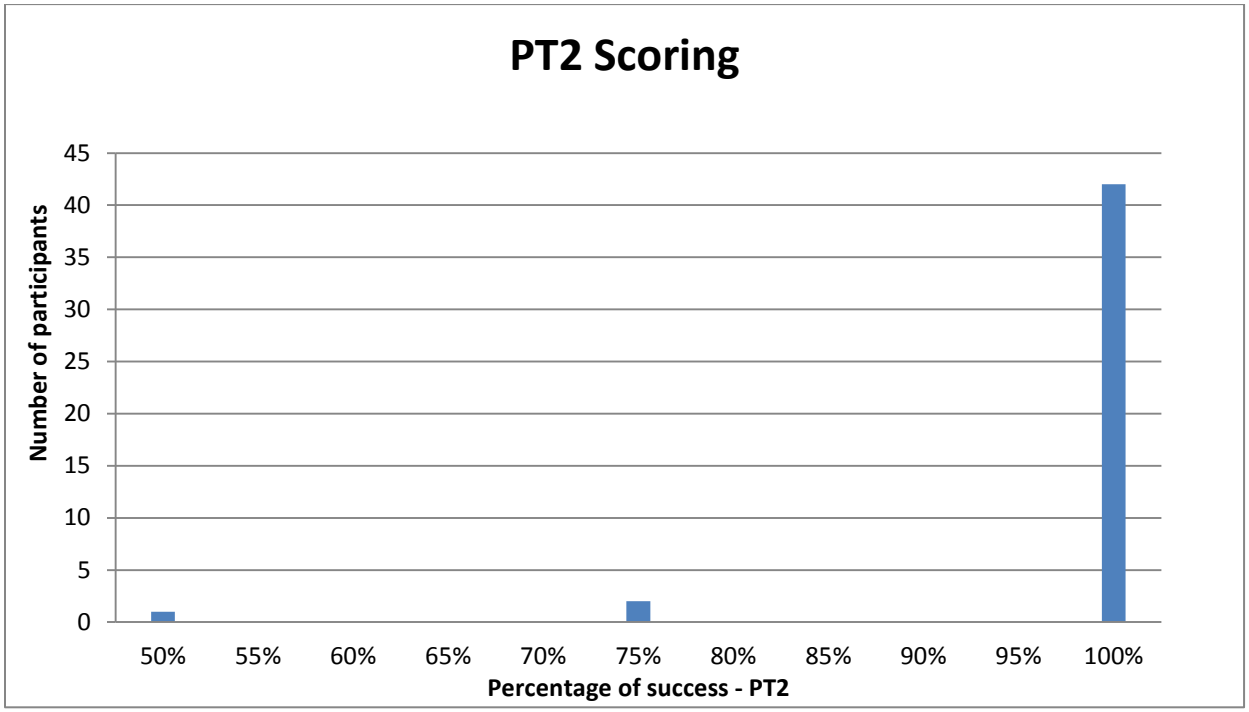


Figure 10 "a" and "b" Success-rate of participating laboratories 2015

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 one 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) and the conventional PCR protocol described by Bercovier et *al*. (2005), the SAV real-time RT-PCR protocol described by Hodneland et al. (2006) and the ISAV real-time RT-PCR protocol described by Snow et *al*. (2006) and conventional RT-PCR protocol described by Mjaaland et *al*. (2002).

The details of the virus isolates used in the proficiency test 2 are outlined in table 9.

Table 9. Content in each ampoule with reference to culture conditions and major publications of the included pathogens.

Code	Specifications/References
Ampoule VI	**KHV, (CyHV-3)** Koi Herpesvirus (CyHV-3): KHV-TP 30 (syn: KHV-T (for Taiwan)). KHV-TP 30 was isolated from koi in Taiwan and cloned for producing large plaques by Dr. Peiyu Lee, Institute of Medical Biotechnology, Central Taiwan University of Science and Technology, Dakeng, BeiTung District, TaiChung City 406, Taiwan in-2005. The isolate was provided by Dr. Sven M. Bergmann, Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Südufer 10, 17393 Greifswald-Insel Riems, Germany
Ampoule VII	**SAV 6** * Pancreas Disease Virus, Ireland F104596 GenBank accession numbers: EF675499 (nsp3 gene); EF675547 (E2 gene) *: In the result sheet submitted by the EURL one week after deadline for submission of the PT-2 results the SAV isolate was nominated as SAV1- this is not correct, according to E Fringuelli, H M Rowley, J C Wilson, R Hunter, H Rodger and D A Graham Journal of Fish Diseases 2008, 31, 811–823 doi:10.1111/j.1365-2761.2008.00944.x the correct name is SAV6
Ampoule VIII	**BF-2, cells** Supernatant from NON Infected BF-2 cells
Ampoule IX	**ISAV,** FO/01/01/HPR13. ISAV virus kindly provided by Debes Christiansen, The Faroe Islands Genotype: HPR13 GenBank accession numbers: AJ440970 Cunningham CO et al., 2002 Christiansen et al. J., 2011

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 PCR (Bercovier et *al.* (2005)) and real-time PCR (Gilad et *al.* (2004)) for KHV, by RT-PCR (Mjaaland et *al.* (1997)) and real-time RT-PCR (Snow et *al.* (2006)) for ISAV and by RT-PCR (Fringuelli et al. (2008)) and real-time RT PCR (Hodneland et al. (2006)) 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, 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 10 and Figure 12).
Conventional PCR/RT-PCR fragments were sequenced and so was the HPR region of the ISAV isolate.

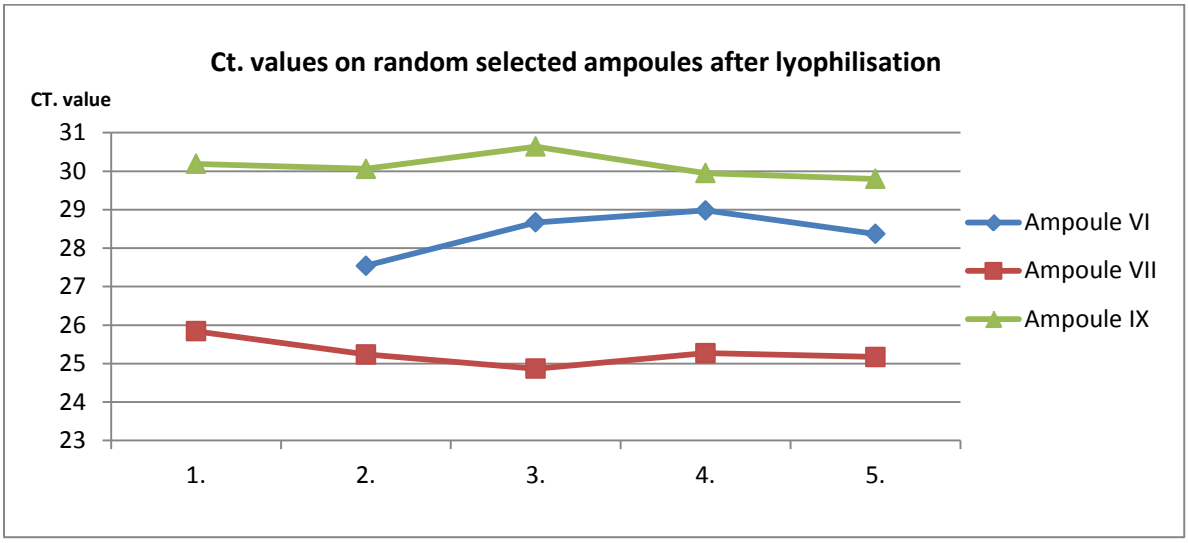


Figure 11, Ampule VI (CyHV-3), VII (SAV) and IX (ISAV) tested shortly after lyophilisation to assess homogeneity of the content.

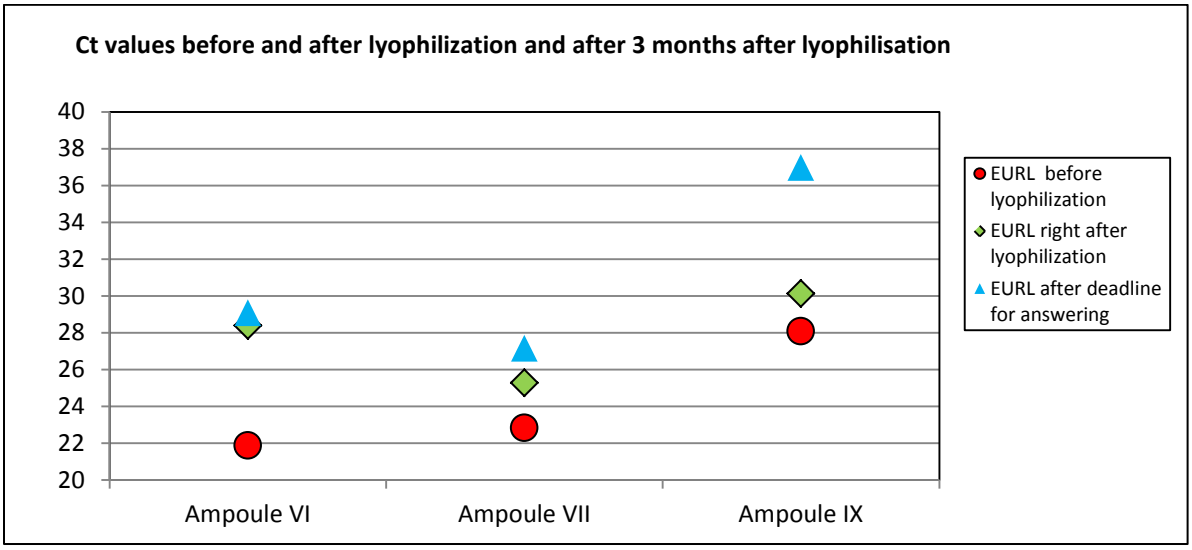


Figure 12, Ampule VI, VII and IX tested before and after lyophilisation and after deadline for handling in results.

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

Ampoule No.	Content		EURL Ct. value before lyophilization	EURL Ct. value right after lyophilization	EURL Ct. value after deadline for answering
Ampoule VI	KHV	a	21,87	27,54	29,08
		b			
		c		28,67	
		d		28,98	
		e		28,37	
	Average		**21,87**	**28,39**	**29,08**
Ampoule VII	SAV	a	22,83	25,84	27,16
		b		25,24	
		c		24,87	
		d		25,27	
		e		25,17	
	Average		**22,83**	**25,28**	**27,16**
Ampoule IX	ISAV	a	28,08	30,19	36,98
		b		30,06	
		c		30,64	
		d		29,95	
		e		29,80	
	Average		**28,08**	**30,13**	**36,98**

The lyophilisation procedure caused a significant virus reduction (mainly in ampule VI and IX) 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) if present in the ampoules, 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. This year the panel of pathogens to be investigated were expanded to include SAV – Salmonid Alpha Virus. Since this is not a listed disease in the European legislation the participation was voluntary and therefor 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 OIE manual chapter 2.3.5b – Infection with Salmonid alpha virus. In order to obtain uniform answers, participants were requested to download a spreadsheet available from the EURL web page, insert results in this and return by email. The results from participating laboratories are shown in table 11.

Table 11. Inter-Laboratory Proficiency Test, PT2, 2015 - Virus identification.

Laboratory code number	Score	Answer recieved at EURL	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
			KHV (CyHV-3)	SAV	Sterile	ISAV (HPR13)
46	8-8	09.11.15	KHV	SAV	Negative	ISAV
45	8-8	06.11.15	KHV	SAV	no virus detected	ISAV
44	8-8	12.11.15	KHV	no pathogen found	no pathogen found	ISAV
43[2]		30.11.15	0	0	0	0
42[1]	6-8	13.11.15	KHV	NO VIRUS	RNA VIRUS, no rhabdo or birna virus	ISAV
41	8-8	11.11.15	KHV	SAV	Negative	ISAV
40[1]	8-8	13.11.15	KHV	not KHV nor ISAV	not KHV nor ISAV	ISAV
39	6-8	11.11.15	KHV	0	0	ISAV + KHV
38	8-8	13.11.15	KHV	SAV	Not ISAV, not KHV, not SAV	ISAV
37[1]	8-8	12.11.15	KHV	no KHV no ISAV	no KHV no ISAV	ISAV
36	8-8	13.11.15	KHV	SAV	No virus detected	ISAV
35	8-8	12.11.15	KHV	SAV	no virus	ISAV HPR13
34	8-8	26.10.15	KHV	SAV	negative for all viruses tested	ISAV
33	8-8	12.11.15	KHV	SAV	0	ISAV
32	8-8	13.11.15	KHV	SAV	NO KHV NO ISA NO SAV	ISAV
31	8-8	13.11.15	KHV	SAV	NEGATIV	ISAV
30[3]	8-8	11.11.15	No virus detected	SAV	No virus detected	ISAV
28	8-8	13.11.15	KHV	SAV	Negative	ISAV
27	8-8	16.11.15	KHV	SAV	Negative	ISAV
26	8-8	13.11.15	KHV	SAV	-	ISAV
25[1]	8-8	12.11.15	KHV	Not KHV, Not ISAV	Not KHV, Not ISAV	ISAV
24[1]	8-8	12.11.15	KHV	Negative for KHV and ISAV	Negative for KHV and ISAV	ISAV
23	8-8	13.11.15	KHV	SAV	No KHV ISAV or SAV detected	ISAV
22	8-8	13.11.15	KHV viable virus detected	SAV viable virus detected	No viruses detected	ISAV viable virus detected
21	8-8	10.11.15	KHV	SAV6	No virus detected	ISAV
20	8-8	13.11.15	KHV	SAV	0	ISAV
19	8-8	13.11.15	KHV	SAV	No Virus	ISAV
18	8-8	13.11.15	KHV	SAV	negativ for KHV, ISAV, SAV	ISAV
17[1]	8-8	13.11.15	KHV	Negative	Negative	ISAV
16[1]	8-8	13.11.15	KHV	Not ISAV Not KHV	Not ISAV Not KHV	ISAV
15	8-8	10.11.15	KHV	SAV	Negative	ISA
14[4]	8-8	13.11.15	KHV	KHV negative	KHV negative	KHV negative
13	8-8	13.11.15	KHV	SAV	-	ISAV
12	8-8	13.11.15	KHV	SAV	Not detected	ISAV
11	8-8	12.11.15	KHV	SAV	negative	ISAV
10[1]	8-8	12.11.15	KHV	Negative	Negative	ISAV
9	8-8	12.11.15	KHV	SAV	Not virus detected	ISAV HPR-deleted
8	8-8	13.11.15	KHV	SAV	Negativ	ISAV
7	4-8	10.11.15	KHV	no ISAV, SAV or KHV	SAV	ISA
6	8-8	12.11.15	KHV	SAV	Negative	ISAV
5	8-8	13.11.15	KHV	SAV	Virus was not detected.	ISAV
4	8-8	13.11.15	KHV +	Alphavirus +	Negative (for ISAV/SAV and cyprinid herpes virus inc. KHV)	ISAV +
3	8-8	06.11.15	KHV	SAV	Negative	ISAV
2	8-8	13.11.15	KHV	SAV	negative	ISAV
1	8-8	12.11.15	KHV	SAV	not ISAV, KHV, SAV	ISAV

[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 were encouraged to sequence the HPR region of ISAV isolates. However, this was not a mandatory task.

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

- 44 laboratories submitted results
- 31 laboratories correctly identified all four ampoules
- 42 laboratories tested for both two listed pathogens
- 43 laboratories tested for ISAV
- 43 laboratories tested for KHV
- 33 laboratories tested for SAV
- 1 laboratory tested for KHV only
- 1 laboratory that did participate in PT 1 did not participate in PT2

Ampoule VI – *KHV*

- 43 laboratories correctly identified KHV
- 1 laboratory did not examine for KHV

Ampoule VII – SAV

- 32 laboratories correctly identified SAV
- 11 laboratories did not examined for SAV

Ampoule VIII – BF2 cell supernatant

- 42 laboratories correctly identified 'Not KHV or ISAV'
- 2 laboratory found a virus

Ampoule IX – ISAV

- 42 laboratories correctly identified only ISAV
- 1 laboratories identified ISAV and KHV

Scores

We have assigned a score of 2 for each correct answer (Table 11), giving the possibility for obtaining a maximum score of 8. Incorrectly finding of pathogens not present in the ampoules gives the score 0.

Of the 44 laboratories submitting results 41 laboratories obtained maximum score. The laboratories (there did not test for all viruses) obtained maximum score if they did not find the viruses they tested for in other ampules than where the virus actual were present. E.g. one laboratory examined for KHV only and found KHV in ampoule VI and 'not KHV' in VII, VIII and IX, this laboratory obtained the score 8 out of 8 possible.
Genotyping of ISAV HPR region and submission of sequencing results was not a mandatory part of the test and is not included in the score of participants.

Methods applied

The following methods were used by the participants:

- 33 laboratories used KHV PCR, among these approx. 21 used the protocol provided by Bercovier et al 2005.
- 26 laboratories used KHV Real-time PCR among these approx. 21 used the protocol from Gilad et al 2004.
- 16 laboratories used both KHV real-time PCR and KHV PCR.

- 25 laboratories used SAV RT-PCR among these approx. 17 used the protocol from Fringuelli et al. 2008.
- 18 laboratories used SAV real-time RT-PCR among these approx. 13 used the protocol from Hodneland et al. 2006.
- 8 laboratories used both SAV real-time RT-PCR and SAV RT-PCR.

- 33 laboratories used ISAV RT-PCR among these the two most used protocols were the ones from Mjaaland et al 2002 and OIE Manual for diagnostic tests for aquatic animals 2009.
- 24 laboratories used ISAV real-time RT-PCR among these approx. 18 used the protocol from Snow et al., 2006.
- 14 laboratories used both ISAV real-time RT-PCR and ISAV RT-PCR

Genotypning and sequencing
Participants were encouraged to sequence the HPR region of possible ISAV isolates though it was not a mandatory task.

- 22 laboratories performed sequencing for KHV
- 33 laboratories performed sequencing for SAV
- 27 laboratories performed sequencing for ISAV

AMPOULE VI KHV:
22 laboratories sequenced the KHV isolate included in Ampoule VI

- 15 laboratories sequenced the Thimidine kinase region using primer sets described in Bercovier et al.2005
- 2 laboratory used primer sets according described in Engelsma et al.,2013
- 1 laboratory provided primer set without describing the protocol
- 1 laboratory did not provide primer sets nor protocol
- 1 laboratory sequenced the sphl gene using primersets described in Gray et al. 2002
- 2 laboratories sequenced the polymerase gene using primersets described in protocol by Stone and Way from CEFAS 2010

AMPOULE VII SAV:
33 laboratories participated in testing ampoules for SAV, which was included in PT2 2015 on a volunteer basis.

Of these, 20 laboratories sequenced the SAV isolate included in Ampoule VII.

- 13 laboratories sequenced the E2 and nsP3 gene using primer sets described in Fringuelli et al.,2008
- 4 laboratories sequenced the E2 region according to Hodneland et al., 2006
- 1 laboratory targeted the RNA polymerase without specifying the protocol
- 2 laboratories provided primersets without reporting the protocol

AMPOULE IX ISAV:
27 laboratories sequenced the ISAV isolate included in Ampoule IX

- 12 laboratories targeted the HPR region using primersets described in Mjaaland et al.,2002
- 1 laboratory targeted the HPR region using primer set described in Cunningham et al.,2002
- 4 laboratories sequenced the partial HA gene according to Kibenge et al.,2009
- 1 laboratory sequenced the HA region according to McBeath et al.,2009
- 3 laboratories sequenced the region using primerset by Christiansen et al. 2011
- 6 laboratories used in house developed primersets protocols

Concluding remarks PT2

This was the first time that the EURL provided a proficiency test on SAV identification. Considering that 33 laboratories participated (of which 32 correctly identified SAV in ampoule VII) this was regarded as a proper initiative that strengthen the diagnostic capacities of the NRLs in detecting emerging pathogens, and it will be included in the coming years as well.
All 43 laboratories testing for KHV identified the virus in ampoule VI.
Out of the 33 laboratories that tested for SAV 32 laboratories identified SAV in ampoule VII.
Out of 44 laboratories 42 laboratories identified Not *KHV or* ISAV in ampoule VIII and there were "only" two false positive.
All 43 laboratories testing for ISAV identified the virus in ampoule IX, though one laboratory also wrongly identified KHV in ampoule IX.
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).
Of the 27 laboratories sequencing the ISAV virus all found that the isolate was with deletion in segment 6 and thus not belong to HPR0.

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 20th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 31th of May-1st of June 2016 in Copenhagen, Denmark.

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European Union Reference laboratory for Fish diseases
National Veterinary Institute, Technical University of Denmark, February 2016

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