European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2020 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

DTU

Table of contents

Introduction **1**

Distribution of the test **3**

Shipment and handling 3

Participation 3

Proficiency test 1, PT1 .... **4**

Content of ampoules **5**

Testing of the PT1 test **8**

Virus identification and titration **10**

Identification of content 29

Scores 30

Cells applied for solving the test 30

Ct. values 31

Genotyping and sequencing 35

Résumé and concluding remarks PT1 .... **38**

Proficiency test 2, PT2 .... **40**

Content of ampoules **40**

Testing of the PT2 test **42**

Pathogen identification **43**

Identification of content 45

Scores 46

Ct. values 47

Genotyping and sequencing 49

Concluding remarks PT2 .... **52**

Remarks for ILPT 2019 .... **54**

References **55**

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 assess the ability of participating laboratories in quantifying and identifying 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). The laboratory procedures for isolating and titrating these pathogens is primarily based on cell culture methods, however recently the use of molecular methods (Real Time PCR based) has been implemented for their detection and identification.
PT2 was designed to assess 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).
44 laboratories participated in PT1 while 43 participated in PT2.
Regarding PT1 and PT2, 41 and 38 laboratories respectively participated in identifying all viruses included.
The tests were sent from the EURL 25th of September 2020.
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.
The EURL use Eurofins Genomics as subcontractor for sequencing the amplicons of viral isolates included in the PTs and DTU – National Food Institute for lyophilisation of the ampoules.
This report covers both the results of PT1 and PT2.

PT1 consisted of five coded ampoules (I-V). These ampoules contained VHSV, IHNV/IPNV, SVCV, non-infected BF-2 cell supernatant and EHNV, respectively (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. Finally, participants have to consider that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). The participants were also 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 further characterize VHSV and IHNV isolates by means of genotyping. It was recommended to use the genotyping procedure described in Einer-Jensen et *al*. (2004) [5] for VHSV and ; for IHNV we suggest to follow procedure provided in the latest IHNV chapter of the OIE manual on Aquatic Animal Diseases (primer references are given in Emmenegger et al. (2000), Diseases of Aquatic Organisms 40 (3), 163-

176 and PCR conditions are given in Garver et al. (2003), Diseases of Aquatic Organisms 55;187-203. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of four coded ampoules (VI-IX). These ampoules contained BF-2 cell supernatant, ISAV, SAV/ISAV, and KHV, respectively (see table 11). In order to introduce a novelty in the test, this year one ampoule contained a mixed infection with SAV and ISAV. 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 38 of 43 laboratories tested for SAV in 2020.
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 in theory, it should be possible to replicate them in cell cultures.

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 10 and 15) and display the genotyping results provided by all participants.

For the first time this year, the EURL has compiled and presented the Ct values reported by the different laboratories (table 9 and figure 10 for PT1; table 14 and figure 13 for PT2).

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 has 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 (https://www.eurl-fish-crustacean.eu/) to be used for reporting results and to be submitted to the EURL electronically. Participants were asked to reply latest December 4th 2020. The results of the inter-laboratory proficiency test for listed fish diseases 2020 and plans and idea for future inter-laboratory tests will be presented at the 25th Annual Workshop of the NRLs for Fish Diseases on May 31st, June 1st 2021, due to COVID-19 travel restriction the meeting will be held online. Furthermore a specific online meeting on March 25th will be organized to discuss the report and receive comments, inputs and feedback from the participating laboratory.

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 test parcels were delivered by courier, with the exception of participant whom themselves arranged the pick-up of the test. When possible participants were provided with a tracking number so they were able to follow the shipment.

Shipment and handling

The parcels were picked-up on a Friday. The following Monday, the tests were delivered to 23 participants; 13 more parcels were delivered within the first week; 95% were delivered within the first two weeks and 100% within three weeks (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 proficiency test reports 2007,2010,2011.

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

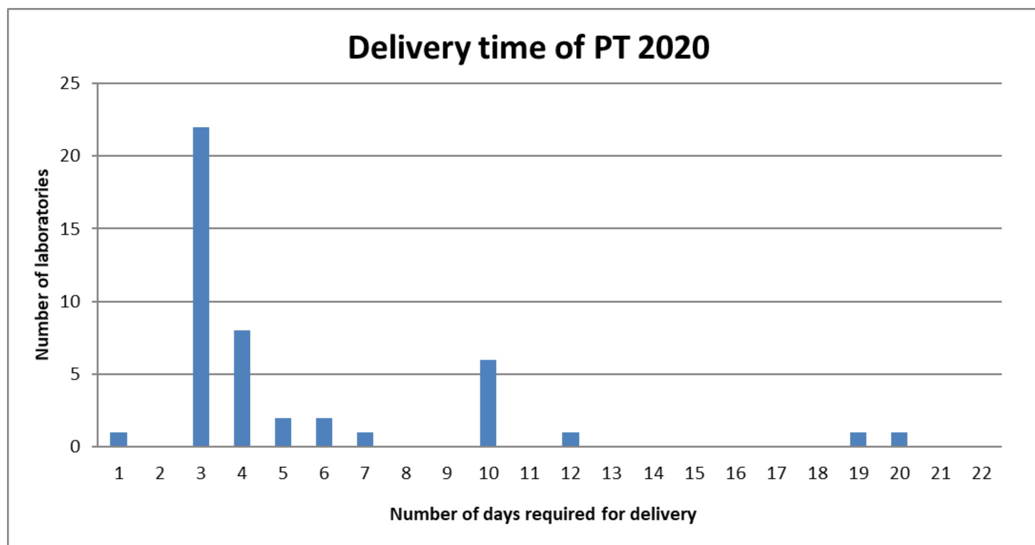


Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 45 laboratories received the annual proficiency test. 44 participants submitted the full spreadsheet within the deadline; 1 participant did receive the parcel but did not submit the spreadsheet. Figure 2 show the numbers of participants in the proficiency test from 2007 to 2020.

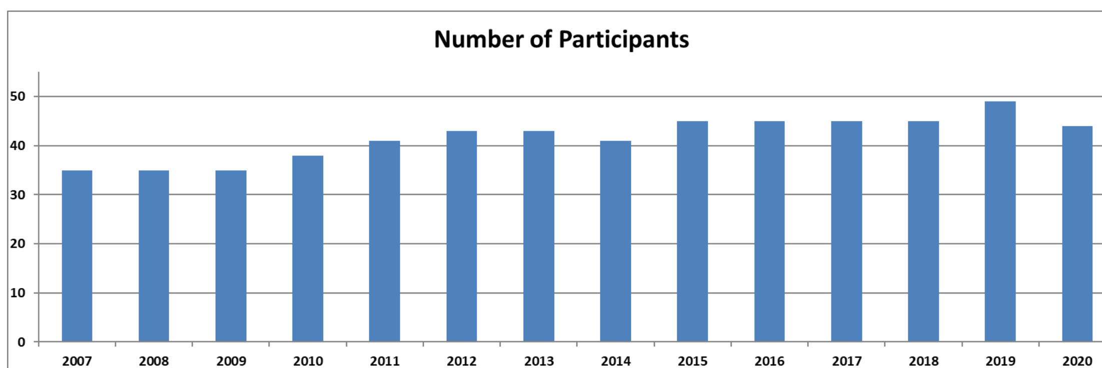


Figure 2. Participants in the EURL proficiency test over the years.

Proficiency test 1, PT1

Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States, including Denmark, to the United Kingdom (NRLs in England and Wales, Scotland, Northern Ireland)*, and likewise to the NRLs in Australia, Bosnia and Herzegovina, Canada, Faroe Islands, Iceland, Japan, New Zealand, Norway, Republic of North Macedonia, Russia, Serbia, Switzerland, Tunisia, Turkey and to two laboratories in South Korea and USA, respectively.
The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy and Cyprus for identification of all listed diseases. Figure 3 shows the worldwide distribution of the participating NRLs. This year five participating laboratories which are normally enrolled in this exercise, were not able to participate, possibly in relation to consequences on accessibility to laboratories due to COVID-19 pandemics.

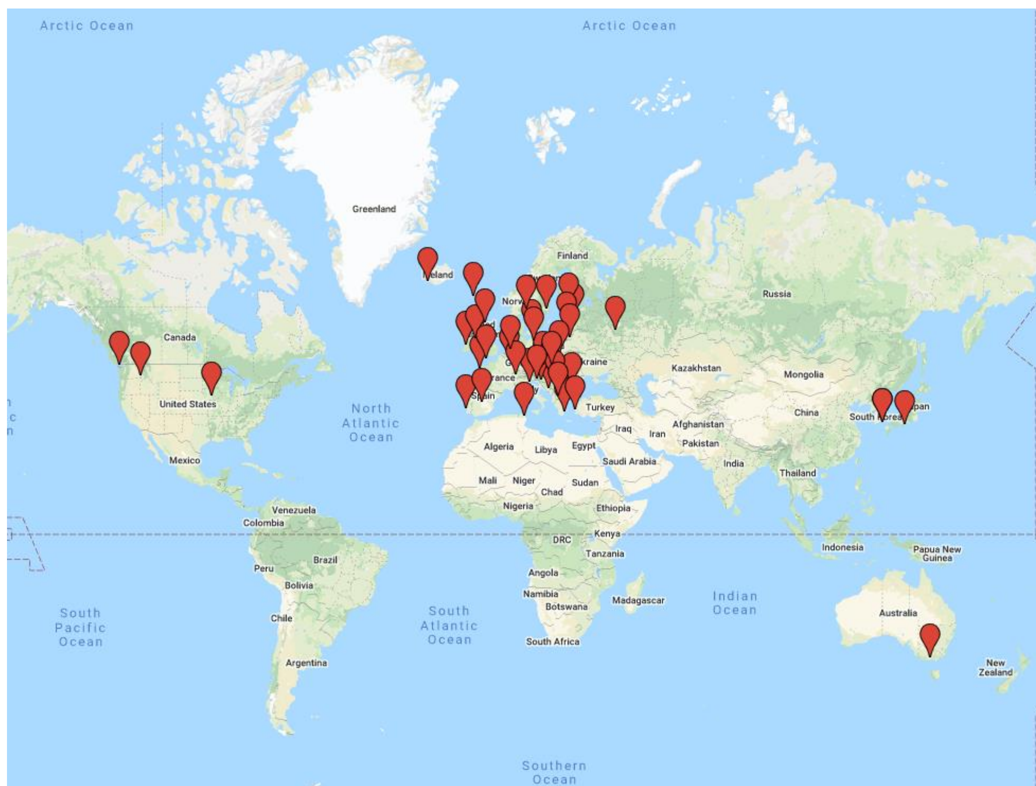


Figure 3. Worldwide distribution of the participants in the EURL proficiency test 2020

* In accordance with the Agreement on the withdrawal of the United Kingdom of Great Britain and Northern Ireland from the European Union and the European Atomic Energy Community ('Withdrawal Agreement'), EU law applies to and in the United Kingdom during the transition period from 1 February 2020 to 31 December 2020.

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: VHSV	**VHSV Isolate DK-9895174** Viral haemorragic septicaemia virus isolated in 1998, FP.VHSV.269 in Denmark from *Oncorhynchus mykiss* (Rainbow trout) **Genotype**: Ia **Reference on isolate**: Skall et al. (2004) **Reference on G protein sequence**: Toplak et al.,2010 https://www.int-res.com/articles/dao_oa/d092p021.pdf **Reference on full genome sequence** : Panzarin et al.,2020 https://www.frontiersin.org/articles/10.3389/fmicb.2020.01984/full Accession number **MK829413**

Code	Specifications/References
Ampoule II: **IHNV** **+** **IPNV**	**IHNV Isolate 32/87** First French isolate (April 1987) from rainbow trout. **Genotype:** Genogroup E **GenBank accession number:** J265717 and AY524121 (G-gene), FJ265711 (N-gene). **Reference on isolate:** Baudin Laurencin F (1987) IHN in France. Bulletin of the European Association of Fish Pathologists 7, 104. **Reference on sequence:** Kolodziejek J., Schachner O., Dürrwald R., Latif M. & Nowotny N. (2008) "Mid-G" region sequences of the glycoprotein gene of Austrian infectious hematopoietic necrosis virus isolates form two lineages within European isolates and are distinct from American and Asian lineages. Journal of Clinical Microbiology 46, 22-30. Johansson T., Einer-Jensen K., Batts W., Ahrens P., Björkblom C., Kurath G., Björklund H. & Lorenzen N. (2009) Genetic and serological typing of European infectious haematopoietic necrosis virus (IHNV) isolates. Diseases of Aquatic Organisms 86, 213-221. --- **IPNV SP isolate Sp** The Sp (Spjarup) reference strain of Infectious Pancreatic Necrosis (IPN) virus from farmed rainbow trout in Denmark, isolated in 1969 by Dr. Vestergaard Jørgensen. **Genotype:** Genogroup 5 **Received from:** National Veterinary Institute, Technical University of Denmark. **GenBank accession numbers:** AM889221 Segment B; AF342728 Segment A **Reference on isolate:** Jørgensen PEV & Bregnballe F (1969) Infectious pancreatic necrosis in rainbow trout in Denmark. *Nordisk Veterinærmedicin* **21**, 142-148. Jørgensen PEV & Grauballe PC (1971) Problems in the serological typing of IPN virus. *Acta Veterinaria Scandinavica* **12**, 145-147. **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

Code	Specifications/References
Ampoule III: SVCV	**SVCV isolate DK-203273** Spring Viraemia of Carp Virus isolated from Koi Carp in Denmark June 2003 **Genotype:** 1a **Received from:** National Veterinary Institute, Technical University of Denmark. **GenBank accession numbers:** The isolate is unpublished (in 2019)
Ampoule IV: BLANK	**Cell supernatant from BF-2 cells** **01/18** **Passage No.: 36. Passage date:12.02.20** **Tested negative for Mycoplasma: 13.01.20**
Ampoule V: 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. **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. 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. 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.

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 in the viral titre especially for VHSV. Previous experience reported during the past Proficiency tests demonstrated a rather high stability for SVCV, EHNV and IPNV serotype Sp. Lyophilised viral supernatant mixed in freeze drying medium preserved in glass sealed ampoules is stable for more than half a year when kept at room temperature (Inter-Laboratory Proficiency Test report 2007) ; it can survive exposure to 30°C for 24 hours (Inter-Laboratory Proficiency Test report 2010) And a temperature raise from 20 to 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, 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 all of the ampoules, the reduction of the titre was between 1-3 log in the same cell line. 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: VHSV DK-9895174	BF-2	5,9E+07	1,3E+05	5,9E+04
	EPC	2,7E+05	2,7E+03	2,7E+03
	RTG-2	8,6E+07	1,9E+05	1,3E+05
	FHM	8,6E+07	4,0E+05	5,9E+05
Ampoule II: IHNV 32/87 + IPNV Sp	BF-2	1,3E+09	1,3E+07	1,3E+07
	EPC	1,9E+08	5,9E+06	4,0E+06
	RTG-2	8,6E+08	5,9E+06	1,3E+06
	FHM	1,9E+08	1,3E+06	2,7E+05
Ampoule III: SVCV DK-203273	BF-2	2,7E+08	1,9E+06	1,3E+06
	EPC	2,7E+07	4,0E+05	8,6E+05
	RTG-2	2,7E+07	2,7E+04	1,3E+04
	FHM	8,6E+07	1,3E+06	8,6E+05
Ampoule IV: Blank	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: EHNV 86/8774	BF-2	1,9E+06	1,3E+05	8,6E+05
	EPC	2,7E+05	8,6E+04	5,9E+04
	RTG-2	5,9E+05	5,9E+04	5,9E+04
	FHM	2,7E+03	8,6E+02	4,0E+02

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 approx. 3 months after shipment (1 ampoule), (stored at 4°C).

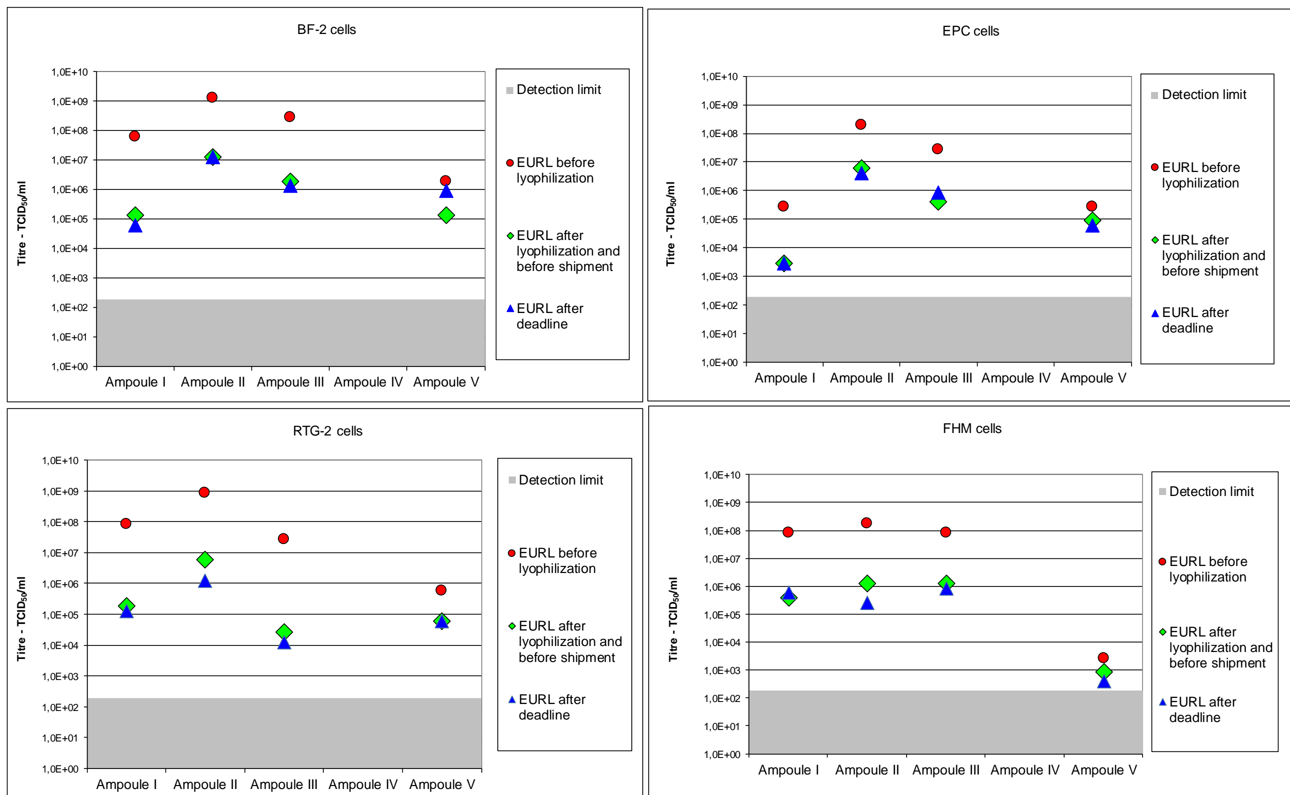


Figure 4. Virus titres in different cell lines: before lyophilisation, before shipment and after deadline for handling in results (storage 4°C in the dark).

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 were encouraged to identify the genotype of the virus isolates.

Table 3. Inter-Laboratory Proficiency Test, PT1, 2020 - Virus identification and score obtained by participants.

Laboratory code number	Score	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
		VHSV DK-9895174	IHNV 32/87 + IPNV Sp	SVCV DK-203273	Blank	EHNV 86/8774
1	10/10	VHSV	IPNV and IHNV	SVCV	Negative	EHNV
2	10/10	VHSV	IHNV & IPNV	SVCV	no virus detected	EHNV
3	10/10	VHSV	IHNV IPNV	SVCV	No Virus growth	EHNV
4[1)]	8/8	VHSV	IPNV + IHNV	SVC	NO CPE	NO IHNV,VHSV,SVCV,IPNV
5[2)]	9/10	VHSV	IHNV+IPNV	SVCV	no virus	Ranavirus
6	10/10	VHSV	IHNV + IPNV	SVCV	0	EHNV
7	10/10	VHSV	IHNV and IPNV	SVCV	Not VHSV, IHNV,EHNV, not Ranavirus, IPNV nor SVCV	EHNV
8	10/10	VHSV	IHNV, IPNV	SVCV	negative	EHNV
9	10/10	VHSV	IHNV and IPNV	SVCV	Not VHSV, not IHNV, not Ranavirus	Ranavirus, EHNV
10[1)]	8/8	VHSV	IHNV and IPNV	SVCV	negative	no VHSV; no IHNV;no IPNV, no SVCV
11	10/10	VHSV	IHNV + IPNV	SVCV	no virus detected	EHNV
12	10/10	VHSV	IHNV, IPNV	SVCV	No virus	EHNV
13	10/10	VHSV	IPNV+IHNV	SVCV	negative	EHNV
14	10/10	VHSV	IHNV and IPNV	SVCV	negative	EHNV
15	10/10	VHSV	IHNV & IPNV	SVCV	NO VHSV NO IHNV NO EHNV NO RANAVIRUS NO IPNV NO SVCV	EHNV
16	10/10	VHSV	IPNV and IHNV	SVCV	negativ	EHNV
17	10/10	VHSV genotype 1a	IPNV and IHNV	SVCV	0	EHNV
18	10/10	VHSV	IPNV and IHNV	SVCV	Blank	EHNV
19	10/10	VHSV	IHNV + IPNV	SVCV	NEG	EHNV
20	10/10	VHSV	IHNV, IPNV	SVCV	-	Ranavirus (EHNV)
21	10/10	VHSV	IHNV/IPNV	SVCV	0	Ranavirus
22	10/10	VHSV	IHNV,IPNV	SVCV	NEGATIVE	EHNV
23	10/10	VHSV	IHNV/ IPNV	SVCV	No virus detected	Ranavirus/EHNV
24	10/10	VHSV viable virus	IHNV + IPNV viable virus	SVCV viable virus	Negative	EHNV viable virus
25	10/10	VHSV	IHNV and IPNV	SVCV	No virus detected	EHNV

26	**10/10**	VHSV	IHNV, IPNV	SVCV	-	EHNV
27	**10/10**	VHSV	IHNV + IPNV	SVCV	-	EHNV
28 [1)]	**8/8**	VHSV	IHNV, IPNV	SVCV	Negative	Negative
29	**10/10**	VHSV	IHNV IPNV	SVCV	Negative	EHNV
30 [2)]	**9/10**	VHSV	IHNV, IPNV	SVCV	Not VHSV, Not IHNV, Not EHNV, Not IPNV, Not SVCV	EHNV
31	**10/10**	VHSV	IPNV+IHNV	SVCV	No virus	EHNV
32	**10/10**	VHSV	IHNV, IPNV	SVCV	-	EHNV
33	**10/10**	VHSV	IHNV, IPNV	SVCV	Negative/Not detected	EHNV
34	**10/10**	VHSV	IHNV, IPNV	SVCV	NEGATIVE	EHNV
35	**10/10**	VHSV	IHNV/IPNV	SVCV	-	EHNV
36	**10/10**	VHSV	IHNV + IPNV	SVCV	NO VIRUS	EHNV
37	**10/10**	VHSV	IPNV, IHNV	SVCV	no virus	EHNV
38	**10/10**	VHSV	IHNV & IPNV	SVCV	NO VHSV, IHNV,SVCV,IPNV,EHNV,PRV	EHNV
39	**10/10**	VHSV	IHNV & IPNV	SVCV	Negative	EHNV
41	**10/10**	VHSV	IPNV, IHNV	SVCV	-	Ranavirus was identified by conventional PCR and than REA was applied as given by OIE manuel to identified EHNV
42	**10/10**	VHSV	IHNV/IPNV	SVCV	Negative	EHNV
43	**10/10**	VHSV	IHNV, IPNV	SVCV	Negative	EHNV
44	**10/10**	VHSV	IHNV + IPNV	SVCV	Blank	EHNV
45	**10/10**	VHSV	IHNV & IPNV	SVCV	not VHSV, IHNV, EHNV, Ranavirus, IPNV, or SVCV	EHNV

1) Do not test for Ranavirus
2) Did not corroborate the findings in ampoule V by sequencing or REA

Table 4. Inter-Laboratory Proficiency Test, PT1, 2020 – Results of titration of **ampoule I**.

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

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

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

VHSV DK-9895174	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	41	16	12
Median titre	2,7E+04	2,7E+03	1,9E+04	4,0E+03
Maximum titre	5,9E+09	4,0E+06	2,7E+05	5,9E+05
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	8,6E+02
25% quartile titre	1,3E+04	1,1E+03	8,3E+03	1,3E+03
75% quartile titre	1,1E+05	1,3E+04	3,4E+04	2,1E+05

Table 5. Inter-Laboratory Proficiency Test, PT1, 2020 – Results of titration of **ampoule II**.

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IPNV and IHNV	8,6E+06	4,0E+06	5,9E+05	N/A
2	IHNV & IPNV	4,0E+07	5,9E+06	N/A	N/A
3	IHNV IPNV	8,6E+06	1,9E+07	1,3E+07	N/A
4	IPNV + IHNV	5,9E+06	1,9E+06	N/A	N/A
5	IHNV+IPNV	1,9E+08	1,9E+08	N/A	N/A
6	IHNV + IPNV	1,9E+07	1,3E+06	4,0E+05	4,0E+05
7	IHNV and IPNV	8,6E+07	1,9E+05	N/A	N/A
8	IHNV, IPNV	N/A	8,6E+07	4,0E+07	8,6E+07
9	IHNV and IPNV	1,9E+07	5,9E+06	1,3E+07	1,3E+06
10	IHNV and IPNV	5,9E+06	1,9E+06	N/A	N/A
11	IHNV + IPNV	N/A	N/A	N/A	N/A
12	IHNV, IPNV	2,7E+07	1,3E+06	N/A	N/A
13	IPNV+IHNV	5,9E+08	5,9E+07	1,3E+07	N/A
14	IHNV and IPNV	2,7E+07	8,6E+06	1,9E+05	N/A
15	IHNV & IPNV	5,9E+07	1,3E+06	N/A	N/A
16	IPNV and IHNV	8,6E+07	1,3E+07	N/A	N/A
17	IPNV and IHNV	4,0E+06	1,3E+06	N/A	N/A
18	IPNV and IHNV	5,9E+07	4,0E+06	N/A	N/A
19	IHNV + IPNV	4,0E+07	8,6E+06	N/A	N/A
20	IHNV, IPNV	8,6E+06	5,9E+06	1,3E+06	2,7E+05
21	IHNV/IPNV	1,9E+07	2,7E+07	N/A	N/A
22	IHNV,IPNV	N/A	2,7E+06	2,7E+06	N/A
23	IHNV/ IPNV	1,9E+06	5,9E+05	N/A	N/A
24	IHNV + IPNV viable virus	5,9E+06	8,6E+04	N/A	5,9E+04
25	IHNV and IPNV	4,0E+05	4,0E+05	N/A	N/A
26	IHNV, IPNV	1,9E+08	4,0E+08	5,9E+07	5,9E+07
27	IHNV + IPNV	N/A	N/A	N/A	N/A
28	IHNV, IPNV	N/A	N/A	N/A	N/A
29	IHNV IPNV	1,9E+06	1,3E+06	N/A	N/A
30	IHNV, IPNV	8,6E+05	5,9E+05	1,3E+07	N/A
31	IPNV+IHNV	1,9E+04	2,7E+04	N/A	N/A
32	IHNV, IPNV	8,6E+06	5,9E+06	2,7E+06	2,7E+05
33	IHNV, IPNV	5,9E+09	5,9E+09	N/A	N/A
34	IHNV, IPNV	2,7E+06	1,3E+06	2,7E+04	1,9E+06
35	IHNV/IPNV	1,9E+07	5,9E+06	N/A	N/A
36	IHNV + IPNV	1,9E+07	1,3E+07	5,9E+06	8,6E+06
37	IPNV, IHNV	1,3E+07	N/A	N/A	5,9E+06
38	IHNV & IPNV	8,6E+06	5,9E+06	N/A	N/A
39	IHNV & IPNV	4,0E+07	N/A	N/A	4,0E+06
41	IPNV, IHNV	8,6E+06	1,9E+06	N/A	N/A
42	IHNV/IPNV	4,0E+07	5,9E+06	N/A	N/A

43	IHNV, IPNV	8,6E+04	4,0E+05	N/A	N/A
44	IHNV + IPNV	2,7E+06	5,9E+06	N/A	8,6E+06
45	IHNV & IPNV	8,6E+06	8,6E+06	1,3E+05	N/A

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

IHNV 32/87 + IPNV Sp	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	41	16	13
Median titre	1,3E+07	5,9E+06	2,7E+06	2,9E+06
Maximum titre	5,9E+09	5,9E+09	5,9E+07	8,6E+07
Minimum titre	1,9E+04	2,7E+04	2,7E+04	5,9E+04
25% quartile titre	5,9E+06	1,3E+06	4,9E+05	3,7E+05
75% quartile titre	4,0E+07	8,6E+06	1,3E+07	8,6E+06

Table 6. Inter-Laboratory Proficiency Test, PT1, 2020 – Results of titration of **ampoule III**.

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	SVCV	5,9E+05	5,9E+04	<1,9E+02	N/A
2	SVCV	1,9E+06	2,7E+05	N/A	N/A
3	SVCV	<1,9E+02	1,9E+04	<1,9E+02	N/A
4	SVC	1,86E+05	2,73E+05	N/A	N/A
5	SVCV	1,9E+04	8,6E+05	N/A	N/A
6	SVCV	1,9E+03	2,7E+05	<1,9E+02	5,9E+05
7	SVCV	5,9E+05	1,9E+06	N/A	N/A
8	SVCV	N/A	5,9E+04	5,9E+04	8,6E+04
9	SVCV	5,9E+06	1,3E+06	5,9E+04	5,9E+05
10	SVCV	1,3E+05	1,9E+06	N/A	N/A
11	SVCV	N/A	N/A	N/A	N/A
12	SVCV	2,7E+05	8,6E+05	N/A	N/A
13	SVCV	1,9E+05	5,9E+05	2,7E+05	N/A
14	SVCV	4,0E+04	1,9E+04	<1,9E+02	N/A
15	SVCV	1,3E+05	2,7E+05	N/A	N/A
16	SVCV	5,9E+02	5,9E+03	N/A	N/A
17	SVCV	1,3E+05	4,0E+04	N/A	N/A
18	SVCV	1,3E+06	8,6E+05	N/A	N/A
19	SVCV	5,9E+05	1,3E+05	N/A	N/A
20	SVCV	1,3E+03	8,6E+05	1,3E+03	8,6E+05
21	SVCV	1,9E+05	5,9E+04	N/A	N/A
22	SVCV	N/A	5,9E+04	<1,9E+02	N/A
23	SVCV	5,9E+04	4,0E+05	N/A	N/A
24	SVCV viable virus	N/A	1,86E+05	N/A	2,7E+05
25	SVCV	8,6E+03	1,3E+05	N/A	N/A
26	SVCV	5,9E+05	4,0E+06	4,0E+06	4,0E+06
27	SVCV	N/A	N/A	N/A	N/A
28	SVCV	N/A	N/A	N/A	N/A
29	SVCV	1,3E+04	<1,9E+02	N/A	N/A
30	SVCV	<1,9E+02	<1,9E+02	<1,9E+02	N/A
31	SVCV	1,3E+05	1,3E+05	N/A	N/A
32	SVCV	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
33	SVCV	1,3E+03	1,9E+05	N/A	N/A
34	SVCV	8,62E+03	4,00E+04	<1,9E+02	N/A
35	SVCV	1,26E+05	2,73E+03	N/A	1,26E+04

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

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

SVCV DK-203273	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	41	41	16	13
Median titre	7,2E+04	1,3E+05	<1,9E+02	1,1E+05
Maximum titre	5,9E+06	4,0E+06	4,0E+06	4,0E+06
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	2,7E+03	4,0E+04	<1,9E+02	3,3E+04
75% quartile titre	1,9E+05	4,9E+05	5,9E+04	5,9E+05

Table 7. Inter-Laboratory Proficiency Test, PT1, 2020 – Results of titration of **ampoule IV**.

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	Negative	<1,9E+02	<1,9E+02	<1,9E+02	N/A
2	no virus detected	<1,9E+02	<1,9E+02	N/A	N/A
3	No Virus growth	<1,9E+02	<1,9E+02	<1,9E+02	N/A
4	NO CPE	<1,9E+02	<1,9E+02	N/A	N/A
5	no virus	<1,9E+02	<1,9E+02	N/A	N/A
6	0	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
7	Not VHSV, IHNV,EHNV, not Ranavirus, IPNV nor SVCV	<1,9E+02	<1,9E+02	N/A	N/A
8	negative	N/A	<1,9E+02	<1,9E+02	<1,9E+02
9	Not VHSV, not IHNV, not Ranavirus	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
10	negative	<1,9E+02	<1,9E+02	N/A	N/A
11	no virus detected	N/A	N/A	N/A	N/A
12	No virus	<1,9E+02	<1,9E+02	N/A	N/A
13	negative	<1,9E+02	<1,9E+02	<1,9E+02	N/A
14	negative	<1,9E+02	<1,9E+02	<1,9E+02	N/A
15	NO VHSV, NO IHNV, NO EHNV, NO RANAVIRUS, NO IPNV, NO SVCV	1,3E+03	<1,9E+02	N/A	N/A
16	negativ	<1,9E+02	<1,9E+02	N/A	N/A
17	0	<1,9E+02	<1,9E+02	N/A	N/A
18	Blank	<1,9E+02	<1,9E+02	N/A	N/A
19	NEG	<1,9E+02	<1,9E+02	N/A	N/A
20	-	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
21	0	<1,9E+02	<1,9E+02	N/A	N/A
22	NEGATIVE	N/A	<1,9E+02	<1,9E+02	N/A
23	No virus detected	<1,9E+02	<1,9E+02	N/A	N/A
24	Negative	N/A	N/A	N/A	N/A
25	No virus detected	<1,9E+02	<1,9E+02	N/A	N/A
26	-	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
27	-	N/A	N/A	N/A	N/A
28	Negative	N/A	N/A	N/A	N/A

29	Negative	<1,9E+02	<1,9E+02	N/A	N/A
30	Not VHSV, Not IHNV, Not EHNV, Not IPNV, Not SVCV	<1,9E+02	<1,9E+02	<1,9E+02	N/A
31	No virus	<1,9E+02	<1,9E+02	N/A	N/A
32	-	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
33	Negative/Not detected	<1,9E+02	<1,9E+02	N/A	N/A
34	NEGATIVE	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
35	-	<1,9E+02	<1,9E+02	N/A	N/A
36	NO VIRUS	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
37	no virus	<1,9E+02	N/A	N/A	<1,9E+02
38	NO VHSV, IHNV,SVCV,IPNV,EHNV,PRV	<1,9E+02	<1,9E+02	N/A	N/A
39	Negative	<1,9E+02	N/A	N/A	<1,9E+02
41	-	<1,9E+02	<1,9E+02	N/A	N/A
42	Negative	<1,9E+02	<1,9E+02	N/A	N/A
43	Negative	<1,9E+02	<1,9E+02	N/A	N/A
44	Blank	<1,9E+02	<1,9E+02	N/A	<1,9E+02
45	not VHSV, IHNV, EHNV, Ranavirus, IPNV, or SVCV	<1,9E+02	<1,9E+02	<1,9E+02	N/A

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

Supernatant from BF-2 cells	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	41	40	16	12
Median titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
Maximum titre	1,3E+03	<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, 2020 – Results of titration of **ampoule V**.

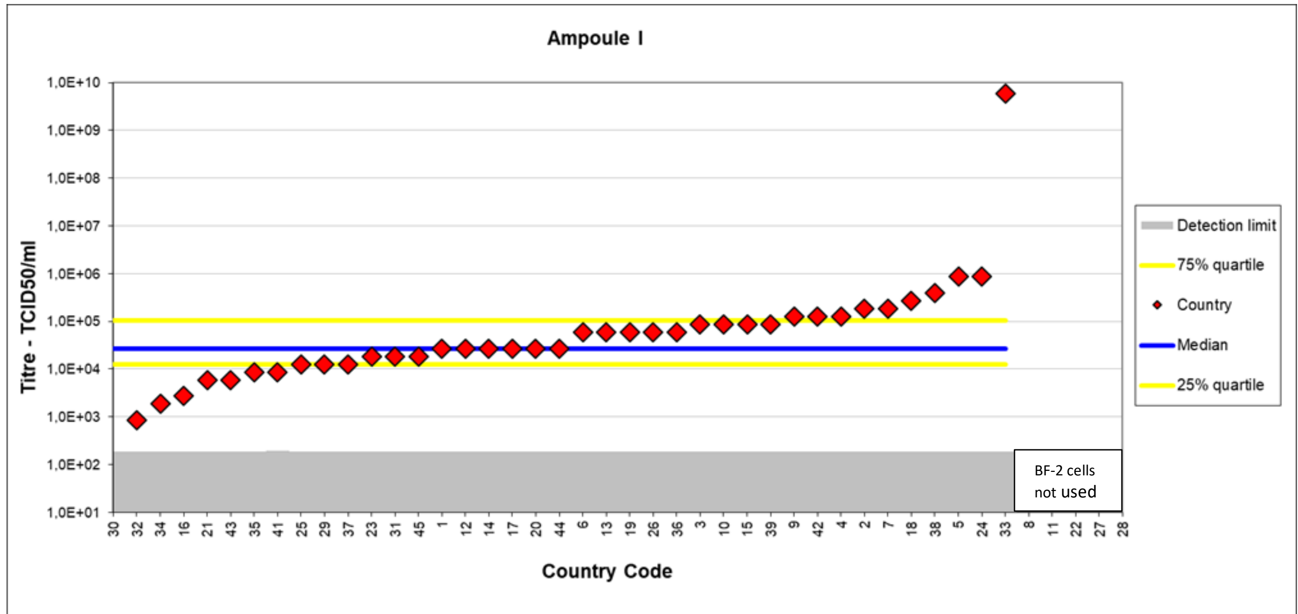
Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	EHNV	1,3E+05	1,9E+05	1,9E+03	N/A
2	EHNV	1,3E+06	2,7E+05	N/A	N/A
3	EHNV	4,0E+05	4,0E+05	1,9E+05	N/A
4	NO IHNV,VHSV,SVCV,IPNV	5,9E+05	4,0E+05	N/A	N/A
5	Ranavirus	1,9E+04	2,7E+04	N/A	N/A
6	EHNV	1,9E+05	1,3E+05	8,6E+02	8,6E+03
7	EHNV	1,9E+05	2,7E+04	N/A	N/A
8	EHNV	N/A	5,9E+04	8,6E+03	4,0E+04
9	Ranavirus, EHNV	8,6E+05	5,9E+04	8,6E+04	1,3E+03
10	no VHSV; no IHNV;no IPNV, no SVCV	1,9E+03	8,6E+04	N/A	N/A
11	EHNV	N/A	N/A	N/A	N/A
12	EHNV	8,6E+04	5,9E+03	N/A	N/A
13	EHNV	5,9E+05	2,7E+04	2,7E+04	N/A
14	EHNV	1,9E+04	< 1,9E+02	< 1,9E+02	N/A
15	EHNV	8,6E+04	5,9E+04	N/A	N/A
16	EHNV	8,6E+03	5,9E+03	N/A	N/A
17	EHNV	2,7E+03	8,6E+03	N/A	N/A
18	EHNV	8,6E+05	8,6E+03	N/A	N/A
19	EHNV	8,6E+05	5,9E+05	N/A	N/A
20	Ranavirus (EHNV)	4,0E+06	4,0E+05	5,9E+05	4,0E+05
21	Ranavirus	5,9E+03	1,3E+03	N/A	N/A
22	EHNV	N/A	5,9E+03	< 1,9E+02	N/A
23	Ranavirus/EHNV	1,9E+03	1,9E+03	N/A	N/A
24	EHNV viable virus	4,0E+05	4,0E+04	N/A	N/A
25	EHNV	1,9E+04	1,3E+04	N/A	N/A
26	EHNV	1,3E+05	8,6E+04	4,0E+06	1,9E+05
27	EHNV	N/A	N/A	N/A	N/A
28	Negative	N/A	N/A	N/A	N/A
29	EHNV	1,3E+04	4,0E+03	N/A	N/A
30	EHNV	1,9E+05	4,0E+05	2,7E+06	N/A
31	EHNV	1,9E+05	2,7E+05	N/A	N/A
32	EHNV	1,9E+04	5,9E+03	1,9E+04	5,9E+03
33	EHNV	8,6E+05	4,0E+05	N/A	N/A
34	EHNV	5,9E+03	1,3E+03	< 1,9E+02	1,3E+03

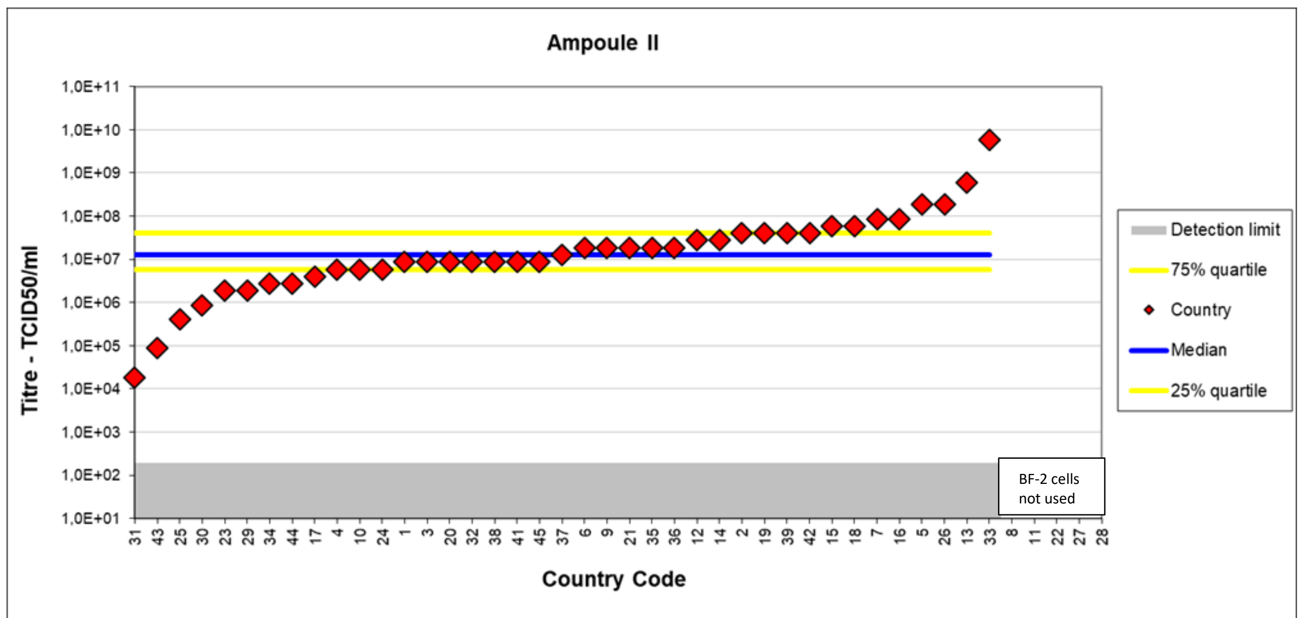
35	EHNV	1,3E+05	2,7E+04	N/A	N/A
36	EHNV	1,3E+05	2,7E+05	4,0E+05	8,6E+04
37	EHNV	1,9E+05	N/A	N/A	1,9E+03
38	EHNV	1,3E+06	1,9E+05	N/A	N/A
39	EHNV	2,7E+05	N/A	N/A	2,7E+02
41	Ranavirus was identified by conventional PCR and than REA was applied as given by OIE manuel to identified EHNV	1,3E+05	1,3E+05	N/A	N/A
42	EHNV	1,3E+06	4,0E+05	N/A	N/A
43	EHNV	1,9E+05	1,3E+05	N/A	N/A
44	EHNV	2,7E+05	1,3E+05	N/A	1,9E+04
45	EHNV	1,3E+06	8,6E+04	1,9E+05	N/A

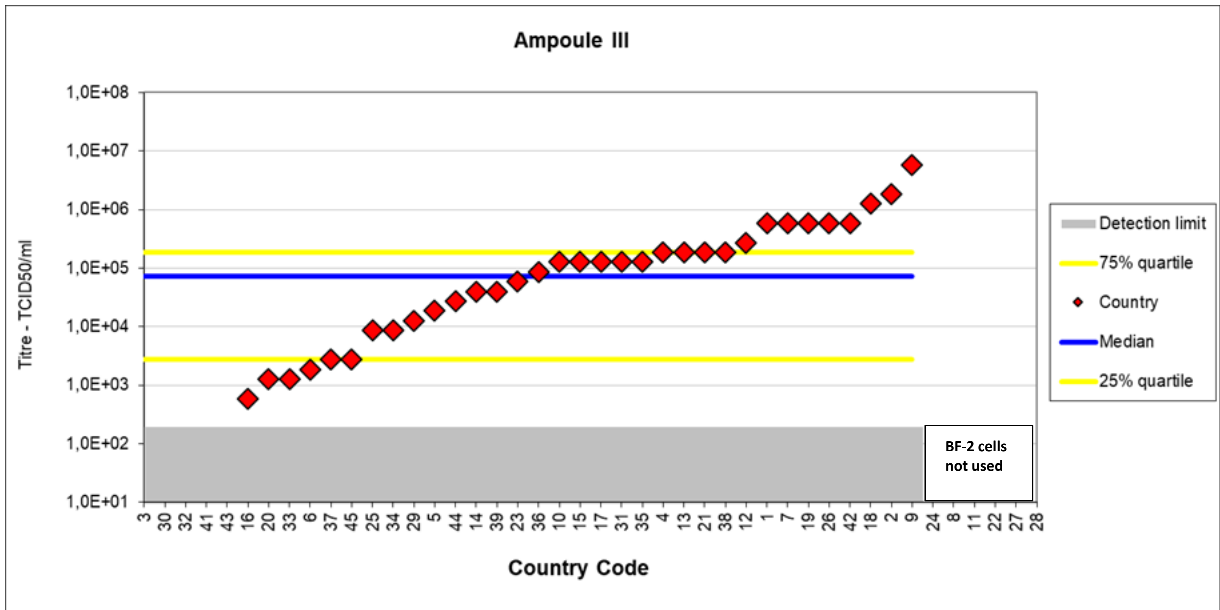
N/A: Cell line not applied by the participating laboratory for titration of the virus

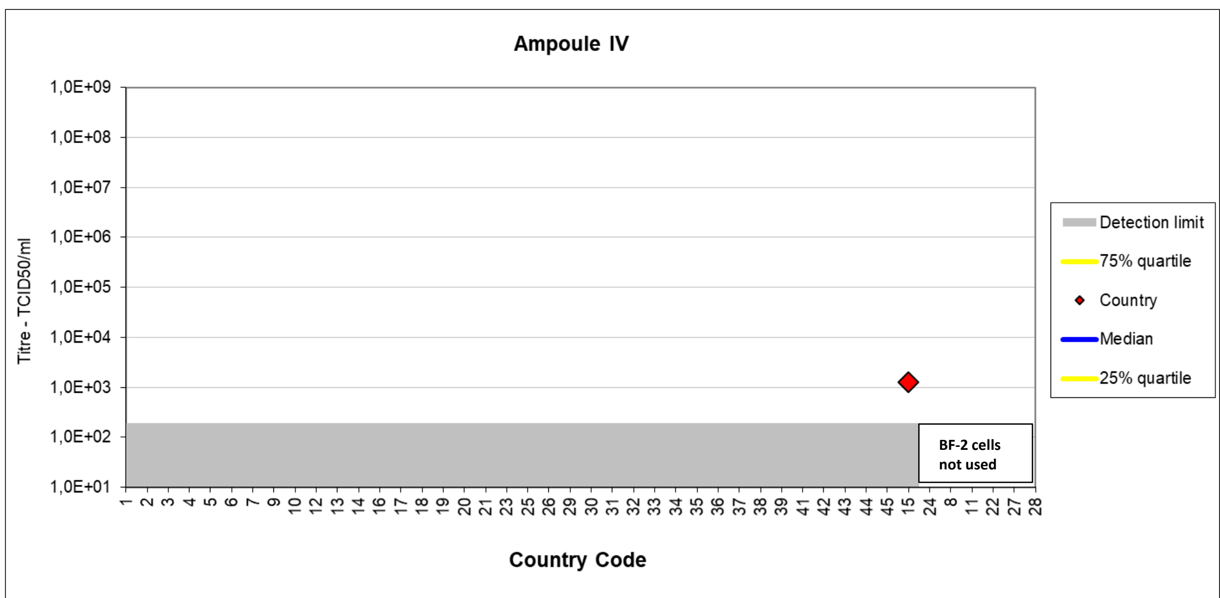
EHNV 86/8774	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	41	16	12
Median titre	1,9E+05	5,9E+04	2,7E+04	8,6E+03
Maximum titre	4,0E+06	5,9E+05	4,0E+06	4,0E+05
Minimum titre	1,9E+03	<1,9E+02	<1,9E+02	2,7E+02
25% quartile titre	1,9E+04	8,6E+03	1,4E+03	1,6E+03
75% quartile titre	5,9E+05	2,3E+05	2,9E+05	6,3E+04

Figure 5. Virus titres obtained in BF-2 cells. The titre (red diamond) of each participating laboratory (country code on the x axis) using BF-2 cells illustrated for ampoule I, II, III, IV and V. The detection limit (grey shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line) are plotted on all graphs. Participants failing to obtain any titre are listed on the x axis under the grey zone but no red diamond is plotted; participants who did not use a specific cell line are listed under the white text box.






Ampoule III
Titre - TCID50/ml
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
Detection limit
75% quartile
Country
Median
25% quartile
BF-2 cells not used
3 30 32 41 43 16 20 33 6 37 45 25 34 29 5 44 14 39 23 36 10 15 17 31 35 4 13 21 38 12 1 7 19 26 42 18 2 9 24 8 11 22 27 28
Country Code


Ampoule IV
Titre - TCID50/ml
1,0E+09
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
Detection limit
75% quartile
Country
Median
25% quartile
BF-2 cells not used
1 2 3 4 5 6 7 9 10 12 13 14 16 17 18 19 20 21 23 25 26 29 30 31 32 33 34 35 36 37 38 39 41 42 43 44 45 15 24 8 11 22 27 28
Country Code

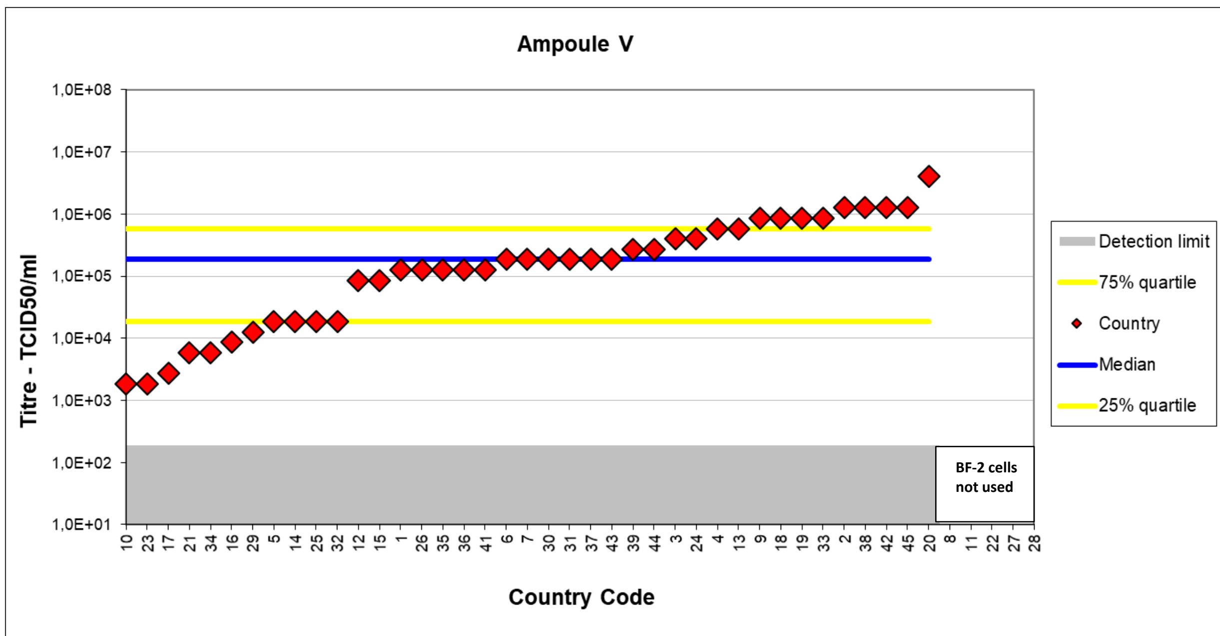
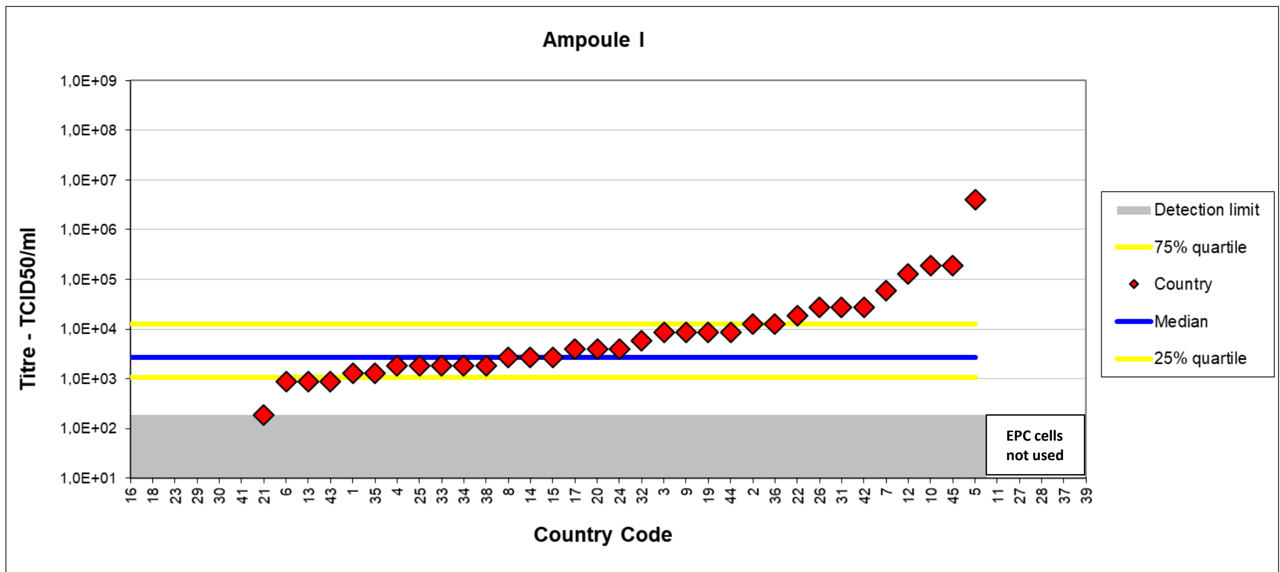
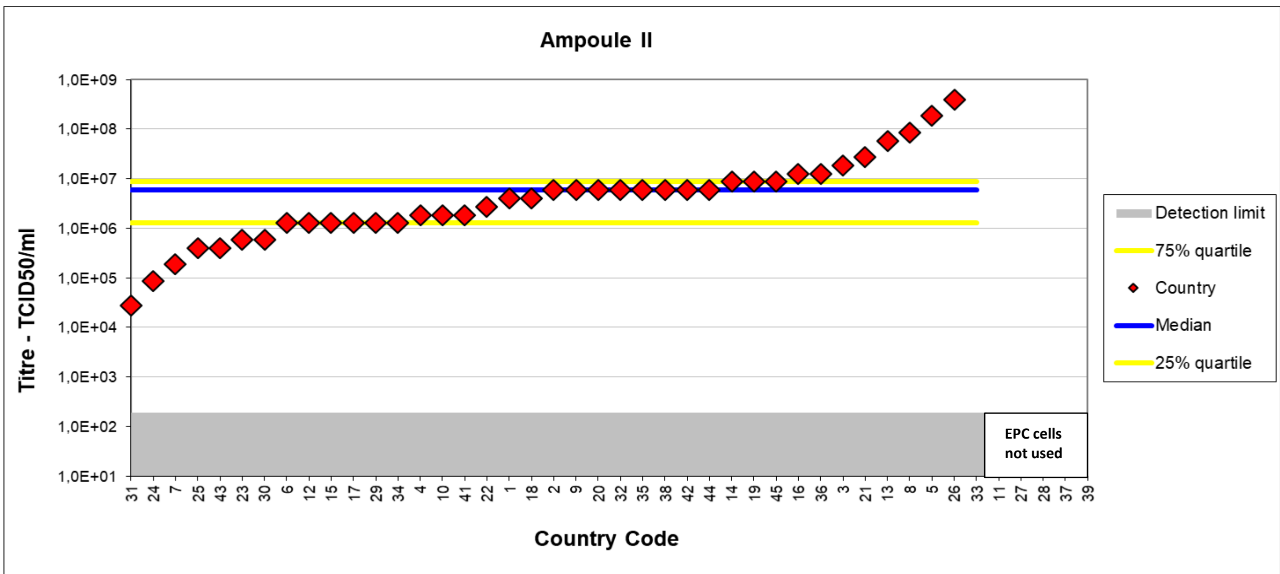
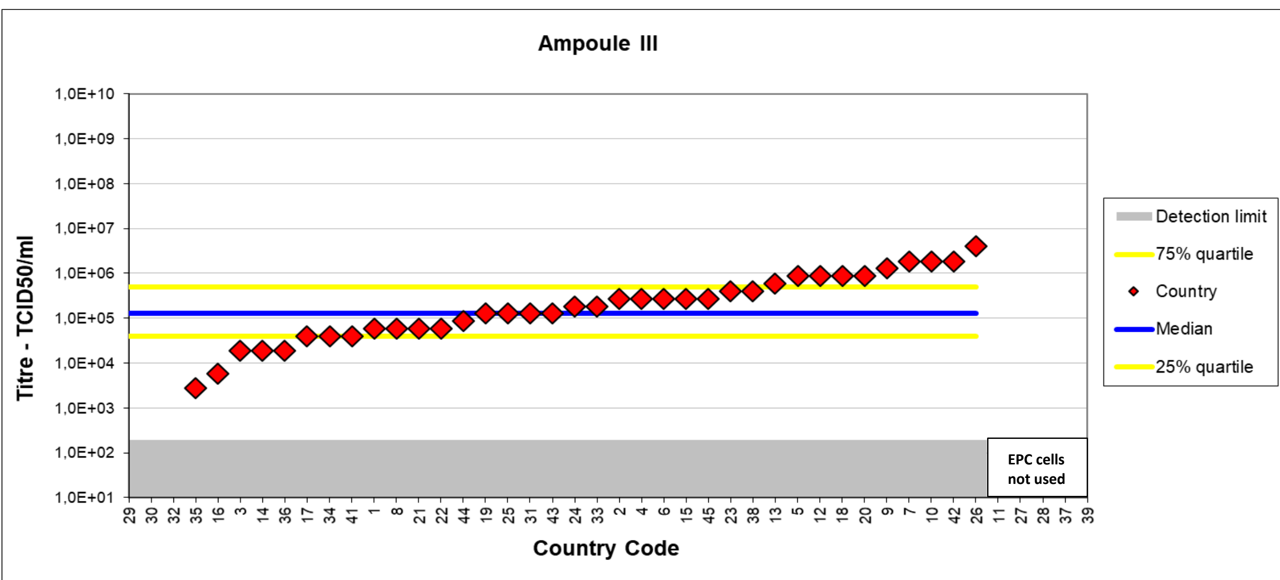

Ampoule V
Titre - TCID50/ml
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
Detection limit
75% quartile
Country
Median
25% quartile
BF-2 cells not used
10 23 17 21 34 16 29 5 14 25 32 12 15 1 26 35 36 41 6 7 30 31 37 43 39 44 3 24 4 13 9 18 19 33 2 38 42 45 20 8 11 22 27 28
Country Code

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







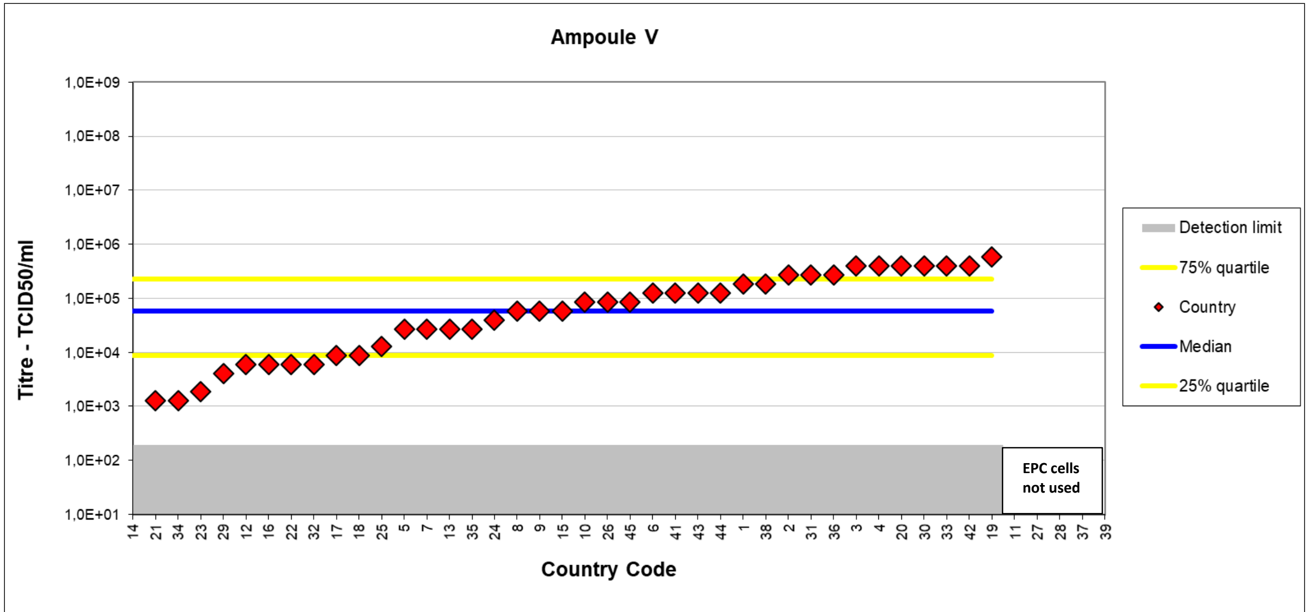
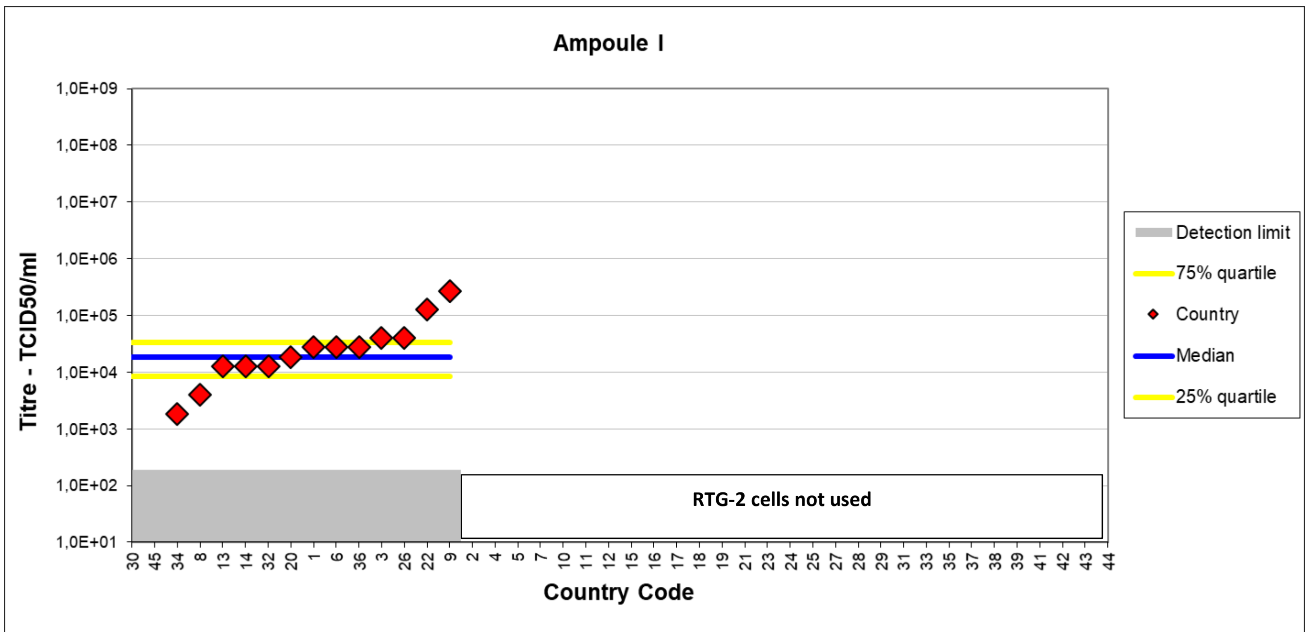
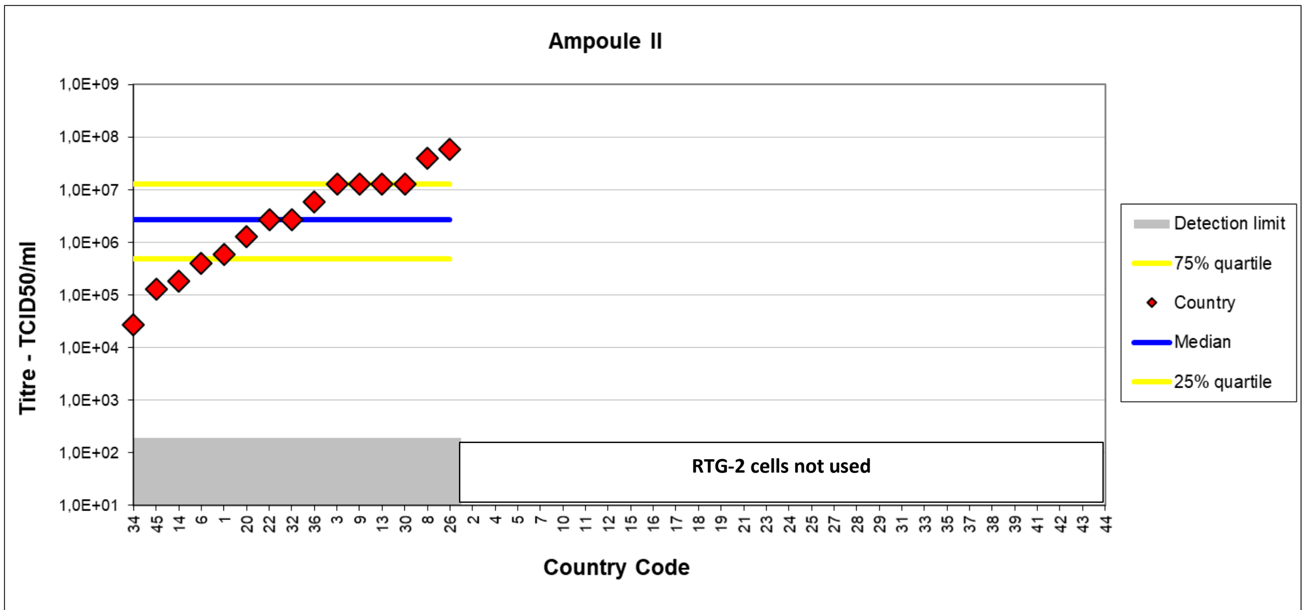
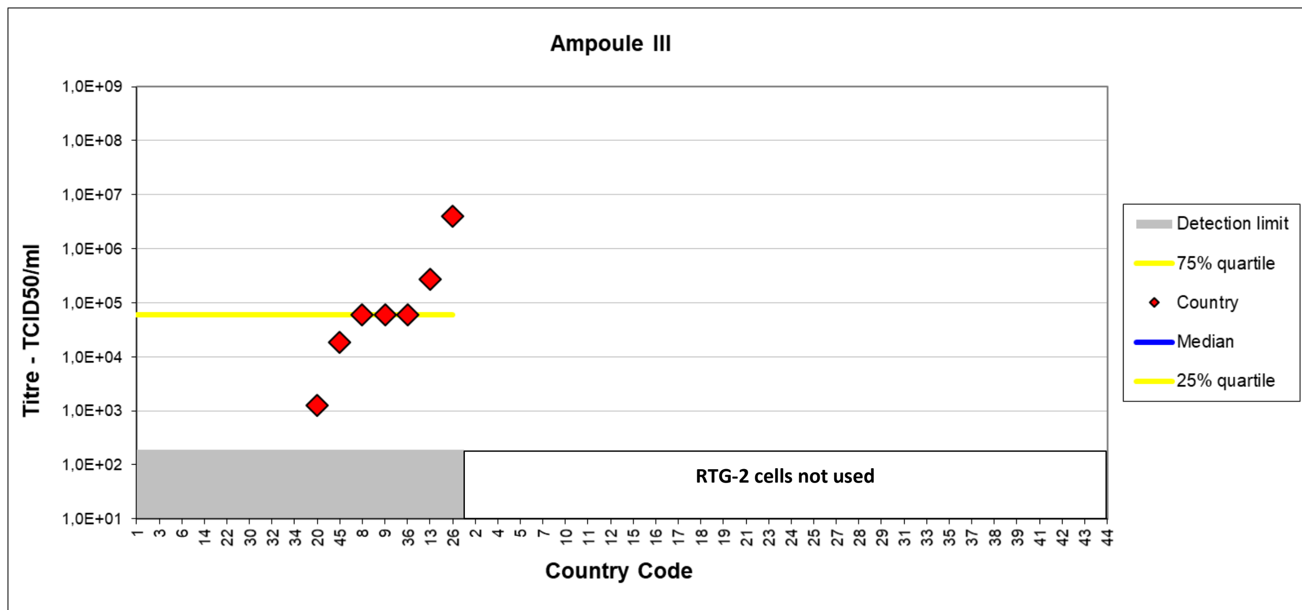


Figure 7. Virus titre obtained in RTG-2 cells. For further details see description at Figure 5




Ampoule II
Titre - TCID50/ml
1,0E+09
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
RTG-2 cells not used
34 45 14 6 1 20 22 32 36 3 9 13 30 8 26 2 4 5 7 10 11 12 15 16 17 18 19 21 23 24 25 27 28 29 31 33 35 37 38 39 41 42 43 44
Country Code
Detection limit
75% quartile
Country
Median
25% quartile


Ampoule III
Titre - TCID50/ml
1,0E+09
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
RTG-2 cells not used
1 3 6 14 22 30 32 34 20 45 8 9 36 13 26 2 4 5 7 10 11 12 15 16 17 18 19 21 23 24 25 27 28 29 31 33 35 37 38 39 41 42 43 44
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

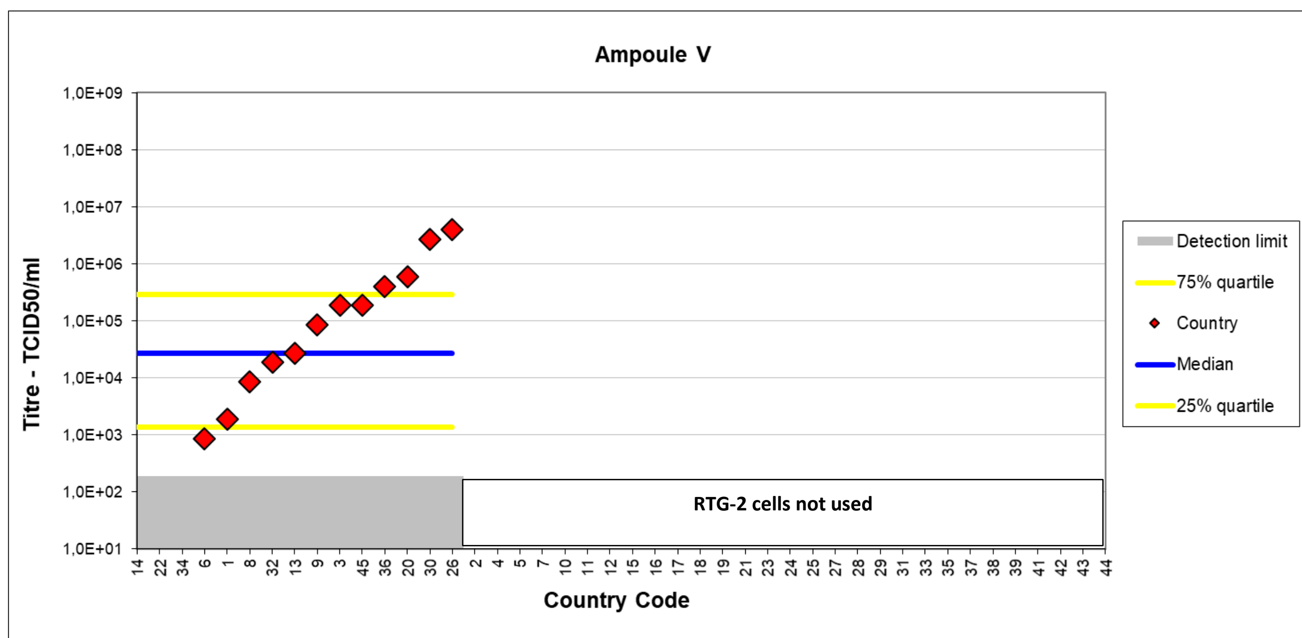
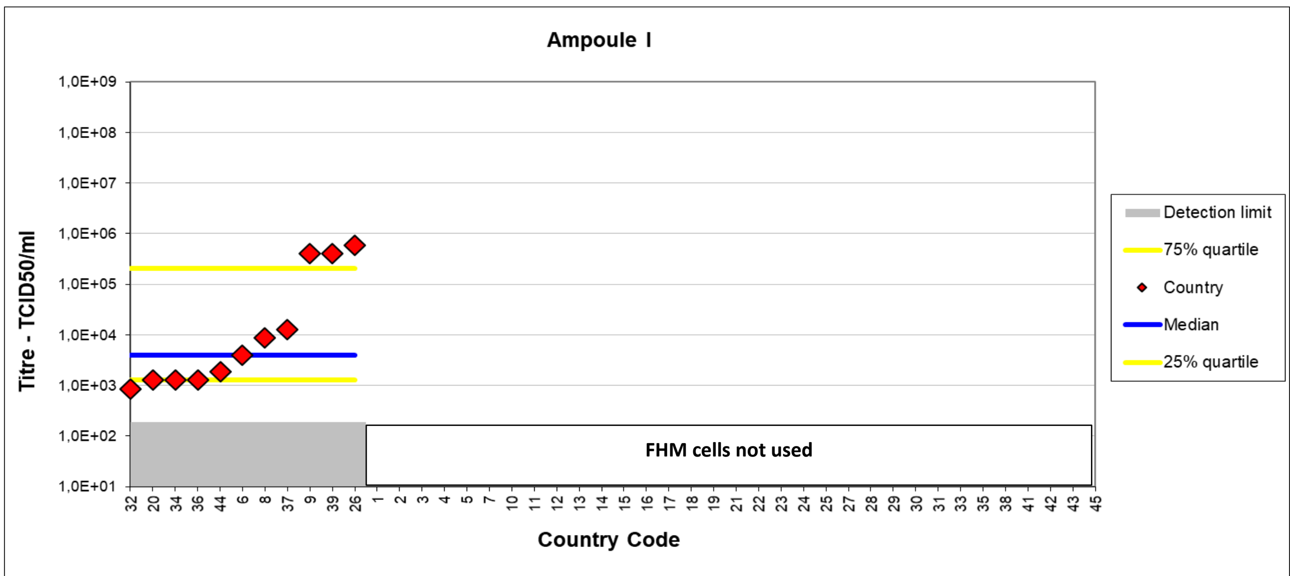
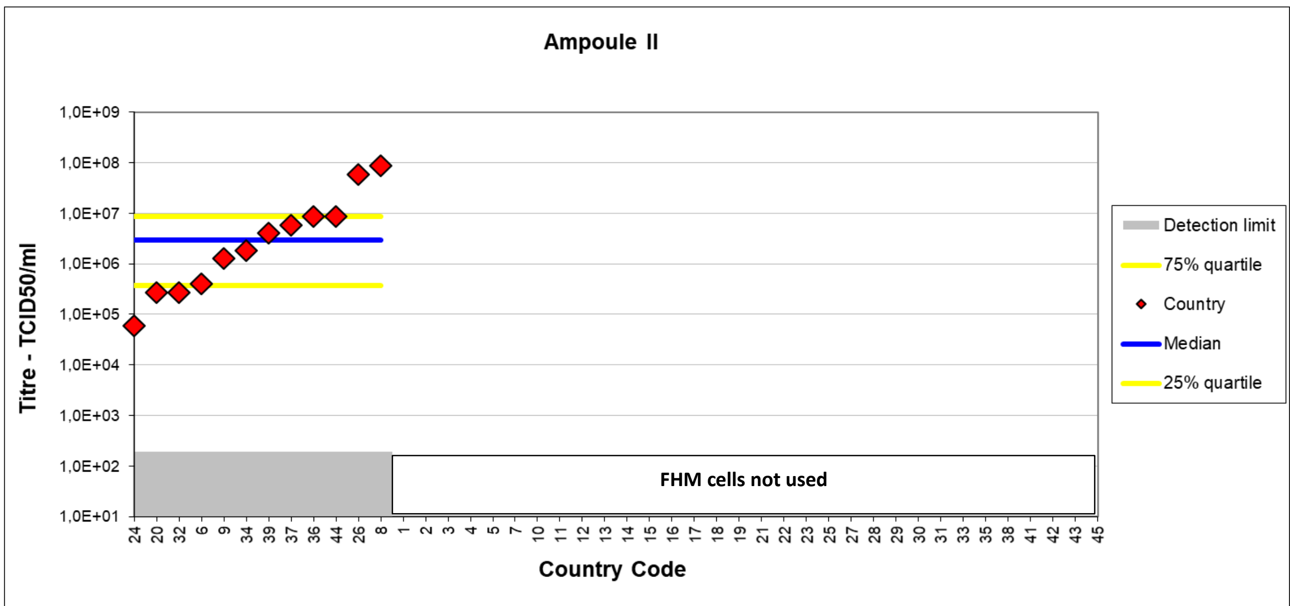
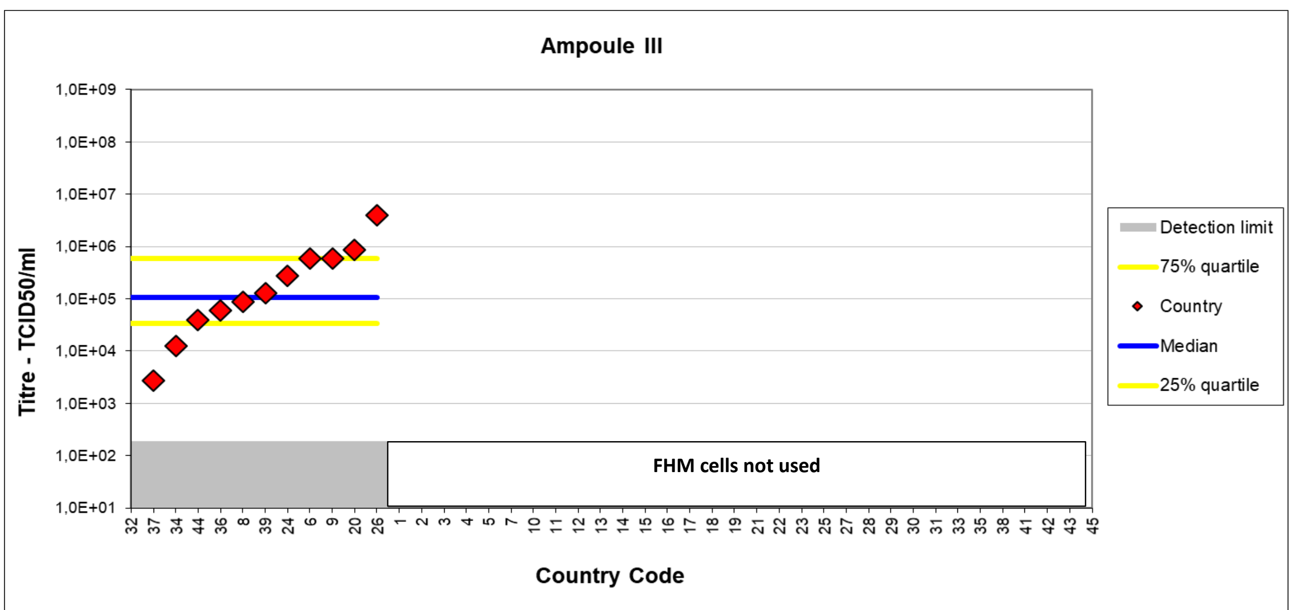
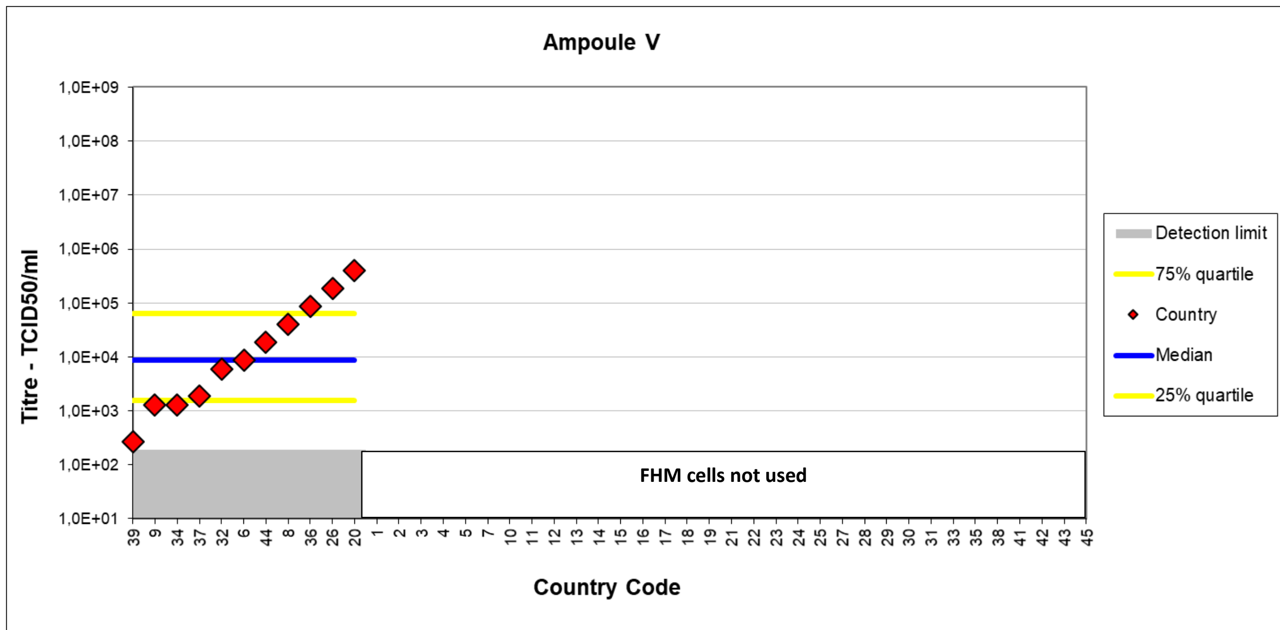

Ampoule V
Titre - TCID50/ml
1,0E+09
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
RTG-2 cells not used
14 22 34 6 1 8 32 13 9 3 45 36 20 30 26 2 4 5 7 10 11 12 15 16 17 18 19 21 23 24 25 27 28 29 31 33 35 37 38 39 41 42 43 44
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

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









Identification of content

- 41 laboratories out of 44 participants analysed for all viruses; 39 of these laboratories correctly identified all viruses in all ampoules.
- 3 laboratories out of 44 participants did not test for Rana; all three laboratories correctly identified all other viruses in the ampoules.

Ampoule I – VHSV (DK-9895174)

- All 44 laboratories correctly identified VHSV in ampoule I.

Ampoule II – IHNV (32/87) + IPNV (Sp)

- All 44 laboratories correctly identified both IHNV and IPNV in ampoule II.

Ampoule III – SVCV (DK-203273)

- All 44 laboratories correctly identified SVCV in ampoule III.

Ampoule IV – Blank (Cell supernatant from BF-2 cells)

- All 44 laboratories correctly did not isolate any virus in ampoule IV.

Ampoule V – EHNV (86/8774)

- 39 laboratories correctly identified the isolate as the listed EHNV in ampoule V by sequencing or REA. One of the laboratory answered Ranavirus even though the sequence showed EHNV.
- One laboratory identified Ranavirus but did not specify if the isolate was the listed EHNV or not by sequencing or REA. One laboratory reported the use of pathogen specific qPCR kit, but documentation for validation of the kit has not been made accessible.
- 3 laboratories do not test for Ranavirus

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: VHSV identification was given the score 2.
- Ampoule II: IHNV and IPNV identification was given the score 2, identification of only IHNV would have given 1 point and identification of IPNV only would have given 0 points to this ampoule.
- Ampoule III: SVCV identification was given the score 2.
- Ampoule IV: Identification “No virus”, “Blank”, “Not IHNV, not VHSV, not IPNV, not SVCV, not EHNV” or similar answer was given the score 2
- Ampoule V: EHNV identification was given the score 2. No identification of the Ranavirus by sequencing or REA was given the score 1.

Out of 44 laboratories participating in the PT 1 2020, 39 obtained score 10/10. The score 8/8 was assigned to 3 participants as they did not test Ranavirus. Two laboratories scored below 100% due to no identification by either sequencing or REA of the Ranavirus.

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
- 39 laboratories used EPC cells
- 15 laboratories used RTG-2 cells
- 12 laboratories used FHM cells
- 9 laboratories used CHSE-214 cells
- 3 laboratories 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 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 (VHSV, DK-9895174) replicates on all four cell lines however it grows a little less efficiently on EPC and FHM but within one log difference.
- Ampoule II (IHNV 32/87 + IPNV Sp) replicate equally on all four cell lines (BF-2 cells, EPC, FHM and RTG-2).
- Ampoule III (SVCV, DK-203273) replicates equally on BF-2 cells, EPC and FHM but it does not replicate on RTG-2.
- Ampoule IV (Non infected BF-2 cell supernatant) does not replicates on any of the four cell lines.
- Ampoule V (EHNV, 86/8774) replicates on all four cell lines, however it grows best on BF-2 cells, a less efficiently on FHM.

As from Table 3-8 the variations in titres between laboratories was high – with more than 6 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 ones 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.

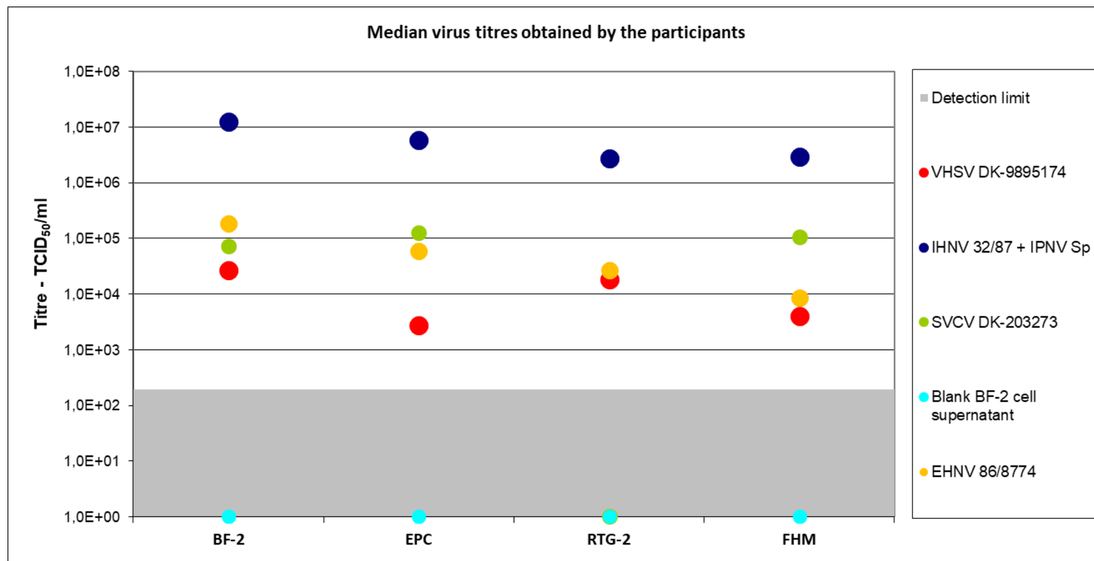


Figure 9. Median virus titres obtained by the participants in 4 different cell lines.

Ct. values

This year we have encouraged participants to insert the Ct value in the spreadsheet if they have performed a real-time (RT-) PCR.
The Ct values obtained by the participating laboratories are summarised in tables 9. The Ct. values obtained from each participating laboratory are represented graphically in Figures 9. On these figures, the median value and the 25% and 75% inter-quartile range is displayed.

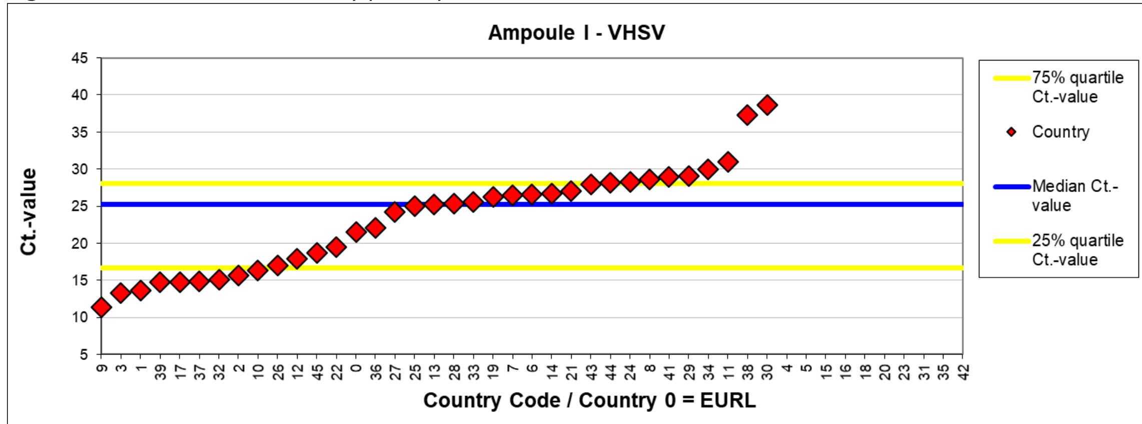
The Ct values cannot be directly compered due to the use of different methods, reagents and equipment for nucleic acid extraction and (RT)-qPCR. Additionally, some participants may have tested directly from the ampoule provided whereas others used cell supernatant from inoculation on cell culture.

Table 9. Inter-Laboratory Proficiency Test, PT1, 2020 – Ct.-values.

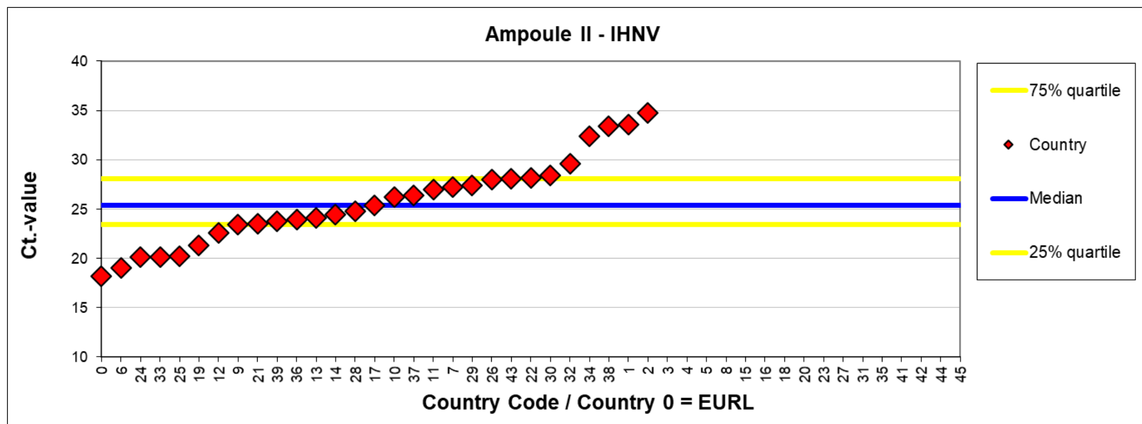
Laboratory Code number	Ct. value Ampoule I (VHSV)	Ct. value Ampoule II (IHNV)	Ct. value Ampoule II (IPNV)	Ct. value Ampoule III (SVCV)	Ct. value Ampoule V (EHNV)
0	21,5	18,2	21,1	N/A	25,90
1	13,6	33,6	17,9	13,3	15,5
2	15,7	34,8	N/A	12,7	N/A
3	13,3	N/A	N/A	N/A	N/A
4	N/A	N/A	N/A	N/A	N/A
5	N/A	N/A	N/A	N/A	N/A
6	26,6	19,0	25,6	24,0	N/A
7	26,5	27,2	N/A	N/A	N/A
8	28,7	N/A	N/A	N/A	N/A
9	11,3	23,4	15,5	N/A	N/A
10	16,4	26,2	N/A	N/A	N/A
11	31,0	27,0	21,0	N/A	N/A
12	17,9	22,6	21,2	N/A	N/A
13	25,3	24,1	23,2	N/A	N/A
14	26,7	24,5	27,8	N/A	N/A
15	N/A	N/A	N/A	N/A	N/A
16	N/A	N/A	N/A	N/A	N/A
17	14,8	25,4	17,3	N/A	12,6
18	N/A	N/A	N/A	N/A	N/A
19	26,3	21,3	24,5	N/A	N/A
20	N/A	N/A	N/A	N/A	N/A
21	27,0	23,5	N/A	N/A	N/A
22	19,5	28,1	N/A	31,3	12,3
23	N/A	N/A	N/A	N/A	N/A
24	28,3	20,1	28,5	28,4	26,2
25	25,0	20,2	17,2	N/A	N/A
26	17,0	28,0	N/A	N/A	N/A
27	24,2	N/A	N/A	N/A	N/A
28	25,3	24,8	N/A	20,4	N/A
29	29,0	27,4	N/A	26,5	N/A
30	38,7	28,4	N/A	25,4	30,2
31	N/A	N/A	N/A	N/A	N/A
32	15,1	29,6	N/A	N/A	N/A
33	25,6	20,1	N/A	N/A	N/A
34	30,0	32,4	N/A	N/A	N/A
35	N/A	N/A	N/A	N/A	N/A
36	22,1	23,9	N/A	N/A	N/A
37	14,9	26,4	11,9	N/A	11,2
38	37,3	33,4	29,9	N/A	N/A
39	14,8	23,8	5,8	13,3	N/A
41	29,0	N/A	N/A	N/A	N/A
42	N/A	N/A	N/A	N/A	N/A
43	28,0	28,1	27,6	N/A	N/A
44	28,2	N/A	N/A	N/A	N/A
45	18,7	N/A	N/A	N/A	N/A

N/A: No Ct-value given by the participating laboratory.

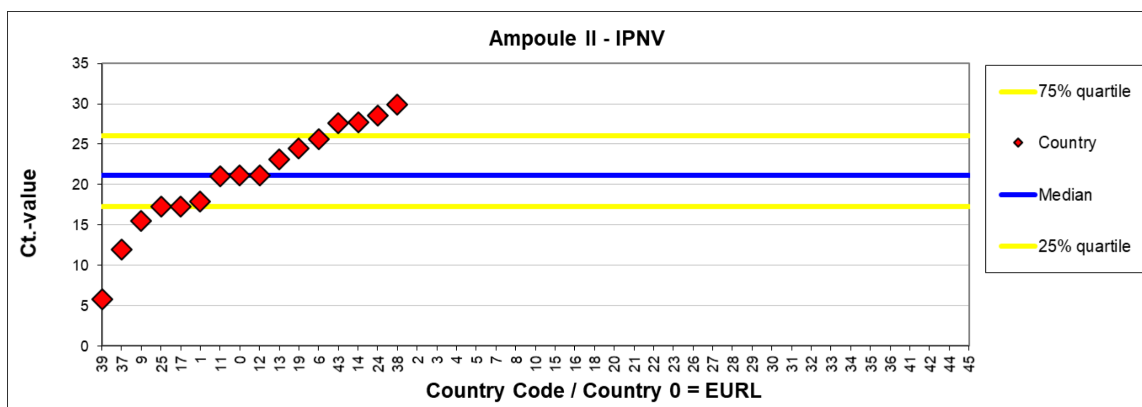
Figure 10. Ct. values obtained by participants.



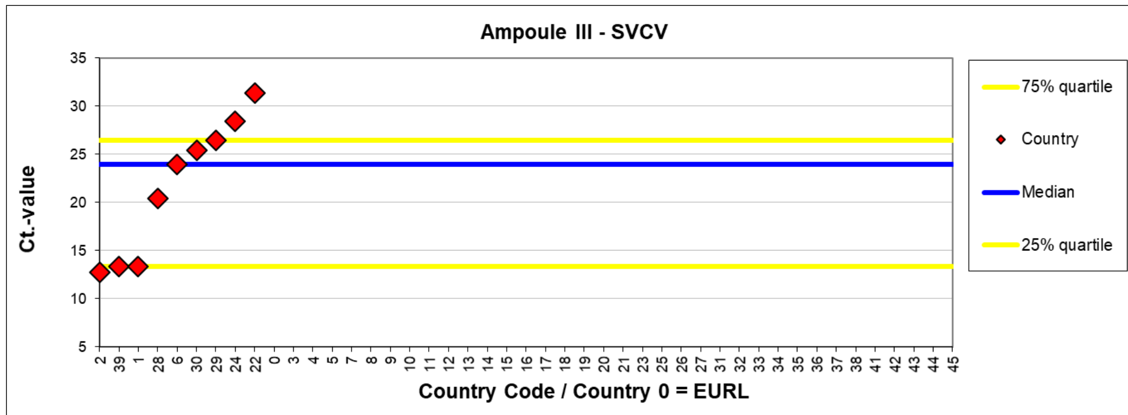
Median Ct.-value	25,3
Maximum Ct.-value	38,7
Minimum Ct.-value	11,3
25% quartile Ct.-value	16,7
75% quartile Ct.-value	28,1



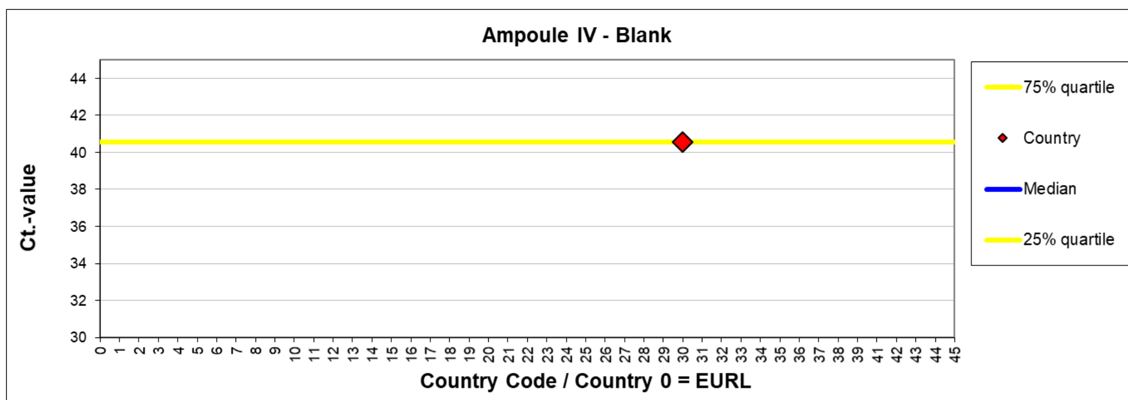
Median Ct.-value	25,4
Maximum Ct.-value	34,8
Minimum Ct.-value	18,2
25% quartile Ct.-value	23,4
75% quartile Ct.-value	28,1



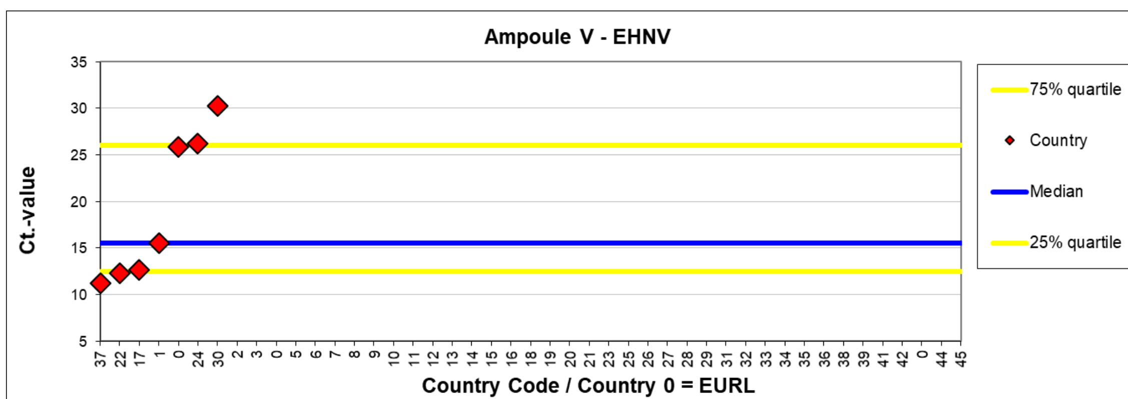
Median Ct.-value	21,2
Maximum Ct.-value	29,9
Minimum Ct.-value	5,8
25% quartile Ct.-value	17,3
75% quartile Ct.-value	26,1



Median Ct.-value	24,0
Maximum Ct.-value	31,3
Minimum Ct.-value	12,7
25% quartile Ct.-value	13,3
75% quartile Ct.-value	26,5



Median Ct.-value	40,6
Maximum Ct.-value	40,6
Minimum Ct.-value	40,6
25% quartile Ct.-value	40,6
75% quartile Ct.-value	40,6



Median Ct.-value	15,5
Maximum Ct.-value	30,2
Minimum Ct.-value	11,2
25% quartile Ct.-value	12,5
75% quartile Ct.-value	26,1

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 protocols described in Einer-Jensen et *al*. (2004) [5] for VHSV and in Kurath et *al*. (2003) [7]and for IHNV we suggest to follow the procedures provided in the latest IHNV chapter of the OIE manual on Aquatic Animal Diseases (primer references are given in Emmenegger et al. (2000), Diseases of Aquatic Organisms 40 (3), 163-176 and PCR conditions are given in Garver et al. (2003), Diseases of Aquatic Organisms 55;187-203
An overview of the genotyping results obtained for PT1 by all participants is displayed in the following table 10.
The EURL has disclosed the content of the ampoules after deadline for delivering results.
Sequencing of Ampoule V (EHNV) is already considered in the scoring system for pathogen identification and therefore not further considered for this scoring. The scoring on genotyping is based on what each participating laboratory has reported in the cell "genotype" of the spreadsheet at the page "Sequencing results".
In case of uncertainty the evaluation has been based on the cell "Additional information".

Two points per ampoule have been given, meaning that maximum score is 6.

- For Ampoule I: 2 points for the correct identification of VHSV genotype Ia
- For ampoule II: 1 point was given for the correct identification of IHNV genotype E and 1 point for the correct identification of IPNV genogroup 5, 1 point was also given to participants who correctly identified IPNV as SP but provided serogroup instead of genogroup.
- For Ampoule III: 2 points for the correct identification of SVCV genotype Ia.

Table 10. The genotyping results obtained for PT1 by all 44 participants

Code number	Score	Ampoule I	Ampoule II	Ampoule III
		VHSV gen. Ia	IHNV gen E IPNV Geno group 5	SVCV gen. Ia
1	6/6	1a	IPNV Genogroup 5; IHNV Genogroup E	Genogroup 1a
2 [1]	5/6	VHS genotype Ia	IPN: Genotype 5; IHN: Genotype:N	Genotype Iaii MO, USA
3		0	0	0
4		0	0	0
5		0	0	0
6	6/6	Ia	E (IHNV) Genogroup 5 (IPNV)	Genogroup Ia
7	6/6	Genogroup I a	IHNV Genotype M and IPNV genogroup 5	Genogroup I a
8	6/6	I a	IHNV: E, IPNV: 5	1a
9	6/6	VHS: Genotype Ia	IHNV: E IPNV: Genogroup 5	SVCV: Genogroup 1a
10		0	0	0
11 [2]	4/6	VHSV_Genotype=1a	IPNV serogroup = A, serotype Sp	SVCV_genogroup=Ia
12 [3]	4/6	Ia	IHNV: E, IPNV: genogroup 5	0

13	6/6	Ia	IPNV: 5 IHNV: E	Ia
14	6/6	Ia	IHNV E; IPNV 5	Ia
15	3/3	Ia	E	N/A
16		0	0	0
17	5/5	Genotype 1a	IPNV strain Sp, genogroup 5	Genotype 1a
18 [3]	3/5	Geno 1a	Geno E	0
19	6/6	Ia1	E + genogroup 5 (pVP2)	Genogroup Ia
20	6/6	Ia	IHNV: E IPNV: genogroup 5	Genogroup 1a
21	3/3	Ia	E	0
22		0	0	0
23 [4]	3/4	1a	IHNV - M, IPNV	N/A
24	6 /6	1a	M IPN Genogroup: 5, serotype: Sp, serogroup: A	1a
25	6 /6	Ia	E and 5	1a
26 [5]	3/6	Ia	IHNV - Genotype M-EUR, IPNV genotype Sp	Genotype Id
27 [6]	5/6	I	IHNV - M IPNV - 5	1a
28 [7]	1/3	III	E	0
29	2/2	0	M-Eur1 for IHNV Genogroup 5 for IPNV	0
30		0	0	0
31 [7]	2/4	Genotype II	IPNV Genogroup 5 IHNV Genogroup E	0
32	6/6	Ia	IHNV - M; IPNV - 5	Ia
33 [8]	3/6	I a	IPNV: genotype 5; IHNV	I d
34	6/6	Ia	E (IHNV), Genogroup 5 (IPNV Sp, by Blake et al., 2001)	Ia
35	6/6	Ia	IHNV :E IPNV: 5	Ia
36	6/6	Ia	IHNV: M IPNV: Genogroup 5	Genogrup 1a
37	4/4	Ia	Genogroup 5, E	N/A
38 [9]	3/5	I, Ia	IHN: M-Eur1	0
39		0	0	0
41		0	0	0
42 [10]	4/6	Genogroup I	IHNV - Genogroup M IPNV N/A	Genotype Ia (Asian SVCV)
43	6/6	VHSV 1a	IHNV Type M, IPNV A2	SVCV 1a
44	4/4	0	IHNV genogroup M IPNV Serotype=Sp genogroup 5	Type Ia
45 [6]	5/6	VHSV I	IPNV- genogroup 5: IHNV-E	I-a

No. of participants performing sequencing	**33**	**35**	**25**
No. of participants getting full score	**28**	**23**	**23**
No. of correct sequences provided without genotype assigned or incomplete sequence	**3**	**10**	**0**
No. of incorrect genotype provided	**2**	**2**	**2**

[1] Genotype of IHNV is indicated as "N"
[2] The genotype of IHNV is not reported, for IPNV serogroup is reported instead of genotype
[3] The genotype for SVCV isolate is not reported
[4] The genotype of IPNV isolate is not reported
[5] For IPNV serogroup is reported instead of genotype the genotype of SVCV is not correct
[6] The genotype for VHSV is partial.
[7] The genotype for VHSV is incorrect
[8] The genotype of IHNV is not reported, the genotype of SVCV is incorrect
[9] The genotype for SVCV is not reported, no sequencing of IPNV performed
[10] The genotype for VHSV is incomplete, the genotype/genogroup for IPNV not reported

Résumé and concluding remarks PT1

The parcels were submitted on a Friday and 51% of parcels were delivered by the shipping companies the following Monday, 80% was delivered within 1 week and 100% was delivered within 3 weeks. Overall 42 out of 44 participants scored 100% success rate and 2 participants scored 90% due to sequencing of the content in ampoule V (EHNV). This point will be addressed directly with the participants that has underperformed.

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 rather high – with more than 6 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.

Although the direct comparison of Ct Values cannot be done due to specific differences in laboratory, reagents, assay setup etc. the tables included in this report may provide valuable information for the participating laboratories, in assessing their results with other laboratories as well as with the EURL, and evaluate the working pipeline in the molecular laboratory, in case of significant differences in the results are obtained. Further specifications both on the assay set up and on the working pipeline will be provided at the specific meeting on March 25th .

We have for the second time scored the sequencing results of all participating laboratories.
For each ampoules 2 points were given. Ampoule V, which contained the ranavirus, was not included in this exercise being the sequence of virus already assessed in the main scoring and of course ampoule IV, which contained non infected BF-2 cell supernatant was not included either.
Overall it is acknowledged that the majority of participants are putting more and more effort in this exercise, however it appears that sequencing efforts across the laboratories varies significantly spanning from laboratories which sequenced and genotyped all isolates in the panel of PT1 (29 out of 44) to laboratories which do not sequence any of the isolates included in the ampoule (5 out of 44).
Within the results collected there is also somewhat a variation in the nomenclature, highlighting the need for further harmonization on nomenclature used in genotyping and this is reflected by examples of providing serotype identification instead of genotype in the case of IPN virus or using genotype M instead of E in case of IHNV. These points will be addressed with all participating laboratories at the meeting on 25th of March, to ensure harmonization of reporting the data from the future proficiency test.
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. 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.

The results presented in this report will be further presented and discussed at the 25th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 31st of May and 1st of June, 2021 .

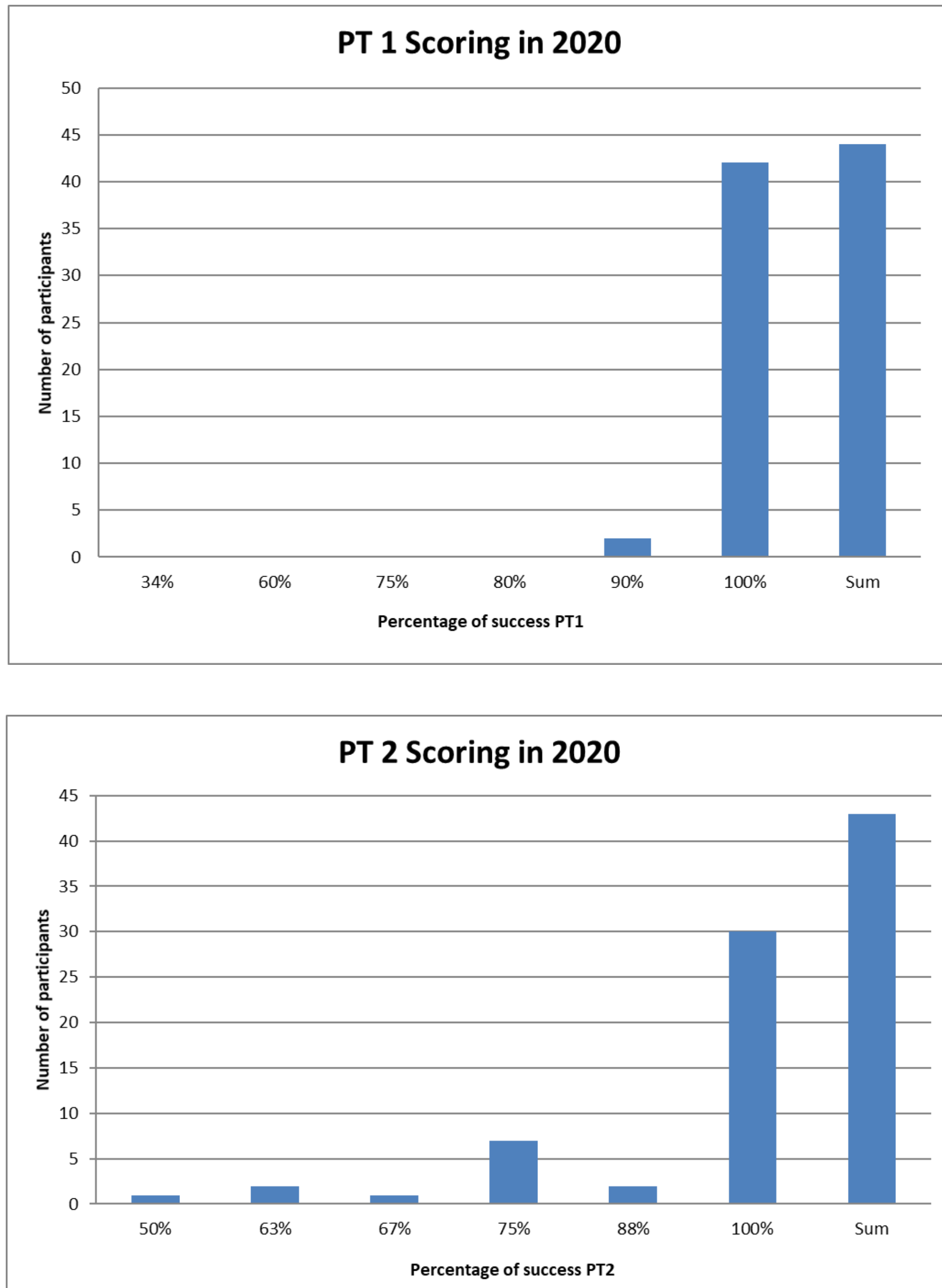


Figure 11 "a" and "b" Success-rate of participating laboratories 2020

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 laboratory 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 11.

Table 11. Content in each ampoule with reference to culture conditions and major publications of the included pathogens.

Code	Specifications/References
Ampoule VI: Blank	**Cell supernatant from BF-2 cells** **01/18** **Passage No.: 36. Passage date:12.02.20** **Tested negative for Mycoplasma: 13.01.20**
Ampoule VII: ISAV	**Infectious Salmon Anaemia Virus ISAV 390/98, HPRΔ** ISAV HPRΔ isolated from Atlantic salmon in Scotland in 1998. **Received from:** Marine Scotland Science . **Genbank accession number** AJ276859 **Reference on sequence and isolate** Rimstad,E., Mjaaland,S., Snow,M., Mikalsen,A.B. and Cunningham, C.O. Characterization of the infectious salmon anemia virus genomic segment that encodes the putative hemagglutinin J. Virol. 75 (11), 5352-5356 (2001)
Ampoule VIII: SAV + ISAV	**Salmonid alpha virus (SAV) 3, Pancreas Disease Virus (PD)** Norway – R-1_2007 **Received from**: Dr. Hilde Sindre, Norwegian Veterinary Institute, Norway **Reference on isolate:** Taksdal et al. 2015 **Gene Bank Ref**.: 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. -- **Infectious Salmon Anaemia Virus. ISAV 2016-70-1297_Vir4415** ISAV HPRΔ isolate from Atlantic salmon in Norway. Hestholmen in 2016. **Received from** Norwegian Veterinary Institute. **Genbank accession number** MK216307
Ampoule IX: KHV	**Koi Herpes Virus, NRIA 0301** Virus isolated from Common carp farmed in japan – province of Ibaragi in 2003. Carp, the sequence of the isolate is unpublished. **Genotype** : CyHV-3 **Received from:** Kei Juasa . **Genbank accession number: N/A** **Reference on the isolate** Sano et al (2004), Fish Pathology, 39,165-167

Testing of the PT2 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 12). 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 12 and Figure 13). Conventional PCR/RT-PCR fragments were sequenced and so was the HPR region in segment 6 of the ISAV isolates.

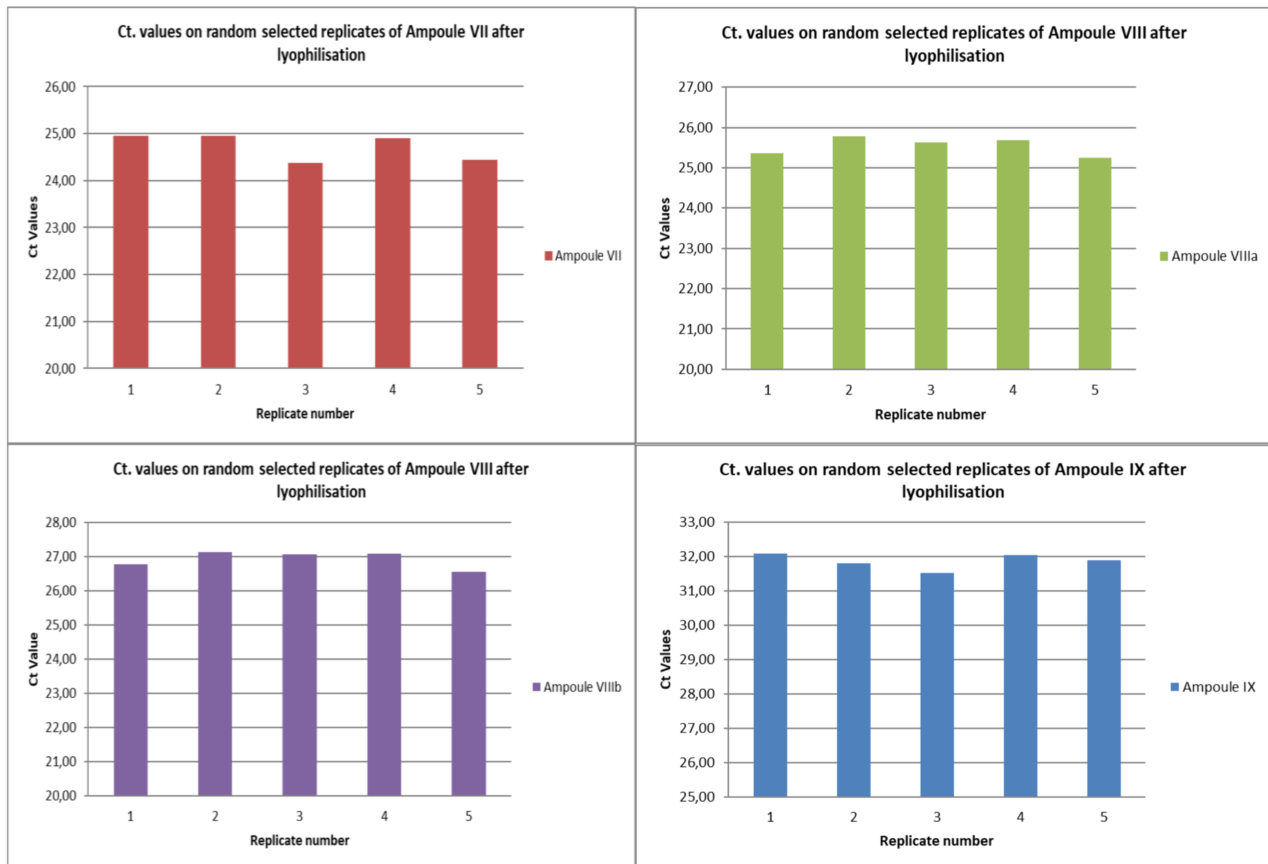


Figure 12, Ampule VII (ISAV), VIIa (SAV), VIIIb (ISAV) and IX (KHV) tested shortly after lyophilisation to assess homogeneity of the content.

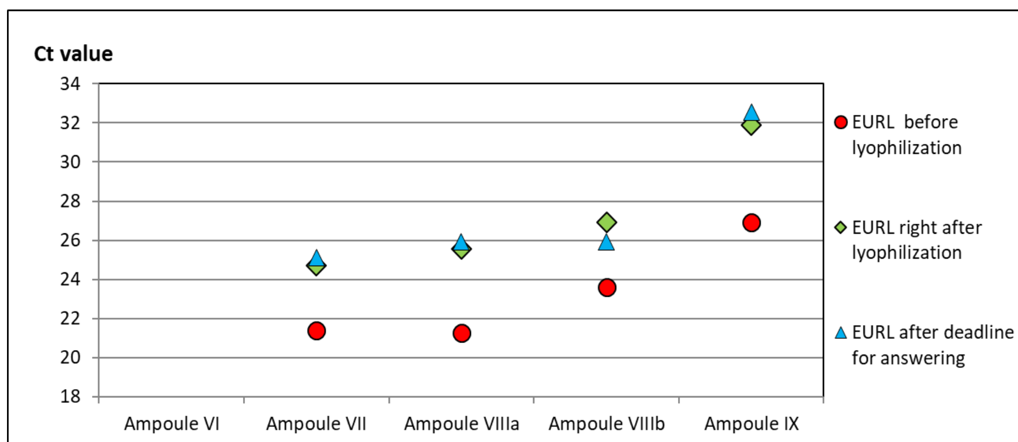


Figure 13, Ampoule VI, VII, VIII and IX tested before and after lyophilisation and after deadline for handling in results.

Table 12: 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 VII	**ISAV**	a	21,41	24,96	25,12
		b		24,95	
		c		24,38	
		d		24,90	
		e		24,45	
			21,41	**24,73**	**25,12**
Ampoule VIIIa	**SAV**	a	21,26	25,37	25,92
		b		25,79	
		c		25,63	
		d		25,68	
		e		25,25	
			21,26	**25,54**	**25,92**
Ampoule VIIIb	**ISAV**	a	23,61	26,78	27,54
		b		27,13	
		c		27,06	
		d		27,09	
		e		26,54	
			23,61	**26,92**	**27,54**
Ampoule IX	**KHV**	a	26,92	32,08	32,54
		b		31,80	
		c		31,52	
		d		32,05	
		e		31,90	
			26,92	**31,87**	**32,54**

The lyophilisation procedure caused a significant virus reduction in all four ampoules (especially in ampule IX with KHV) 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, so, theoretically, the isolates should 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 https://www.eurl-fish-crustacean.eu, insert results in this and return by email.
The results from participating laboratories are shown in table 13.

Table 13. Inter-Laboratory Proficiency Test, PT2, 2020 - Virus identification.

Laboratory code number	Score	Ampoule VI BF-2 cell supernatant	Ampoule VII ISAV, HPRΔ	Ampoule VIII SAV 3, PD ISAV, HPRΔ	Ampoule IX KHV, (CyHV-3)
1	8/8	Negative	HPR-deleted ISAV	HPR-deleted ISAV and SAV	KHV
2	8/8	no virus detected	ISAV	ISAV, SAV	KHV
3	6/8	No ISAV No KHV No SAV	HPR deleted ISAV	HPR deleted ISAV SAV	No ISAV No KHV No SAV
4[2)]	-	0	0	0	0
5	5/8	no virus	ISAV	ISAV	KHV
6	8/8	0	HPR-deleted ISAV	HPR-deleted ISAV + SAV	KHV
7	6/8	not KHV, not ISAV nor SAV	HPR0 ISAV	HPR0 ISAV and SAV	KHV
8	8/8	negative	HPR-deleted ISAV	HPR-deleted ISAV, SAV	KHV
9	8/8	Not ISAV, not KHV, not SAV	ISAV	ISAV+SAV	KHV
10[1)]	8/8	no ISAV; no KHV	HPR-deleted ISAV	HPR-deleted ISAV	KHV
11	8/8	No virus detected	ISAV	ISAV + SAV	KHV
12	8/8	No virus	HPR-deleted ISAV	HPR-deleted ISAV, SAV	KHV
13	8/8	no ISAV, no KHV, no SAV	HPR-deleted ISAV	HPR-deleted ISAV + SAV	KHV Japanese lineage
14	8/8	0	ISAV (HPRdel)	ISAV (HPRdel), SAV	KHV
15	8/8	NO ISAV NO KHV NO SAV	ISA HPRΔ	ISA HPRΔ & SAV	KHV
16[1)]	6/8	NEGATIV	ISAV	ISAV	KHV
17[3)]	4/6	0	ISAV	ISAV and SAV	0
18	6/8	Blank	ISAV	ISAV and SAV	KHV
19	8/8	NEG	ISAV	ISAV + SAV	KHV
20	8/8	-	ISAV	ISAV, SAV	KHV
21[1)]	8/8	0	ISAV	ISAV	KHV
22	5/8	NEGATIVE	ISAV	ISAV	KHV
23	8/8	No virus detected	ISAV	SAV + ISAV	KHV
24	8/8	Negative	ISAV	ISAV + SAV	KHV
25	8/8	No virus detected	HPR-deleted ISAV	HPR-deleted ISAV and SAV	KHV
26	7/8	-	ISAV	ISAV	KHV
27[1)]	8/8	0	ISAV	ISAV	KHV
28[4)]	4/4	Negative	Negative	Negative	KHV
29	7/8	Negative	ISAV	ISAV, SAV	KHV
30	4/8	Not ISAV, Not KHV, Not SAV	ISAV	ISAV, SAV	Not ISAV, Not KHV, Not SAV
31	6/8	No virus	HPR-deleted ISAV	SAV	KHV

32	8/8	-	HPR-deleted ISAV	HPR-deleted ISAV,SAV	KHV
33	6/8	Negative/Not detected	ISAV	ISAV, SAV	KHV
34	8/8	NEGATIVE	ISAV	ISAV, SAV	KHV
35	8/8	-	ISAV	ISAV/SAV	KHV
36	8/8	NO VIRUS	HPR-deleted ISAV	HPR-deleted ISAV + SAV	KHV
37	8/8	No virus	ISAV	ISAV, SAV	KHV
38	8/8	NO ISAV, SAV, KHV	ISAV	ISAV & Salmon Pancreas Disease Virus	KHV
39	8/8	negative	HPR-deleted ISAV	HPR-deleted ISAV and SAV	KHV
41	6/8	-	ISAV	ISAV, SAV	KHV
42	8/8	Negative	ISAV (HPR deletion; HPR7)	ISAV (HPR deletion; HPR type not described) and SAV	KHV
43	8/8	Negative	ISAV - HPR deleted	ISAV - HPR deleted, SAV	CyHV-3
44	8/8	Blank	ISAV	SAV + ISAV	KHV
45	8/8	not ISAV, KHV, or SAV	HPR deleted ISAV	HPR deleted ISAV and SAV	KHV

[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 distinguish 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

- 43 laboratories submitted results
- 26 laboratories correctly identified all four ampoules (ISAV, KHV, SAV, ISAV)
- 41 laboratories tested for the two listed pathogens
- 42 laboratories tested for ISAV
- 42 laboratories tested for KHV
- 38 laboratories tested for SAV
- 1 laboratory that did participate in PT 1 did not participate in PT2

Ampoule VI – Blank

- 43 laboratories ruled out the presence of pathogens they were testing for, the answers varied from ‘Not KHV, Not ISAV, Not SAV’ to leaving the field empty.

Ampoule VII – ISAV

- 41 laboratories correctly identified ISAV hereof 7 laboratories did not sequenced and 2 laboratories found the wrong genotype; 3 did provide an incomplete genotype.
- 1 laboratories did not participate for ISAV and answered ‘Negative’

Ampoule VIII – SAV + ISAV

- 34 laboratories correctly identified both ISAV and SAV hereof 5 laboratories did not sequenced ISAV and 2 laboratories found the wrong genotype.
- 4 laboratories correctly identified ISAV and did not participate for SAV hereof 1 laboratory did not sequenced ISAV
- 3 laboratories correctly identified ISAV and participated for SAV but did not find SAV hereof 2 laboratories did not sequenced ISAV
- 1 laboratory did only find SAV and did not find ISAV
- 1 laboratories did not participate for ISAV and SAV and answered 'Negative'

Ampoule IX – KHV

- 40 laboratories correctly identified KHV
- 2 laboratories did not find the virus and answered 'Not ISAV, Not KHV, Not SAV'
- 1 laboratory did not participate for KHV and answered '0'

Scores

We have assigned a score of 2 points for each ampoule (Table 13), giving the possibility for obtaining a maximum score of 8. Identifying the correct pathogen or the correct combination of pathogens in one ampoule gives score of 2 points.

Special criteria were applied in ampoule VIII with co-infection of SAV and ISAV:

- Detecting both pathogens and correctly identifying ISAV as HPRΔ gave full score of 2.
- Not detecting SAV and correctly detecting and typing ISAV as HPRΔ gave 1 point.
- Detecting SAV and ISAV but incorrectly typing ISAV as HPR0 gave 1 point.
- Detecting SAV only and not ISAV gave 0 point.
- Detecting only ISAV but incorrectly typing ISAV as HPR0 gave 0 point.

Of the 43 laboratories submitting results 30 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 if tested the two listed pathogens KHV and ISAV. If a laboratory did not test for one of the listed pathogens KHV or ISAV but tested for SAV the maximum score was 6 points.
One laboratory did test only for KHV therefore the maximum score was 4 points.

Ct. values

This year we have encouraged participants to insert the Ct value in the spreadsheet if they have performed a real-time (RT-) PCR.
The Ct. values obtained by the participating laboratories are summarised in tables 14. The Ct. values obtained from each participating laboratory are represented graphically in Figures 13. On these figures, the median Ct value and the 25% and 75% inter-quartile range is displayed.

The Ct-values cannot be directly compered due to the use of different methods, reagents and equipment nucleic acid extraction and (RT)-qPCR.

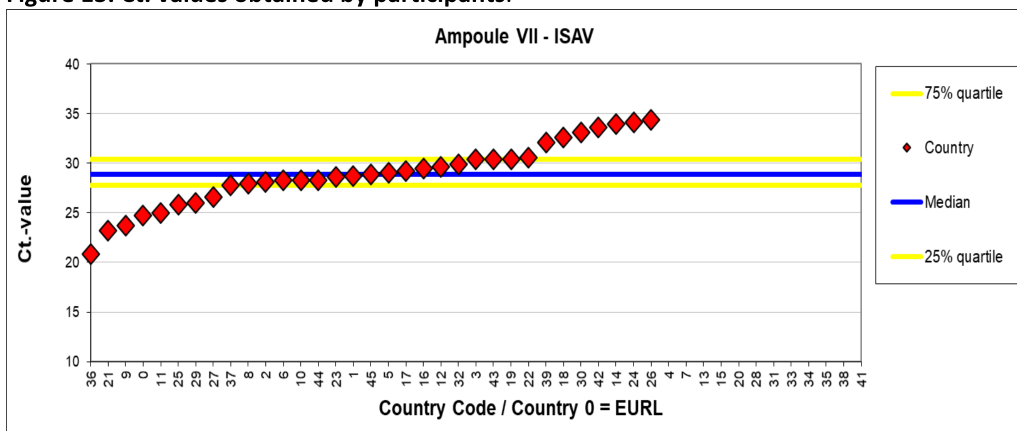
Table 14. Inter-Laboratory Proficiency Test, PT2, 2021 – Ct.-values.

Laboratory Code number	Ct. value Ampoule VII (ISAV)	Ct. value Ampoule VIIIa (SAV)	Ct. value Ampoule VIIIb (ISAV)	Ct. value Ampoule IX (KHV)
0	24,73	25,54	26,92	31,87
1	28,7	32,0	30,9	34,4
2	28,1	30,9	29,3	33,7
3	30,4	28,3	33,0	N/A
4	N/A	N/A	N/A	N/A
5	29,0	N/A	29,0	N/A
6	28,3	28,7	30,7	30,0
7	N/A	N/A	N/A	31,1
8	28,0	N/A	29,0	33,5
9	23,7	24,6	25,3	33,0
10	28,3	N/A	31,6	33,7
11	25,0	28,0	26,0	N/A
12	29,6	33,8	32,6	34,9
13	N/A	31,8	N/A	32,4
14	33,9	36,5	36,5	33,0
15	N/A	N/A	N/A	N/A
16	29,4	N/A	28,9	32,4
17	29,2	30,3	32,5	N/A
18	32,6	34,4	33,7	35,7
19	30,4	34,7	32,3	27,7
20	N/A	N/A	N/A	N/A
21	23,2	N/A	26,6	28,7
22	30,6	N/A	32,3	33,0
23	28,6	N/A	30,1	33,7
24	34,1	37,6	34,8	34,1
25	25,9	30,0	26,8	N/A
26	34,4	N/A	35,6	35,6
27	26,6	N/A	28,1	35,1
28	N/A	N/A	N/A	30,5
29	26,0	30,3	29,0	32,1
30	33,1	35,9	33,4	No Ct found
31	N/A	N/A	N/A	30,2
32	29,9	N/A	31,2	35,7
33	N/A	N/A	N/A	33,6
34	N/A	N/A	N/A	N/A
35	N/A	N/A	N/A	N/A

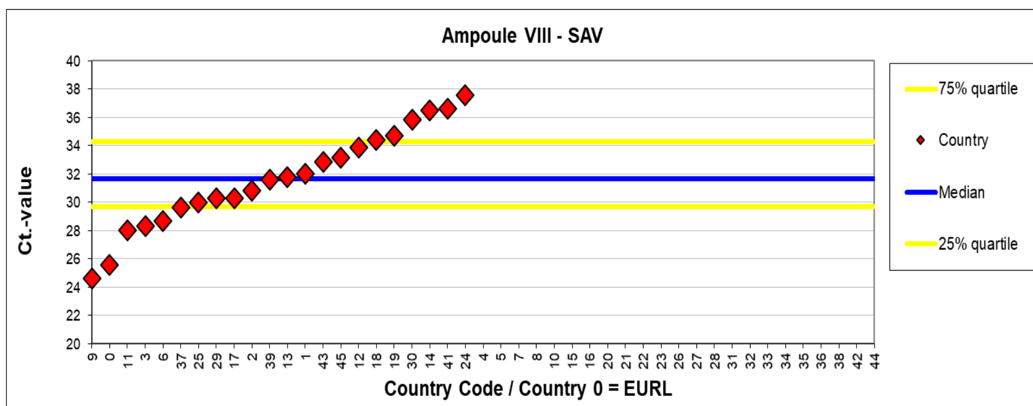
36	20,8	N/A	22,5	29,9
37	27,8	29,6	30,1	30,9
38	N/A	N/A	N/A	33,0
39	32,1	31,6	33,0	32,6
41	N/A	36,6	N/A	N/A
42	33,6	N/A	35,0	N/A
43	30,4	32,9	32,6	35,5
44	28,3	N/A	32,2	N/A
45	28,9	33,1	31,1	38,7

Laboratory code 0 in this table refer to results obtained by EURL
N/A: No Ct-value given by the participating laboratory.

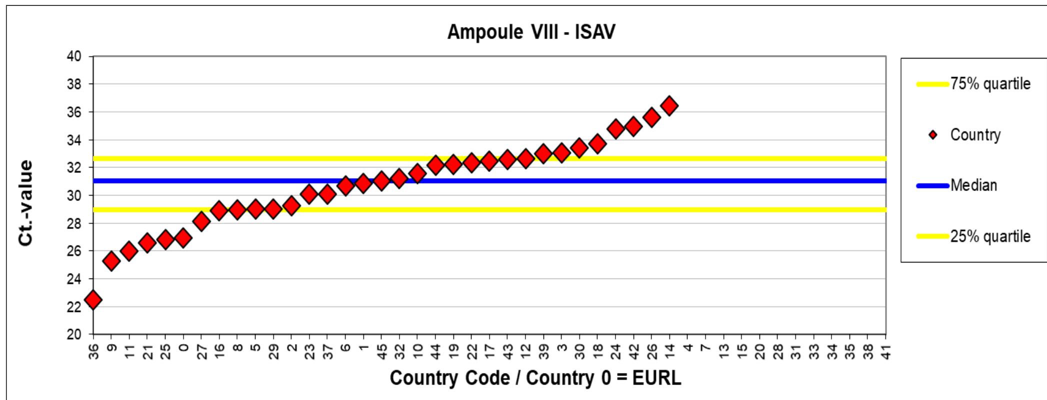
Figure 13. Ct. values obtained by participants.



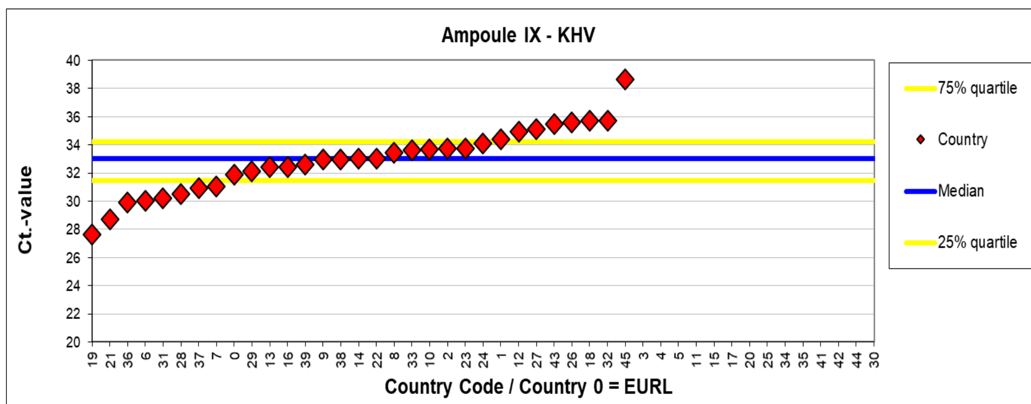
Median Ct.-value	28,9
Maximum Ct.-value	34,4
Minimum Ct.-value	20,8
25% quartile Ct.-value	27,8
75% quartile Ct.-value	30,4



Median Ct.-value	31,7
Maximum Ct.-value	37,6
Minimum Ct.-value	24,6
25% quartile Ct.-value	29,7
75% quartile Ct.-value	34,3



Median Ct.-value	31,1
Maximum Ct.-value	36,5
Minimum Ct.-value	22,5
25% quartile Ct.-value	29,0
75% quartile Ct.-value	32,6



Median Ct.-value	33,0
Maximum Ct.-value	38,7
Minimum Ct.-value	27,7
25% quartile Ct.-value	31,5
75% quartile Ct.-value	34,2

Genotyping and sequencing

Participants were asked to sequence the HPR region of possible ISAV isolates and determine whether isolates included in the ampoules were ISAV HPRΔ currently listed in EU legislation or non listed ISAV HPR0. For KHV the correct sequencing definition based on the blast analysis was “CyHV-3” or “KHV”. Finally sequencing of SAV isolates was assessed from the participants who did actually tested for SAV in PT2.

An Overview of the genotyping results obtained for PT2 by all participants is displayed in the following table 15.

- For Ampoule VII 2 points were given respectively once the isolate was determined as HPR deleted, whereas 0 point were given if the virus was typed as HPR0.
- For Ampoule VIII 1 point was given for determining the SAV isolate as type 3 and 1 point was given respectively once the ISA isolate was determined as HPR deleted.
- For Ampoule IX 2 points were given once the viral isolate as CyHV-3 or KHV.

When the results reported in the “genotype” cell of the spread sheet were not conclusive or not reported it was investigate the information reported under “additional information to the isolate”.

Table 15 The genotyping results obtained for PT2 by all 44 participants

Code number	Score	Ampoule VII	Ampoule VIII	Ampoule IX
		ISAV 390/98, HPRΔ	SAV3 Norway-R-1_2007, (PD) / ISAV 2016-70-1297_Vir4415, HPRΔ	KHV, NRIA CyHV-3
1	6/6	HPR-deleted	ISAV HPR-deleted and SAV Genotype 3	CyHV3
2	6/6	HPR:7b-IN	ISAV: HPR3; SAV: subtype 3	CyHV-3; Mexico
3	3/3	ISAV HPRΔ	ISAV HPRΔ	0
4		0	0 0	
5		0	0 0	
6	6/6	HPR-deleted	HPR-deleted (ISAV) 3 (SAV)	CyHV-3
7[1]	3/6	HPR0	HPR0 and SAV, subtype 3	CyHV3
8	6/6	HPR-deleted	ISAV: HPR-deleted, SAV: 3	CyHV3
9	6/6	HPR-deleted	HPR-deleted + SAV3	CyHV3
10	3/3	HPR7	HPR9	0
11[2]	1/4	ISAV_genotype=G3	ISAV_Clade=CIIIa SAV_subtype 3	0
12	5/5	HPR-deleted	ISAV: HPR-deleted	CyHV-3
13	6/6	HPR-deleted	ISAV: HPR-deleted SAV: 3	CyHV3 Japanese lineage
14	6/6	Clade CVI; HPR7b	ISAV: HPR9 SAV3A	KHV 100% TK; KHV-100% DNA polymerase
15				
16				
17				
18				
19	6/6	HPR Deleted	HPR Deleted + SAV3	CYHV 3
20	6/6	HPR Deleted -HPR7b	ISAV: HPR Deleted - HPR9 SAV: Subtype 3	CyHv-3
21	5/5	HPR7b	HPR9	CyHV3
22				
23[3]	3/4	HPR deleted	E2 (III), ISAV	N/A
24[2]	3/6	HPR	HPR SAV subtype 3	CyHV Type 3
25	6/6	HPR-deleted	HPR-deleted and 3	CyHV-3
26	3/3	Genotype HPR13	HPRΔ	0
27	3/3	HPR-deleted ISAV	HPR-deleted ISAV	0

28	2/2	0	0	Cyprinid herpesvirus 3
29	4/4	HPR deleted	0	CyHV-3
30				
31[4]	4/5	ISAV HPR deleted	SAV 4	CyHV-3
32	6/6	HPR-deleted	ISAV: HPR-deleted; SAV: 3	CyHV-3
33[1]	3/6	HPR0	HPR0 (ISAV); Subtype 3 (SAV)	CyHV 3
34	6/6	HPR14	ISAV HPR1, SAV 3	CyHV 3
35	6/6	ISA-HPRΔ(14)	ISA-HPRΔ(1) SAV3	CyHV-3
36	6/6	HPR-deleted	ISAV: HPR-deleted SAV: 3	CyHV 3
37	4/4	HPR-deleted	HPR-deleted, 3	N/A
38	3/3	ISAV_HPRΔ	ISAV_HPRΔ 0	
39	3/3	HPR-deleted	HPR-deleted 0	
41				
42[5]	2/6	ISAV-HPR	iSAV-HPR and SAV	KHV wildtype
43	6/6	ISAV HPR 7	ISAV HPR 9, SAV TYPE III	CyHV-3
44	3/3	ISAV: HPR Deleted Clade CIVa	ISAV: HPR Deleted Genotype: 2; SAV not sequenced	0
45[6]	1/6	EU-ISAV	EU-ISAV; SAVIII	0

No. of sequences performed	**34**	**33**	**24**
No. of correct genotypes given	**29**	**16**	**24**
No. of correct sequences provided without genotype assigned	**3**	**16**	**0**
No. of incorrect genotype provided	**2**	**1**	**0**

[1] ISAV isolates in ampoule VII and VIII are incorrectly defined as HPR0
[2] ISAV isolates in ampoule VII and VIII are sequenced but the HPR type is not defined
[3] ISAV isolate in ampoule VIII is not typed
[4] SAV genotype in ampoule VIII is incorrectly assigned to type 4
[5] ISAV isolates in ampoules VII and VIII are not assigned an HPR type, SAV is not genotyped
[6] ISAV isolates in ampoules VII and VIII are not assigned an HPR type, KHV is not assigned

Concluding remarks PT2

43 laboratories participated in PT2.
41 out of the 43 laboratories correctly identified the ISA virus in both ampoule VII and VIII, hereof 7 laboratories did not sequenced and 2 laboratories found the wrong genotype. 1 laboratory did not test for ISAV and 1 did not find the ISAV in ampoule VIII.
40 laboratories correctly identified the KHV in ampoule IX, 1 did not test for KHV and 2 did not find the KHV in ampoule IX.
38 laboratories tested for SAV and 35 correctly identified the virus in Ampoule VIII, 3 laboratories did not find the virus.

It is highly appreciated that many laboratories are putting efforts in performing genetic analysis and further 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]. Starting from 2020, participants will have to report in the "concluding result" if the ampoule contain ISAV HPR0 or ISAV HPRΔ, as per methods provided in the diagnostic manual for ISA. Concluding ISAV HPR0 instead of ISAV HPRΔ will reduce the score to 0 , considering that ISAV HPR0 is not listed according to EU legislation. The EURL will update the example in the spreadsheet accordingly.

It is acknowledged that more and more laboratories have increased efforts in genotyping the viral isolates included in the ampoules, and therefore we have decided to score the genotyping results.
Out of 43 participating laboratories, 35 perform sequencing of ISAV to determine HPR type, 26 for KHV and 25 for SAV.
33 laboratories out of 35 correctly sequenced the ISAV in ampoule VII and VIII as type HPRΔ and only 2 as HPR0.
It has to be observed that the sequencing of the ISAV isolate in both ampoule VII and VIII has created some challenges for the participants, since 2 laboratories typed it as HPR0.
Regardless instructions provided with the proficiency test on the nomenclature there is still some heterogeneity on how the genotype is provided from each participants. These points will be addressed again at the upcoming ad hoc meeting on the Proficiency test results and at the annual workshop
It is generally highly appreciated the development observed in the quality of the analysis provided by the NRL and with small improvements in the nomenclature an harmonized system will be implemented within the NRL network.

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. 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 25th Annual Workshop of National Reference Laboratories for Fish Diseases to be held May 31st and June 1st 2021, the meeting will be held online due to travel restrictions due to COVID-19 pandemic.

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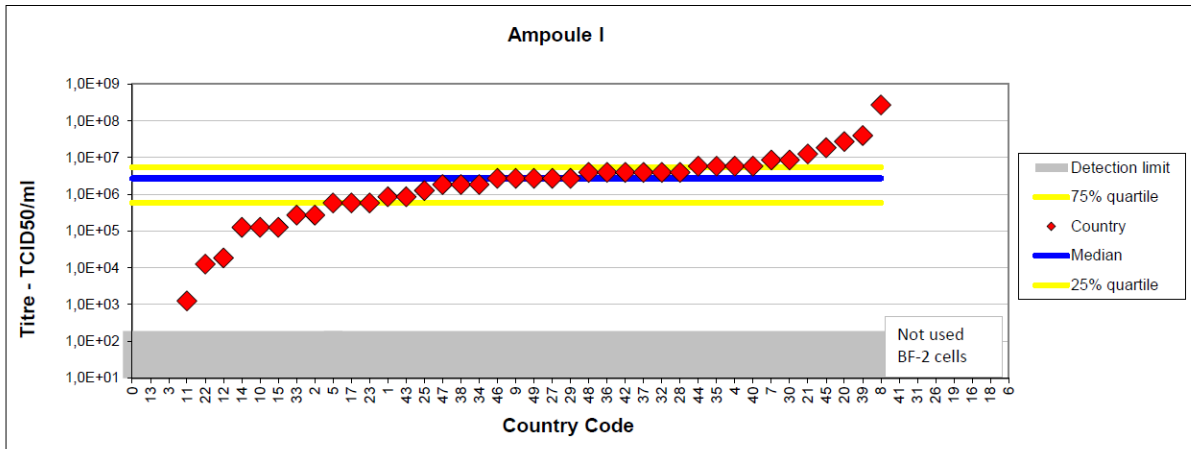
Remarks for ILPT 2019

Thanks to the feedback evaluation provided by participating laboratories, it has appeared that a mistake occurred in the report for the ILPT for fish diseases 2019. One of the graph plotting titration data was duplicated. However numerical values of titer were correctly reported in the respective tables.
We take the opportunity to include in this report the correct graph that was missing from the report 2019.



Correction to 'Report of the Inter-Laboratory Proficiency Test 2019 for identification and titration of VHSV, IHNV, EHNV, SVCV and IPNV (PT1) and identification of CyHV-3 (KHV), SAV and ISAV (PT2) on figure 5 for BF-2 titer obtained from ampoule I.

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