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 2019

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
European Union Reference Laboratory for Fish and Crustacean Diseases,
National Institute of Aquatic Resources, Technical University of Denmark,
Kgs. Lyngby, Denmark

DANAK
PT Reg. no.: 515

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Introduction

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
PT1 was designed to primarily assess the identification of the fish viruses causing notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and in addition other fish pathogenic viruses as pike fry rhabdovirus (PFR), spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) by cell culture based methods.
PT2 was designed for assessing the ability of participating laboratories to identify the fish viruses: infectious salmon anaemia virus (ISAV), salmonid alphavirus (SAV) and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by bio molecular methods (PCR based).
49 laboratories participated in PT1 while 45 participated in PT2.
Regarding PT1 and PT2, 42 and 38 laboratories respectively participated in identifying all viruses included.
The tests were sent from the EURL 27th of September 2019.
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 and DTU – National Food Institute for lyophilisation.
This report covers both the results of PT1 and PT2.

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

If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals [4]. Laboratories were encouraged to identify VHSV and IHNV isolates by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al*. (2004) [5] for VHSV and ; 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). One ampoule contained CyHV-3 (KHV), two ampoules contained ISAV and one contained SAV, see table 9. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV and KHV (listed in Council Directive 2006/88/EC, Annex IV and Commission Implementing Directive 2014/22/EU) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test for SAV or not. The EURL team would then take care of calculating the score accordingly, overall 39 of 47 laboratories tested for SAV in 2019.
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.

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

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

Participants were asked to download an excel sheet from the EURL web site (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 1st 2019. The results of the inter-laboratory proficiency test for listed fish diseases 2019 and plans and idea for future inter-laboratory tests will be presented at the 24th Annual Workshop of the NRLs for Fish Diseases on June 2nd and 3rd 2020 in Kgs. Lyngby.

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 2 participants 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 a Friday. The following Monday, the tests were delivered to 25 participants; 17 more parcels were delivered within the first week; 90% were delivered within the first two weeks and 96% within three weeks; due to internal problems in the receiving country, 2 parcels were more than 3 weeks to deliver. (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 4°C in order to be able to provide fast substitutes in case of damage during transport.

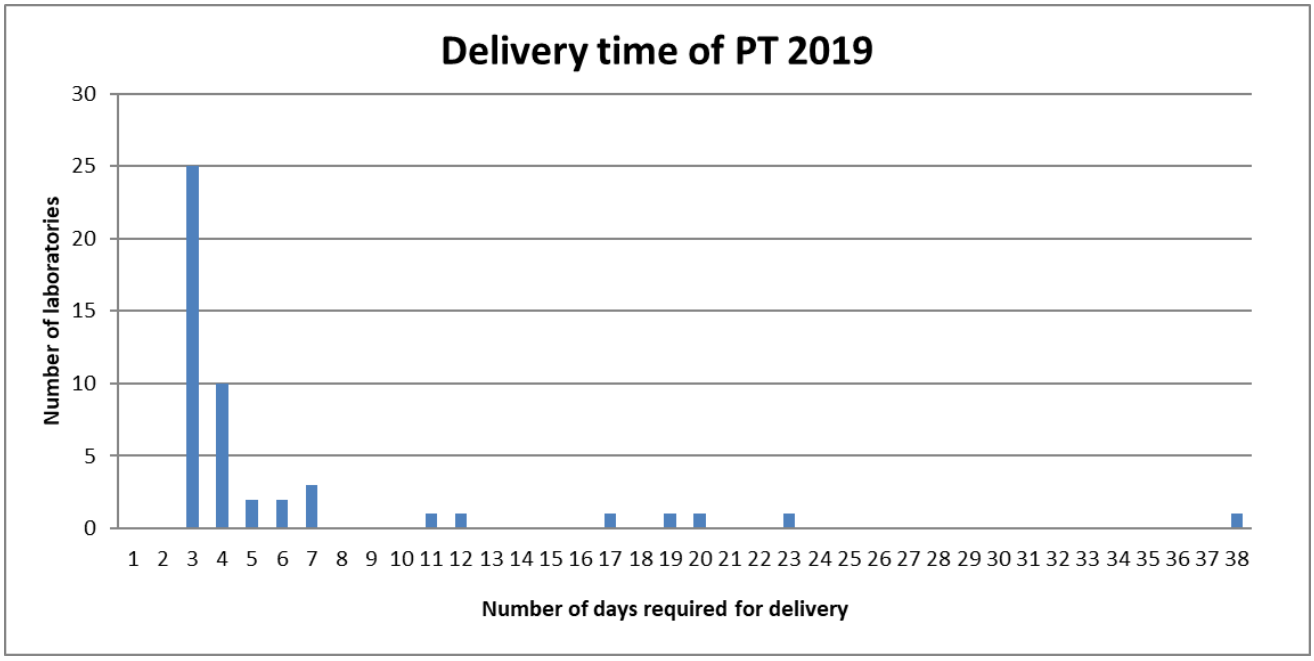


Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 49 laboratories received the annual proficiency test. 48 participants submitted the full spreadsheet within the deadline; 1 participant got the deadline extended due to delivery problems. Figure 2 show the numbers of participants in the proficiency test from 1996 to 2019.

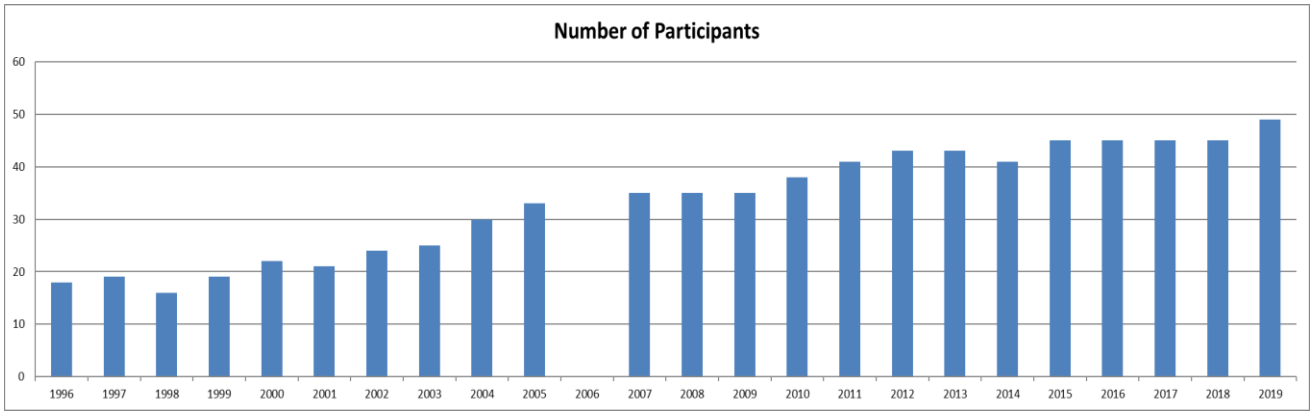


Figure 2. Participants in the EURL proficiency test over the years.

Proficiency test 1, PT1

Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States, including Denmark, and likewise to the NRLs in Australia, Bosnia and Herzegovina, Canada, Chile, Faroe Islands, Iceland, Iran, Japan, New Zealand, Norway, Republic of North Macedonia, Russia, Serbia, Switzerland, Tunisia, Turkey and to two laboratories in P.R. China, South Korea and USA, respectively.

The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy, Cyprus and Malta for identification of all listed diseases. Figure 3 shows the worldwide distribution of the participating NRLs. This year there were two new participating countries: the National Reference laboratories of Tunisia and Republic of North Macedonia.

Figure 3. Worldwide distribution of the participants in the EURL proficiency test 2019

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 + IHNV	**VHSV DK-9695377** **Genotype** Ia VHSV isolate from asymptomatic farmed Rainbow trout in brackish water in Denmark in 1996 **GenBank accession number** AY546597 **Reference on isolate and sequence:** Einer-Jensen K., Ahrens P., Forsberg R. and Lorenzen N.(2004) Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virusJournal of General Virology, 85, 1167–1179 **+** **IHNV 32/87.** **Genotype** E First French isolate (April 1987) from rainbow trout. **GenBank accession number**: FJ265717 and AY524121 (truncated 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.
Ampoule II: ECV	**European catfish virus 562/92.** Italian isolate from catfish suffering high mortality. **Received from** Dr. G. Bovo, ISZ-Ve, Padova, Italy. **GenBank accession number**: FJ358608 or KT989884.1 or KT989885.1 or JQ724856.1 **Reference on isolate**: Bovo G, Comuzi M, De Mas S, Ceschia G, Giorgetti G, Giacometti P & Cappellozza E (1993). Isolamento di un agente virale irido-like da pesce gatto (Ictalurus melas) dallevamento. Bollettino Societa Italiana di Patologia Ittica 11, 3–10. **Reference on sequence**: Holopainen R., Ohlemeyer S., Schütze H., Bergmann S.M. & Tapiovaara H. (2009) Ranavirus phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofilament triplet H1-like protein genes. *Diseases of Aquatic Organisms* 85, 81-91.

Code	Specifications/References
Ampoule III: VHSV	**VHSV 4p101.** **Genotype: IIIa** Marine isolate from whiting (*Merlangius merlangus*) caught in Skagerrak (1997). **GenBank accession number**: AY546581 (G-gene) and AJ130918 (N-gene). Mortensen HF, Heuer OE, Lorenzen N, Otte L, Olesen NJ (1999) Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. Virus Research 63, 95-106. Einer-Jensen K., Ahrens P., Forsberg R. and Lorenzen N.(2004) Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virusJournal of General Virology, 85, 1167–1179 Snow M., Bain N, Black J, Taupin V, Cunningham CO, King JA,, Skall HF, Raynard RS (2004) Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). Diseases of Aquatic Organisms 61, 11-21.
Ampoule IV: SVCV	**SVCV Isolate DK-203273** **Genotype: 1a** Spring Viraemia of Carp Virus isolated from Koi Carp in Denmark June 2003, the isolate is unpublished **Received from:** National Veterinary Institute, Technical University of Denmark. **GenBank accession numbers: unpublished**
Ampoule V: IPNV	**IPNV strain 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 Phylogenetic relationships of aquatic birnaviruses based on deduced amino acid sequences of genome segment A cDNA. Blake S, Ma JY, Caporale DA, Jairath S, Nicholson BL.

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, serum neutralisation tests (SNT), PCR and/or qPCR and RT-PCR and/or RT-qPCR. After shipment the stability of the content in the ampoules were assessed by titrating the virus on cell cultures, and identifying it by ELISA, furthermore PCR based tests were performed on the original content of all the ampoules. This year reductions of the titres after lyophilisation were observed. For the major part of the ampoules, the reduction of the titre was between 1-3 log in the same cell line. The reduction of the titre in ampoule V (containing IPNV) was close to 4 log in EPC and RTG-2 cells. This highlights the importance of using two heterologous cell lines according to Commission Decision 2015-1554 [3] for conducting surveillance for listed viral disease in fish. No significant reductions were observed after long term storage (Table 2 and figure 4)

Ampoul No.	Cell line	Titre before Lyophilisation	Titre after Lyophilisation and before shipment	Titre after deadline for handling in results (storage 4°C in the dark)
		$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I: VHSV DK-9695377 + IHNV 32/87	BF-2	2,7E+08	4,0E+06	2,7E+06
	EPC	5,9E+07	1,9E+06	1,3E+06
	RTG-2	4,0E+08	4,0E+06	1,9E+06
	FHM	4,0E+08	1,3E+07	8,6E+06
Ampoule II: ECV 562/92	BF-2	2,7E+07	4,0E+05	1,3E+06
	EPC	1,9E+06	1,3E+04	4,0E+04
	RTG-2	1,3E+08	1,9E+05	5,9E+05
	FHM	2,7E+04	1,9E+02	< 1,9E+02
Ampoule III: VHSV 4p101	BF-2	1,3E+09	1,3E+07	8,6E+06
	EPC	8,6E+08	4,0E+07	1,3E+07
	RTG-2	8,6E+04	5,9E+03	4,0E+03
	FHM	8,6E+08	8,6E+06	4,0E+06
Ampoule IV: SVCV DK-203273	BF-2	1,3E+09	8,6E+06	8,6E+06
	EPC	1,9E+08	5,9E+06	1,9E+06
	RTG-2	8,6E+07	2,7E+05	1,3E+05
	FHM	5,9E+08	1,3E+07	1,3E+07
Ampoule V: IPNV strain Sp	BF-2	2,7E+07	4,0E+04	5,9E+04
	EPC	1,9E+07	4,0E+03	1,3E+04
	RTG-2	4,0E+07	8,6E+03	1,3E+04
	FHM	5,9E+04	2,7E+03	5,9E+03

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).

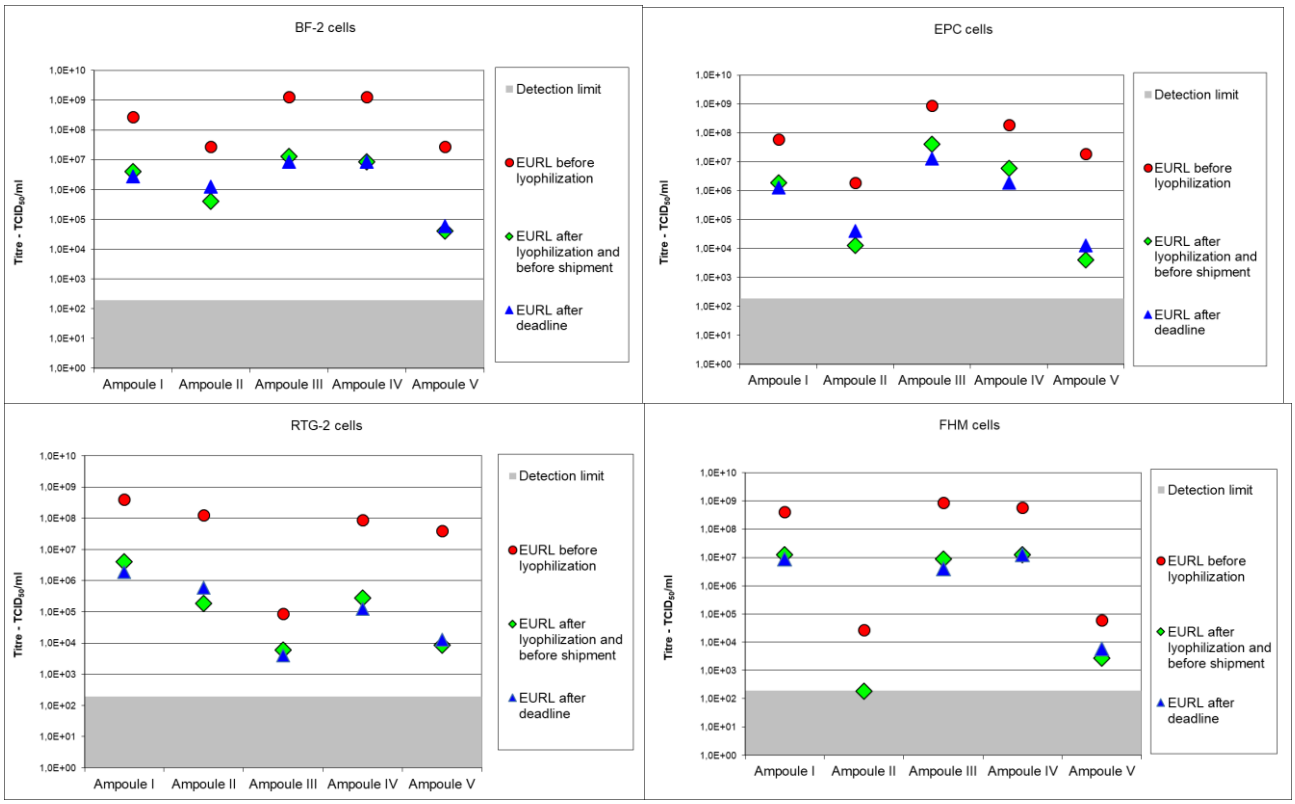


Figure 4. Virus titres in different cell lines: before lyophilisation, before shipment and after deadline for handling in results (storage 4°C in the dark).

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 strongly encouraged to identify the genotype of the virus isolates.

Table 3. Inter-Laboratory Proficiency Test, PT1, 2019 - Virus identification and score obtained by participants.

Laboratory code number	Score	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
		VHSV DK-9695377 + IHNV 32/87	ECV	VHSV 4p101	SVCV 203273	IPNV sp
1	10/10	VHSV and IHNV	ESV	VHSV	SVCV	IPNV
2	10/10	VHSV + IHNV	Ranavirus	VHSV	SVCV	IPNV
3	10/10	VHSV/IHNV	Ranavirus (not EHNV)	VHSV	SVCV	IPNV
4	10/10	+ IHNV and +VHSV	+ European catfish virus	+VHSV	+SVCV	+ IPNV
5	10/10	VHSV, IHNV	Ranavirus was identified by conventional PCR and than REA was applied as given by OIE manuel to identified ECV	VHSV	SVCV	IPNV
6[3)]	2/6	VHSV	IHNV	VHSV	0	0
7	10/10	IHNV & VHSV	ECV	VHSV	SVCV	IPNV
8	10/10	VHSV & IHNV	Ranavirus – NOT EHNV	VHSV	SVCV	IPNV
9	10/10	VHSV, IHNV	Ranavirus - NOT EHNV	VHSV	SVCV	IPNV
10	10/10	VHSV -IHNV	Ranavirus- NOT EHNV	VHSV	SVCV	IPNV
11	8/10	IHNV	Ranavirus	VHSV	SVCV	IPNV
12	10/10	VHSV, IHNV	Ranavirus (European catfish virus)	VHSV	SVCV	IPNV
13	8/10	VHSV, IHNV	ECV	VHSV	0	IPNV
14	10/10	VHSV,IHNV	RANA virus (ECV)	VHSV	SVCV	IPNV
15	10/10	VHSV/IHNV	Ranavirus	VHSV	SVCV	IPNV
16[1)]	6/8	VHSV	0	VHSV	SVCV	IPNV
17	6/10	VHSV	ECV / ESV	VHSV	SVCV	IHNV IPNV
18[1)]	8/8	VHSV,IHNV	Negative	VHSV	SVCV	IPNV
19	10/10	VHSV/IHNV	Rana - NOT EHNV	VHSV	SVCV	IPNV
20	10/10	VHS/IHN	Ranavirus - NOT EHNV	VHS	SVC	IPN
21	9/10	IHNV+VHSV	ECV	VHSV+IHNV	SVCV	IPNV
22	9/10	VHSV and IHNV	Ranavirus – NOT EHNV	VHSV and IHNV	SVCV	IPNV
23	10/10	IHNV and VHSV	Ranavirus - NOT EHNV	VHSV	SVCV	IPNV
24	10/10	VHSV + IHNV viable virus	ECV viable virus	VHSV viable virus	SVCV viable virus	IPNV viable virus
25	10/10	VHSV + IHNV	Ranavirus	VHSV	SVCV	IPNV
26[1)]	8/8	VHSV,IHNV	No VHSV, IHNV, SVCV, IPNV	VHSV	SVCV	IPNV
27	10/10	VHSV/IHNV	Ranavirus	VHSV	SVCV	IPNV

28	10/10	IHNV / VHSV	Ranavirus	VHSV	SVCV	IPNV
29	10/10	VHSV + IHNV	ECV/ESV	VHSV	SVCV	IPNV
30	10/10	VHSV and IHNV	ECV	VHSV	SVCV	IPN
31[1)]	8/8	VHSV, IHNV	N/A	VHSV	SVCV	IPNV
32	10/10	VHSV and IHNV	ECV	VHSV	SVCV	IPNV
33	6/10	IHNV and VHSV	ECV European Catfish Virus and VHSV	VHSV	SVCV	SVCV
34	10/10	IHN &VHS	ECV/ESV	VHS	SVC	IPN
35	10/10	IHNV, VHSV	Ranavirus, ECV not EHNV	VHSV	SVCV	IPNV
36	10/10	VHSV+IHNV	ECV	VHSV	SVCV	IPNV
37	10/10	VHSV, IHNV	Ranavirus - NOT EHNV	VHSV	SVCV	IPNV
38	10/10	VHSV + IHNV	ECV or ESV	VHSV	SVCV	IPNV
39[1)]	8/8	VHSV, IHNV	No VHSV, IHNV, IPNV, SVCV	VHSV	SVCV	IPNV
40	10/10	VHSV + IHNV	Ranavirus, not EHNV	VHSV	SVCV	IPNV
41	10/10	VHSV + IHNV	Ranavirus – NOT EHNV	VHSV	SVCV	IPNV
42	9/10	VHSV and IHNV	EHNV	VHSV	SVCV	IPNV
43	10/10	VHSV Genotype Ia IHNV genogroup M	European Catfish Virus	VHSV Genotype III	SVCV	IPNV
44	10/10	IHNV and VHSV	ECV	VHSV	SVCV	IPNV
45[2)]	9/10	VHSV + IHNV	Ranavirus	VHSV	SVCV	IPNV
46[1)]	8/8	IHNV + VHSV	NO IHNV,VHSV,SVCV,IPNV	VHSV	SVCV	IPNV
47	10/10	VHSV IHNV	Ranavirus NOT EHNV	VHSV	SVCV	IPNV
48	10/10	VHSV & IHNV	Ranavirus - NOT EHNV	VHSV	SVCV	IPNV
49	10/10	IHNV/VHSV	Ranavirus (ECV/ESV)	VHSV	SVCV	IPNV

1) Do not test for Ranavirus
2) Did not corroborate the findings in ampoule IV by sequencing or REA
3) Do not test for Ranavirus and IPNV

Table 4. Inter-Laboratory Proficiency Test, PT1, 2019 – Results of titration of **ampoule I**.

VHSV DK-9695377 + IHNV 32/87					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	VHSV and IHNV	8,6E+05	4,0E+06	5,9E+05	N/A
2	VHSV + IHNV	2,7E+05	1,9E+05	N/A	1,9E+06
3	VHSV/IHNV	4,0E+06	5,9E+06	N/A	N/A
4	+ IHNV and +VHSV	5,9E+06	8,6E+06	N/A	N/A
5	VHSV, IHNV	5,9E+05	1,3E+06	N/A	N/A
6	VHSV	N/A	N/A	N/A	N/A
7	IHNV & VHSV	8,6E+06	N/A	N/A	1,9E+07
8	VHSV & IHNV	2,7E+08	4,0E+08	N/A	N/A
9	VHSV, IHNV	2,7E+06	N/A	N/A	8,6E+06
10	VHSV -IHNV	1,3E+05	2,7E+06	8,6E+05	2,7E+06
11	IHNV	1,3E+03	1,9E+04	N/A	2,7E+04
12	VHSV, IHNV	1,9E+04	2,7E+04	4,0E+03	4,0E+03
13	VHSV, IHNV	4,0E+03	N/A	N/A	1,9E+05
14	VHSV,IHNV	1,3E+05	2,7E+04	2,7E+03	1,3E+04
15	VHSV/IHNV	1,3E+05	1,3E+05	N/A	N/A
16	VHSV	N/A	1,9E+04	2,7E+04	N/A
17	VHSV	5,9E+05	1,3E+03	N/A	N/A
18	VHSV,IHNV	N/A	N/A	N/A	N/A
19	VHSV/IHNV	N/A	< 1,9E+02	N/A	N/A
20	VHS/IHN	2,7E+07	8,6E+06	5,9E+07	1,3E+08
21	IHNV+VHSV	1,3E+07	1,3E+05	N/A	N/A
22	VHSV and IHNV	1,3E+04	N/A	N/A	1,3E+08
23	IHNV and VHSV	5,9E+05	5,9E+05	N/A	N/A
24	VHSV + IHNV viable virus	8,6E+04	2,7E+04	N/A	2,7E+04
25	VHSV + IHNV	1,3E+06	5,9E+05	N/A	N/A
26	VHSV,IHNV	N/A	2,7E+04	5,9E+06	N/A
27	VHSV/IHNV	2,7E+06	1,9E+06	N/A	N/A
28	IHNV / VHSV	4,0E+06	5,9E+06	2,7E+06	5,9E+06
29	VHSV + IHNV	2,7E+06	5,9E+05	N/A	N/A
30	VHSV and IHNV	8,6E+06	1,9E+06	N/A	N/A
31	VHSV, IHNV	N/A	N/A	N/A	N/A
32	VHSV and IHNV	4,0E+06	4,0E+06	N/A	N/A
33	IHNV and VHSV	2,7E+05	5,9E+03	N/A	N/A

34	IHN &VHS	1,9E+06	8,6E+05	N/A	N/A
35	IHNV, VHSV	5,9E+06	5,9E+06	1,3E+06	N/A
36	VHSV+IHNV	4,0E+06	1,3E+07	8,6E+06	N/A
37	VHSV, IHNV	4,0E+06	2,7E+06	N/A	N/A
38	VHSV + IHNV	1,9E+06	4,0E+06	N/A	N/A
39	VHSV, IHNV	4,0E+07	5,9E+05	N/A	N/A
40	VHSV + IHNV	5,9E+06	2,7E+06	5,9E+06	1,9E+07
41	VHSV + IHNV	N/A	1,9E+06	1,3E+05	5,9E+04
42	VHSV and IHNV	4,0E+06	1,3E+05	N/A	N/A
43	VHSV Genotype Ia IHNV genogroup M	8,6E+05	4,0E+05	4,0E+05	N/A
44	IHNV and VHSV	5,9E+06	1,9E+06	1,3E+06	1,9E+06
45	VHSV + IHNV	1,9E+07	1,9E+07	N/A	N/A
46	IHNV + VHSV	2,7E+06	1,3E+06	N/A	N/A
47	VHSV IHNV	1,9E+06	2,7E+07	5,9E+05	N/A
48	VHSV & IHNV	4,0E+06	5,9E+06	N/A	N/A
49	IHNV/VHSV	2,7E+06	8,6E+05	8,6E+05	N/A

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

VHSV DK-9695377 + IHNV 32/87	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	42	16	15
Median titre	2,7E+06	1,3E+06	8,6E+05	1,9E+06
Maximum titre	2,7E+08	4,0E+08	5,9E+07	1,3E+08
Minimum titre	1,3E+03	<1,9E+02	2,7E+03	4,0E+03
25% quartile titre	5,9E+05	1,3E+05	3,3E+05	4,3E+04
75% quartile titre	5,4E+06	4,0E+06	3,5E+06	1,4E+07

Table 5. Inter-Laboratory Proficiency Test, PT1, 2019 – Results of titration of **ampoule II**.

ECV					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	ESV	2,7E+06	4,0E+06	1,3E+04	N/A
2	Ranavirus	1,3E+06	1,9E+05	N/A	1,9E+05
3	Ranavirus (not EHNV)	4,0E+06	8,6E+05	N/A	N/A
4	+ European catfish virus	4,0E+06	2,7E+06	N/A	N/A
5	Ranavirus was identified by conventional PCR and than REA was applied as given by OIE manuel to identified ECV	5,9E+06	8,6E+05	N/A	N/A
6	IHNV	N/A	N/A	N/A	N/A
7	ECV	4,0E+05	N/A	N/A	< 1,9E+02
8	Ranavirus – NOT EHNV	1,3E+08	8,6E+06	N/A	N/A
9	Ranavirus - NOT EHNV	1,9E+06	N/A	N/A	1,9E+03
10	Ranavirus- NOT EHNV	1,9E+06	2,7E+06	1,9E+06	1,9E+06
11	Ranavirus	1,86E+04	1,86E+04	N/A	8617,73876
12	Ranavirus (European catfish virus)	5,87E+06	5,87E+05	< 1,9E+02	5,87E+05
13	ECV	8,6E+07	N/A	N/A	1,9E+05
14	RANA virus (ECV)	2,7E+05	1,3E+05	1,3E+03	1,3E+04
15	Ranavirus	8,6E+04	1,9E+05	N/A	N/A
16	0	N/A	5,9E+04	1,9E+04	N/A
17	ECV / ESV	8,6E+05	1,3E+04	N/A	N/A
18	Negative	N/A	N/A	N/A	N/A
19	Rana - NOT EHNV	N/A	< 1,9E+02	N/A	N/A
20	Ranavirus - NOT EHNV	5,9E+05	1,3E+05	2,7E+05	4,0E+05
21	ECV	1,3E+05	1,3E+05	N/A	N/A
22	Ranavirus – NOT EHNV	1,3E+04	N/A	N/A	1,3E+06
23	Ranavirus - NOT EHNV	1,9E+05	8,6E+04	N/A	N/A
24	ECV viable virus	1,9E+05	< 1,9E+02	N/A	5,9E+04
25	Ranavirus	5,9E+02	2,7E+02	N/A	N/A
26	No VHSV, IHNV, SVCV, IPNV	N/A	8,6E+02	4,0E+02	N/A
27	Ranavirus	5,9E+05	1,3E+05	N/A	N/A
28	Ranavirus	4,0E+06	1,3E+07	2,7E+03	1,9E+06
29	ECV/ESV	4,0E+06	8,6E+05	N/A	N/A
30	ECV	5,9E+06	4,0E+05	N/A	N/A
31	N/A	N/A	N/A	N/A	N/A

32	ECV	5,9E+04	5,9E+04	N/A	N/A
33	ECV European Catfish Virus and VHSV	4,0E+03	< 1,9E+02	N/A	N/A
34	ECV/ESV	1,3E+05	5,9E+04	N/A	N/A
35	Ranavirus, ECV not EHNV	4,0E+05	2,7E+05	< 1,9E+02	N/A
36	ECV	1,9E+06	1,9E+06	< 1,9E+02	N/A
37	Ranavirus - NOT EHNV	4,0E+06	1,9E+05	N/A	N/A
38	ECV or ESV	4,0E+06	< 1,9E+02	N/A	N/A
39	No VHSV, IHNV, IPNV, SVCV	1,3E+07	5,9E+03	N/A	N/A
40	Ranavirus, not EHNV	4,0E+06	8,6E+05	4,0E+06	2,7E+04
41	Ranavirus – NOT EHNV	N/A	5,9E+04	1,3E+04	8,6E+04
42	EHNV	2,7E+05	4,0E+04	N/A	N/A
43	European Catfish Virus	2,7E+06	1,9E+05	4,0E+04	N/A
44	ECV	4,0E+06	1,9E+05	< 1,9E+02	1,9E+04
45	Ranavirus	5,9E+05	5,9E+05	N/A	N/A
46	NO IHNV,VHSV,SVCV,IPNV	5,87E+05	1,86E+05	N/A	N/A
47	Ranavirus NOT EHNV	1,3E+07	4,0E+05	2,7E+02	N/A
48	Ranavirus - NOT EHNV	4,0E+06	1,9E+05	N/A	N/A
49	Ranavirus (ECV/ESV)	8,6E+05	1,9E+06	< 1,9E+02	N/A

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

ECV	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	42	16	15
Median titre	1,6E+06	1,9E+05	2,0E+03	8,6E+04
Maximum titre	1,3E+08	1,3E+07	4,0E+06	1,9E+06
Minimum titre	5,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	2,7E+05	5,9E+04	<1,9E+02	1,6E+04
75% quartile titre	4,0E+06	7,9E+05	2,4E+04	4,9E+05

Table 6. Inter-Laboratory Proficiency Test, PT1, 2019 – Results of titration of **ampoule III**.

VHSV 4p101					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	VHSV	2,7E+06	1,3E+07	1,9E+05	N/A
2	VHSV	1,3E+06	4,0E+06	N/A	1,3E+07
3	VHSV	1,3E+07	5,9E+07	N/A	N/A
4	+VHSV	1,3E+07	5,9E+07	N/A	N/A
5	VHSV	5,9E+05	1,3E+07	N/A	N/A
6	VHSV	N/A	N/A	N/A	N/A
7	VHSV	5,9E+07	N/A	N/A	2,7E+07
8	VHSV	1,9E+08	5,9E+08	N/A	N/A
9	VHSV	5,9E+06	N/A	N/A	2,7E+07
10	VHSV	1,3E+07	8,6E+07	4,0E+06	4,0E+07
11	VHSV	8,62E+02	1,26E+05	N/A	2,73E+06
12	VHSV	5,87E+05	1,86E+06	< 1,9E+02	5,87E+02
13	VHSV	1,9E+04	N/A	N/A	1,3E+07
14	VHSV	2,7E+03	2,7E+03	1,3E+03	1,3E+03
15	VHSV	1,3E+06	2,7E+06	N/A	N/A
16	VHSV	N/A	1,9E+05	8,6E+04	N/A
17	VHSV	1,9E+06	1,3E+04	N/A	N/A
18	VHSV	N/A	N/A	N/A	N/A
19	VHSV	N/A	< 1,9E+02	N/A	N/A
20	VHS	4,0E+08	1,9E+08	1,3E+04	5,9E+07
21	VHSV+IHNV	1,3E+07	1,3E+09	N/A	N/A
22	VHSV and IHNV	1,3E+04	N/A	N/A	4,0E+06
23	VHSV	1,9E+06	8,6E+06	N/A	N/A
24	VHSV viable virus	8,62E+03	4,00E+03	N/A	< 1,9E+02
25	VHSV	5,9E+06	5,9E+06	N/A	N/A
26	VHSV	N/A	2,7E+08	2,7E+04	N/A
27	VHSV	5,9E+06	5,9E+06	N/A	N/A
28	VHSV	1,9E+07	5,9E+07	1,9E+03	2,7E+07
29	VHSV	1,3E+07	4,0E+06	N/A	N/A
30	VHSV	4,0E+07	4,0E+07	N/A	N/A
31	VHSV	N/A	N/A	N/A	N/A
32	VHSV	1,9E+07	2,7E+07	N/A	N/A
33	VHSV	2,7E+05	2,7E+06	N/A	N/A
34	VHS	2,7E+06	5,9E+05	N/A	N/A

35	VHSV	8,6E+06	2,7E+07	< 1,9E+02	N/A
36	VHSV	1,9E+06	1,3E+07	1,3E+04	N/A
37	VHSV	1,3E+07	4,0E+06	N/A	N/A
38	VHSV	8,6E+06	1,3E+08	N/A	N/A
39	VHSV	5,9E+07	4,0E+06	N/A	N/A
40	VHSV	4,0E+07	1,3E+07	1,9E+03	5,9E+06
41	VHSV	N/A	1,9E+07	5,9E+06	1,9E+06
42	VHSV	5,9E+06	4,0E+06	N/A	N/A
43	VHSV Genotype III	4,0E+06	1,9E+07	1,3E+03	N/A
44	VHSV	8,6E+06	1,9E+07	1,3E+03	5,9E+06
45	VHSV	4,0E+08	4,0E+07	N/A	N/A
46	VHSV	5,87E+06	1,26E+07	N/A	N/A
47	VHSV	8,6E+05	8,6E+06	1,9E+04	N/A
48	VHSV	8,6E+06	1,9E+07	N/A	N/A
49	VHSV	5,9E+06	8,6E+07	8,6E+02	N/A

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

VHSV 4p101	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	42	16	15
Median titre	5,9E+06	1,3E+07	7,3E+03	5,9E+06
Maximum titre	4,0E+08	1,3E+09	5,9E+06	5,9E+07
Minimum titre	8,6E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	1,4E+06	4,0E+06	1,3E+03	2,3E+06
75% quartile titre	1,3E+07	4,0E+07	4,2E+04	2,7E+07

Table 7. Inter-Laboratory Proficiency Test, PT1, 2019 – Results of titration of **ampoule IV**.

SVCV 203273					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	SVCV	4,0E+04	1,9E+06	4,0E+04	N/A
2	SVCV	2,7E+05	4,0E+05	N/A	5,9E+06
3	SVCV	< 1,9E+02	8,6E+06	N/A	N/A
4	+SVCV	4,0E+06	8,6E+06	N/A	N/A
5	SVCV	4,0E+04	2,7E+05	N/A	N/A
6	0	N/A	N/A	N/A	N/A
7	SVCV	5,9E+05	N/A	N/A	5,9E+06
8	SVCV	2,7E+06	2,7E+07	N/A	N/A
9	SVCV	2,7E+05	N/A	N/A	4,0E+05
10	SVCV	4,0E+05	1,9E+05	4,0E+05	2,7E+05
11	SVCV	4,00E+02	4,00E+03	N/A	4,0E+03
12	SVCV	8,6E+05	1,86E+06	1,3E+04	5,9E+05
13	0	< 1,9E+02	N/A	N/A	< 1,9E+02
14	SVCV	5,9E+04	2,7E+03	1,3E+03	1,3E+03
15	SVCV	1,9E+06	1,3E+06	N/A	N/A
16	SVCV	N/A	2,7E+03	5,9E+03	N/A
17	SVCV	2,7E+05	1,3E+03	N/A	N/A
18	SVCV	N/A	N/A	N/A	N/A
19	SVCV	N/A	< 1,9E+02	N/A	N/A
20	SVC	4,0E+07	4,0E+07	4,0E+04	5,9E+07
21	SVCV	1,3E+03	1,3E+06	N/A	N/A
22	SVCV	1,3E+04	N/A	N/A	2,7E+06
23	SVCV	5,9E+05	1,3E+06	N/A	N/A
24	SVCV viable virus	< 1,9E+02	2,7E+04	N/A	12649,11064
25	SVCV	2,7E+05	1,3E+06	N/A	N/A
26	SVCV	N/A	8,6E+04	1,3E+03	N/A
27	SVCV	2,7E+06	4,0E+05	N/A	N/A
28	SVCV	5,9E+06	2,7E+06	2,7E+05	1,9E+06
29	SVCV	5,9E+06	8,6E+05	N/A	N/A
30	SVCV	8,6E+06	1,3E+07	N/A	N/A
31	SVCV	N/A	N/A	N/A	N/A
32	SVCV	4,0E+06	1,3E+06	N/A	N/A
33	SVCV	4,0E+03	2,7E+05	N/A	N/A
34	SVC	4,0E+06	1,3E+06	N/A	N/A

35	SVCV	5,9E+05	1,3E+07	< 1,9E+02	N/A
36	SVCV	2,7E+05	1,3E+06	1,9E+05	N/A
37	SVCV	8,6E+05	4,0E+06	N/A	N/A
38	SVCV	4,0E+05	1,3E+07	N/A	N/A
39	SVCV	4,0E+04	8,6E+05	N/A	N/A
40	SVCV	8,6E+06	4,0E+06	2,7E+04	1,9E+06
41	SVCV	N/A	5,9E+04	2,7E+04	8,6E+04
42	SVCV	8,6E+05	1,9E+05	N/A	N/A
43	SVCV	2,7E+06	2,7E+05	4,0E+03	N/A
44	SVCV	1,3E+04	4,0E+06	< 1,9E+02	1,9E+06
45	SVCV	4,0E+06	8,6E+03	N/A	N/A
46	SVCV	1,3E+05	4,00E+05	N/A	N/A
47	SVCV	2,7E+03	1,9E+06	< 1,9E+02	N/A
48	SVCV	1,9E+05	1,3E+05	N/A	N/A
49	SVCV	5,9E+05	1,9E+06	1,3E+03	N/A

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

SVCV 203273	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	42	16	15
Median titre	4,0E+05	1,3E+06	9,3E+03	5,9E+05
Maximum titre	4,0E+07	4,0E+07	4,0E+05	5,9E+07
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	4,0E+04	1,9E+05	1,3E+03	4,9E+04
75% quartile titre	2,7E+06	2,5E+06	4,0E+04	2,3E+06

Table 8. Inter-Laboratory Proficiency Test, PT1, 2019 – Results of titration of **ampoule V**.

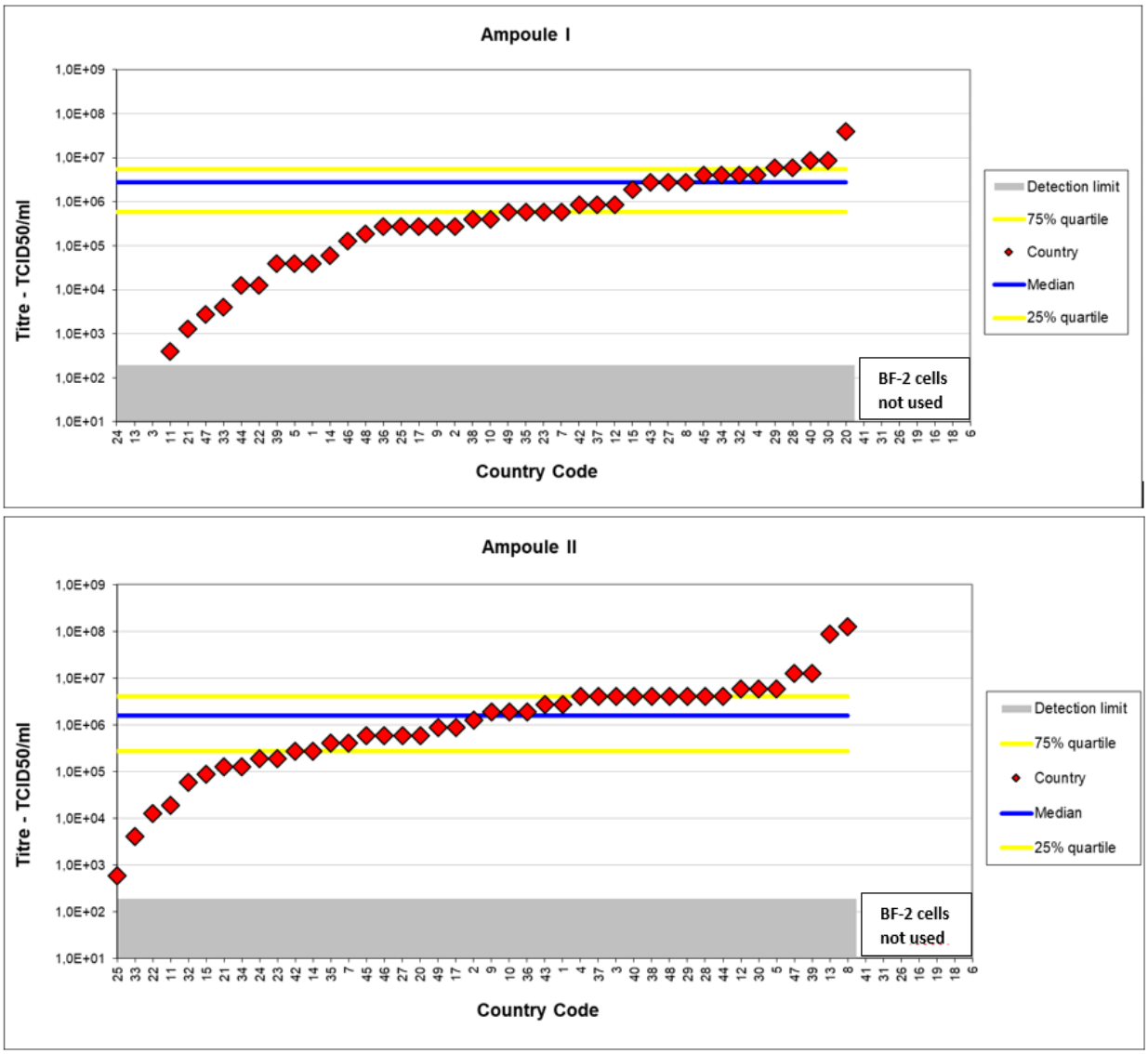
IPNV sp					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	IPNV	4,0E+04	4,0E+04	2,7E+02	N/A
2	IPNV	1,3E+04	8,6E+04	N/A	1,9E+03
3	IPNV	1,9E+05	2,7E+04	N/A	N/A
4	+ IPNV	1,3E+05	2,7E+04	N/A	N/A
5	IPNV	4,0E+04	4,0E+04	N/A	N/A
6	0	N/A	N/A	N/A	N/A
7	IPNV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

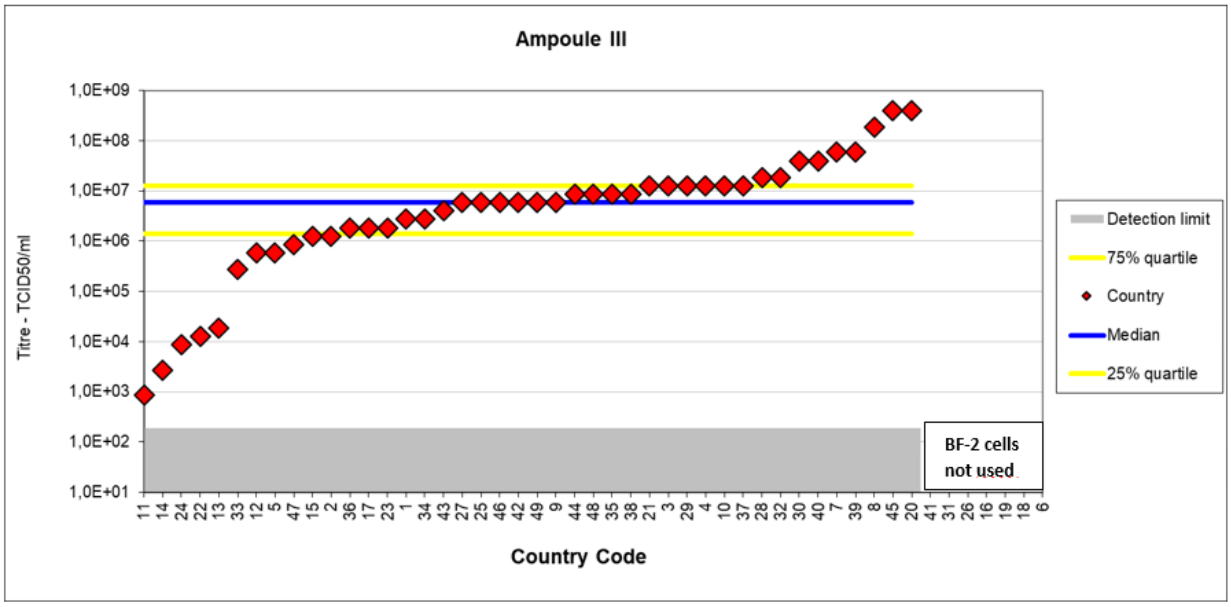
35	IPNV	2,7E+05	2,7E+05	8,6E+03	N/A
36	IPNV	4,0E+04	1,9E+05	4,0E+04	N/A
37	IPNV	4,0E+04	2,7E+04	N/A	N/A
38	IPNV	8,6E+04	1,3E+04	N/A	N/A
39	IPNV	5,9E+05	5,9E+03	N/A	N/A
40	IPNV	1,3E+05	1,3E+05	8,6E+03	2,7E+04
41	IPNV	N/A	1,3E+04	1,9E+04	1,3E+04
42	IPNV	2,7E+05	1,3E+04	N/A	N/A
43	IPNV	5,9E+04	1,9E+04	1,9E+02	N/A
44	IPNV	2,7E+04	4,0E+04	< 1,9E+02	< 1,9E+02
45	IPNV	1,9E+07	5,9E+05	N/A	N/A
46	IPNV	1,3E+05	1,9E+04	N/A	N/A
47	IPNV	1,9E+05	2,7E+05	2,7E+04	N/A
48	IPNV	2,7E+05	4,0E+04	N/A	N/A
49	IPNV	5,9E+04	1,3E+04	5,9E+03	N/A

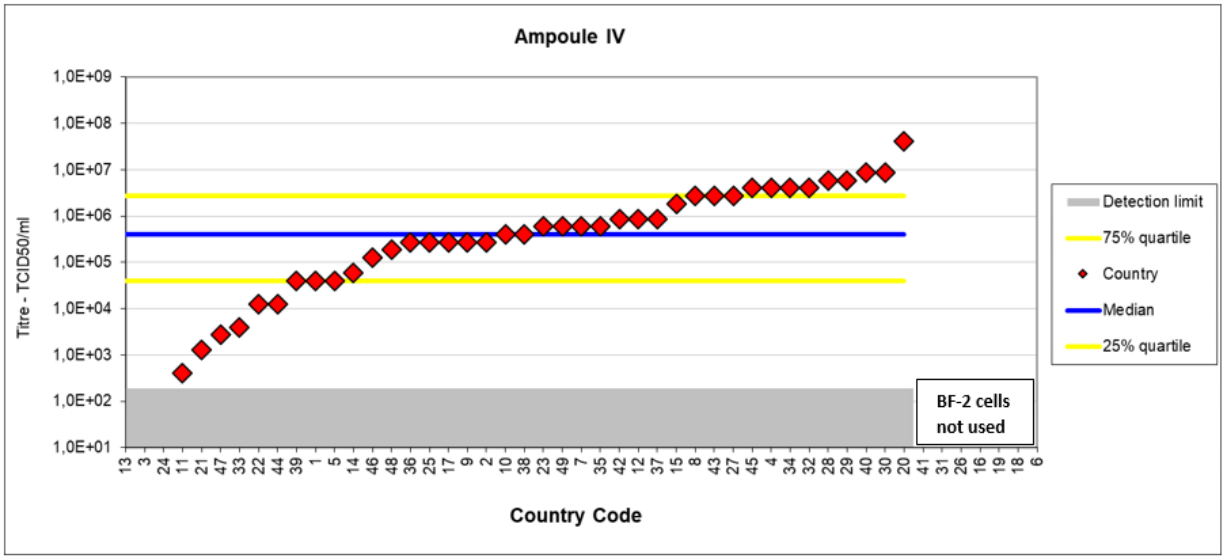
N/A: Cell line not applied by the participating laboratory for titration of the virus

IPNV sp	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	42	42	17	15
Median titre	1,3E+05	2,7E+04	1,3E+04	1,3E+04
Maximum titre	1,9E+07	5,9E+05	4,0E+04	1,9E+05
Minimum titre	5,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	4,0E+04	1,3E+04	2,7E+02	1,6E+03
75% quartile titre	1,9E+05	5,9E+04	2,7E+04	3,4E+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+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
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
Country Code

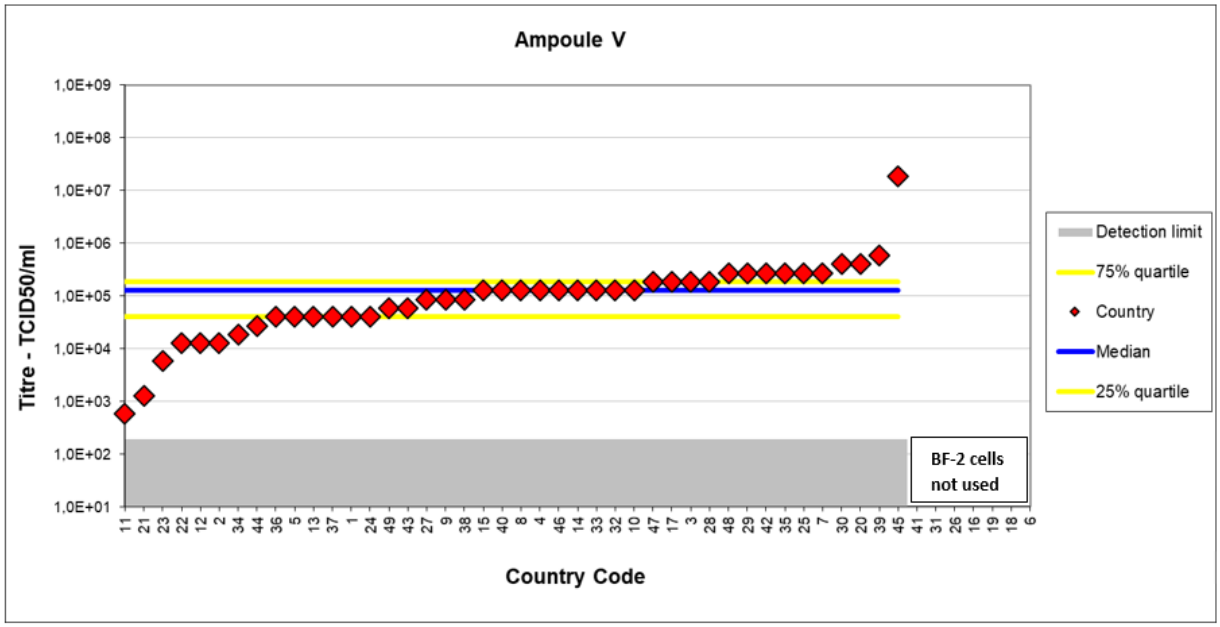
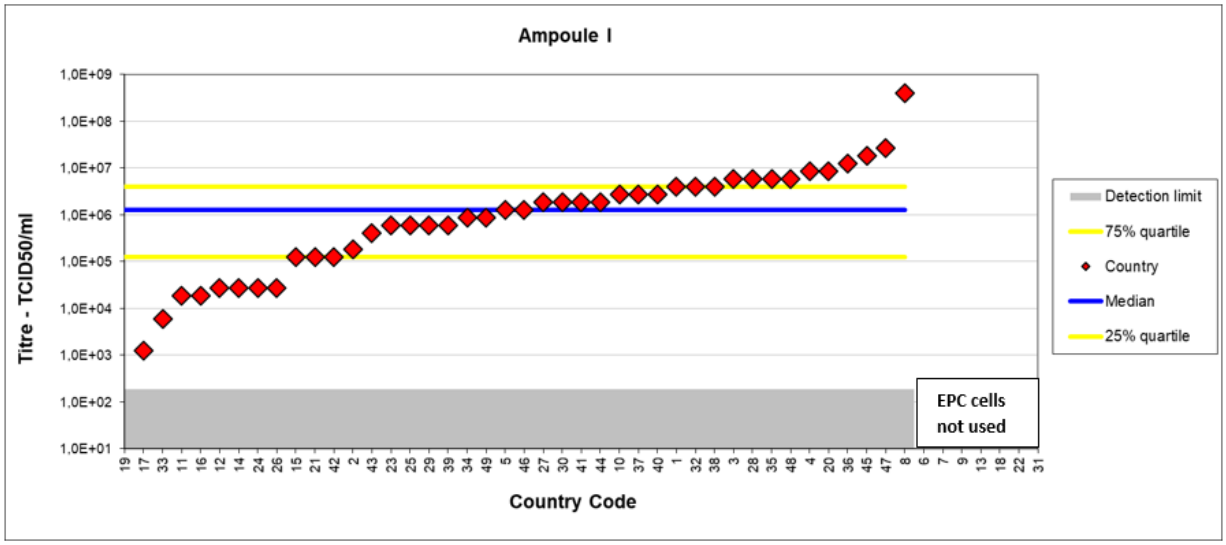
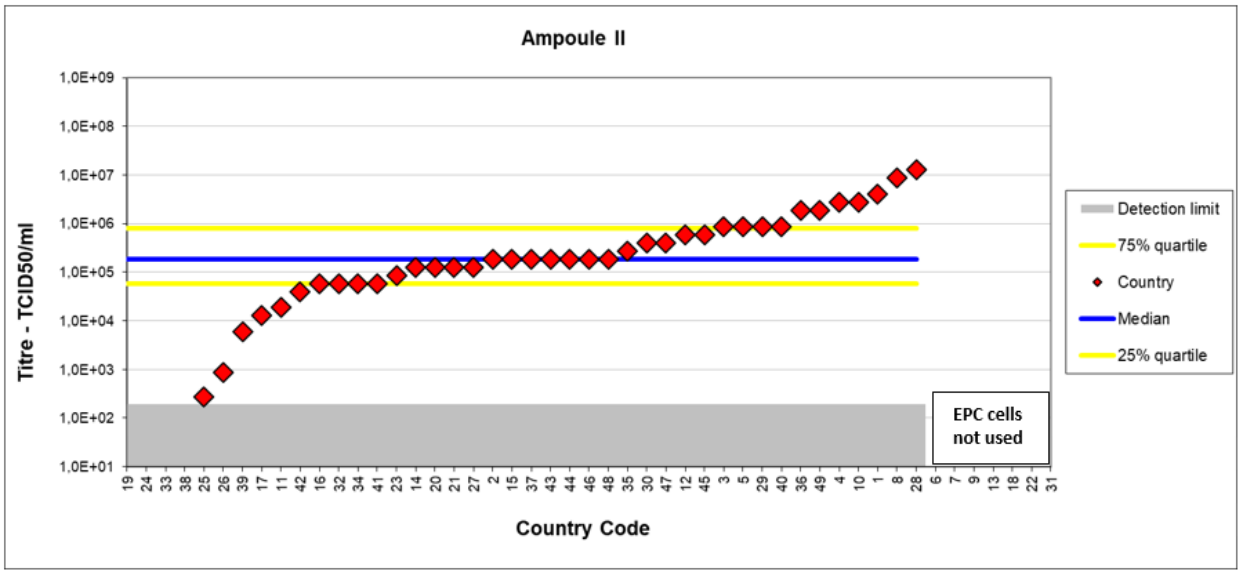
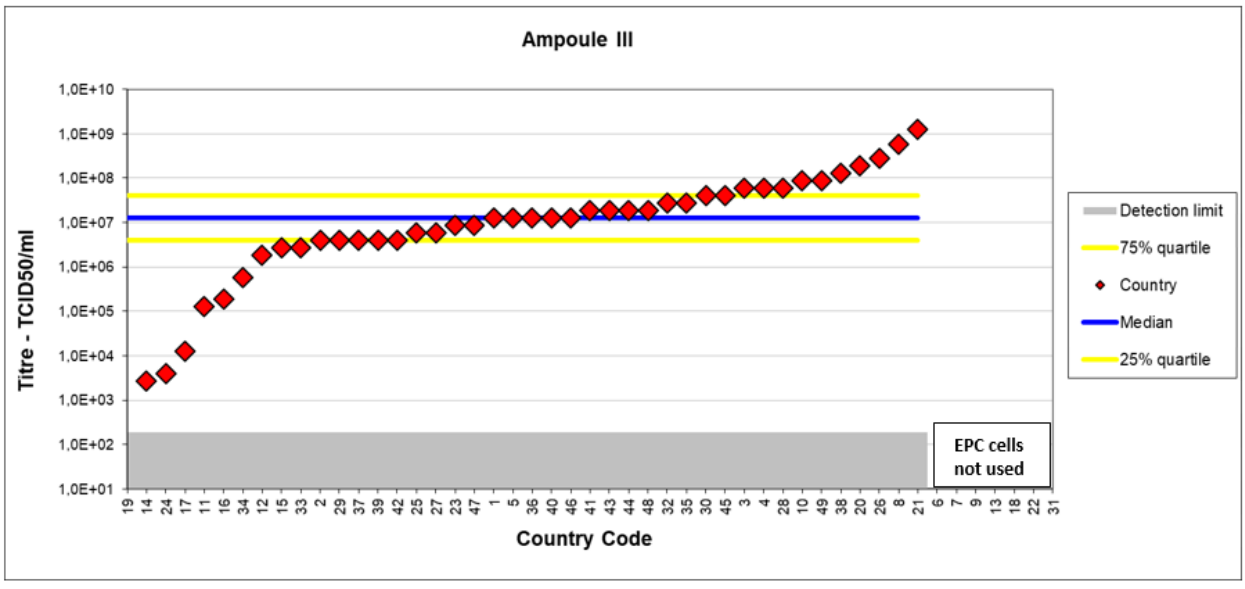
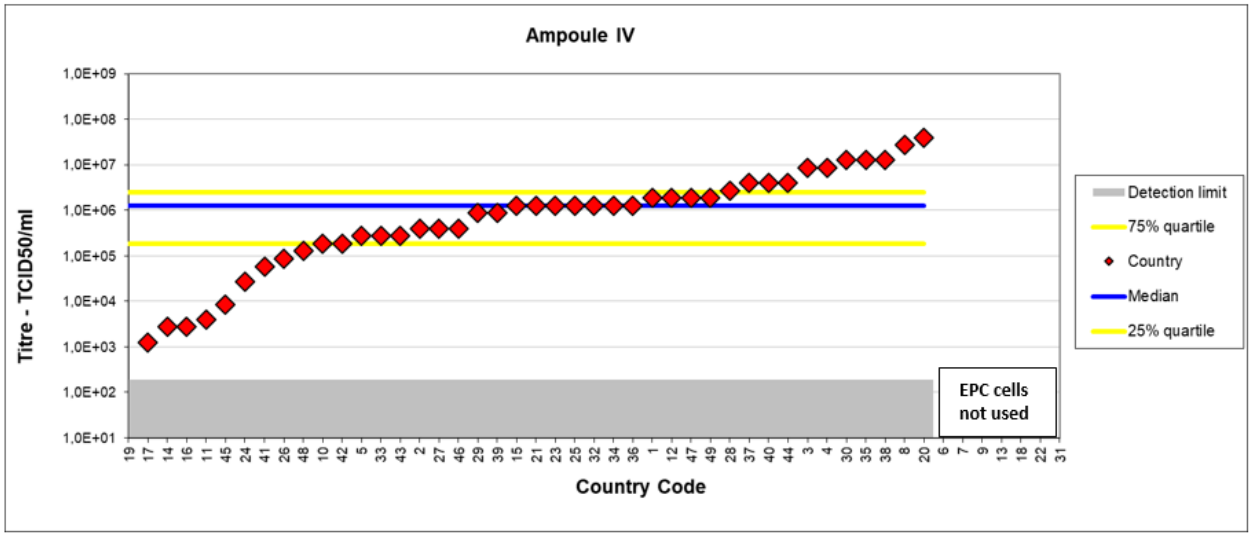

Ampoule V
Titre - TCID50/ml
1,0E+09
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
Detection limit
75% quartile
Country
Median
25% quartile
BF-2 cells not used
Country Code

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








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
19 17 14 16 11 45 24 41 26 48 10 42 5 33 43 2 27 46 29 39 15 21 23 25 32 34 36 1 12 47 49 28 37 40 44 3 4 30 35 38 8 20 6 7 9 13 18 22 31
Country Code
EPC cells not used
Detection limit
75% quartile
Country
Median
25% quartile

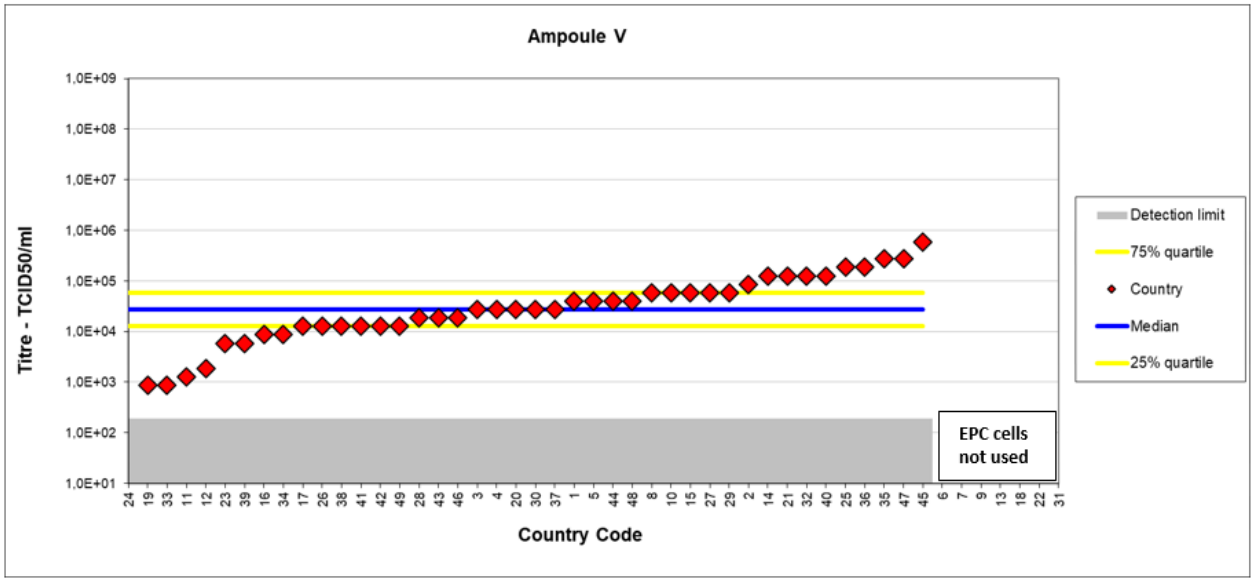
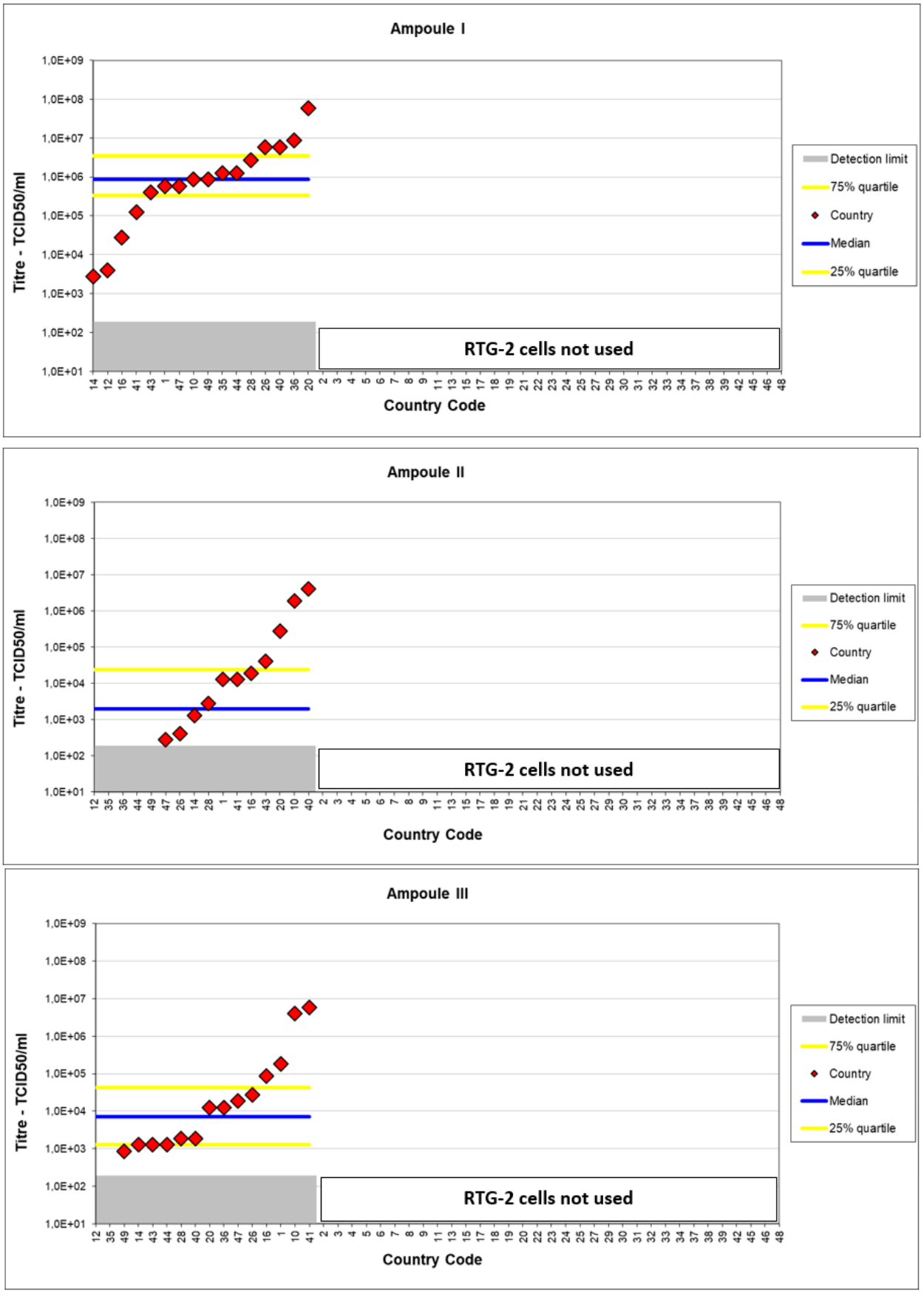
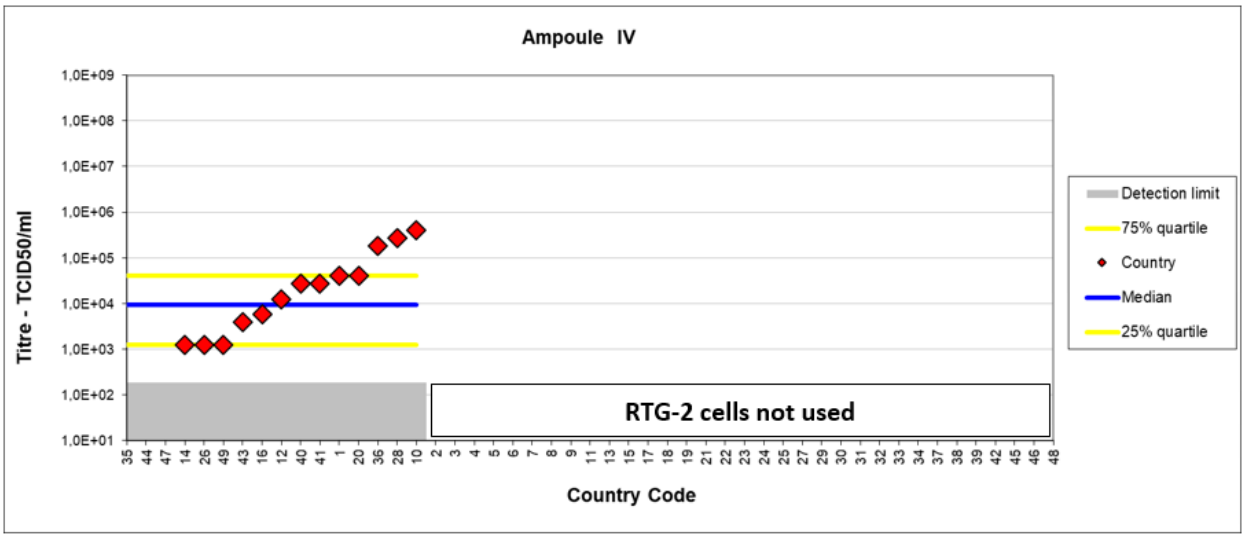

Ampoule V
Titre - TCID50/ml
1,0E+09
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
24 19 33 11 12 23 39 16 34 17 26 38 41 42 49 28 43 46 3 4 20 30 37 1 5 44 48 8 10 15 27 29 2 14 21 32 40 25 36 35 47 45 6 7 9 13 18 22 31
Country Code
EPC cells not used
Detection limit
75% quartile
Country
Median
25% quartile

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




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
RTG-2 cells not used
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

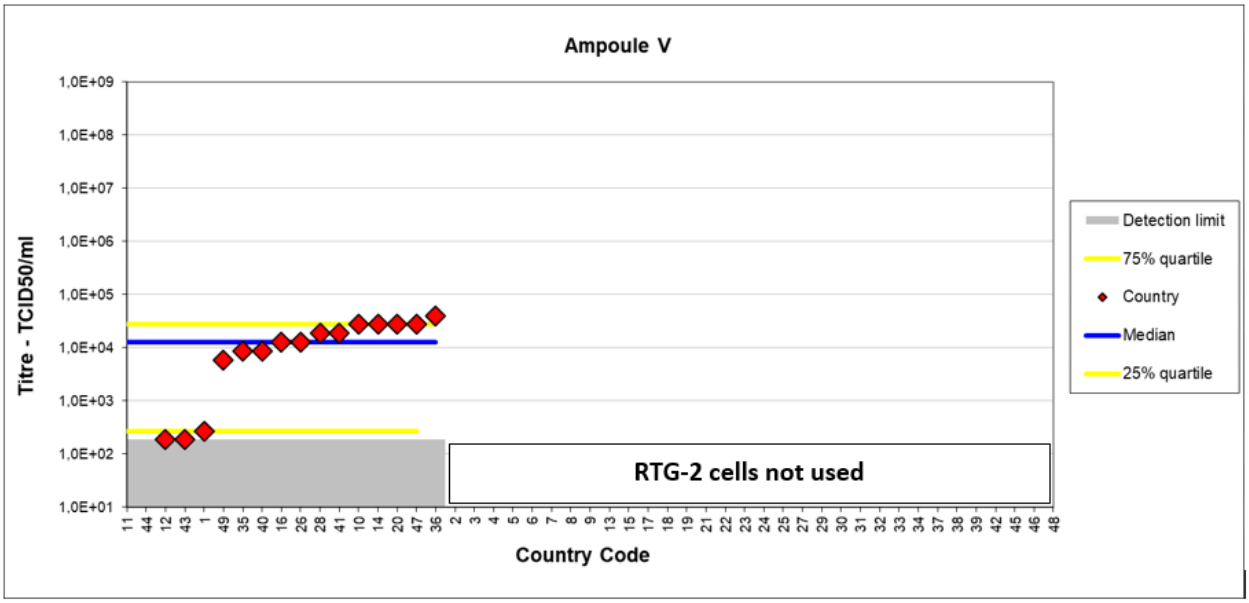
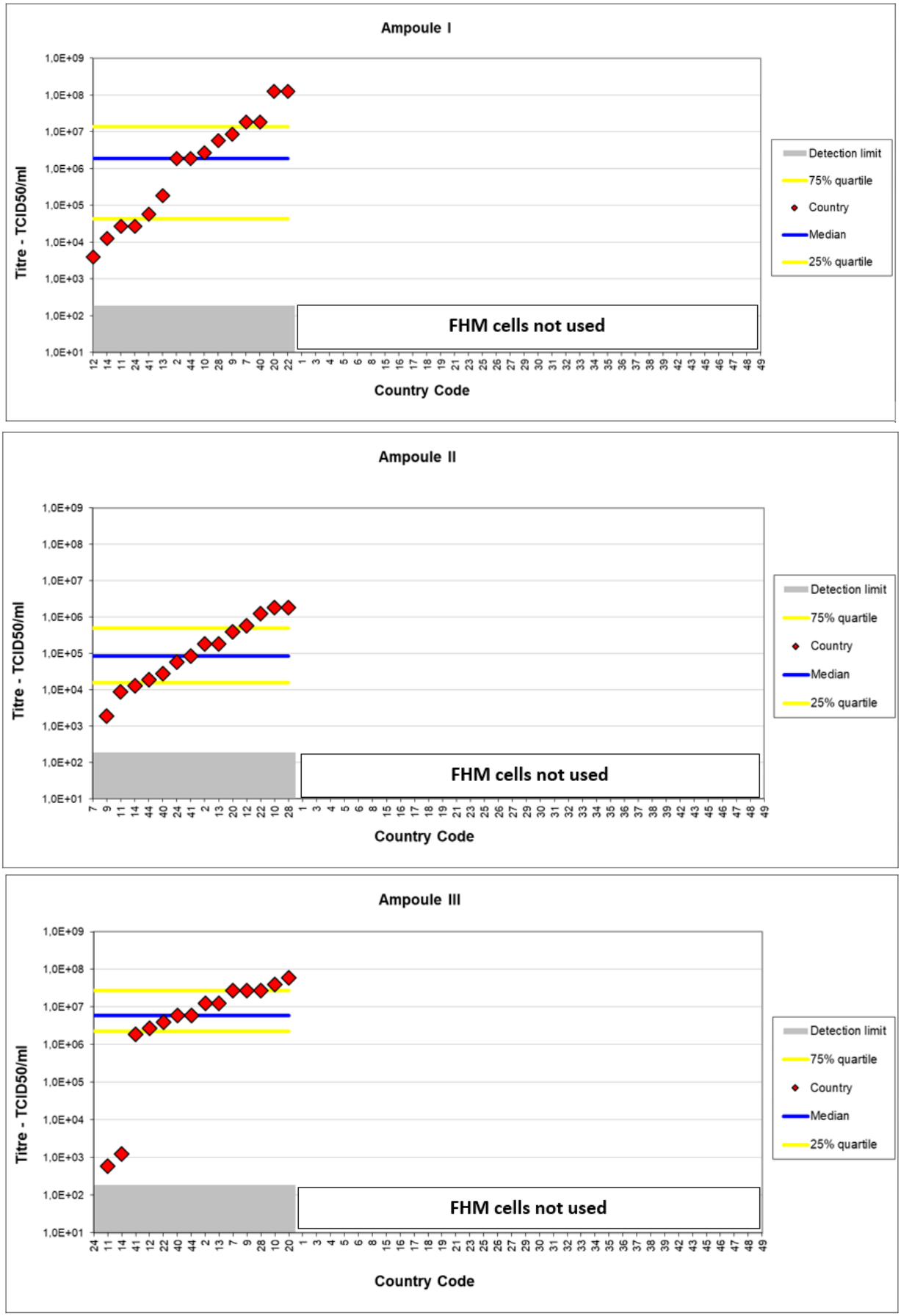
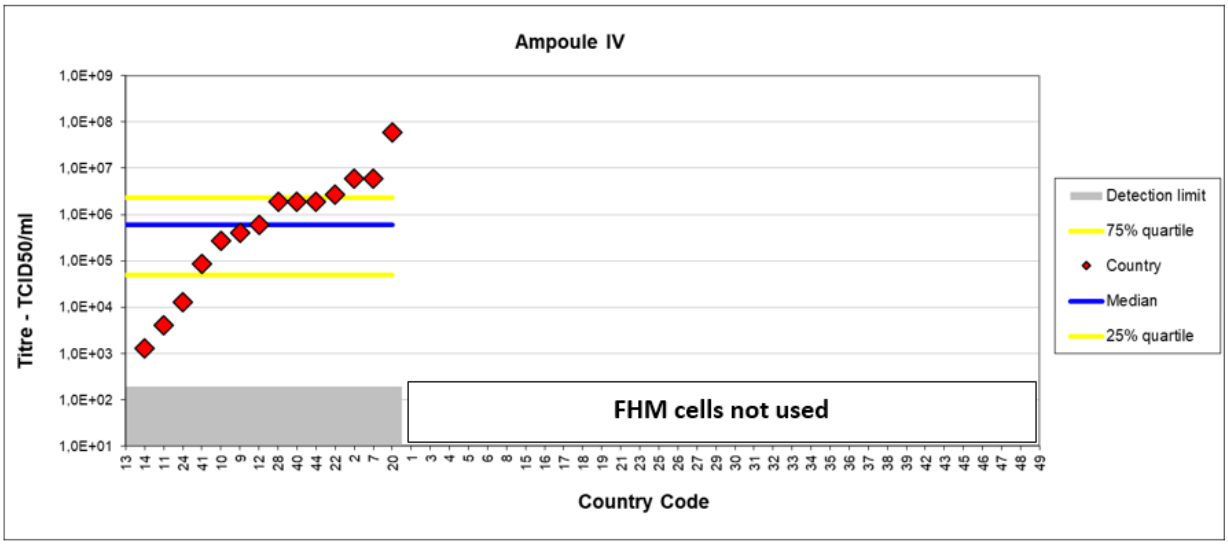
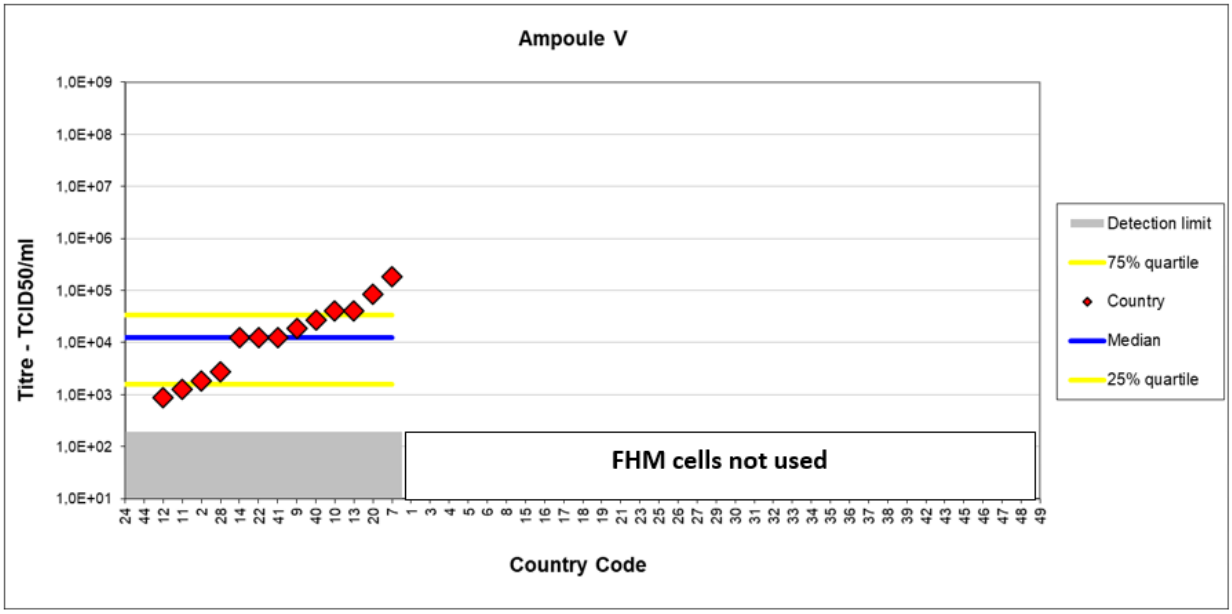

Ampoule V
Titre - TCID50/ml
1,0E+09
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
RTG-2 cells not used
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

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




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
13 14 11 24 41 10 9 12 28 40 44 22 2 7 20 1 3 4 5 6 8 15 16 17 18 19 21 23 25 26 27 29 30 31 32 33 34 35 36 37 38 39 42 43 45 46 47 48 49
Country Code
FHM cells not used
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
24 44 12 11 2 28 14 22 41 9 40 10 13 20 7 1 3 4 5 6 8 15 16 17 18 19 21 23 25 26 27 29 30 31 32 33 34 35 36 37 38 39 42 43 45 46 47 48 49
Country Code
FHM cells not used
Detection limit
75% quartile
Country
Median
25% quartile

Identification of content

- 42 laboratories out of 49 participants analysed for all viruses; 34 of these laboratories correctly identified all viruses in all ampoules

Ampoule I – VHSV (DK-9695377) + IHNV (32/87)

- 45 laboratories correctly identified both VHSV and IHNV in ampoule I
- 3 laboratory did not identify IHNV
- 1 laboratory did not identify VHSV

Ampoule II – ECV

- 39 laboratories correctly identified ECV or 'Not EHNV' by sequencing or REA (restriction enzyme analysis) in ampoule II
- 1 laboratory identified Ranavirus but did not specify if the isolate was the listed EHNV or not by sequencing or REA
- 1 laboratory answered EHNV even though the sequence showed ECV.
- 1 laboratory correctly identified ECV by sequencing but contaminated the ampoule with another virus (VHSV)
- 1 laboratory incorrectly identified IHNV in the ampoule.
- 6 laboratories do not test for Ranavirus

Ampoule III – VHSV (4p101)

- 47 laboratories correctly identified VHSV
- 2 laboratories correctly identified VHSV but contaminated the ampoule with another virus (IHNV)

Ampoule IV – SVCV (203273)

- 47 laboratories correctly identified SVCV in ampoule IV
- 2 laboratory did not identified SVCV and answered '0'

Ampoule V – IPNV (sp)

- 46 laboratories correctly identified IPNV in ampoule V
- 1 laboratory did not identified IPNV but incorrectly identified SVCV in the ampoule.
- 1 laboratory correctly identified IPNV but contaminated the ampoule with another virus (IHNV)
- 1 laboratory do not test for IPNV

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 and IHNV identification was given the score 2. Finding of only one of the listed viruses included in the ampoule scored 0.

Ampoule II: ECV or 'Not EHNV' identification was given the score 2. The answer Ranavirus with no identification was given the score 1. Incorrectly finding of other type of viruses than the one included in the ampoule scored 0. Identification of ECV (non-listed virus) but also finding of additional type of viruses scored 0 if the contamination was with a listed pathogen. The answer EHNV but with sequencing identification of ECV was given the score 1.

Ampoule III: VHSV identification was given the score 2. Identification of VHSV (listed virus) but also finding of additional type of viruses scored 1.

Ampoule IV: SVCV identification was given the score 2. Finding of "no virus" in the ampoule scored 0.

Ampoule V: IPNV identification was given the score 2. Incorrect finding of other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen.

Out of 49 laboratories participating in the PT 1 2019, 34 obtained score 10/10. The score 8/8 was assigned to 5 participants as they did not test Ranavirus. Scores below 100% were obtained due to above-mentioned causes.

Cells applied for solving the test

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

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

- 1 laboratory used only one cell line (EPC) and 3 laboratory did not titrate.

The combination of EPC and FHM cells or BF-2 and RTG 2 alone is not valid according to Commission Decision 2015-1554. The laboratories 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-9695377 + IHNV 32/87) replicates equally on all four cell lines (EPC, BF-2, FHM and RTG-2).
- Ampoule II (ECV) replicate on all four cell lines, however it grows best on BF-2 cells, a little less efficiently on EPC and FHM and less on RTG-2.
- Ampoule III (VHSV, 4p101) replicates on all four cell lines, equally on cell lines EPC, BF-2 and FHM and less on RTG-2.
- Ampoule IV (SVCV, 203273) replicates on all four cell lines, equally on cell lines EPC, BF-2 and FHM and less on RTG-2.
- Ampoule V (IPNV, Sp) replicates on all four cell lines, however it grows best on BF-2 cells, a little less efficiently on EPC, FHM and RTG-2.

As it appears from Table 3-8 the variations in titres between laboratories was very high – with more than 7 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved and the ones with a high titre should ensure to follow the correct titration procedure.

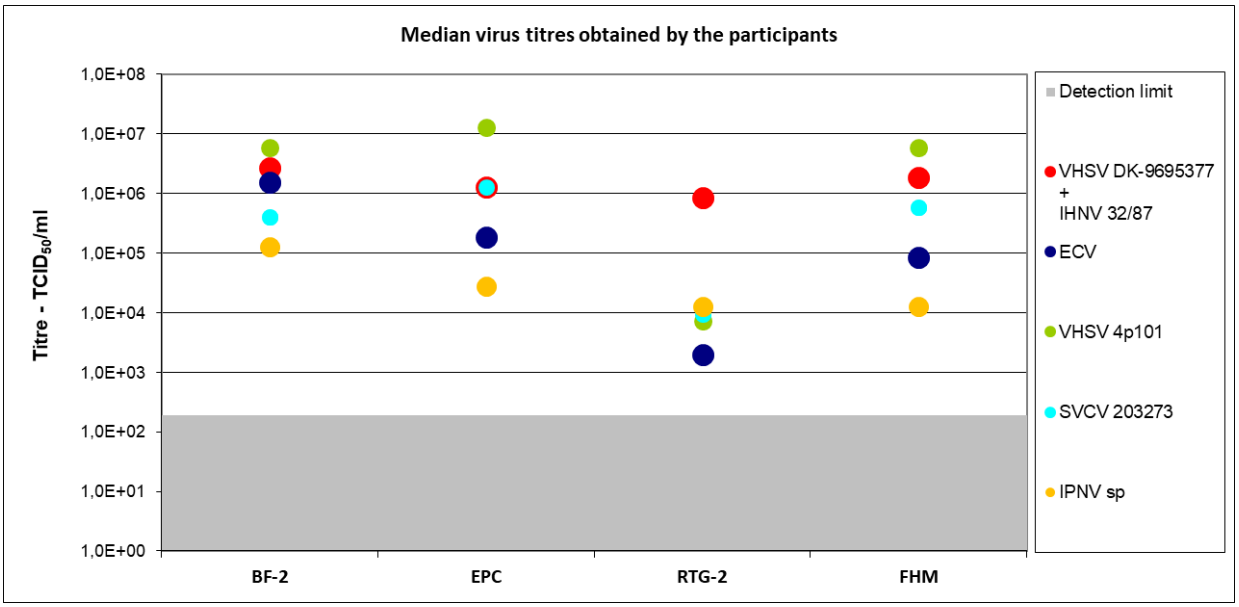


Figure 9. Median virus titres obtained by the participants in 4 different cell lines.

Genotyping and sequencing

In previous proficiency tests provided by the EURL, we have encouraged participants to genotype the identified viruses though it has not been a mandatory task. As ranaviruses are included in the test, it is mandatory to do sequence or REA analysis in order to discriminate EHNV from the non-listed ranaviruses. For IHNV and VHSV we still encouraged participants to genotype isolates according to the notification ; described in Einer-Jensen et *al*. (2004) [5]for VHSV and in Kurath et *al*. (2003) [7]and 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 9.

The EURL has disclosed the content of the ampoules after deadline for delivering results. In that occasion a Typo mistake occurred, as the SVCV isolate was incorrectly described as belonging to genotype “Id”. This has been now amended and the correct genotype “Ia” is now reported in the isolate description at page 6 of this report.

Sequencing of Ampoule II 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 8.

For Ampoule I, 1 point was given for the correct identification of VHSV genotype Ia and 1 point for the correct identification of IHNV genotype E or M

For ampoule III, 2 points for the correct identification of VHSV genotype III

For Ampoule IV, 2 points for the correct identification of SVCV genotype Ia

For Ampoule V, 2 points for the correct identification of IPNV genogroup 5; 1 point was given to participants who correctly identified IPNV as SP but provided serogroup instead of genogroup.

Table 9. The genotyping results obtained for PT1 by all 49 participants

Code number	Score	Ampoule I	Ampoule III	Ampoule IV	Ampoule V
		VHSV gen. Ia IHNV gen. E	VHSV gen IIIa	SVCV gen. Ia	IPNV genogroup 5
1	6/8 [1]	VHSV genotype Ia; IHNV European M based on N gene sequence	VHSV genotype III	SVCV Ia	0
2	5/8 [2]	IHNV genogroup M	VHSV 1a-1	type 1a	Serotype=Sp genogroup 5
3	7/8 [21]	IHN: M VHS: 1a	III	1A	A2
4	7/8 [3]	IHNV - M VHSV - 1a	III	1a (Asian)	N/A
5	n/a	0	0	0	0
6	n/a	0	0	0	0
7	n/a	0	0	0	0
8	6/6	IHN: Genotype: M Subgroupe: M-Eur1 VHS: Genotype: I, Genotype Subtype: Ia	Genotype: III Genotype Subtype: III	Ia	0

9	6/6	VHSV Ia, IHNV E	III	0	5
10	8/8 [4]	VHSV Ia IHNV M	III	Genogroup I a	0
11	5/8 [5]	Genogroup-E	Ia	Ia	Genogroup-5
12	8/8 [6]	IHNV : European isolates (E–1–l–1) VHSV : Ia	III	Ia	0
13	n/a [7]	0	0	0	0
14	7/8 [8]	VHSV - Ia; IHNV - M	III	Ia	strain Sp
15	n/a [9]	0	0	0	0
16	n/a	0	0	0	0
17	n/a	0	0	0	0
18	n/a	0	0	0	0
19	4/4 [10]	VHSV: I-a IHNV: M or E	III	0	0
20	4/4	IHN: M , VHS: Ia	III	-	-
21	8/8	IHNV: E ; VHSV: Ia	VHSV:III;	Ia	V
22	4/6[11]	VHSV:Genotype I IHNV:Genotype M	VHSV:Genotype I IHNV:Genotype M	genogroup I	0
23	6/6 [12]	IHNV: E; VHSV: Ia	III	0	5
24	5/6 [13]	IHN is type M. VHS is 1a	3	0	Serotype Sp, serogroup A
25	2/4 [14]	VHS-1a IHNV- M	1a	N/A	IPNV
26	n/a	0	0	0	0
27	4/4	VHSV - Ia; IHNV - E	III	0	0
28	7/8 [15]	IHNV: M VHSV: Ia	III	Ia	Serptype: Sp
29	8/8	VHSV: Genotype Ia (sublineage: Ia1); IHNV: Genogroup E	Genotype III	Genogroup Ia	VP1: Genogroup 2; pVP2: Genogroup V
30	7/8 [16]	VHSV 1a	III	Genogroup I (from Stone et al., 2003)	Genogroup 5 (Sp) (Buyukekiz JFD 2017)
31	n/a	0	0	0	0
32[5]	n/a[17]	Blast analysis of the sequence showed the highest sequence identity with accession nr. MK829413.1 for VHSV and LT627085.1 for IHNV	Blast analysis of the sequence showed the highest sequence identity with accession nr. MK829397.1	Blast analysis of the sequence showed the highest sequence identity with accession nr. DQ227504.1	0
33	n/a	0	0	0	0
34	4/4	Ia	III	0	0
35	8/8	Ia, E	III	Ia	5
36	8/8	VSHV : genotype Ia IHNV : genotype E	Genotype III	Genotype Ia	Genogroup 5
37	6/8 [18]	VHSV Ia, IHNV genotype M	III	0	Genogroup 5
38	7/8 [19]	VHSV_Genotype=Ia. IHNV_Genotype=E	VHSV_Genotype=III	SVCV_genogroup=Ia	IPNV=Sp

39	n/a	0	0	0	0
40	8/8	IHNV genotype E VHSV genotype Ia	Genotype III	Genotype Ia	Genotype 5
41	5/8 [20]	VHSV: III , IHNV: E	I a	I a	5
42	8/8	VHSV I a IHNV E	III	1a	Genogroup 5
43	4/4	VHSV Genotype I subtype Ia. IHNV Genogroup M	III	0	0
44	8/8	IHNV - genotype E VHSV - Ia	III	Ia	5
45	n/a	0	0	0	0
46	n/a	0	0	0	0
47	n/a	0	0	0	0
48	8/8	VHS genotype Ia IHN genotype: M	Genotype III	Genotype Iaii MO, USA	Genotype 5
49	8/8	IHNV Genogroup M; VHSV Genotype 1a	VHSV Genotype III	SVCV Genogroup 1a	IPNV Genogroup 5
No. of participants performing sequencing		**44**	**34**	**24**	**22**
No. of participants getting full score		**41**	**28**	**23**	**16**
No. of correct sequences provided without genotype assigned or incomplete sequence		**2**	**1**	**1**	**6**
No. of incorrect genotype provided		**1**	**5**	**0**	**0**

[1] did sequence all ampoules but did not provide genotype for Ampoule V
[2] did not provide genotype for VHSV isolate in Ampoule I and gave wrong Genotype for Ampoule III
[3] did sequence ampoule V but did not provide its genotype
[4] did sequence Ampoule V but did not provide its genotype
[5] did not sequence VHSV in Ampoule I and gave the wrong genotype for Ampoule III
[6] did sequence ampoule V but did not provide its genotype
[7] did sequence isolates in ampoule I, III and V but did not provide genotype for those
[8] did sequence isolate in ampoule V and identified the right isolate but provided the wrong genotype
[9] did sequence ampoule 2 and 4 but did not provide genotype for Ampoule IV
[10] did sequence all ampoules in PT1 but provided genotype only for Ampoule I,II and III
[11] did identify the wrong isolates in ampoule III
[12] did not sequence ampoule IV
[13] did sequence ampoule IV but did not provide a genotype, and described the genotype of ampoule V as serotype
[14] did sequence all ampoule in PT 1 but provided genotype only for Ampoule I,II and III. Genotype provided for ampoule III is incorrect.
[15] described the genotype of ampoule V as serotype
[16] did not sequence the IHNV isolate in Ampoule I
[17] did sequence 4 isolates out of 5 in PT1 but did not provide any genotype
[18] did sequence ampoule IV but did not provide its genotype
[19] did sequence ampoule V and described the genotype of ampoule V as serotype
[20] provide an incorrect genotype for VHSV in ampoule I and in ampoule III
[21] did sequence ampoule V and described the genotype of ampoule V as serotype

Résumé and concluding remarks PT1

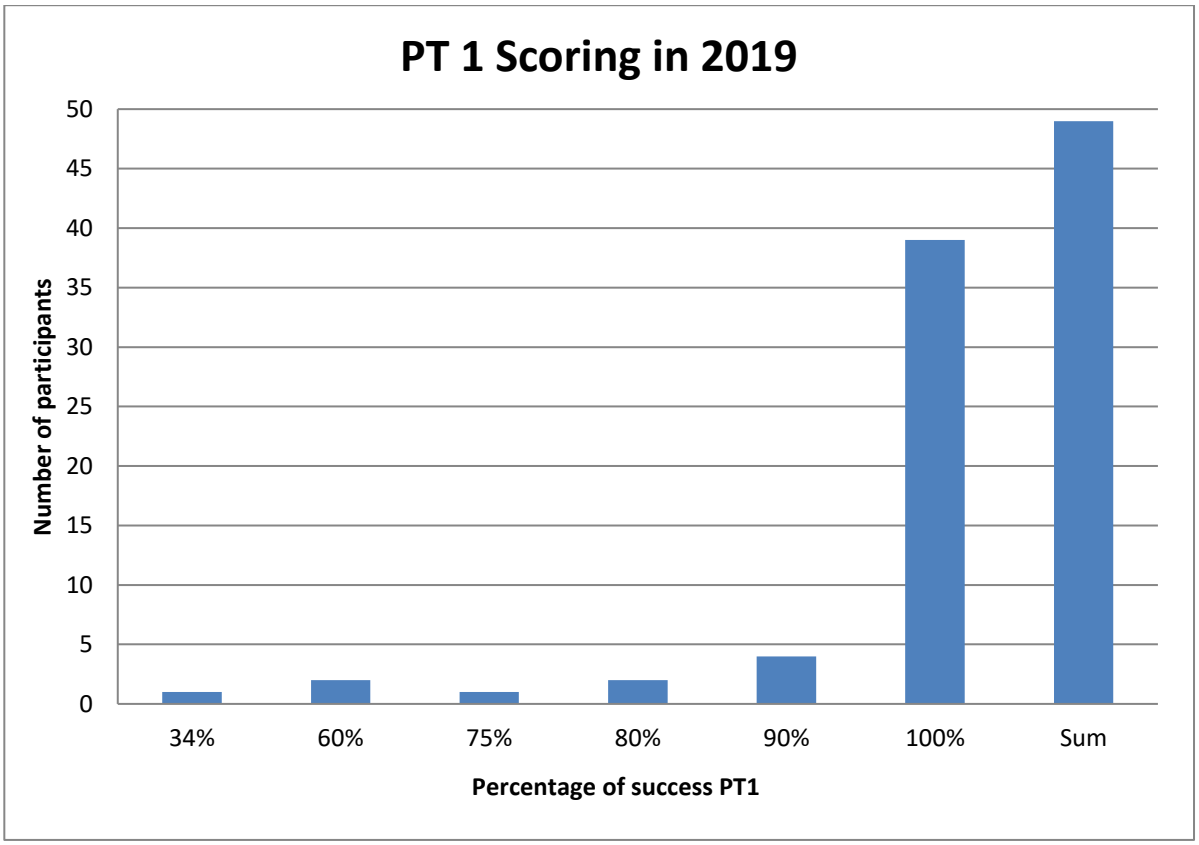
The parcels were submitted on a Friday and 51% of parcels were delivered by the shipping companies the following Monday, 86% was delivered within 1 week and 98% was delivered within 23 days. One parcels took 38 days due to internal clearance problems.
Overall 39 out of 49 participants scored 100% success rate; 4 participant scored 90% due to sequencing of the content in ampoule II (ECV) or contamination in ampoule III (VHSV) and 2 participants scored 80% due to not finding one of the virus. 4 participants scored below 80% due to not finding one of the virus or/and contamination of the ampoule with another virus.
These points will be addressed directly with the single 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 very high – with more than 7 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved and the laboratories scoring very high titres should ensure that the titration procedure is properly implemented.

For the first time this year we have scored the sequencing results of all participating laboratories.
For each ampoules 2 points were given. Ampoule II, which contained the ranavirus, was not included in this exercise being the sequence of virus already assessed in the main scoring.
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 (15 out of 49) to laboratories which do not sequence any of the isolates included in the ampoule (13 out of 49).
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.

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.

The results presented in this report will be further presented and discussed at the 24th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 2nd and 3rd of June, 2020 in Kgs. Lyngby, Denmark.



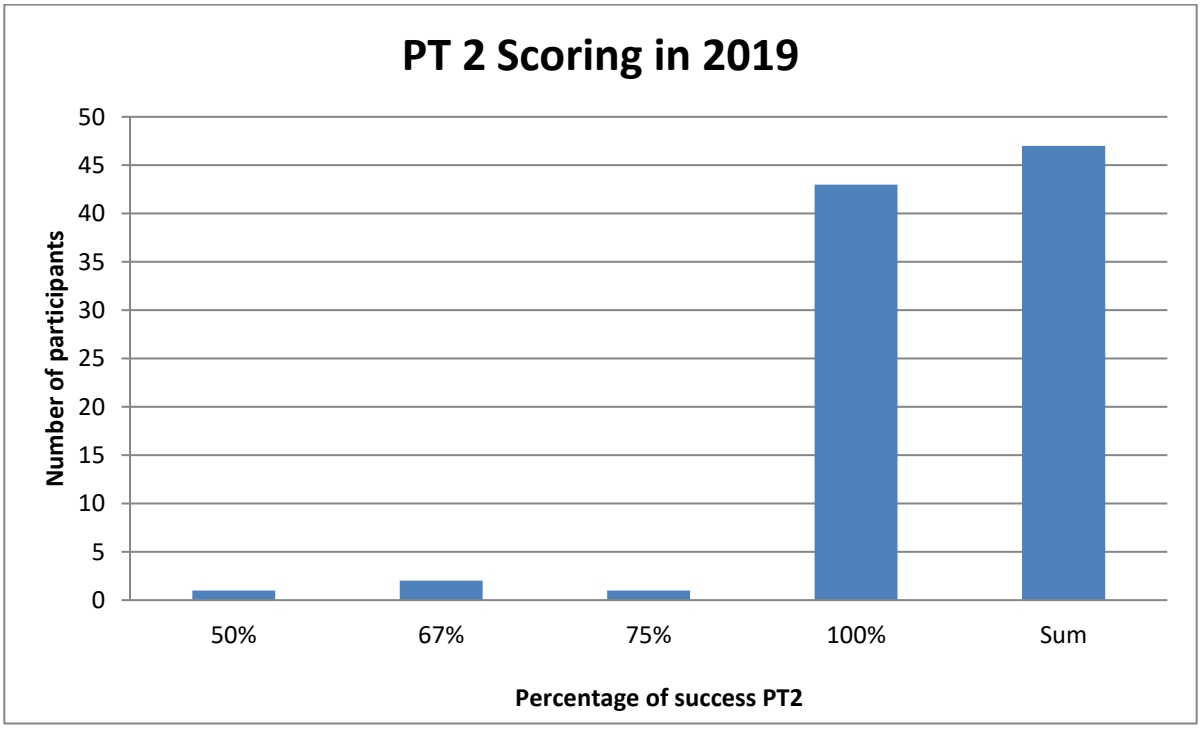


Figure 10 "a" and "b" Success-rate of participating laboratories 2019

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 two laboratories that participated only in PT1.

Content of ampoules

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

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

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

Code	Specifications/References
Ampoule VI: ISAV	**Infectious Salmon Anaemia Virus** 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 VII: 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
Ampoule VIII: SAV	**Salmonid AlphaVirus, (SAV2), MR-N1-2011**. Isolated from Atlantic salmon original name **Genbank accession number** LT630445 **Received from:** Norwegian Veterinary Institute, OIE ref lab for SAV dr. Hilde Sindre **Reference on isolate and sequence** Taksdal,T., Jensen,B.B., Bockerman,I., McLoughlin,M.F.,Hjortaas,M.J., Ramstad,A. and Sindre,H. Mortality and weight loss of Atlantic salmon, Salmon salar L., experimentally infected with salmonid alphavirus subtype 2 and subtype 3 isolates from Norway Journal of fish diseases 38 (12), 1047-1061 (2015)
Ampoule IX: ISAV	**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

Testing of the test

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

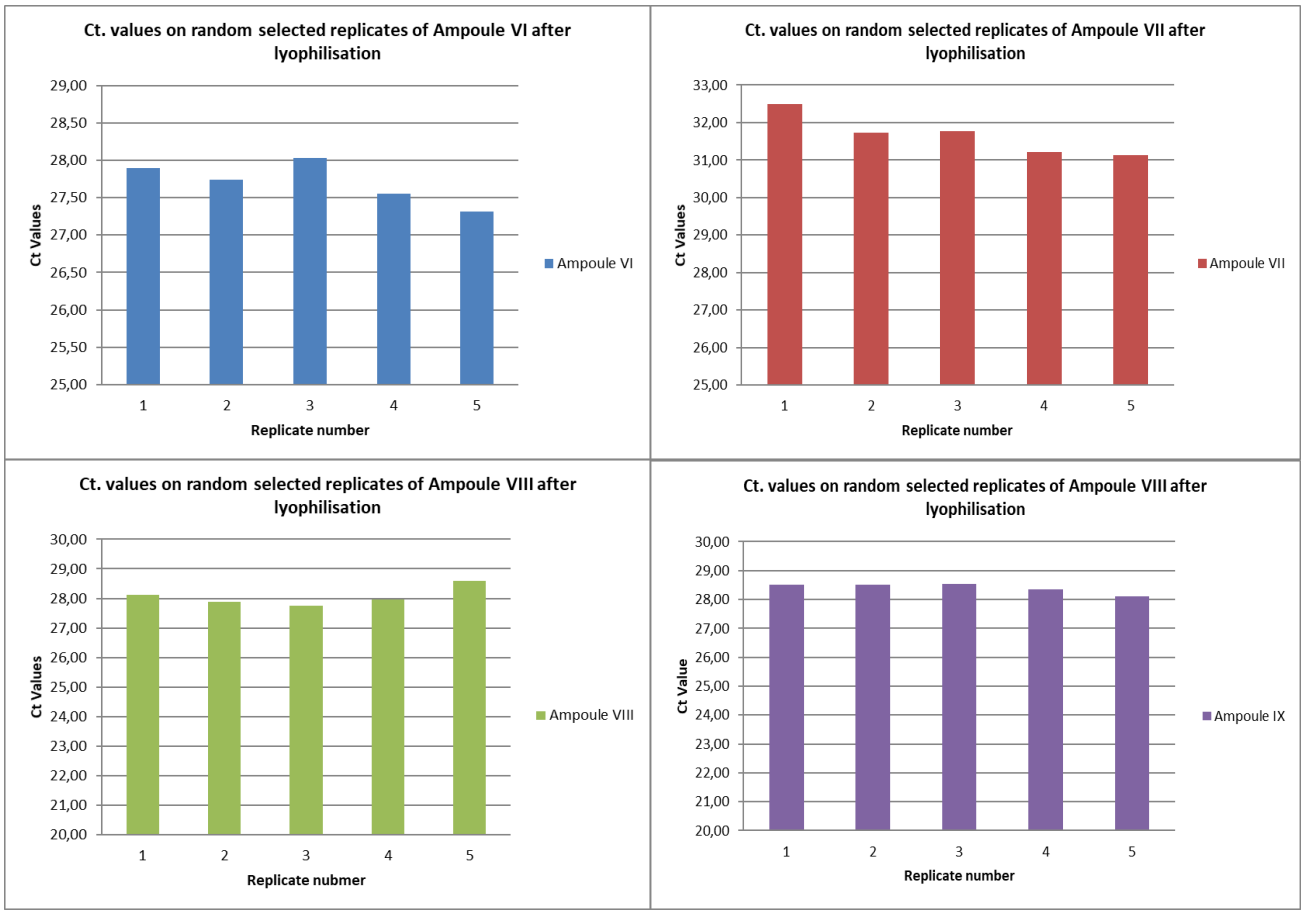


Figure 11, Ampule VI (ISAV), VII (KHV), VIII (SAV) and IX (ISAV) tested shortly after lyophilisation to assess homogeneity of the content.

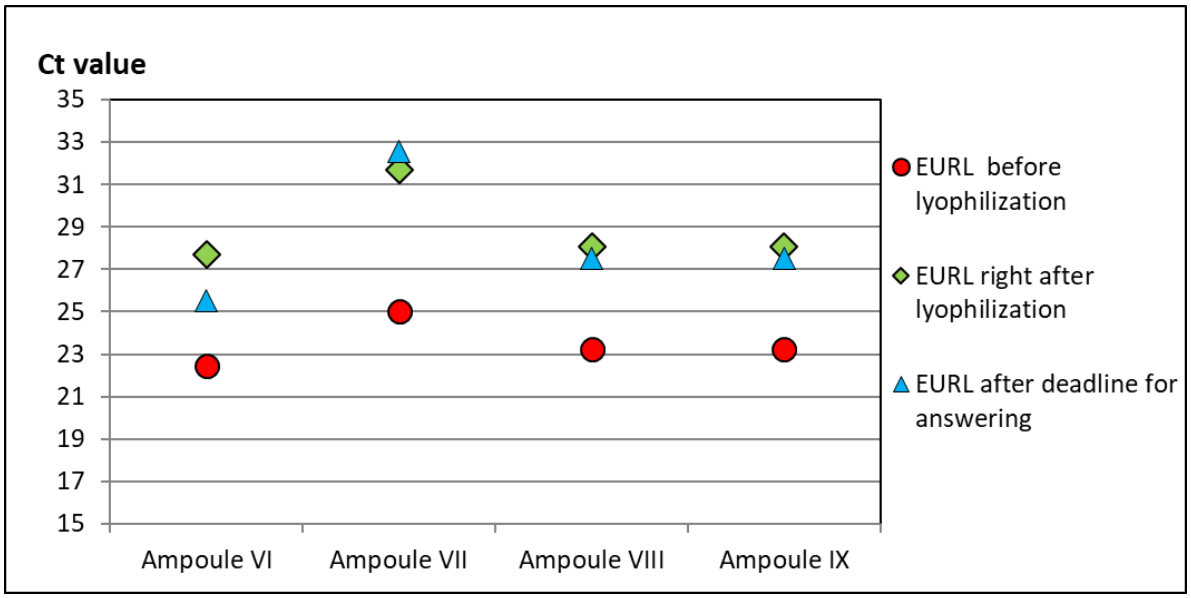


Figure 12, Ampoule VI, VII, VIII and IX tested before and after lyophilisation and after deadline for handling in results.

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

Ampoule	Content	Cell line	EURL before lyophilization	EURL right after lyophilization	EURL after deadline for answering
Ampoule VI	**ISAV**	a	22,44	27,90	25,53
		b		27,74	
		c		28,03	
		d		27,55	
		e		27,31	
			22,44	**27,71**	**25,53**
Ampoule VII	**KHV**	a	25,01	32,50	32,55
		b		31,73	
		c		31,77	
		d		31,21	
		e		31,14	
			25,01	**31,67**	**32,55**
Ampoule VIII	**SAV**	a	23,26	28,13	27,53
		b		27,87	
		c		27,74	
		d		27,95	
		e		28,58	
			23,26	**28,05**	**27,53**
Ampoule IX	**ISAV**	a	23,17	28,51	27,23
		b		28,50	
		c		28,53	
		d		28,34	
		e		28,11	
			23,17	**28,40**	**27,23**

The lyophilisation procedure caused a significant virus reduction in all four ampoules (especially in ampule VII 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 and should thus be viable.
Also this year, the panel of pathogens to be investigated included SAV – salmonid alpha virus. Since this is not a listed disease in the European legislation the participation was voluntary and therefore the participants were asked to declare if the ampoules were tested for SAV or not. Regarding methods for detection of SAV the participants were notified that they could refer to the chapter 2.3.5 – Infection with salmonid alpha virus OIE Manual of Diagnostic Tests for Aquatic Animals.
In order to obtain uniform answers, participants were requested to download a spreadsheet available from the https://www.eurl-fish-crustacean.eu, insert results in this and return by email.
The results from participating laboratories are shown in table 12.

Table 12. Inter-Laboratory Proficiency Test, PT2, 2019 - Virus identification.

Laboratory code number	Score	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
		ISAV 390/98 HPRΔ	KHV NRIA 0301 (CyHV-3)	SAV 2 SD	ISAV HPRΔ
1	8/8	ISAV	KHV	SAV	ISAV
2	8/8	ISAV	KHV	SAV	ISAV
3	8/8	ISAV	KHV	SAV	ISAV
4	8/8	+ ISAV (HPR 7)	+ KHV	+SAV	+ ISAV (Novel HPR deletion)
5	8/8	ISAV	KHV	SAV	ISAV
6[4)]	2/2	0	KHV	0	0
7	8/8	ISAV	KHV	SAV	ISAV
8	8/8	ISAV	KHV	SD/PD, Sleeping Disease/ Salmon Pancreas Disease	ISAV
9	8/8	ISAV	KHV	SAV	ISAV
10	8/8	ISAV	KHV	SAV	ISAV
11	8/8	ISAV	KHV	SAV	ISAV
12	8/8	ISAV	KHV	SAV	ISAV
13	8/8	ISAV	KHV	SAV	ISAV
14	8/8	ISAV	KHV	SAV	ISAV
15[1)]	6/6	ISAV	KHV	0	ISAV
16[2)]		0	0	0	0
17	8/8	ISAV	KHV	SAV	ISAV
18[4)]	1/2	0	0	0	0
19[1)]	4/6	ISAV	KHV	0	0
20	8/8	ISAV	KHV	SAV	ISAV
21	8/8	ISAV	KHV	SAV	ISAV
22	8/8	ISAV	KHV	SAV	ISAV
23	8/8	ISAV	KHV	SAV	ISAV
24	8/8	ISAV viable virus	KHV	SAV viable virus	ISAV viable virus
25	8/8	ISAV	KHV	SAV	ISAV
26[1)]	6/6	ISAV	KHV	-	ISAV
27[1)]	6/6	ISAV	KHV	Not ISAV/Not KNV	ISAV
28	8/8	ISAV	KHV	SAV	ISAV
29	8/8	ISAV	KHV	SAV	ISAV
30	6/8	ISAV	KHV	Blank	ISAV
31	8/8	ISA	KHV	SAV	ISA
32[3)]	4/6	0	0	SAV	ISAV
33[1)]	6/6	ISAV	KHV	NEGATIVE	ISAV
34	8/8	ISAV	KHV	SAV	ISA-HPROV
35	8/8	ISAV (HPRdel)	KHV	SAV	ISAV (HPRdel)
36	8/8	ISAV	KHV Japanese lineage	SAV	ISAV
37	8/8	ISAV	KHV	SAV	ISAV
38	8/8	ISAV	KHV	SAV	ISAV
39[1)]	6/6	ISAV	KHV	No KHV and no ISAV	ISAV
40	8/8	ISAV	KHV	SAV	ISAV
41	8/8	ISAV	KHV	SAV	ISAV
42	8/8	ISAV	KHV	SAV	ISAV
43	8/8	ISAV HPR7b	KHV	SAV	ISAV HPR9
44	8/8	ISAV	CyHV-3 (KHV)	SAV	ISAV
45	8/8	ISAV	KHV	SAV	ISAV
46[2)]		0	0	0	0
47	8/8	ISA	KHV	SAV	ISA
48	8/8	ISA	KHV	SAV	ISA
49	8/8	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 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

- 47 laboratories submitted results
- 37 laboratories correctly identified all four ampoules (ISAV, KHV, SAV, ISAV)
- 44 laboratories tested for the two listed pathogens
- 45 laboratories tested for ISAV
- 46 laboratories tested for KHV
- 39 laboratories tested for SAV
- 2 laboratories that did participate in PT 1 did not participate in PT2

Ampoule VI – ISAV

- 44 laboratories correctly identified ISAV
- 2 laboratories did not participate for ISAV and answered '0'
- 1 laboratory did not find the virus and answered '0'

Ampoule VII – KHV

- 46 laboratories correctly identified KHV
- 1 laboratory did not participate for KHV and answered '0'

Ampoule VIII – SAV

- 38 laboratories correctly identified SAV
- 8 laboratories did not participated in identifying SAV and 2 correctly ruled out the other two listed pathogens by answering 'Not KHV/Not ISAV'. 1 Laboratory answered 'Negative' and 5 laboratories answered '0' or '-'.
- 1 laboratory did not find the virus and answered 'Blank'

Ampoule IX – ISAV

- 44 laboratories correctly identified ISAV
- 2 laboratories did not participate for ISAV and answered '0'
- 1 laboratory did not find the virus and answered '0'

Scores

We have assigned a score of 2 for each correct answer (Table 12), giving the possibility for obtaining a maximum score of 8.
Not finding of the pathogen present in the ampoule gives the score 0.
Finding of correct pathogen present in the ampoule but without filling in concluding results gives the score 1.

Of the 47 laboratories submitting results 43 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).

If a laboratory did not test for SAV the maximum score was 6 points.
If a laboratory did not test for one of the pathogens KHV or ISAV but tested for SAV the maximum score was 6 points.
1 laboratory did neither test for ISAV or SAV therefor the maximum score was 2 points.

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 13.
For Ampoule VI and IX, 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 VII 2 points were given once the viral isolate as CyHV-3 or KHV, finally for ampoule VIII 2 points were given for determining the SAV isolate as type 2.
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 13. The genotyping results obtained for PT2 by all 42 participants

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

Code number	Score	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
		ISAV HPRΔ isolated from Atlantic salmon in Scotland in 1998	KHV CyHV-3	Salmonid AlphaVirus, (SAV2), MR-N1-2011	ISAV HPRΔ 2016-70-1297_Vir4415
1	8/8 [1]	European- deleted	0	Subtype 2	European- deleted
2	6/8 [2]	HPRΔ Clade CIVa	CyHV-3	subtype 3	HPR Genotype: 2
3	8/8	HPR 7	CyHV-3	Type 2	HPR 9
4	8/8	HPR 7	Wildtype KHV	SAV-2	Novel HPR deletion
5	n/a	0	0	0	0
6	n/a	0	0	0	0
7	4/4	HPR genotype PR14	0	0	HPR genotype PR03
8	6/6	HPRΔ, CIVe, CVI, CIVa	0	0	HPRΔ
9	6/6	ISAV HPR deleted (HPR36)	0	SAV2	ISAV HPR deleted (HPR9)
10	8/8	HPR Deleted -HPR7b	CyHV 3	2	HPR Deleted - HPR9
11	2/8 [3]	HPR0	CyHV-3	Ia	HPR0
12	8/8 [4]	HPR7b	0	0	HPR9
13	n/a [5]	0	0	0	0
14	8/8	HPRΔ, strain NVI-50-274/2018	Cyprinid Herpesvirus 3, isolate E8-UCD-NC	2	HPR2, isolate 47/99
15	n/a [6]	0	0	0	0
16	n/a	0	0	0	0
17	2/4 [7]	0	CyHV-3	0	PR-0
18	n/a	0	0	0	0
19	2/4 [8]	EU-G3	CyHV-3	0	0
20	4/6 [9]	HPR-delated	0	SAV-2	HPR0
21	6/6	HPR-deleted	0	SAV 2	HPR-deleted
22	6/8 [22]	HPR-deleted ISAV	CyHV-3	Salmonid alphavirus subtype 3	HPR-deleted ISAV
23	8/8	HPR deleted	CyHV3	2	HPR deleted
24	6/6 [10]	HPR	0	SAV 2	HPR
25	6/6	HPR7	N/A	E2 (III)	HPR6
26	n/a	0	0	0	0
27	6/6	HPR7b	CyHV3	0	HPR9
28	8/8 [11]	HPR7b	CyHv-3	subtype II	Unknown
29	6/8 [12]	N/A	CyHV-3	Subtype SAV2	N/A

30	6/6 [13]	0	CyHv-3	0	HPR2
31	n/a	0	0	0	0
32	n/a	0	0	0	0
33	2/2 [14]	0	KHV	0	0
34	n/a [15]	0	0	0	0
35	8/8 [16]	EU-G1	-	PDV/SAV-2	EU-G1
36	8/8	Genotype HPR deleted	CyHV3 Japanese lineage	SAV2	Genotype HPR deleted
37	6/6 [17]	HPR Deleted	CyHV-3	0	HPR Deleted
38	8/8 [18]	ISAV_genotype=G3	0	SAV_genotype=SAV2	ISAV_genotype=G1
39	4/4	HPR Deleted	0	0	HPR Deleted
40	8/8	HPR Deleted	CyHV 3	genotype 2	HPR Deleted
41	8/8	HPR deleted	CyHV-3	2	HPR deleted
42	8/8 [19]	HRP Genotype I, genogroup 2	CyHV 3	SAV 2	0
43	6/6	ISAV HPR deleted HPR7b	0	SAV2	ISAV HPR deleted HPR9
44	8/8 [20]	EU-G3	CyHV-3	2	EU-G1
45	n/a	0	0	0	0
46	n/a	0	0	0	0
47	2/4 [21]	ISA HPR Deleted	CyHV3	0	ISA HPR O
48	8/8	HPR7	CyHV-3; USA	SAV2; Norway	HPR3
49	8/8	ISAV HPR7b	KHV	SAV Genotype 2	ISAV HPR9
No. of sequences performed		33	20	26	33
No. of correct genotypes given		28	16	23	27
No. of correct sequences provided without genotype assigned		4	4	0	2
No of incorrect genotype provided		1	0	3	4

[1] The participant sequenced the KHV in Ampoule VII but did not provide genotype
[2] The participant did identify as subtype 3 the SAV 2 isolate
[3] The participant has incorrectly identified as HPR0 the ISAV isolates in ampoule VI and IX, furthermore the participant has identified as type Ia the SAV isolates in ampoule VII
[4] The participant has sequenced all isolates in PT2, the ISAV isolates in Ampoule VI and IX have been identified as ISAV HPRΔ, the genotype of ampoule VII and VIII is not included in the "genotype" cell of the spreadsheet, however the correct identification is reported in the cell additional information
[5] The participant has sequenced all ampoules in PT2 but has not provided any genotype
[6] The participant has sequenced ampoule VI in PT2 but has not provided the Genotype nor if it is HPR deleted or HPR0
[7] The participant has correctly identified CyHV-3 in ampoule VII, and incorrectly identified as HPR0 the isolate in Ampoule IX.
[8] The participant has sequenced the ISAV isolate in ampoule VI, but has not determined whether is HPRΔ or HPR0
[9] The participant has incorrectly identified as HPR0 the isolate in ampoule IX.

[10] The participant has not determined in "genotype"cell of the spreadsheet if the ISAV isolates are HPRΔ or HPR0, however the correct identification has been reported in the "additional information"
[11] The participant has identified the right isolate in ampoule IX but not reported it in the "genotype" cell of the spreadsheet
[12] The participant has sequenced all isolates in PT2. The isolates in ampoule VII and VIII have been correctly genotyped, however regardless the sequence provided identify the correct ISAV isolates in ampoule VI and IX it has not been determined whether the isolates are HPRΔ or HPR0
[13] The participant identified the right isolate in ampoule VI but did not provide HPR characterization
[14] The participant has sequenced only CyHV-3 isolate in ampoule VII
[15] The participant did not report the genotype nor the HPR classification in the genotype cell of the spreadsheet, furthermore, has wrongly characterized as HPR0 the isolate in ampoule IX.
[16] The participant has not reported the CyHV type for the isolate in ampoule VII, however has reported the correct identification as KHV in additional information
[17] The participant sequenced all ampoules in PT 2 but did not provide a genotype for SAV isolate in Ampoule VIII
[18] The participant sequenced all ampoules in PT 2 but did not provide a genotype for KHV isolate in Ampoule VII
[19] The participant sequenced all isolates in PT2, despite no HPR type was reported for ISAV isolate in Ampoule IX, the isolate was correctly identified
[20] The participant has sequenced all isolates in PT2. The isolates in ampoule VII and VIII have been correctly genotyped. The sequence provided identify the correct ISAV isolates in ampoule VI and IX however it has not been determined whether the isolates are HPRΔ or HPR0.
[21] The participant has provided sequences for isolates in ampoule VI and IX only. The ISAV isolate in ampoule VI has been correctly determined as HPRΔ, whereas the one in ampoule IX has been incorrectly typed as HPR0
[22] The participant does not provide sequence to support the conclusion of CyHV-3 in ampoule VII

Concluding remarks PT2

47 laboratories participated in PT2.
43 out of the 47 laboratories correctly identified the ISA virus in both ampoule VI and IX, 2 did not test for ISAV and 2 laboratory answered '0' in either ampoule VI or IX.
46 laboratories correctly identified the KHV in ampoule VII, 1 did not test for KHV.
39 laboratories tested for SAV and 38 correctly identified the virus in Ampoule VIII, 1 laboratory 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 for the first time this year, we have decided to score the genotyping results.
Out of 47 participating laboratories, 33 perform sequencing of ISAV to determine HPR type, 23 for KHV and 26 for SAV.
32 laboratories out of 33 correctly sequenced the ISAV in ampoule VI, 28 correctly typed the isolate as HPRΔ and only 1 as HPR0.
It has to be observed that the sequencing of the ISAV isolate in ampoule IX has created some challenges for the participants, in this case 4 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 annual workshop and more detailed instructions will be included in the proficiency test 2020.
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 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 24th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 2nd-3rd of June 2020 in Kgs. Lyngby, Denmark.

Teena Vendel Klinge, Niccolò Vendramin and Niels Jørgen Olesen

European Union Reference laboratory for Fish and Cruestacean diseases
Technical University of Denmark,
National Institute of Aquatic Resources,
Kemitorvet, Building 202,

March 2020.

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2. The European Commission (2014) COMMISSION IMPLEMENTING DIRECTIVE 2014/22/EU of 13 February 2014 amending Annex IV to Council Directive 2006/88/EC as regards infectious salmon anaemia (ISA). Off J Eur Union 45–47
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