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 2023

for identification and titration of

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

and identification of

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

Organised by the
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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 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 in addition other fish pathogenic viruses such as ranaviruses related to EHNV, spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) can be included. The laboratory procedures for isolating and titrating these pathogens is primarily based on cell culture methods, however the use of molecular methods (Real Time PCR based) is also implemented for their detection and identification.
PT2 was designed to assess the ability of participating laboratories to identify by molecular methods (PCR based) the fish viruses causing notifiable disease: infectious salmon anaemia virus (ISAV), and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV); in addition, due to its relevance, salmonid alphavirus (SAV) is also included.
Out of the 43 laboratories participating in PT1, 39 performed analysis to identify all viruses included, also out of the 43 laboratories participating in PT2, 39 attempted to identify all fish viral pathogens included.
The tests were sent from the EURL 3rd of October 2023.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for provision of proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.
The EURL relies on the subcontractor Eurofins Genomics for sequencing the amplicons of viral isolates included in the PTs, DTU – National Food Institute for lyophilisation of the ampoules and the Danish National Reference Laboratory for Fish diseases as provider of cell cultures.
This report covers both the results of PT1 and PT2.

PT1 consisted of five coded ampoules (I-V). These ampoules contained IPNV and VHSV (high titer), EHNV, IHNV and SVCV (medium titer), respectively (see table 1).

The proficiency test is designed to primarily assess the ability of participating laboratories to identify fish viral pathogens causing diseases listed in **Commission Implementing Regulation (EU) 2018/1882** [1] amended in 2024 by CIR (EU) 2024/216.

PT1 include the Category A disease, EHN, for which it is necessary to distinguish by sequencing the causative agent, EHNV, from other ranavirus, and the Category C diseases VHS and IHN. 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 diagnostic manuals for listed fish diseases available on the EURL website https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals and on the instruction to participants delivered along with the parcel [2] and by using fish cell cultures followed by e.g. ELISA, PCR or immunofluorescence (IFAT).

If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis according to the manual provided on the EURL website https://www.eurl-fish-crustacean.eu/fish/diagnostic-manuals. Although sequencing is necessary, it is possible to perform a

corroborative test with 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 2.3.2 in the WOAH Manual of Diagnostic Tests for Aquatic Animals [3]. 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 2004 [4] for VHSV and ; for IHNV, we suggest to follow procedure provided in the latest IHNV chapter of the WOAH manual on Aquatic Animal Diseases (primer references are given in Emmenegger et al. (2000) [5], and PCR conditions are given in Garver et al. (2003) [6]. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of four coded ampoules (VI-IX). These ampoules contained KHV, Eagles-MEM, ISAV and SAV cell supernatant, respectively (see table 11). The test was designed to primarily assess the ability of participating laboratories to identify infection with HPR-deleted ISAV listed as category C disease, , and Koi herpes virus disease listed as category E diseases (Commission Implementing Regulation (EU) 2018/1882**[1]** amended in 2024) 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 37 of 43 laboratories tested for SAV in 2023.
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 2.3.8. of the WOAH Manual of Diagnostic Tests for Aquatic Animals [7]. 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 propagate 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 genotyping results provided by all participants are displayed in Table 10 and 15.

Finally, in the attempt to harmonize the molecular diagnostic methods the EURL has compiled and presented the Ct values reported by the different laboratories (table 9 for PT1; table 14 for PT2 and represented graphically in annex 3 (PT1) and annex 5 (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 15th2023. The results of the inter-laboratory proficiency test for listed fish diseases 2023 and plans and idea for future inter-laboratory tests will be presented at the 28th Annual Workshop of the NRLs for Fish Diseases on May 29th, 2024. The meeting will be held online. Furthermore a specific online meeting in May 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. When possible participants were provided with a tracking number so they were able to follow the shipment.

Shipment and handling
The parcels were delivered to 37 participants within the first week; 93% were delivered within the first two 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.

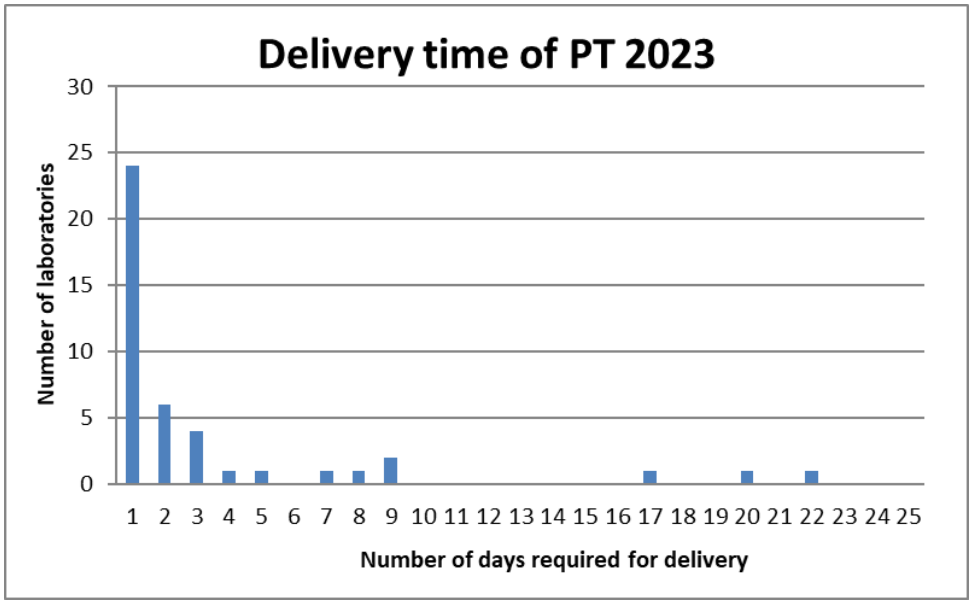

Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 44 laboratories received the annual proficiency test. 43 participants submitted the full spreadsheet within the deadline.

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, India, Japan, New Zealand, Northern Ireland, Norway, Republic of North Macedonia, Serbia, Switzerland, the United Kingdom (Scotland, England and Wales) 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 2 shows the worldwide distribution of the participating NRLs.

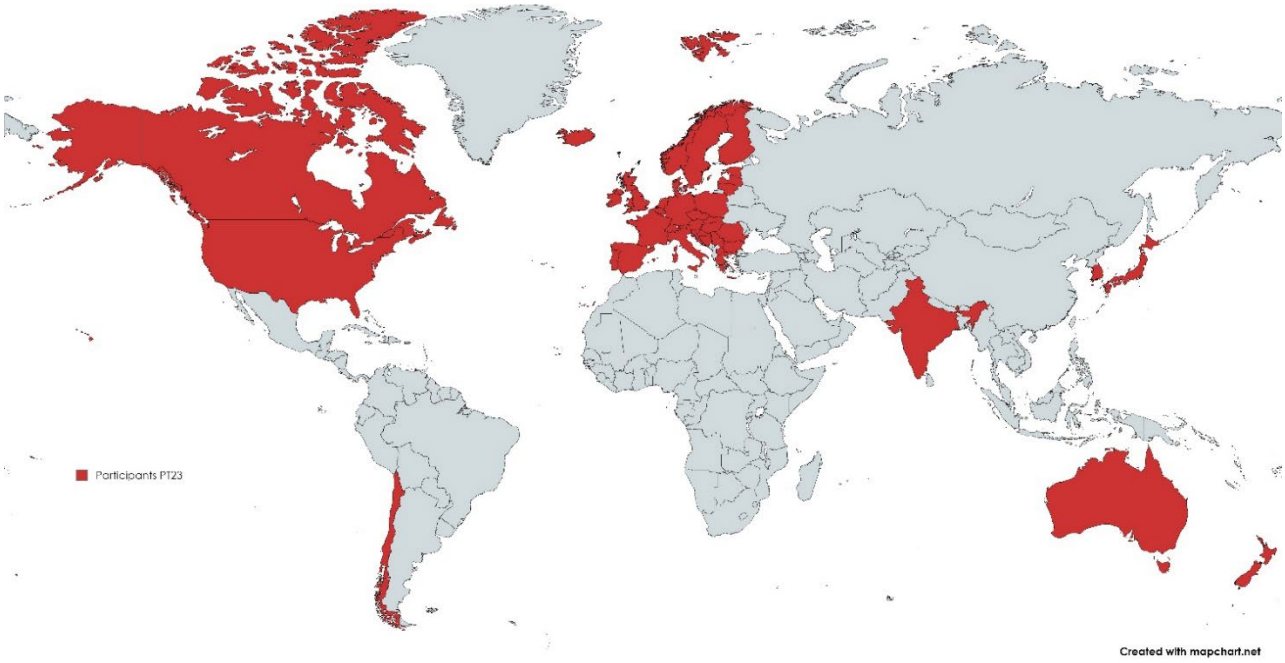

Figure 2. Worldwide distribution of the participants in the EURL proficiency test 2023

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 0.45 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: 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.

Code	Specifications/References
	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. Hick,P.M., Subramaniam,K., Thompson,P.M., Waltzek,T.B., Becker,J.A. and Whittington,R.J. TITLE Molecular epidemiology of Epizootic haematopoietic necrosis virus (EHNV) JOURNAL Virology 511, 320-329 (2017).
Ampoule II: 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:** Geno group 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
Ampoule III: VHSV	**VHS virus**, DK-3592B "**Voldbjerg strain**". **Genotype**: Ia **GenBank accession number**: KC778774.1 Highly pathogenic strain belonging to sero-pattern I. (Olesen, Lorenzen, and Jørgensen 163-70) **Reference on isolate**: Lorenzen N, Olesen NJ, Jørgensen PEV (1993) Antibody response to VHS virus proteins in rainbow trout. Fish Shellfish Immunol 3:461–473

Code	Specifications/References
Ampoule IV: IHNV	**IHNV 32/87.** First French isolate (April 1987) from rainbow trout. **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.
Ampoule V: SVCV	**SVCV strain 56/70** Spring viraemia of carp virus isolate from carp. The isolate is most likely identical to the S/30 isolate described in: Fijan N, Petrinec Z, Sulimanovic D & Zwillenberg LO (1971). Isolation of the viral causative agent from the acute form of infectious dropsy of carp. *Veterinarski Archiv* 41, 125-138. **Received from:** Prof. Fijan (January 1979 in a tube named Rhabdo virus carpio 56/70 and given as the reference strain of SVC virus). **Genotype:** Id **GenBank accession numbers:** Z37505.1 (Fijan), AJ538061.1 (S30) **Reference on sequence (S30) and genotype:** Stone DM, Ahne W, Denham KL, Dixon PF, Liu C-TY, Sheppard AM, Taylor GR & Way K (2003). Nucleotide sequence analysis of the glycoprotein gene of putative spring viraemia of carp virus and pike fry rhabdovirus isolates reveals four genogroups. *Diseases of Aquatic Organisms* 53, 203-210.

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.

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 annex 1).

Ampoul No.	Cell line	Titre before Lyophilisation	Titre after Lyophilisation and before shipment	Titre after deadline for handling in results (min. 120 days of storage 4°C in the dark)
		$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I: EHNV 86/8774	BF-2	8.6E+05	1.3E+05 *(4.0E+04 → 2.7E+05)*	1.3E+05
	RTG-2	4.0E+03	5.9E+04 *(4.0E+04 → 1.3E+05)*	8.6E+04
	EPC	2.7E+05	8.6E+03 *(5.9E+03 → 4.0E+04)*	1.3E+04
	FHM	4.0E+04	1.3E+03 *(5.9E+02 → 1.9E+03)*	1.9E+03
Ampoule II: IPNV Sp	BF-2	2.7E+07	2.7E+05 *(8.4E+04 → 8.6E+05)*	5.9E+05
	RTG-2	1.3E+07	5.9E+04 *(1.9E+04 → 1.9E+05)*	1.9E+05
	EPC	1.3E+07	1.3E+04 *(1.3E+04 → 1.3E+05)*	4.0E+04
	FHM	1.9E+06	4.0E+03 *(1.3E+03 → 1.9E+04)*	2.7E+04
Ampoule III: VHSV DK-3592B	BF-2	1.3E+06	5.9E+04 *(4.0E+04 → 1.9E+05)*	2.7E+04
	RTG-2	1.3E+07	8.6E+04 *(5.9E+04 → 4.0E+05)*	4.0E+04
	EPC	5.9E+05	5.9E+04 *(2.7E+04 → 1.3E+05)*	4.0E+05
	FHM	4.0E+07	1.9E+06 *(8.6E+05 → 1.9E+06)*	1.9E+06

Ampoule IV: IHNV 32/87	BF-2	< 1,9E+02	< 1,9E+02	< 1,9E+02
	RTG-2	5.9E+03	5.9E+02 *(2.7E+02 → 1.3E+03)*	4.0E+02
	EPC	4.0E+05	4.0E+03 *(2.7E+03 → 4.0E+03)*	4.0E+04
	FHM	2.7E+05	1.9E+03 *(1.3E+03 → 1.9E+03)*	8.6E+02
Ampoule V: SVCV 56/70	BF-2	8.6E+06	4.0E+05 *(1.9E+05 → 1.3E+06)*	1.9E+06
	RTG-2	8.6E+04	1.3E+05 *(5.9E+04 → 1.3E+05)*	8.6E+04
	EPC	5.9E+06	1.3E+05 *(1.3E+05 → 4.0E+05)*	4.0E+05
	FHM	5.9E+06	1.3E+05 *(5.9E+04 → 1.3E+05)*	1.9E+05

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. Lowest and highest titre in brackets.
- After deadline for handling in results approx. 3 months after shipment (1 ampoule), (stored at 4°C).

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 the EURL diagnostic manuals [2], i.e. by cell culture followed by ELISA, IFAT 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 also represented graphically in annex 2.
Laboratories were encouraged to identify the genotype of the virus isolates.

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

Table 3. Inter-Laboratory Proficiency Test, PT1, 2023 - Virus identification and score obtained by participants.

Laboratory code number	Score	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
		EHNV 86/8774	IPNV Sp	VHSV DK-3592B	IHNV 32/87	SVCV 56/70
1	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
2	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
3[2)+3)]	6/6	EHNV	-	VHSV	IHNV	-
4	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
5[1)]	9/10	Ranavirus	IPNV	VHSV	IHNV	SVCV
6	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
7	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
8	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
9	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
10	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
11	8/10	EHNV	EHNV, IPNV	VHSV	IHNV	SVCV
12	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
13	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
14	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
15	10/10	EHNV	IPNV	VHSV	IHNV	SVCV

16	8/10	EHNV	IPNV	VHSV	IHNV& EHNV	SVCV
17[2)]	8/8	EHNV	0	VHSV	IHNV	SVCV
18	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
20	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
21	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
22	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
23	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
24[1)]	9/10	EHNV	IPNV	VHSV	IHNV	SVCV
25	10/10	EHNV	IPNV	VHS	IHNV	SVCV
26	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
27	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
28[2)+3)]	6/6	EHNV	Negative for IHNV, Ranavirus and VHSV	VHSV	IHNV	Negative for IHNV, Ranavirus and VHSV
29	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
30	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
31	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
32	10/10	RANAVIRUS	IPNV	VHSV	IHNV	SVCV
33	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
34	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
35	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
36	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
37	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
38	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
39	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
40	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
41	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
42	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
43	10/10	EHNV	IPNV	VHSV	IHNV	SVCV
44	10/10	EHNV	IPNV	VHSV	IHNV	SVCV

1) Did not corroborate the findings in ampoule II by sequencing or REA or have used commercial qPCR kit distinguish between EHNV and other Ranavirus
2) Do not test for IPN
3) Do not test for SVCV

Table 4. Inter-Laboratory Proficiency Test, PT1, 2023 – **Results of titration of ampoule I.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	EHNV	8.6E+04	2.7E+04	1.3E+04	N/A
2	EHNV	4.0E+04	1.9E+04	N/A	N/A
3	EHNV	N/A	N/A	N/A	N/A
4	EHNV	2.7E+04	8.6E+03	N/A	N/A
5	Ranavirus	1.3E+05	4.0E+03	N/A	N/A
6	EHNV	4.0E+04	5.9E+03	1.9E+02	< 1,9E+02
7	EHNV	5.9E+05	8.6E+05	N/A	N/A
8	EHNV	1.9E+04	5.9E+04	N/A	N/A
9	EHNV	8.6E+03	5.9E+03	2.7E+02	1.3E+04
10	EHNV	2.7E+04	5.9E+04	1.9E+04	4.0E+03
11	EHNV	4.0E+05	8.6E+04	N/A	N/A
12	EHNV	N/A	N/A	N/A	N/A
13	EHNV	1.9E+05	5.9E+04	N/A	N/A
14	EHNV	8.6E+05	8.6E+04	2.7E+04	N/A
15	EHNV	1.9E+03	1.9E+03	5.9E+02	N/A
16	EHNV	4.0E+04	4.0E+02	N/A	N/A
17	EHNV	N/A	N/A	N/A	N/A
18	EHNV	8.6E+04	1.3E+04	N/A	N/A
20	EHNV	8.6E+04	1.9E+04	N/A	N/A
21	EHNV	1.9E+05	8.6E+04	N/A	N/A
22	EHNV	8.6E+04	5.9E+04	1.3E+04	8.6E+03
23	EHNV	2.7E+03	5.9E+02	N/A	N/A
24	EHNV	N/A	5.9E+04	1.3E+04	N/A
25	EHNV	5.9E+03	1.9E+03	N/A	N/A
26	EHNV	5.9E+04	1.3E+03	N/A	N/A
27	EHNV	1.3E+05	1.3E+05	4.0E+04	1.9E+03
28	EHNV	N/A	N/A	N/A	N/A
29	EHNV	N/A	N/A	N/A	N/A
30	EHNV	1.3E+04	N/A	N/A	N/A
31	EHNV	N/A	N/A	N/A	N/A

32	RANAVIRUS	4.0E+03	1.3E+04	1.9E+04	N/A
33	EHNV	5.9E+09	2.7E+09	N/A	N/A
34	EHNV	N/A	2.7E+02	2.7E+02	N/A
35	EHNV	1.3E+04	2.7E+04	N/A	4.0E+03
36	EHNV	2.7E+05	1.3E+05	1.9E+05	5.9E+04
37	EHNV	4.0E+04	N/A	N/A	1.9E+02
38	EHNV	1.3E+03	1.3E+03	N/A	N/A
39	EHNV	8.6E+04	N/A	N/A	< 1,9E+02
40	EHNV	1.9E+05	1.3E+05	N/A	N/A
41	EHNV	5.9E+04	N/A	N/A	N/A
42	EHNV	2.7E+05	2.7E+04	N/A	N/A
43	EHNV	2.7E+05	1.9E+04	1.9E+05	N/A
44	EHNV	4.0E+04	4.0E+04	N/A	4.0E+03

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	35	33	13	10
Median titre	5.9E+04	2.7E+04	1.3E+04	4.0E+03
Maximum titre	5.9E+09	2.7E+09	1.9E+05	5.9E+04
Minimum titre	1.3E+03	2.7E+02	1.9E+02	<1,9E+02
25% quartile titre	2.3E+04	5.9E+03	5.9E+02	6.0E+02
75% quartile titre	1.9E+05	5.9E+04	2.7E+04	7.5E+03

Table 5. Inter-Laboratory Proficiency Test, PT1, 2023 – **Results of titration of ampoule II.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IPNV	1.3E+05	4.0E+04	2.7E+04	N/A
2	IPNV	5.9E+05	1.9E+04	N/A	N/A
3	-	N/A	N/A	N/A	N/A
4	IPNV	1.3E+07	1.9E+07	N/A	N/A
5	IPNV	8.6E+05	8.6E+04	N/A	N/A
6	IPNV	8.6E+05	5.9E+04	1.9E+03	2.7E+03
7	IPNV	5.9E+06	4.0E+06	N/A	N/A
8	IPNV	2.7E+05	2.7E+05	N/A	N/A
9	IPNV	5.9E+06	8.6E+06	1.3E+06	4.0E+06
10	IPNV	1.3E+06	8.6E+05	8.6E+05	4.0E+05
11	EHNV, IPNV	5.9E+05	5.9E+04	N/A	N/A
12	IPNV	N/A	N/A	N/A	N/A
13	IPNV	4.0E+05	4.0E+04	N/A	N/A
14	IPNV	5.9E+05	8.6E+05	1.3E+06	N/A
15	IPNV	1.9E+07	1.3E+06	1.9E+06	N/A
16	IPNV	1.9E+06	1.9E+05	N/A	N/A
17	0	N/A	N/A	N/A	N/A
18	IPNV	4.0E+06	5.9E+05	N/A	N/A
20	IPNV	5.9E+02	5.9E+02	N/A	N/A
21	IPNV	1.3E+06	4.0E+05	N/A	N/A
22	IPNV	8.6E+06	<1,9E+02	1.3E+06	1.9E+05
23	IPNV	2.7E+06	4.0E+05	N/A	N/A
24	IPNV	N/A	4.0E+04	1.9E+03	N/A
25	IPNV	2.7E+04	1.3E+04	N/A	N/A
26	IPNV	5.9E+04	1.3E+05	N/A	N/A
27	IPNV	1.9E+06	1.9E+05	2.7E+05	1.9E+05
28	Negative for IHNV, Ranavirus and VHSV	N/A	N/A	N/A	N/A
29	IPNV	N/A	N/A	N/A	N/A
30	IPNV	5.9E+06	N/A	N/A	N/A
31	IPNV	N/A	N/A	N/A	N/A

32	IPNV	2.7E+05	8.6E+05	1.9E+05	N/A
33	IPNV	5.9E+09	4.0E+09	N/A	N/A
34	IPNV	N/A	8.6E+04	1.9E+05	N/A
35	IPNV	<1,9E+02	<1,9E+02	N/A	<1,9E+02
36	IPNV	1.9E+06	8.6E+05	4.0E+05	1.3E+05
37	IPNV	1.3E+06	N/A	N/A	5.9E+04
38	IPNV	8.6E+05	2.7E+05	N/A	N/A
39	IPNV	2.7E+06	N/A	N/A	5.9E+05
40	IPNV	1.9E+06	1.3E+06	N/A	N/A
41	IPNV	1.9E+06	N/A	N/A	N/A
42	IPNV	8.6E+05	1.3E+05	N/A	N/A
43	IPNV	1.3E+05	8.6E+03	5.9E+02	N/A
44	IPNV	1.9E+04	1.3E+04	N/A	5.9E+03

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	35	33	13	10
Median titre	1.3E+06	1.9E+05	2.7E+05	1.6E+05
Maximum titre	5.9E+09	4.0E+09	1.9E+06	4.0E+06
Minimum titre	<1,9E+02	<1,9E+02	5.9E+02	<1,9E+02
25% quartile titre	3.4E+05	4.0E+04	2.7E+04	1.9E+04
75% quartile titre	2.7E+06	8.6E+05	1.3E+06	3.5E+05

Table 6. Inter-Laboratory Proficiency Test, PT1, 2023 – **Results of titration of ampoule III**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	VHSV	1.3E+04	1.9E+04	<1,9E+02	N/A
2	VHSV	5.9E+03	2.7E+03	N/A	N/A
3	VHSV	N/A	N/A	N/A	N/A
4	VHSV	1.26E+03	1.86E+03	N/A	N/A
5	VHSV	1.3E+04	1.9E+03	N/A	N/A
6	VHSV	5.9E+04	1.9E+03	1.3E+03	1.9E+02
7	VHSV	1.3E+05	1.3E+04	N/A	N/A
8	VHSV	1.3E+04	4.0E+04	N/A	N/A
9	VHSV	1.9E+04	4.0E+04	1.3E+04	5.9E+04
10	VHSV	1.9E+04	2.7E+04	2.7E+04	1.9E+06
11	VHSV	1.9E+05	8.6E+04	N/A	N/A
12	VHSV	N/A	N/A	N/A	N/A
13	VHSV	2.7E+04	4.0E+05	N/A	N/A
14	VHSV	1.9E+05	8.6E+03	8.6E+03	N/A
15	VHSV	2.7E+04	1.3E+04	4.0E+02	N/A
16	VHSV	8.6E+03	5.9E+02	N/A	N/A
17	VHSV	N/A	N/A	N/A	N/A
18	VHSV	2.7E+04	4.0E+04	N/A	N/A
20	VHSV	1.9E+02	1.9E+04	N/A	N/A
21	VHSV	1.9E+04	4.0E+04	N/A	N/A
22	VHSV	5.9E+04	<1,9E+02	5.9E+03	8.6E+03
23	VHSV	5.9E+03	1.9E+02	N/A	N/A
24	VHSV	N/A	8.6E+04	8.6E+03	N/A
25	VHS	4.0E+04	8.6E+03	N/A	N/A
26	VHSV	4.0E+03	2.7E+03	N/A	N/A
27	VHSV	1.9E+04	1.3E+04	4.0E+04	4.0E+06
28	VHSV	N/A	N/A	N/A	N/A
29	VHSV	N/A	N/A	N/A	N/A
30	VHSV	5.9E+03	N/A	N/A	N/A
31	VHSV	N/A	N/A	N/A	N/A

32	VHSV	4.0E+02	5.9E+02	1.9E+02	N/A
33	VHSV	8.6E+04	1.3E+07	N/A	N/A
34	VHSV	N/A	1.86E+02	<1,9E+02	8.62E+03
35	VHSV	4.00E+03	1.86E+04	N/A	N/A
36	VHSV	1.9E+03	8.6E+04	1.3E+04	1.9E+04
37	VHSV	1.3E+03	N/A	N/A	4.0E+04
38	VHSV	4.0E+03	1.3E+04	N/A	N/A
39	VHSV	1.3E+05	N/A	N/A	2.7E+06
40	VHSV	5.9E+04	4.0E+04	N/A	N/A
41	VHSV	1.3E+05	8.62E+03	N/A	N/A
42	VHSV	8.6E+03	2.7E+03	N/A	N/A
43	VHSV	5.9E+03	2.7E+05	<1,9E+02	N/A
44	VHSV	5.9E+03	4.0E+04	N/A	1.3E+04

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

VHSV, DK-3592B	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	35	34	13	10
Median titre	1.3E+04	1.3E+04	5.9E+03	2.9E+04
Maximum titre	1.9E+05	1.3E+07	4.0E+04	4.0E+06
Minimum titre	1.9E+02	<1,9E+02	<1,9E+02	1.9E+02
25% quartile titre	5.9E+03	2.7E+03	1.9E+02	9.6E+03
75% quartile titre	4.9E+04	4.0E+04	1.3E+04	1.4E+06

Table 7. Inter-Laboratory Proficiency Test, PT1, 2023 – **Results of titration of ampoule IV.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IHNV	<1,9E+02	2.7E+03	<1,9E+02	N/A
2	IHNV	<1,9E+02	5.9E+02	N/A	N/A
3	IHNV	N/A	N/A	N/A	N/A
4	IHNV	<1,9E+02	5.9E+02	N/A	N/A
5	IHNV	<1,9E+02	1.9E+05	N/A	N/A
6	IHNV	<1,9E+02	8.6E+02	<1,9E+02	<1,9E+02
7	IHNV	<1,9E+02	8.6E+02	N/A	N/A
8	IHNV	1.3E+03	4.0E+04	N/A	N/A
9	IHNV	5.9E+02	5.9E+03	1.3E+03	5.9E+03
10	IHNV	<1,9E+02	1.9E+03	1.9E+03	1.9E+03
11	IHNV	4.0E+02	8.6E+03	N/A	N/A
12	IHNV	N/A	N/A	N/A	N/A
13	IHNV	<1,9E+02	4.0E+03	N/A	N/A
14	IHNV	2.7E+02	1.9E+04	8.6E+02	N/A
15	IHNV	<1,9E+02	1.9E+03	2.7E+03	N/A
16	IHNV& EHNV	1.3E+03	4.0E+02	N/A	N/A
17	IHNV	N/A	N/A	N/A	N/A
18	IHNV	<1,9E+02	5.9E+03	N/A	N/A
20	IHNV	<1,9E+02	1.9E+03	N/A	N/A
21	IHNV	<1,9E+02	2.7E+03	N/A	N/A
22	IHNV	<1,9E+02	1.9E+03	8.6E+02	2.7E+03
23	IHNV	<1,9E+02	<1,9E+02	N/A	N/A
24	IHNV	N/A	1.9E+04	5.9E+02	N/A
25	IHNV	<1,9E+02	4.0E+03	N/A	N/A
26	IHNV	<1,9E+02	1.3E+03	N/A	N/A
27	IHNV	4.0E+03	2.7E+03	5.9E+03	4.0E+03
28	IHNV	N/A	N/A	N/A	N/A
29	IHNV	N/A	N/A	N/A	N/A
30	IHNV	<1,9E+02	N/A	N/A	N/A
31	IHNV	N/A	N/A	N/A	N/A

32	IHNV	5.9E+02	1.3E+03	4.0E+02	N/A
33	IHNV	1.9E+03	4.0E+04	N/A	N/A
34	IHNV	N/A	<1,9E+02	<1,9E+02	N/A
35	IHNV	<1,9E+02	8.6E+03	N/A	<1,9E+02
36	IHNV	<1,9E+02	1.3E+04	<1,9E+02	4.0E+03
37	IHNV	4.0E+02	N/A	N/A	1.9E+02
38	IHNV	<1,9E+02	4.0E+03	N/A	N/A
39	IHNV	<1,9E+02	N/A	N/A	1.9E+03
40	IHNV	<1,9E+02	2.7E+04	N/A	N/A
41	IHNV	N/A	N/A	1.9E+03	4.0E+03
42	IHNV	<1,9E+02	1.9E+03	N/A	N/A
43	IHNV	<1,9E+02	1.9E+03	1.9E+02	N/A
44	IHNV	<1,9E+02	8.6E+02	N/A	1.9E+03

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

IHNV 32/87	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	34	33	14	11
Median titre	<1,9E+02	2.7E+03	7.2E+02	1.9E+03
Maximum titre	4.0E+03	1.9E+05	5.9E+03	5.9E+03
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	1.3E+03	4.7E+01	1.0E+03
75% quartile titre	2.0E+02	8.6E+03	1.7E+03	4.0E+03

Table 8. Inter-Laboratory Proficiency Test, PT1, 2023 – Results of titration of **ampoule V**.

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	SVCV	5.9E+04	2.7E+04	< 1,9E+02	N/A
2	SVCV	5.9E+03	4.0E+04	N/A	N/A
3	-	N/A	N/A	N/A	N/A
4	SVCV	1.3E+03	5.9E+02	N/A	N/A
5	SVCV	5.9E+03	5.9E+04	N/A	N/A
6	SVCV	4.0E+04	5.9E+04	< 1,9E+02	4.0E+03
7	SVCV	1.9E+04	1.9E+04	N/A	N/A
8	SVCV	1.3E+04	2.7E+05	N/A	N/A
9	SVCV	1.9E+03	2.7E+04	4.0E+03	8.6E+04
10	SVCV	1.3E+05	1.9E+04	2.7E+04	1.9E+04
11	SVCV	4.0E+04	2.7E+04	N/A	N/A
12	SVCV	N/A	N/A	N/A	N/A
13	SVCV	1.9E+04	1.3E+05	N/A	N/A
14	SVCV	5.9E+04	1.9E+05	1.3E+03	N/A
15	SVCV	8.6E+03	2.7E+04	< 1,9E+02	N/A
16	SVCV	1.3E+04	1.3E+04	N/A	N/A
17	SVCV	N/A	N/A	N/A	N/A
18	SVCV	1.3E+05	4.0E+05	N/A	N/A
20	SVCV	4.0E+03	5.9E+04	N/A	N/A
21	SVCV	5.9E+04	2.7E+04	N/A	N/A
22	SVCV	1.3E+04	5.9E+04	< 1,9E+02	4.0E+04
23	SVCV	5.9E+02	1.9E+03	N/A	N/A
24	SVCV	N/A	2.7E+04	< 1,9E+02	N/A
25	SVCV	2.7E+03	4.0E+04	N/A	N/A
26	SVCV	5.9E+03	8.6E+03	N/A	N/A
27	SVCV	4.0E+05	1.9E+05	1.3E+04	8.6E+04
28	Negative for IHNV, Ranavirus and VHSV	N/A	N/A	N/A	N/A
29	SVCV	N/A	N/A	N/A	N/A
30	SVCV	2.7E+03	N/A	N/A	N/A

31	SVCV	N/A	N/A	N/A	N/A
32	SVCV	8.6E+02	5.9E+03	5.9E+02	N/A
33	SVCV	4.0E+04	1.3E+06	N/A	N/A
34	SVCV	N/A	1.9E+02	< 1,9E+02	N/A
35	SVCV	1.3E+05	4.0E+04	N/A	1.3E+05
36	SVCV	1.9E+03	2.7E+04	4.0E+04	2.7E+04
37	SVCV	1.9E+03	N/A	N/A	5.9E+03
38	SVCV	4.0E+02	1.9E+04	N/A	N/A
39	SVCV	1.9E+04	N/A	N/A	2.7E+05
40	SVCV	1.3E+05	1.9E+05	N/A	N/A
41	SVCV	N/A	8.6E+04	N/A	N/A
42	SVCV	1.3E+04	1.3E+04	N/A	N/A
43	SVCV	2.7E+03	1.3E+04	< 1,9E+02	N/A
44	SVCV	1.3E+03	2.7E+04	N/A	1.9E+03

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

SVCV 56/70	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	34	34	13	10
Median titre	1.3E+04	2.7E+04	<1,9E+02	3.4E+04
Maximum titre	4.0E+05	1.3E+06	4.0E+04	2.7E+05
Minimum titre	4.0E+02	1.9E+02	<1,9E+02	1.9E+03
25% quartile titre	2.7E+03	1.9E+04	<1,9E+02	9.0E+03
75% quartile titre	4.0E+04	5.9E+04	4.0E+03	8.6E+04

Identification of content

- 40 laboratories out of 43 participants analysed for all viruses; 36 of these laboratories correctly identified all viruses in all ampoules.
- Two laboratories did not test for neither IPNV nor SVCV and one laboratory did not test for IPNV.

Ampoule I – EHNV (86/8774)

- 41 laboratories correctly identified the isolate as EHNV in ampoule I by sequencing or REA.
- 2 laboratories identified Ranavirus but did not specify if the isolate was the listed EHNV or not by sequencing.

Ampoule II – IPNV (Sp)

- 39 laboratories correctly identified the isolate as IPNV in ampoule II
- 1 laboratory correctly identified the virus but also found a non-present virus in the ampoule.
- 3 laboratories do not test for IPNV

Ampoule III – VHSV (DK-3592B)

- 43 laboratories correctly identified the isolate as VHSV in ampoule III

Ampoule IV – IHNV (32/87)

- 42 laboratories correctly identified the isolate as IHNV in ampoule IV
- 1 laboratory correctly identified the virus but also found a non-present virus in the ampoule.

Ampoule V – SVCV (56/70)

- 41 laboratories correctly identified the isolate as SVCV in ampoule V
- 2 laboratories do not test for SVCV

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**: EHNV identification was given the score 2.
  No identification of the Ranavirus by sequencing was given the score 1.
- **Ampoule II**: IPNV identification was given the score 2.
  IPNV identification and detection of a listed virus was given the score 0.
- **Ampoule III**: VHSV identification was given the score 2.
- **Ampoule IV**: IHNV identification was given the score 2.
  IHNV identification and detection of another listed virus was given the score 0.
- **Ampoule V**: SVCV identification was given the score 2.

In relation to the ranaviruses included in the ILPT, full score was given only in case one laboratory could isolate the virus and fully identify the isolate by means of sequencing.
Although it is acknowledged that, theoretically, other methods can be used to discriminate (e.g. specific qPCR assay) these have not been fully validated or the data of such validation are not available, hence we have considered that the result is not corroborated and fully supported from the diagnostic method used.

Out of 43 laboratories participating in the PT 1 2023, 39 obtained a score on 100%.
The score 10/10 was assigned to 36 participants as they did test for all virus.
The score 8/8 was assigned to one participant as they did not test for IPNV SVCV.
The score 6/6 was assigned to two participants as they did not test for IPNV and SVCV.

4 laboratories scored below 100% due to no identification by sequencing of the Ranavirus or due to finding a virus not present in the ampoule.

Cells applied for solving the test

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

- 36 laboratories used BF-2 cells
- 35 laboratories used EPC cells
- 15 laboratories used RTG-2 cells
- 11 laboratories used FHM cells
- 8 laboratories used CHSE-214, SHK-1 or other cells
- 6 laboratories did not titrate

The combination of EPC and FHM cells or BF-2 and RTG 2 alone is not valid according to EURL diagnostic manuals [2] The laboratories are encouraged to include the use of BF-2 cells or RTG 2 cells and EPC cells or FHM cells.

As from Table 4-8 the variations in titres between laboratories was 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 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.

Ct. values comparison

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 and represented graphically in annex 3.

The Ct values cannot be directly compared due to the use of different methods, reagents and equipment for nucleic acid extraction and (RT)-qPCR. In order to align the results, participants have been asked to test the ampoules by molecular methods directly from the re-suspended material and not from the viral isolates, however it seems like a few participants have given the Ct values obtained from the viral isolates.

Table 9. Inter-Laboratory Proficiency Test, PT1, 2023 – Ct.-values.

Laboratory Code number	Ct. value Ampoule I (EHNV)	Ct. value Ampoule II (IPNV)	Ct. value Ampoule III (VHSV)	Ct. value Ampoule IV (IHNV)	Ct. value Ampoule V (SVCV)
EURL	**27.5**	**25.5**	**19.2**	**27.1**	**25.1**
1	23.87	25.64	18.69	27.34	23.79
2	-	-	19.92	28.75	26.32
3	29.54	-	20.68	28.82	-
4	-	-	-	-	25.71
5	-	-	-	-	-
6	-	26.61	20.40	24.61	25.23
7	27.41	26.93	22.13	35.63	26.93
8	-	-	21.48	32.52	-
9	-	-	18.26	-	-
10	27.80	24.46	19.20	25.30	23.85
11	-	-	14.86	16.79	-
12	-	23.97	23.14	31.65	-
13	-	26.42	23.24	32.06	-
14	-	15.65	18.99	27.25	-
15	-	25.57	21.10	30.88	-
16	-	-	-	-	-
17	26.74	-	23.16	31.45	-
18	24.09	26.24	32.33	32.89	-
20	-	-	-	-	-
21	-	24.40	20.66	28.07	-
22	-	-	-	-	-
23	-	-	14.52	35.18	-
24	24.03	-	26.97	30.72	30.88
25	26.64	-	-	27.15	-
26	-	18.15	18.81	27.40	-
27	-	-	18.30	28.91	-
28	-	-	20.71	28.88	-
29	-	22.30	16.80	25.50	22.00
30	-	-	-	-	-
31	-	26.60	23.40	28.50	27.60
32	-	-	19.71	28.03	-
33	-	-	19.56	29.73	-
34	-	-	-	-	-
35	-	-	-	-	-
36	-	16.60	17.30	19.60	-
37	24.40	22.07	20.16	27.56	-
38	-	24.17	20.83	31.35	-
39	24.50	18.20	21.50	24.80	25.80
40	-	-	-	-	-
41	22.86	25.35	25.28	27.14	29.82
42	29.10	20.80	20.55	30.78	-
43	-	-	22.60	-	-
44	-	-	-	-	-

- No Ct-value given by the participating laboratory.

Genotyping and sequencing

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 analysis in order to discriminate EHNV from the non-listed ranaviruses. For IHNV and VHSV we still encouraged participants to genotype isolates. An overview of the genotyping results obtained for PT1 by all participants is displayed in the following table 10. This year the EURL has provided to all participants a set of reference sequences to align genotyping procedures.
The EURL has disclosed the content of the ampoules after deadline for delivering results.

Table 10. Inter-Laboratory Proficiency Test, PT1, 2023 - Genotyping

Code number	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
	EHNV 86/8774	**IPNV Sp Genogroup 5**	**VHSV DK-3592B Genotype Ia**	**IHNV 32/87 Genogroup E**	**SVCV 56/70 Genotype Id**
1	EHNV	Genogroup 5	Ia	E	1d
2	EHNV; Australia: Victoria, Melbourne	Infectious pancreatic necrosis virus; Sp; Genogroup 5	Viral hemorrhagic septicemia virus; Genotype: Ia	Infectious hematopoietic necrosis virus; genotype E	Spring viremia of carp virus; genotype Id; Strain="Fijan"
3[2+3]	N/A	N/A	N/A	N/A	N/A
4	EHNV (seq)	0	0	0	0
5[1]	0	0	0	0	0
6	EHNV	3	Ia	E	Id
7	EHNV	0	Ia	E	SVCV
8	EHNV	Genogroup 5	Genotype: Ia	Genogroup E	Genotype Id
9	EHNV	Genogroup 5	Ia	E	1d
10	0	5	1a	E	I
11	EHNV	EHNV	0	0	0
12	0	Serotype Sp; serogroup A	Genotype Ia	Genogroup E	0
13	EHNV	Genogroup 5	Ia	E	0
14	EHNV	Genogroup 5	Ia	E	Genogroup Id
15	EHNV	5	Ia	E	Id
16	EHNV	Genogroup 5	Genotype Ia	"1) Genogroup E 2) EHNV"	Genotype Id
17[3]	EHNV	0	Ia	E	Id
18	EHNV	Genogroup 5	Ia	M	Genogroup 1d
20	0	5 (Sp)	1b	E	1d
21	EHNV	genogroup 5 (pVP2)	Ia (Ia1)	E	Id
22	EHNV	IPNV	Ia	E	Id

23	EHNV	0	Ia	E	1d
24[1]	0	0	0	0	0
25	EHNV	Genogroup 5	1a	E	Genogroup 1d
26	EHNV	5	Ia	E	0
27	0	5, Sp	Ia	Geno group E	1d
28[2+3]	EHNV	0	Ia	E	0
29	EHNV	0	I-a	E	Id
30	EHNV	Genogroup 5	I a	E	Genogroup 1(d)
31	EHNV	0	IVc	M	0
32	EHNV	Genogroup 5	Ia	E	Genogroup Id
33	EHNV	genogroup 5 (serotype Sp)	genotype Ia	genotype E	genotype 1d
34	EHNV	Genogroup 5 (IPNV Sp, by Blake et al., 2001)	Ia	European	Id
35	EHN-like	Genogroup 5A subtype 1	Ia	Europe Group	Ib
36	EHNV	5	Ia	E	Id
37	EHNV	Genogroup 5	Ia	E	0
38	0	0	Genotype 1a	Genotype E	0
39	EHNV	0	0	0	0
40	N/A	N/A	VHSV Ia	IHNV M	SVCV (1) d
41	EHNV	IPN Genogroup 5, serotype: Sp, serogroup: A.	VHS Genogroup Ia	IHN M.	SVCV Genogroup 1d.
42	EHNV	Genogroup 5	Ia	E	Genogroup 1d
43	EHNV	5	I(a)	E	I(d)
44	EHNV	Strain Sp, Genogroup 5	Type Ia	Genogroup E	Type Id

[1] This laboratory has not provided corroborating data to support the finding of EHNV in ampoule I
[2] This laboratory doesn't test for SVCV
[3] This laboratory doesn't test for IPNV

91% of the participating laboratories obtained 100% success rate in PT1.

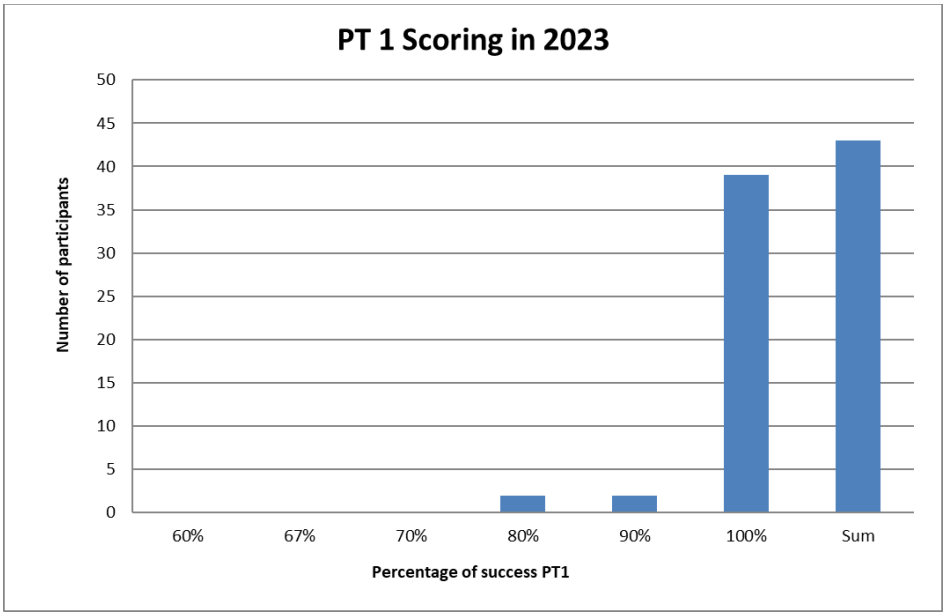

Figure 3 Success-rate of participating laboratories 2023 for PT1

Résumé and concluding remarks PT1

93% of the parcels were delivered by the shipping companies within two week and 100% was delivered within 22 days.
Overall, 39 out of 43 participants scored 100% success rate; out of the 4 laboratories which underperformed two participants scored <100% for the sole reason that they did not back up their concluding results of ampoule I (EHNV) with sequencing. 2 laboratories identified EHNV in another ampoule than the designated one, suggesting a contamination. Suggestions to improve on underperformance will be provided individually to each laboratory. One laboratory never handled in the result.

In this report, 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 as well as with the EURL. 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 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. It has been observed that, in compliance with options provided by the legislative framework, some NRLs are not using cell culture anymore for titration and isolation of viral isolates included in PT1.

Although the direct comparison of Ct Values cannot be done due to specific differences in laboratory, reagents, assay setup etc. the table 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 in May.

Concerning sequence analysis this report can act as tool so that each laboratory can compare its own sequence analysis and genotyping.
The sequencing and genotyping of VHSV and IHNV is well implemented in the network of laboratory participating in this Inter-Laboratory proficiency test, 37 laboratories have sequenced VHSV, and 35 have correctly genotyped the isolate in ampoule III as Genotype Ia. 37 laboratories have sequenced IHNV in ampoule IV, and all 37 have correctly genotyped the IHNV as Genogroup E or M (four M)
Since genogroup “E”, is being one of the first isolate discovered in Europe, it likely to belong to M Genogroup, hence “M” also has been considered correct answer.

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 28th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 29th of May, 2024.The meeting will be held online.

Proficiency test 2, PT2

Four ampoules containing lyophilised cell culture supernatant were delivered to the same laboratories that participated in PT1.

Content of ampoules

The viruses were propagated on each of their preferred cell line and 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 prepared, the concentration of the viral stocks was analysed by the KHV real-time PCR protocol described by Gilad et *al*. (2004) [8], the SAV real-time RT-PCR protocol described by Hodneland et al. (2006) [10], and the ISAV real-time RT-PCR protocol described by Snow et *al*. (2006) [12] .
Each viral stock was further identified by PCR and sequencing. For KHV according to the method described by Bercovier et *al*. (2005) [9] , for SAV according to the conventional PCR targeting segment E2 described by Fringuelli et al. (2008) [11] and for ISAV with conventional RT-PCR protocol described by Mjaaland et *al*. (2002) [13].
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: KHV	**KHV 07/108b** **Received from** Dr. J. Castric, ANSES, France. **Reference on the isolate** Bigarre L., Baud M., Cabon J., Antychowicz J., Bergmann S.M.,Engelsma M., Pozet F., Reichert M. & Castric J. (2009) Differentiation between Cyprinid herpesvirus type-3 lineages using duplex PCR. Journal of Virological Methods 158, 51–57.
Ampoule VII: Blank	Transport medium (US grade) with 10% fetal bovine serum
Ampoule VIII: SAV	**Salmonid alpha virus (SAV) 2, Sleeping disease virus (SD)** **Received from** Dr. J. Castric, ANSES, France in 19. as isolate sp49 **Genotype: 2** **GenBank accession number:** KC593283.1.

Code	Specifications/References
	References on isolate: Castric J., Baudin Laurencin F., Brémont M., Jeffroy J., Le Ven A. & Béarzotti M. (1997) Isolation of the virus responsible for sleeping disease in experimentally infected rainbow trout (Oncorhynchus mykiss). *Bulletin of the European Association of Fish Pathologists* 17, 27–30. Villoing S., Béarzotti M., Chilmonczyk J.C. & Brémont M. (2000) Rainbow trout sleeping disease virus is an atypical alphavirus. *Journal of Virology* 74, 173–183. **Reference on sequence:** E Fringuelli, H M Rowley, J C Wilson, R Hunter, H Rodger, D A GrahamPhylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2 and nsP3 gene nucleotide sequences Journal of fish diseases Volume 31, Issue 11 November 2008 Pages 811–823
Ampoule IX: ISAV	**ISAV Glesvaer/2/90** **Received from:** Dr. B. Dannevig, OIE Reference Laboratory for ISA, Oslo, Norway **HPR Genotype:** 2 **GenBank accession numbers:** HQ259676, or AF220607.1 or DQ785248.1 **References on isolate:** Dannevig BH, Falk K & Namork E (1995). Isolation of the causal virus of infectious salmon anaemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *Journal of General Virology* 76, 1353–1359. Falk K, Namork E, Rimstad E, Mjaaland S & Dannevig BH (1997). Characterization of infectious salmon anemia virus, an orthomyxo-like virus isolated from Atlantic salmon (Salmo salar L.) *Journal of Virology* 71, 9016-9023. **References on sequence:** Mérour E, LeBerre M, Lamoureux A, Bernard J, Brémont M & Biacchesi S (2011). Completion of the full-length genome sequence of the infectious salmon anemia virus, an aquatic orthomyxovirus-like, and characterization of mAbs. *Journal of General Virology* 92, 528-533. **References on genotype:** Table 15. Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety 26.01.07. Which risk factors relating to spread of Infectious Salmon Anaemia (ISA) require development of management strategies? Dok.nr.06/804, 68 pages.

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))[8] for KHV, by real-time RT-PCR (Snow et *al*. (2006)) [12]for ISAV and by real-time RT PCR (Hodneland et al. (2006))[10] for SAV, to ascertain identity and homogeneity of the content in the ampoules (annex 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 annex 4). Conventional PCR/RT-PCR fragments were sequenced and so was the HPR region in segment 6 of the ISAV isolates.

Table 12: Ct-value of ampoules VI to IX tested before and immediately after lyophilisation and after deadline for handling in results**.**

Ampoule	Content	No.	EURL before lyophilization	EURL right after lyophilization	EURL after deadline for answering
Ampoule VI	**KHV**	a	24.40	28.64	26.67
		b		28.39	
		c		28.47	
		d		28.30	
		e		28.32	
				28.42	
Ampoule VII	**Blank**	a	No Ct.	No Ct.	No Ct.
		b		No Ct.	
		c		No Ct.	
		d		No Ct.	
		e		No Ct.	
				No Ct.	
Ampoule VIII	**SAV**	a	26.46	27.11	26.86
		b		27.11	
		c		27.02	
		d		27.09	
		e		27.22	
				27.11	
Ampoule IX	**ISAV**	a	25.86	29.35	29.82
		b		29.30	
		c		29.54	
		d		29.54	
		e		29.25	
				29.39	

The lyophilisation procedure caused a significant virus reduction in the ampoules containing KHV and ISAV (approx. 4 Ct. values) 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 according to diagnostic procedures described in the EURL diagnostic manuals [2]. 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.
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, 2023 - Virus identification.

Laboratory code number	Score	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
		KHV	Blank	SAV	ISAV
1	8/8	KHV	Negative	SAV	HPR-deleted ISAV
2	8/8	KHV	no virus detected	SAV	ISAV
3[1]	6/6	KHV	-	-	ISAV_HPR-deleted
4	6/6	KHV	not KHV, not ISAV	not KHV, not ISAV	ISAV
5[2]	7/8	KHV	no virus	SAV	ISAV
6	8/8	KHV	Neg	SAV	HPR-deleted ISAV
7	8/8	KHV	No Pathogen Detected	SAV	HPR-deleted ISAV
8	8/8	KHV	not KHV, not ISAV nor SAV	SAV	HRP-deleted ISAV
9	8/8	KHV	negative	SAV	HPR-deleted ISAV
10	8/8	KHV	No virus (Not KHV, SAV or ISAV)	SAV	HPR deleted ISAV
11[1]	6/6	KHV	no ISAV, no KHV	no ISAV, no KHV	HPR-deleted ISAV
12	8/8	KHV	Negative	SDV/SAV	ISAV-HPRdeleted
13	8/8	KHV	No virus	SAV	HPR-deleted ISAV
14	8/8	KHV	0	SAV	HPR-deleted ISAV
15	8/8	KHV	negative	SAV	ISAV HPRdel
16[1]	6/6	KHV	NO KHV, NO ISAV	NO KHV, NO ISAV	HPR-deleted ISAV
17[1]	6/6	KHV	0	0	HPR-deleted ISAV
18	8/8	KHV	Not ISAV, Not SAV, Not KHV	SAV	HPR-deleted ISAV

20	8/8	KHV	Blank	SAV	HPR-deleted ISAV
21	8/8	KHV	NEG	SAV	HPR-deleted ISAV
22	4/8	KHV	0	0	0
23[1]	6/6	KHV	0	0	ISAV
24[2]	7/8	KHV	-	SAV	ISAV
25	8/8	KHV	N/A	SAV	ISAV HPR-deleted
26	8/8	KHV	No virus detected	SAV	HPR-deleted ISAV
27	8/8	KHV	-	SAV	HPR-deleted ISAV
28[1]	6/6	KHV	Negative for ISAV and KHV	Negative for ISAV and KHV	HPR-deleted ISAV
29	7/8	KHV	NOT ISAV, NOT KHV, NOT SAV	SAV	ISAV
30	8/8	KHV	NOT ISAV, KHV, SAV	SAV	HPR-deleted ISAV
31	8/8	KHV	Negative	SAV	HPR deleted ISAV
32	8/8	KHV	0	SAV	HPR-deleted ISAV
33	8/8	KHV	Negative/Not detected	SAV	ISAV
34	8/8	KHV	NEGATIVE	SAV	ISAV
35	8/8	KHV	NEGATIVE	SAV	HRP-deleted ISAV
36	8/8	KHV	NEG	SAV	HPR-deleted ISAV
37	8/8	KHV	0	SAV	HPR-deleted ISAV
38	8/8	KHV	No ISAV,No KHV, No SAV	SAV	HPR-deleted ISAV
39	8/8	KHV	negative	SAV	HRP-deleted ISAV
40	8/8	KHV	Negative	SAV	HPR-deleted ISAV
41	8/8	CyHV3 - KHV	NEGATIVE/BLANK	SAV	ISAV
42	8/8	KHV	-	SAV	HPR-deleted ISAV
43	8/8	KHV	Not KHV, SAV, or ISAV	SAV	HPR deleted ISAV
44	8/8	KHV	Blank	SAV	HPR-deleted ISAV

[1)] Did not test for SAV

[2)] Did not sequence ISAV

All laboratories are asked to sequence the HPR region of ISAV isolates to distinguish from the pathogenic HPRΔ variant from ISAV HPR0.

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.
- 32 laboratories correctly identified all four ampoules (KHV, Blank, SAV, ISAV)
- All 43 laboratories tested for the two listed pathogens (KHV, ISAV)
- 36 laboratories tested for SAV.

Ampoule VI – KHV

- All 43 laboratories correctly identified KHV.

Ampoule VII – Blank

- All 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 VIII – SAV

- 35 (out of 36) laboratories correctly identified SAV
- 7 laboratories did not participate for SAV and answered '0' or 'no ISAV; no KHV'

Ampoule IX – ISAV

- 42 laboratories correctly identified ISAV hereof three laboratories did not sequence and one laboratory did not find the pathogen.

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 gives score of 2 points.
For the ISAV isolate in ampoule IX, full score was given if ISAV virus was detected by molecular methods, and if the isolate was sequenced to discriminate between listed HPRΔ ISAV and non-listed HPR0 ISAV.
Of the 43 laboratories submitting results 39 laboratories obtained maximum score. The maximum score was calculated according to the number of pathogens tested by the laboratory.
A laboratory could obtain a maximum score of 8 if tested for all three pathogens included (ISAV, KHV and SAV). A maximum score of 6 is given if only tested for ISAV and KHV.
3 laboratories scored below 100% only due to no identification by sequencing of ISAV in ampoule IX.

Ct. values comparison

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 also represented graphically in annex 5.
A very low Ct may indicate that the sample tested derives from cell culture isolate. A very high Ct may indicate that the assay in use or the procedure reduce the sensitivity of the method.
The Ct-values cannot be directly compared due to the use of different methods, reagents and equipment nucleic acid extraction and (RT)-qPCR.

Table 14. Inter-Laboratory Proficiency Test, PT2, 2023 – Ct.-values.

Laboratory Code number	Ct. value Ampoule VI (KHV)	Ct. value Ampoule VIII (SAV)	Ct. value Ampoule IX (ISAV)
EURL	28.42	27.11	29.39
1	26.53	32.82	29.39
2	27.68	26.47	29.18
3	27.56	-	28.67
4	27.31	-	-
5	-	31.90	30.20
6	24.85	28.26	36.83
7	29.96	28.97	29.27
8	33.56	-	-
9	26.81	-	31.28
10	26.59	25.03	28.37
11	28.62	-	28.69
12	-	26.36	29.18
13	29.69	31.28	30.05
14	27.18	29.82	29.61
15	12.98	33.03	31.22
16	-	-	-
17	30.88	-	32.97
18	-	31.05	34.70
20	29.82	28.41	32.87
21	21.73	28.90	28.62
22	-	-	-
23	24.52	-	23.90
24	24.70	32.61	30.40
25	32.01	32.41	36.97
26	-	26.52	30.63
27	25.97	32.72	35.24
28	28.64	-	28.86
29	24.10	-	-
30	23.22	31.33	-
31	25.80	-	-
32	31.40	-	28.90
33	-	-	-
34	-	-	-
35	-	-	-
36	25.00	-	21.80
37	26.51	29.24	30.46
38	29.40	-	35.64
39	26.89	27.00	29.00
40	-	-	-
41	25.58	29.31	33.40
42	29.70	29.10	31.70
43	28.80	-	32.30
44	31.48	29.94	34.00

- No Ct-value given by the participating laboratory.

Amp.VII was blank (and found blank by all participants) and thereby not included.

Genotyping and sequencing

Participants were asked to sequence the HPR region of possible ISAV isolates and determine whether isolates included in the ampoules were HPRΔ ISAV currently listed in EU legislation or non-listed HPR0 ISAV, the correct characterization of HPRΔ ISAV has been calculated in the general score. Three laboratories did not sequence the ISAV isolate in ampoule IX. The identification of KHV didn't pose particular issues. Finally, regarding sequencing of SAV isolate in ampoule VIII, only 1 out 29 of laboratories which performed genotyping of SAV, assigned the incorrect genotype and four sequenced but did not give any genotype.
An Overview of the genotyping results obtained for PT2 by all participants is displayed in the following table 15.

Table 15 Inter-Laboratory Proficiency Test, PT2, 2023 – Genotyping

Code number	Ampoule VI	Ampoule VIII	Ampoule IX
	KHV	SAV	ISAV
1	CyHV3	2	HPR-deleted
2	Cyprinid herpesvirus 3; genotype: 3	SAV; subtype 2 (SAV2)	ISAV; HPR-deleted
3	N/A	N/A	N/A
4	0	0	Infectious salmon anemia virus isolate Glesvaer/2/90 segment 8
5	0	0	0
6	CyHV-3	2	HPR-deleted
7	0	2	HPR-deleted
8	CyHV-3	Genotype: 2	HPR deleted
9	CyHV 3	2	HPR-deleted
10	0	0	0
11	0	0	HPR2
12	0	Subtype = SAV1	Genotype = G3
13	CyHV-3	0	HPR-deleted
14	CyHV3	2	HPR-deleted
15	CyHV 3	II	2
16	CyHV-3	0	ISA-HPRΔ
17	CyHV 3	0	HPR-deleted

18	CyHV3	2	HPR-deleted
20	CyHV (3)	SAV 2	HPR-deleted
21	CyHV-3	SAV2	HPR-deleted
22	CyHV3	0	0
23	CyHV3	0	HPR2
24	0	0	0
25	CyHV 3	SAV2	HPR2
26	CyHV3	2	HPR-deleted
27	0	0	HPR-deleted
28	CyHV (3)	0	HPR-deleted
29	CyHV 3	II	0
30	CyHV3	0	HPR-deleted
31	0	0	HPR-deleted ISAV
32	CyHV3	SAV2	HPR-deleted
33	CyHV3	SAV-2 , SDV; Genotype 2	ISAV -HPRΔ (Genotype 2)
34	Cyprinid herpesvirus 3	II	PR4
35	CyHV3	Subgroup 2	HPR Group2 - Europe G2
36	CyHV 3	SAV 2	HPR-deleted (PR4)
37	0	2	HPR-deleted
38	0	0	0
39	0	0	HRP-deleted
40	CyHV-3	SAV 2	HPR-deleted
41	CyHV 3	SAV subtype 2	ISA HPR deleted
42	CyHV 3	2	HPR-deleted
43	CyHV-3	2	HPRO-deleted/European
44	0	0	HPR deleted; Genotype 2

91% of the participating laboratories obtained 100% success rate in PT2.

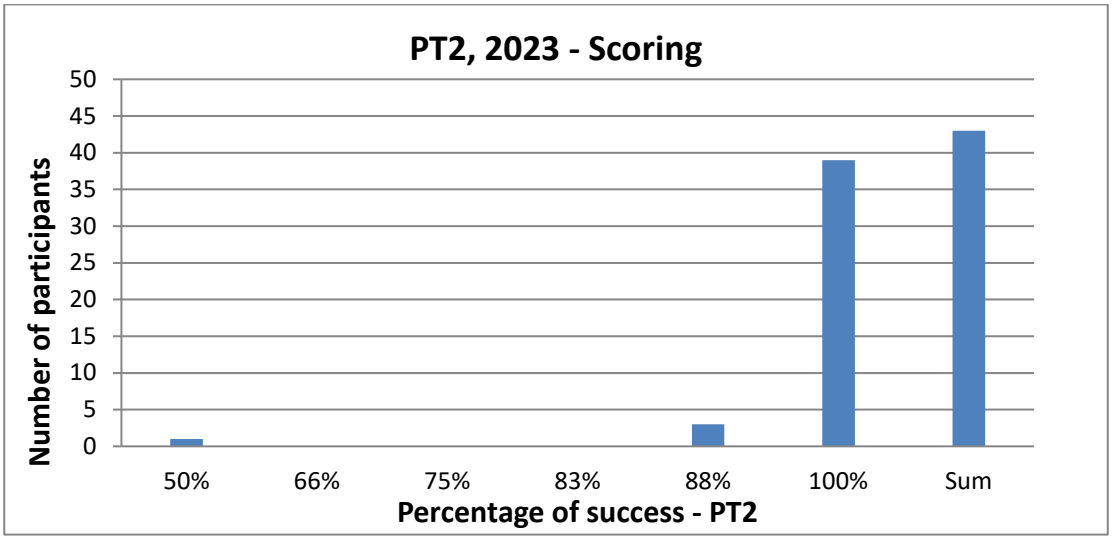

Figure 4 Success-rate of participating laboratories 2023 for PT2

Concluding remarks PT2

43 laboratories participated in PT2, 39 obtained 100% success rate. Out of the 4 laboratories which underperformed, three obtained a lower score do to not providing sequencing-results for the ISAV isolate in ampoule IX. This point will be addressed directly with the participants that has underperformed.

All 43 laboratories correctly identified the CyHV-3 (KHV) in ampoule VI.

42 laboratories correctly identified the ISA virus in ampoule IX, hereof three laboratories did not sequenced. One laboratory did not find the ISAV in this ampoule.

36 laboratories tested for SAV and 35 correctly identified the virus in Ampoule VIII, 7 laboratories did not test for SAV and one laboratory who tested for SAV did not find the SAV in this ampoule.

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.

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 28th Annual Workshop of National Reference Laboratories for Fish Diseases to be held May 29th, 2024. The meeting will be held on-line.

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Denmark

March 2023.

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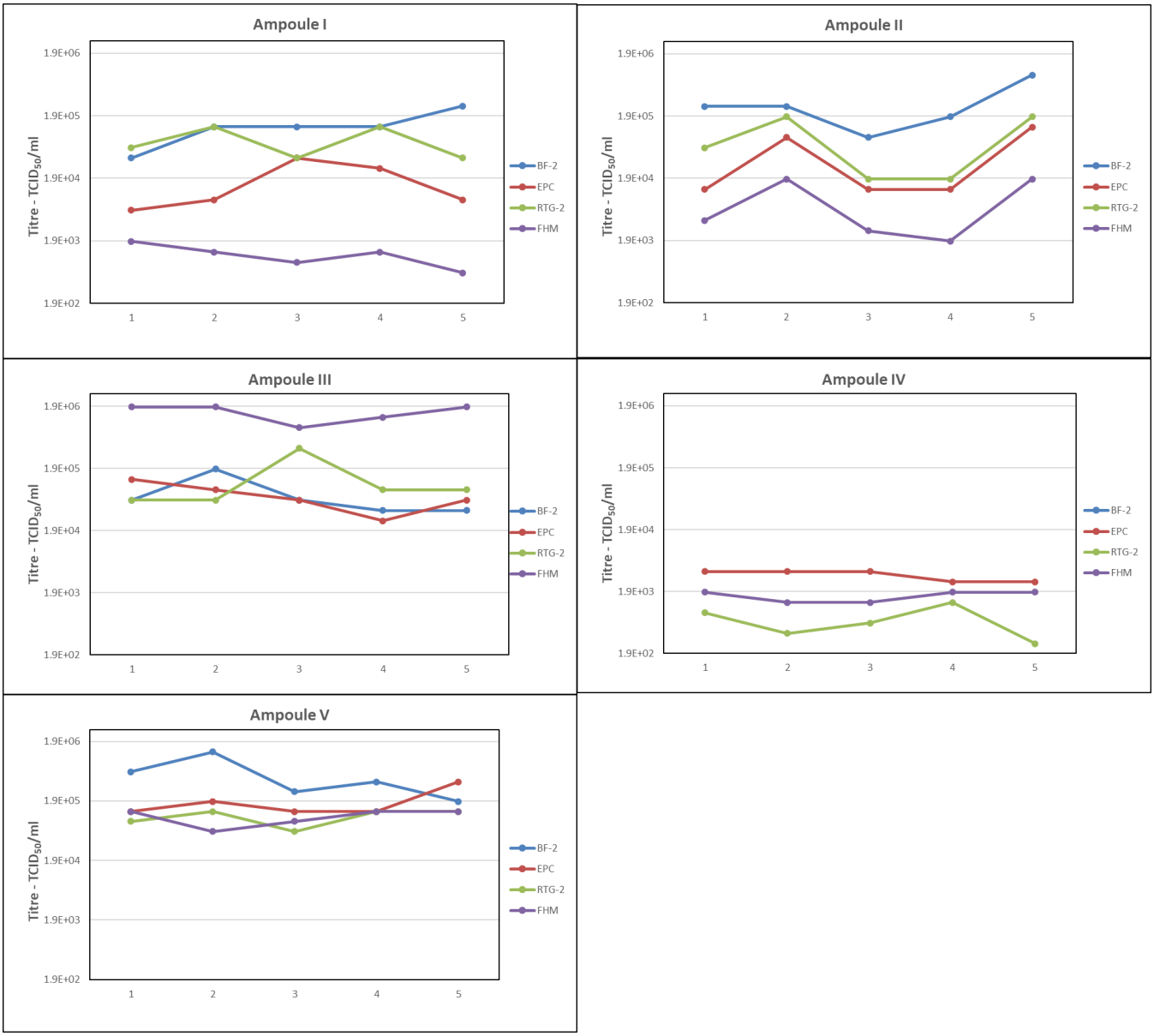
Appendix

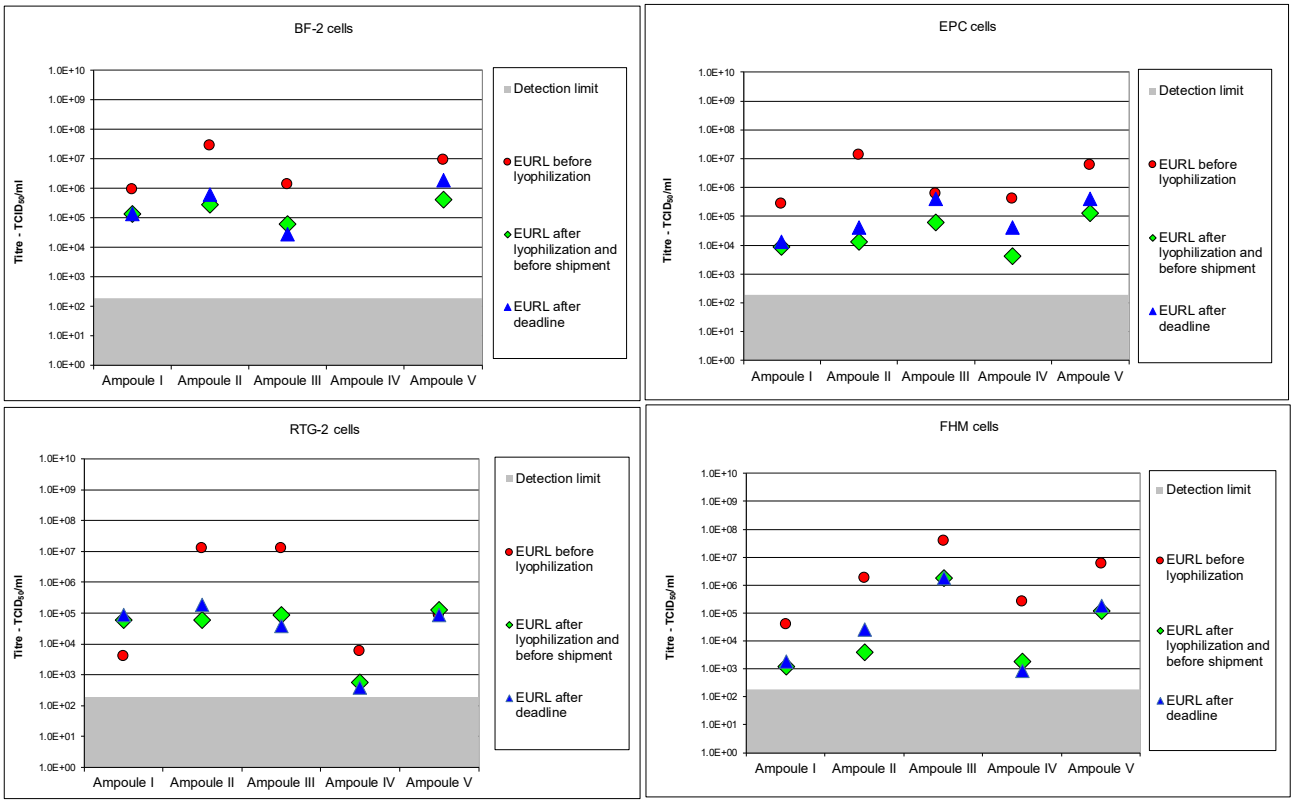
Annex 1: Stability and homogeneity of the content in the ampoules (PT1)

After Lyophilization the stability of the content in the ampoules were assessed by titrating the virus in five ampoules on cell cultures. For all of the ampoules, the reduction of the titre was max. 1 log in the same cell line.

Virus titres for all four cell lines in ampoule I to V after freeze-drying – In five random ampoules.



For all the ampoules, the reduction of the titre before and after freeze-drying was between 1-3 log in the same cell line. No significant reductions were observed after long term storage.



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

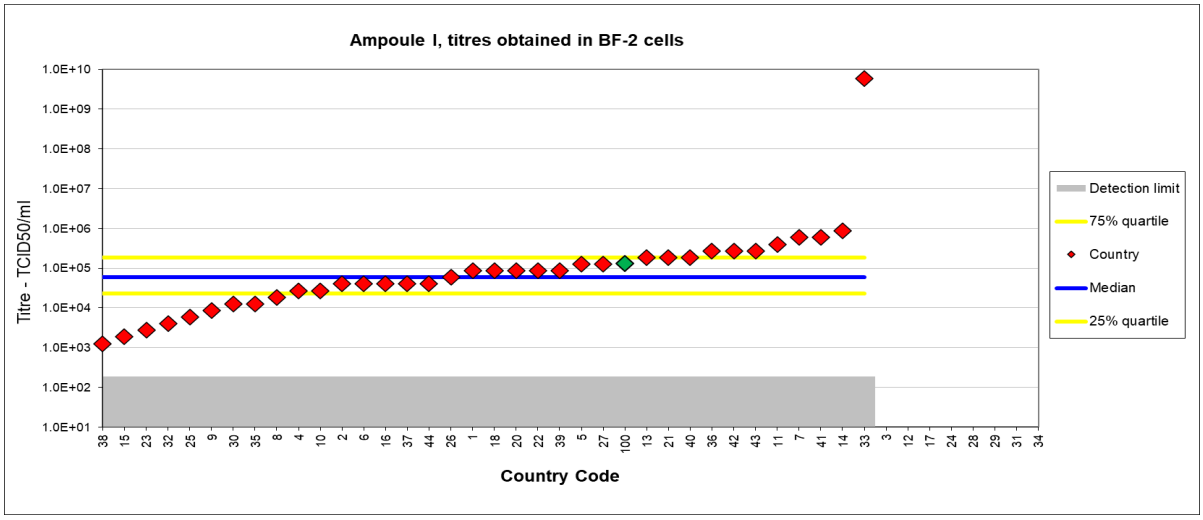
Annex 2: The titres obtained from each participating laboratory represented graphically.

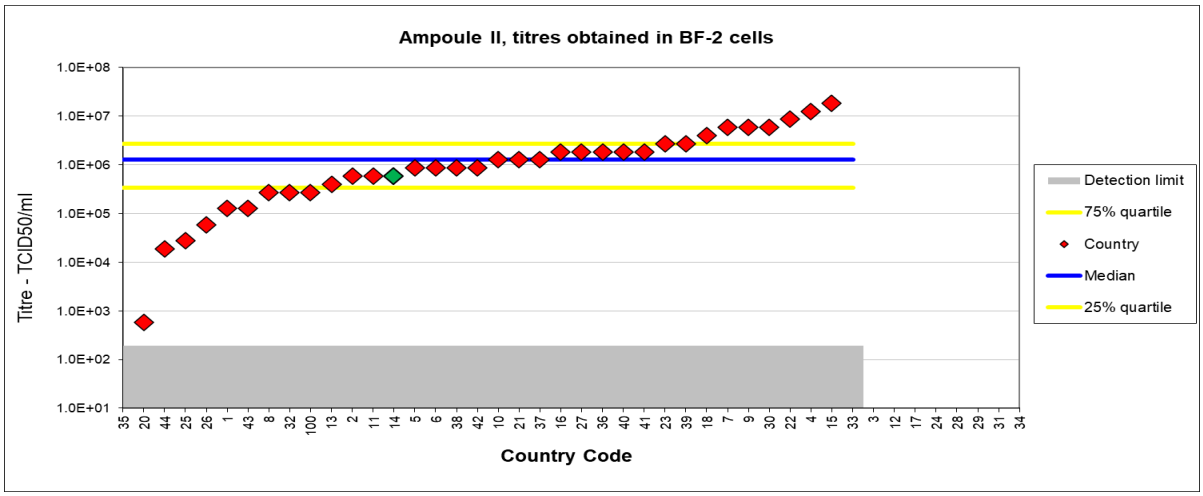
The titres (red diamonds) obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories as well as the titre obtained by the EURL (green diamond and code No. 100).

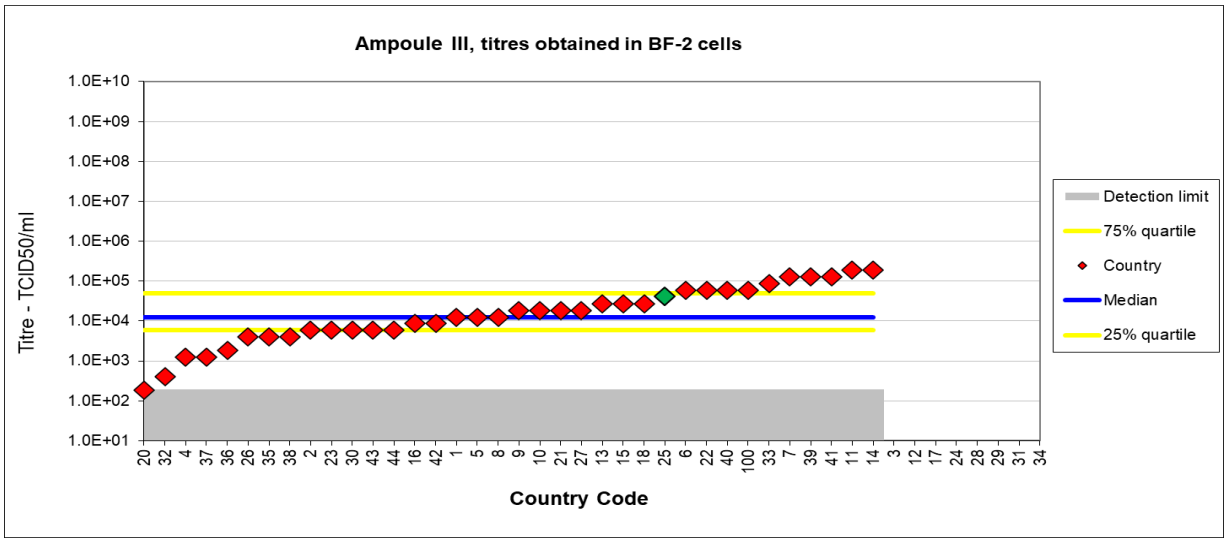
On these figures, The detection limit (grey shadow), the median titre (blue line), the 25% quartile (lower yellow line) and 75% quartile (upper yellow line) are plotted on all graphs.
Participants failing to obtain any titre are listed on the x axis under the grey zone but no red diamond is plotted; participants who did not use a specific cell line are listed after the grey zone.
Only one yellow line shown on the graphs, means the 25% quartile (lower yellow line) are below the detection limit.

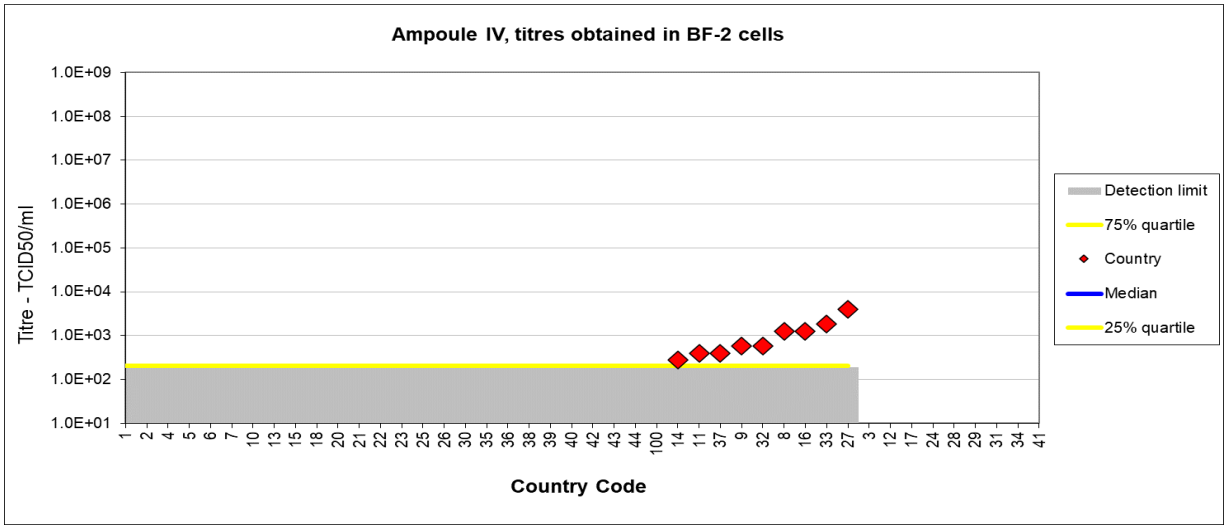
The optimal titre will be within the 25% and 75% inter-quartile range. A low titre, below 25% quartile may be indicator of low sensitivity of the cell culture in use; conversely a very high titre, beyond 75% quartile may indicate errors in assessing CPE.

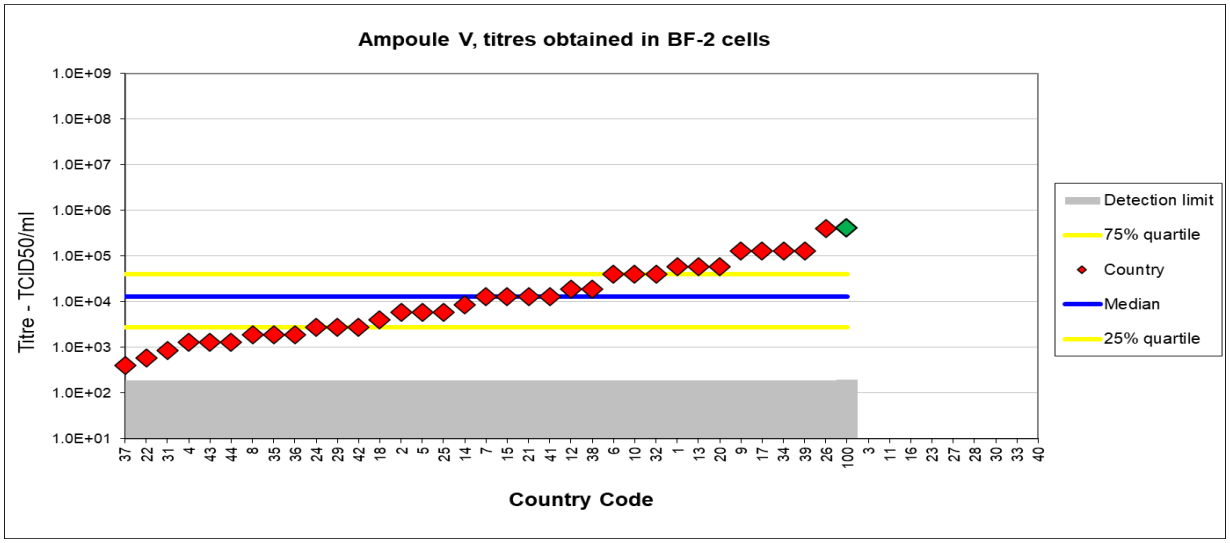
Virus titres obtained in BF-2 cells.



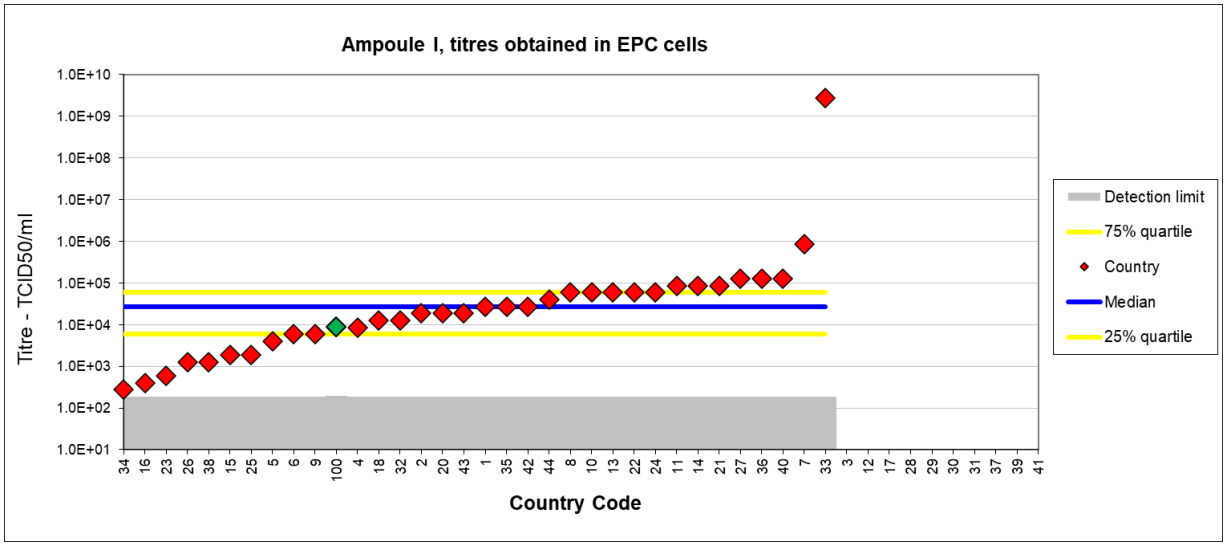


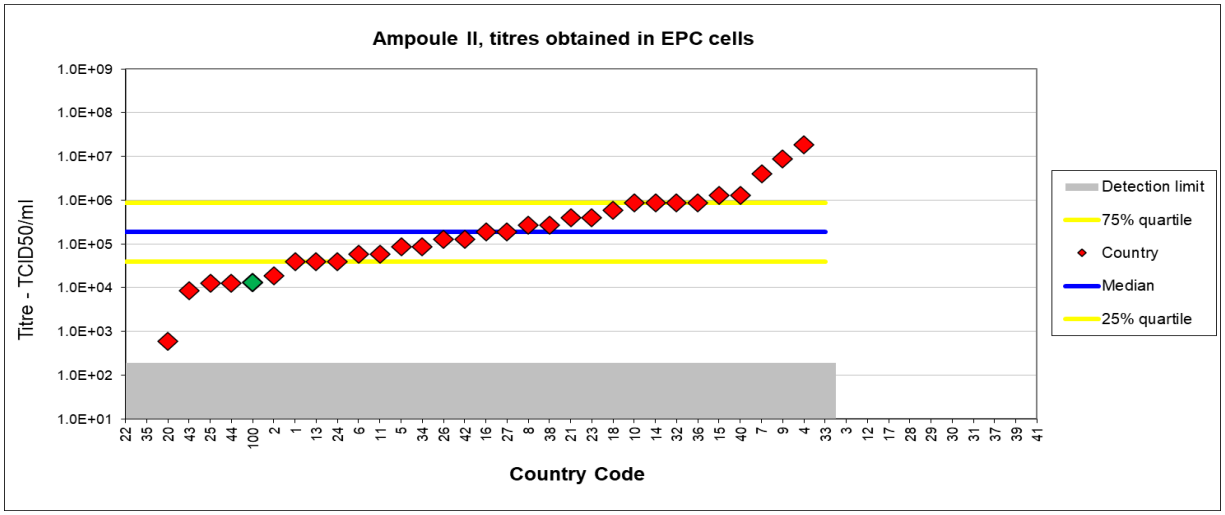

Ampoule III, titres obtained in BF-2 cells
Titre - TCID50/ml
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
20 32 4 37 36 26 35 38 2 23 30 43 44 16 42 1 5 8 9 10 21 27 13 15 18 25 6 22 40 100 33 7 39 41 11 14 3 12 17 24 28 29 31 34
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

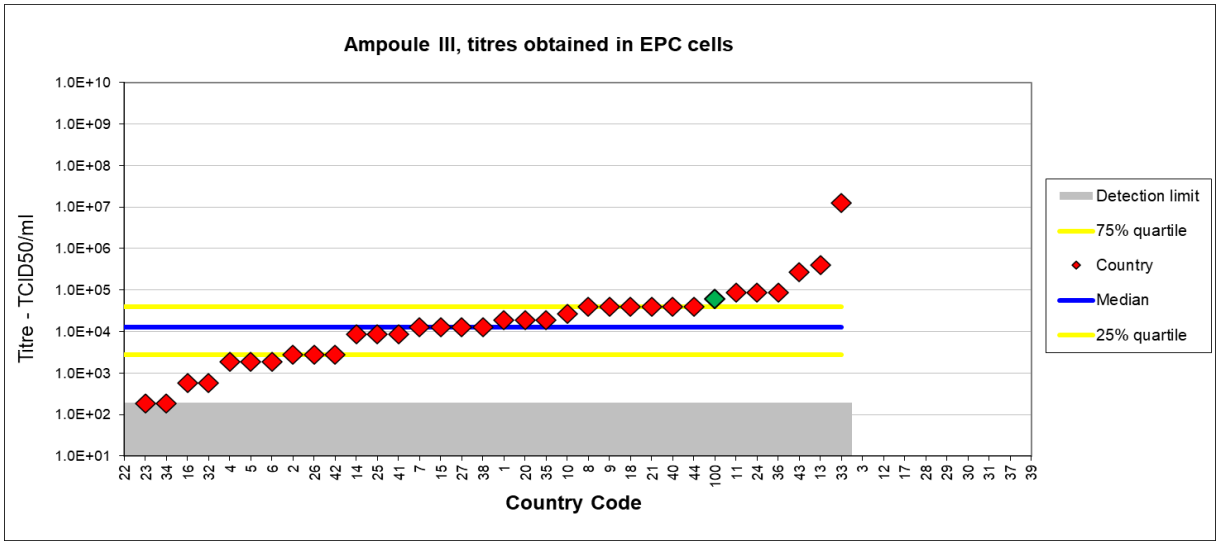

Ampoule IV, titres obtained in BF-2 cells
Titre - TCID50/ml
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1 2 4 5 6 7 10 13 15 18 20 21 22 23 25 26 30 35 36 38 39 40 42 43 44 100 14 11 37 9 32 8 16 33 27 3 12 17 24 28 29 31 34 41
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

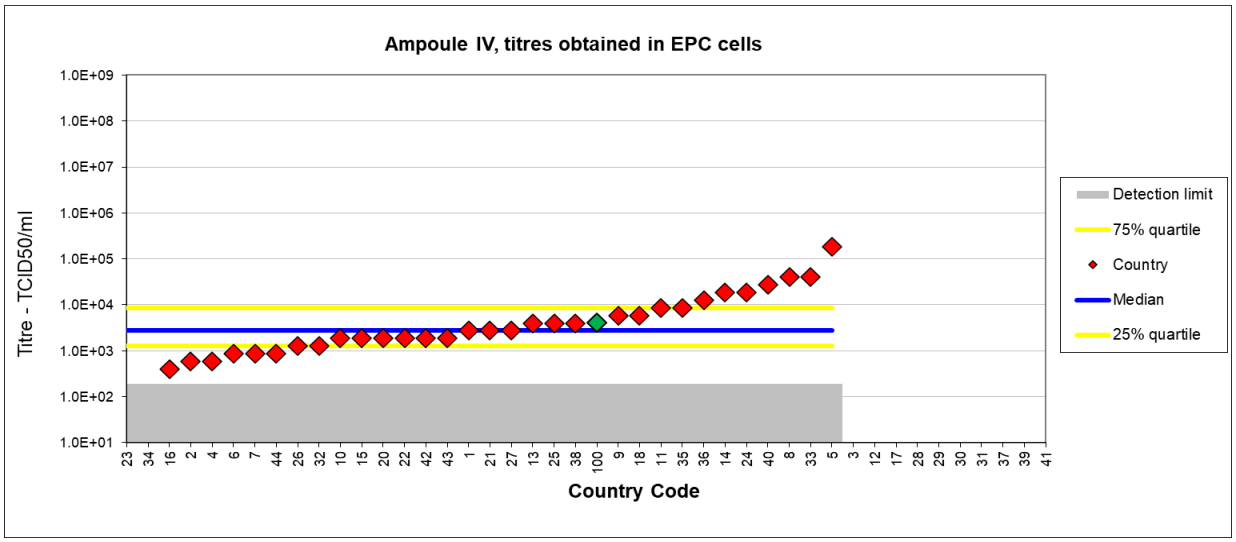

Ampoule V, titres obtained in BF-2 cells
Titre - TCID50/ml
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
37 22 31 4 43 44 8 35 36 24 29 42 18 2 5 25 14 7 15 21 41 12 38 6 10 32 1 13 20 9 17 34 39 26 100 3 11 16 23 27 28 30 33 40
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

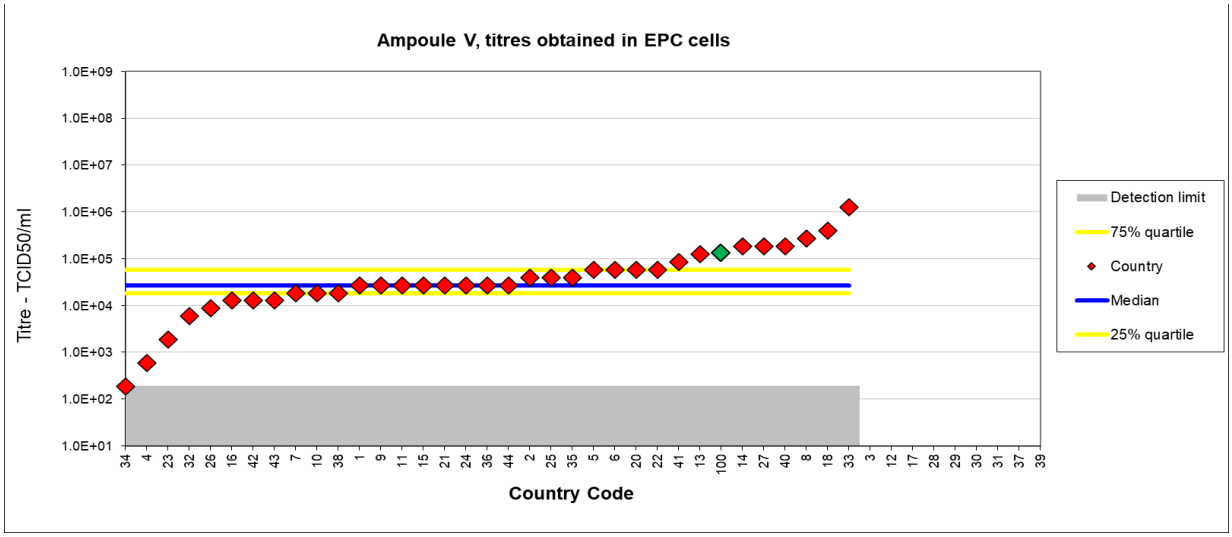
Virus titres obtained in EPC cells.



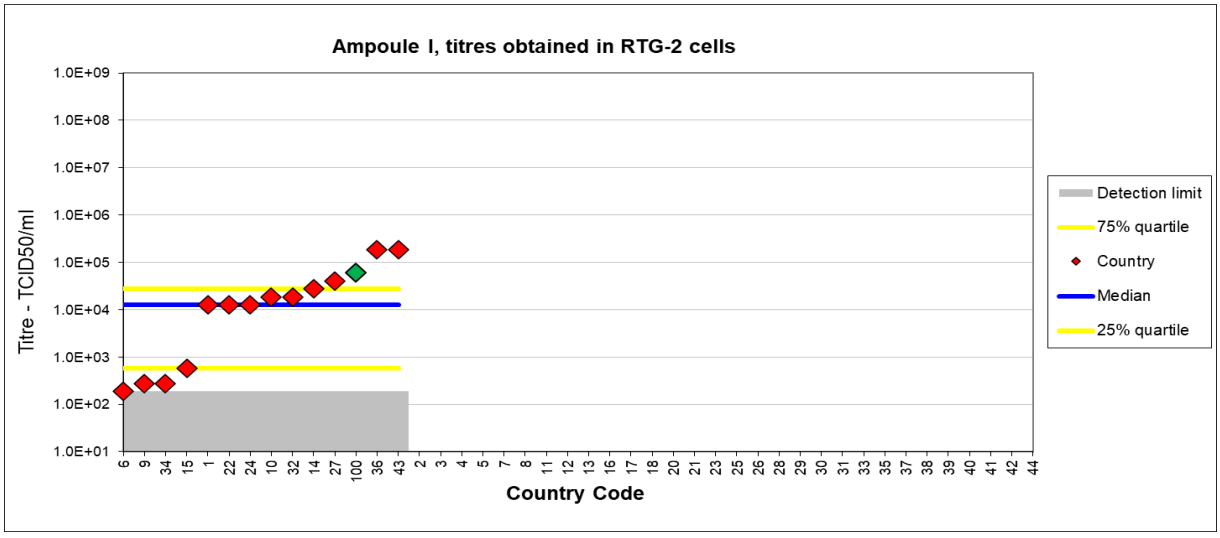


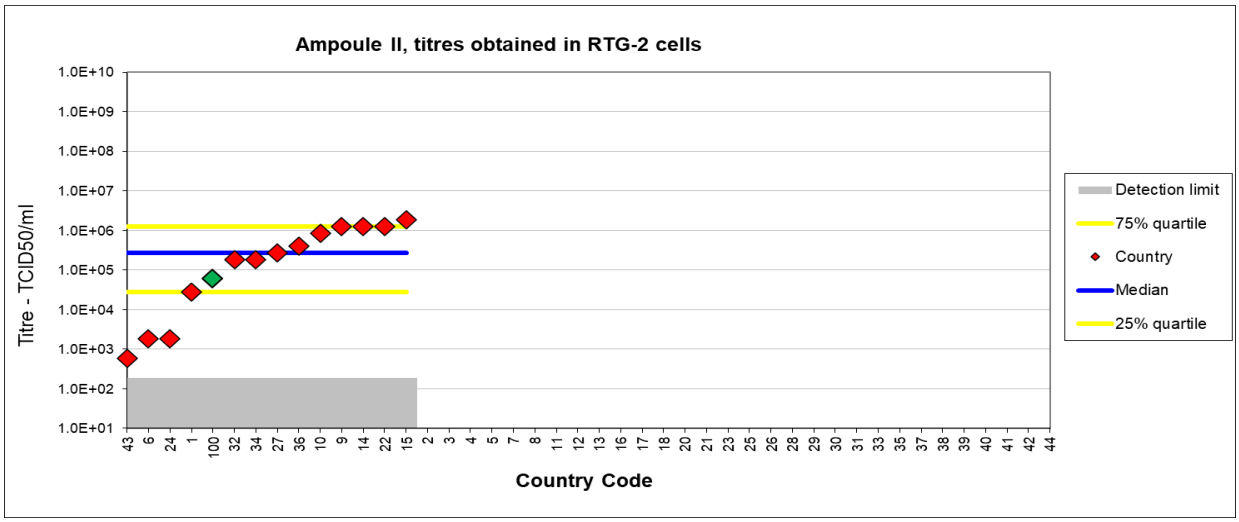


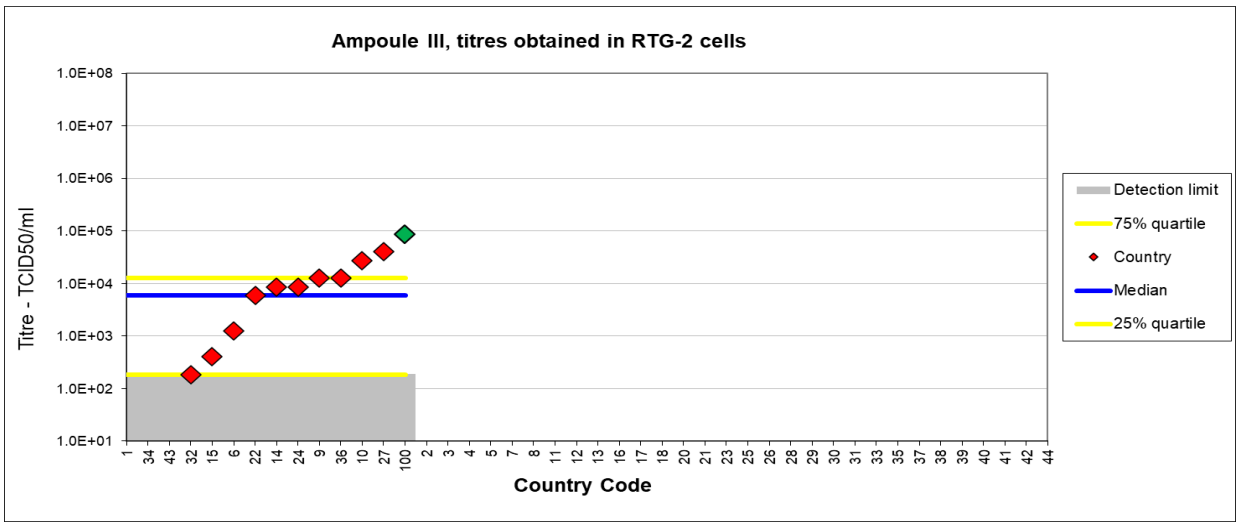


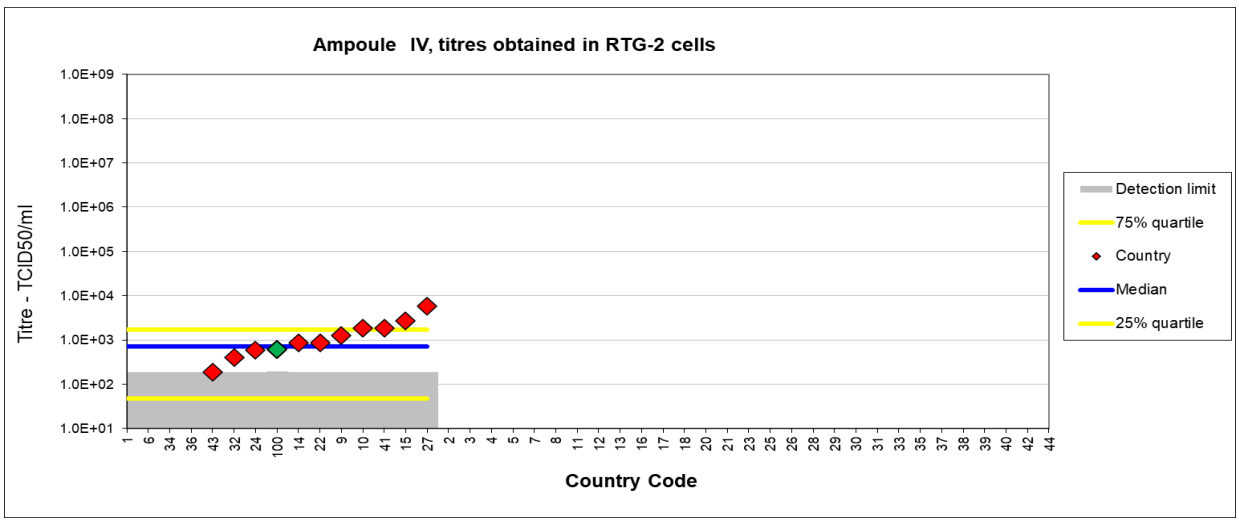


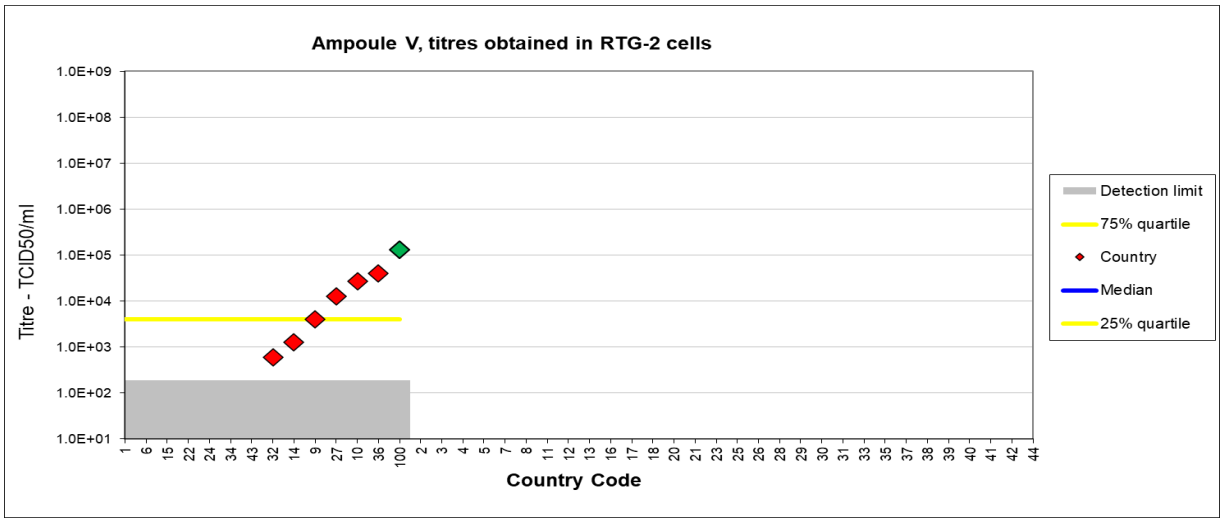
Virus titre obtained in RTG-2 cells.



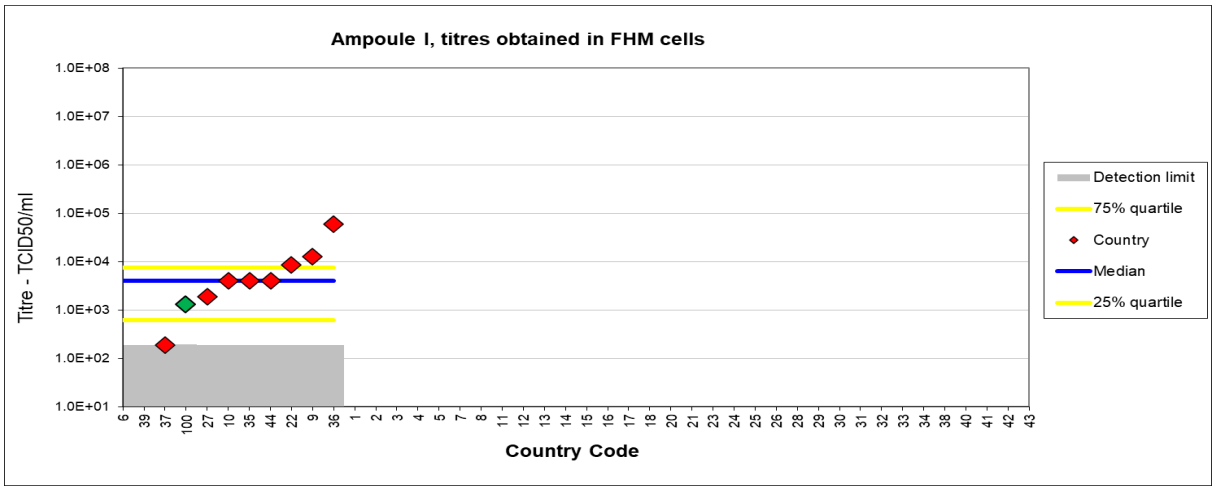

Ampoule II, titres obtained in RTG-2 cells
Titre - TCID50/ml
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
43 6 24 1 100 32 34 27 36 10 9 14 22 15 2 3 4 5 7 8 11 12 13 16 17 18 20 21 23 25 26 28 29 30 31 33 35 37 38 39 40 41 42 44
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

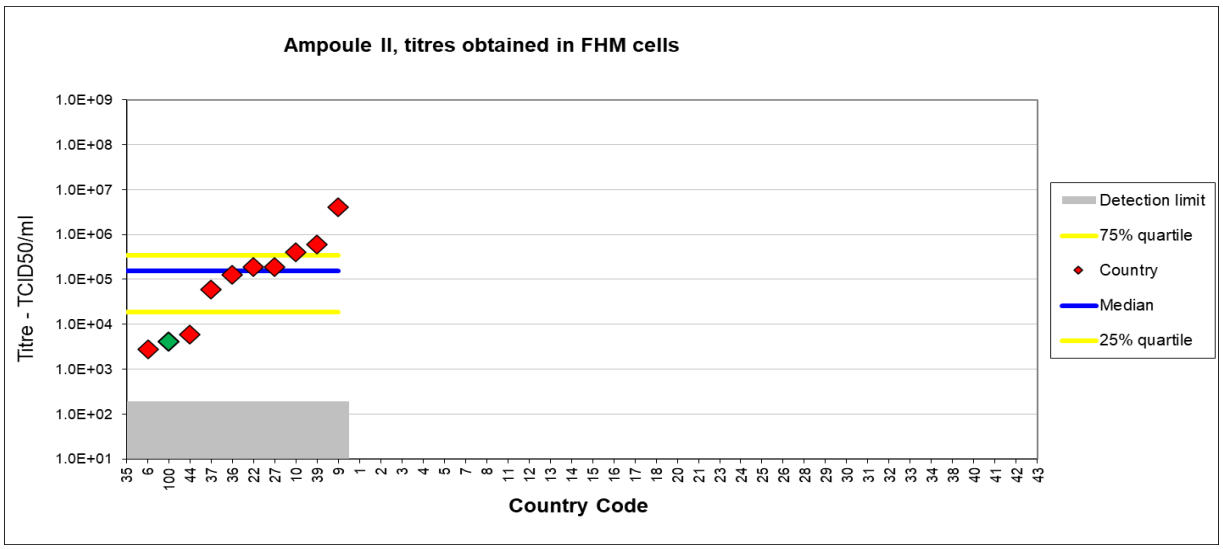

Ampoule III, titres obtained in RTG-2 cells
Titre - TCID50/ml
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1 34 43 32 15 6 22 14 24 9 36 10 27 100 2 3 4 5 7 8 11 12 13 16 17 18 20 21 23 25 26 28 29 30 31 33 35 37 38 39 40 41 42 44
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

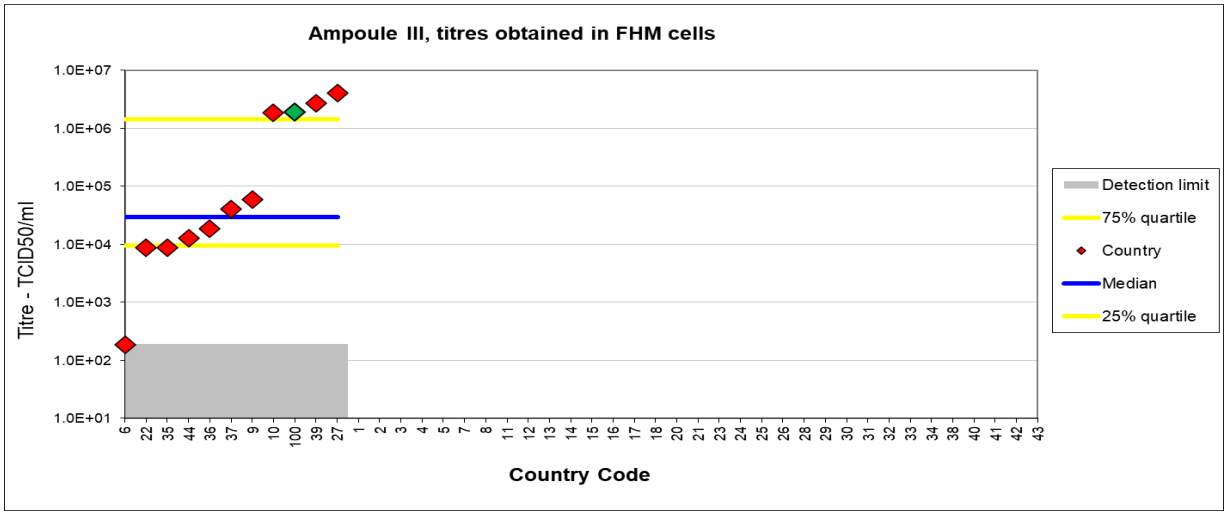

Ampoule IV, titres obtained in RTG-2 cells
Titre - TCID50/ml
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1 6 34 36 43 32 24 100 14 22 9 10 41 15 27 2 3 4 5 7 8 11 12 13 16 17 18 20 21 23 25 26 28 29 30 31 35 37 38 39 40 42 44
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

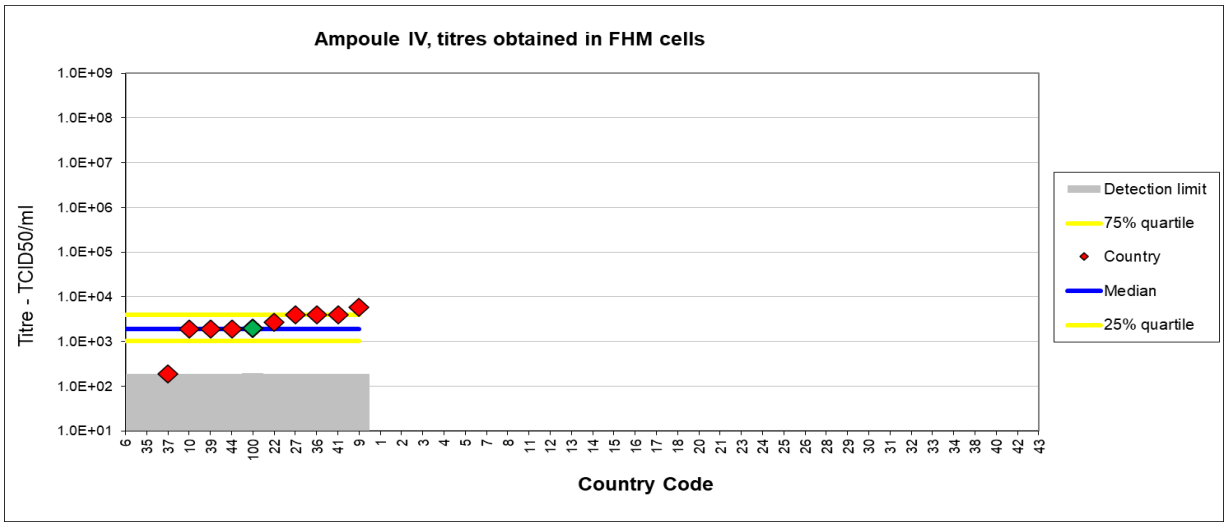


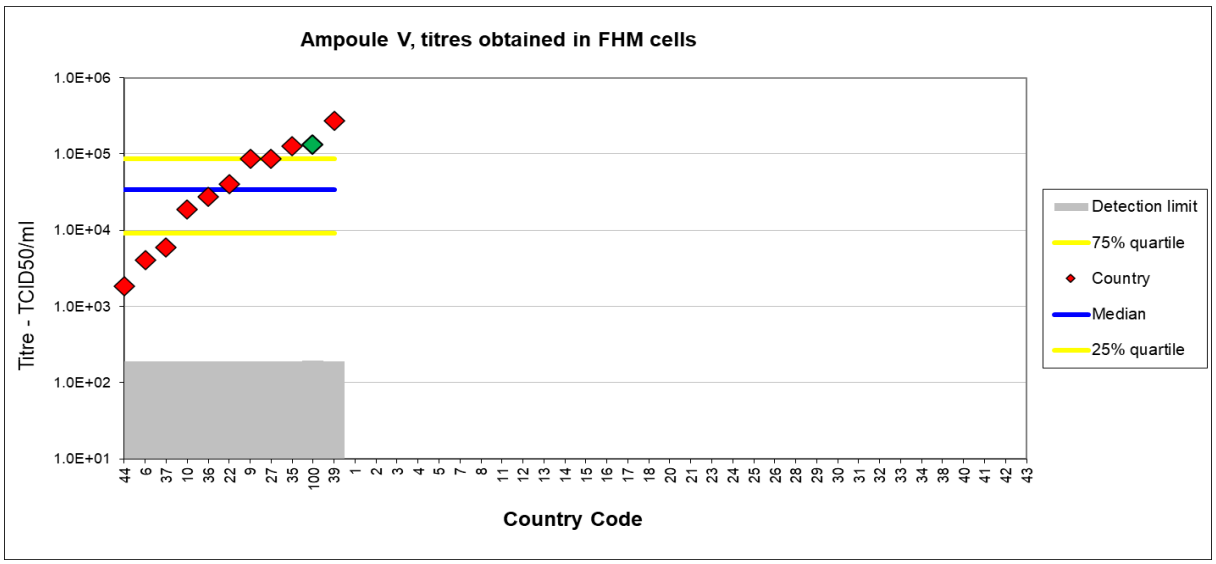
Virus titres obtained in FHM cells.



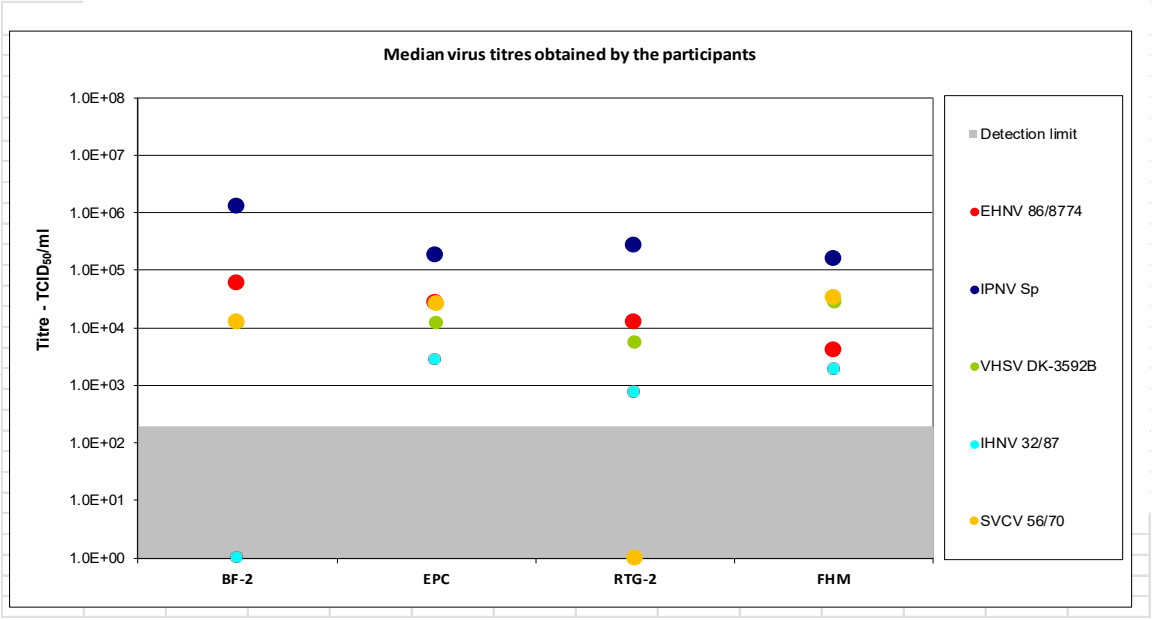



Ampoule III, titres obtained in FHM cells
Titre - TCID50/ml
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
6 22 35 44 36 37 9 10 100 39 27 1 2 3 4 5 7 8 11 12 13 14 15 16 17 18 20 21 23 24 25 26 28 29 30 31 32 33 34 38 40 41 42 43
Country Code
Detection limit
75% quartile
Country
Median
25% quartile


Ampoule IV, titres obtained in FHM cells
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
6 35 37 10 39 44 100 22 27 36 41 9 1 2 3 4 5 7 8 11 12 13 14 15 16 17 18 20 21 23 24 25 26 28 29 30 31 32 33 34 38 40 42 43
Country Code
Detection limit
75% quartile
Country
Median
25% quartile


Ampoule V, titres obtained in FHM cells
Titre - TCID50/ml
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
44 6 37 10 36 22 9 27 35 100 39 1 2 3 4 5 7 8 11 12 13 14 15 16 17 18 20 21 23 24 25 26 28 29 30 31 32 33 34 38 40 41 42 43
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

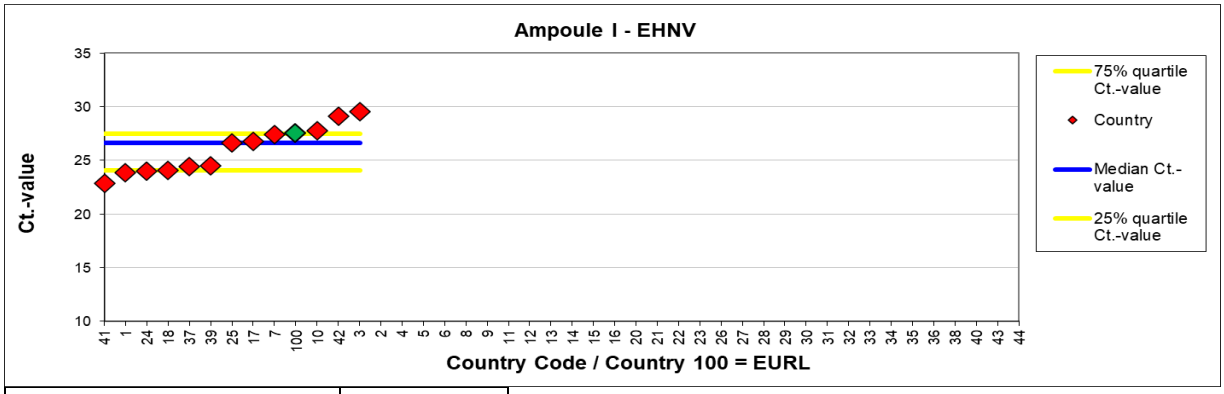
Median virus titres obtained by the participants in 4 different cell lines



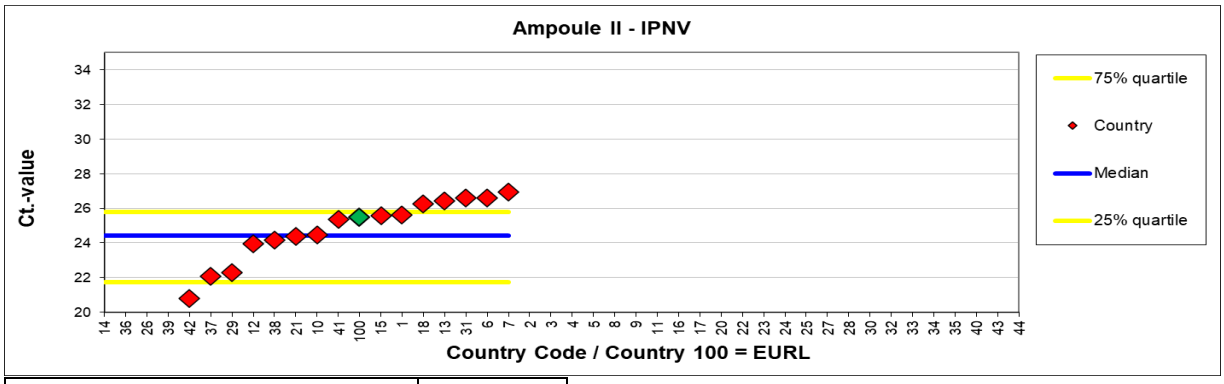
Annex 3: The Ct. values graphically (PT1) obtained from each participating laboratory.

The Ct. values (red diamonds) obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare their Ct. values with the other participating laboratories as well as the Ct. value obtained by the EURL (green diamond and code No. 100), to keep in mind that the Ct values cannot be directly compared due to the use of different methods, reagents and equipment.

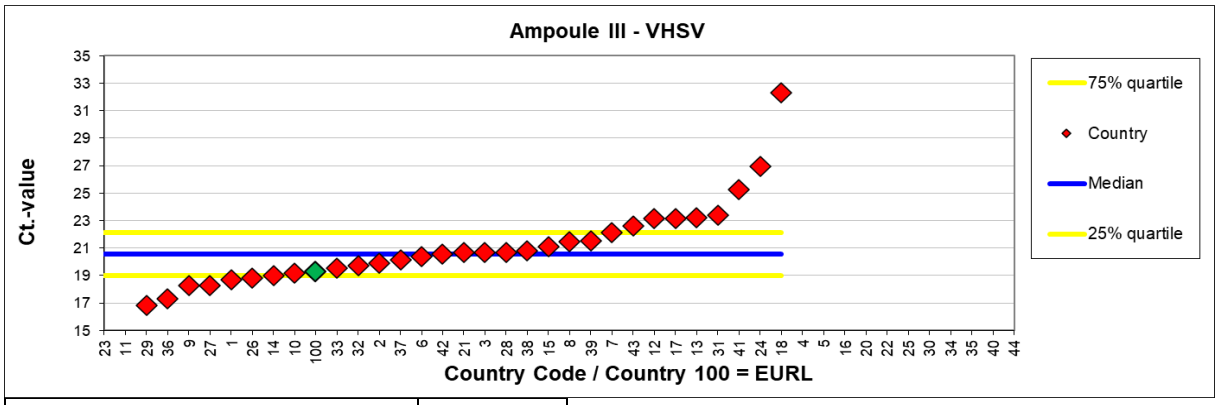
All Ct values submitted by the participants for each ampoule, are compared to each other, No red diamond = No Ct. value given. On these figures, the median values and the 25% and 75% inter-quartile range is displayed, the optimal value will be within these quartiles. A low Ct, below 25% quartile may be indicator of testing the isolate instead of the re-suspended material; conversely a very high Ct, beyond 75% quartile may indicate a lack of sensitivity in the method.



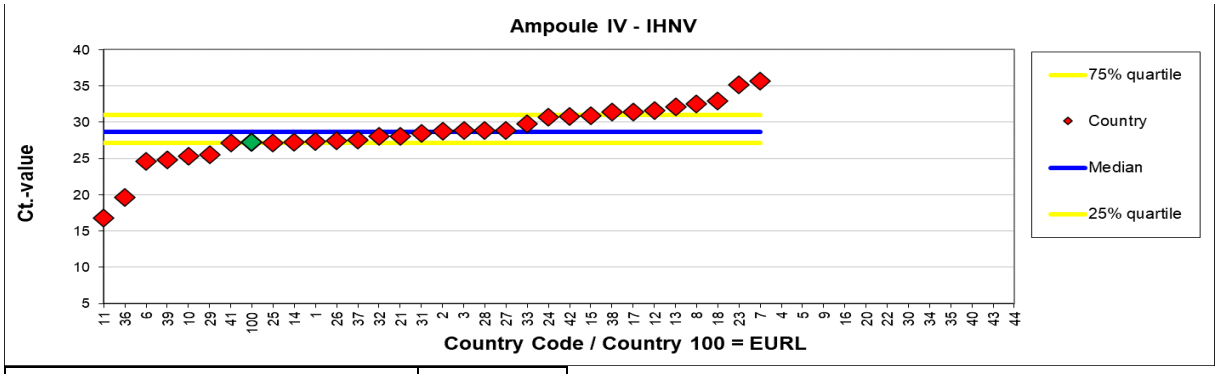
Number of laboratories	13
Median Ct.-value	26.6
Maximum Ct.-value	29.5
Minimum Ct.-value	22.9
25% quartile Ct.-value	24.1
75% quartile Ct.-value	27.5



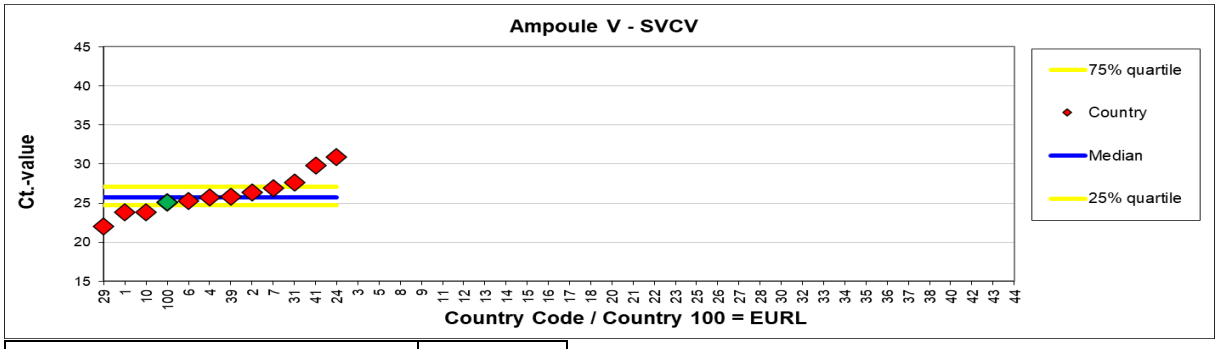
Number of laboratories	20
Median Ct.-value	24.4
Maximum Ct.-value	26.9
Minimum Ct.-value	15.7
25% quartile Ct.-value	21.8
75% quartile Ct.-value	25.8



Number of laboratories	33
Median Ct.-value	20.6
Maximum Ct.-value	32.3
Minimum Ct.-value	14.5
25% quartile Ct.-value	19.0
75% quartile Ct.-value	22.1



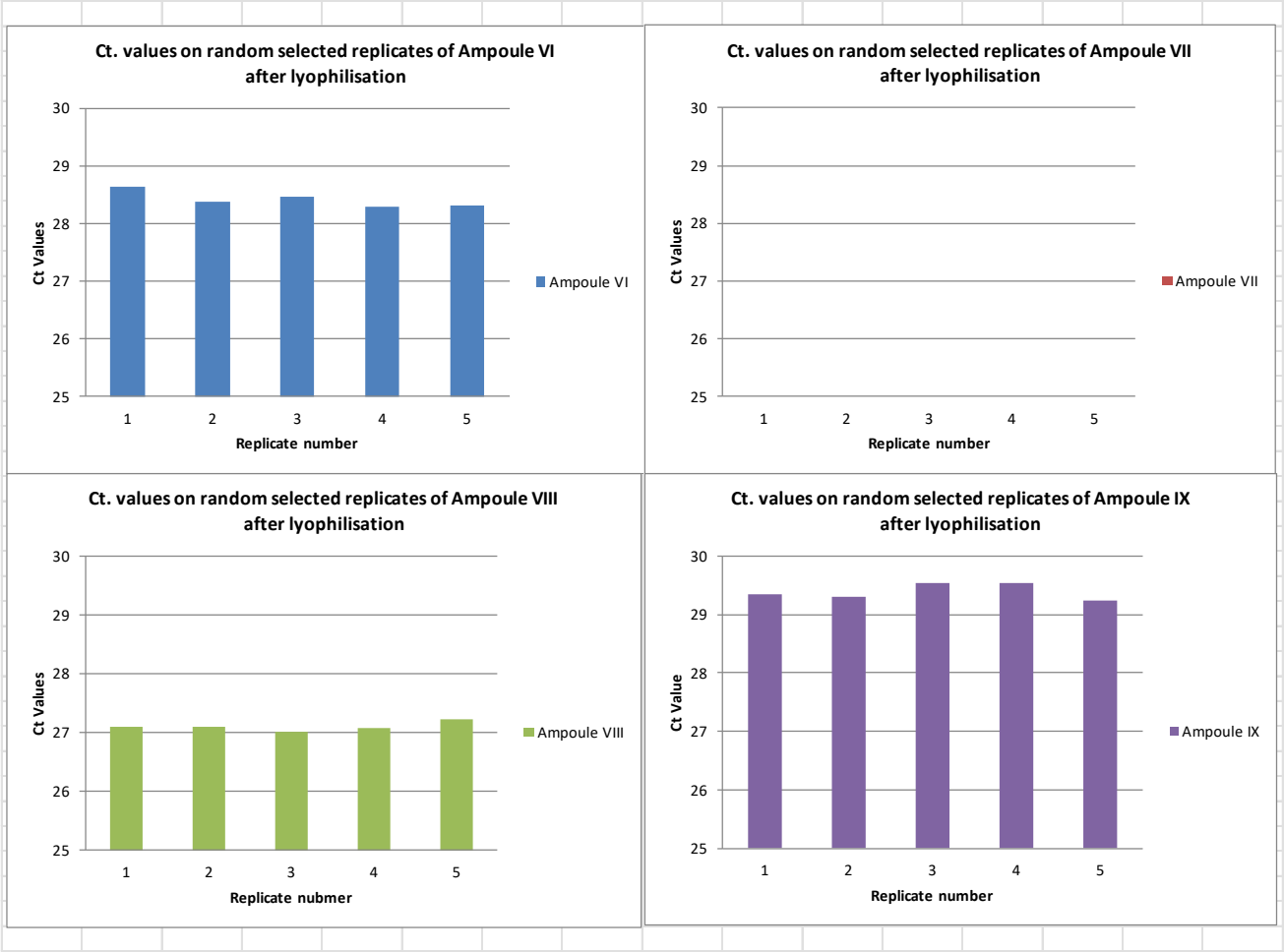
Number of laboratories	32
Median Ct.-value	28.6
Maximum Ct.-value	35.6
Minimum Ct.-value	16.8
25% quartile Ct.-value	27.1
75% quartile Ct.-value	31.0



Number of laboratories	12
Median Ct.-value	25.8
Maximum Ct.-value	30.9
Minimum Ct.-value	22.0
25% quartile Ct.-value	24.8
75% quartile Ct.-value	27.1

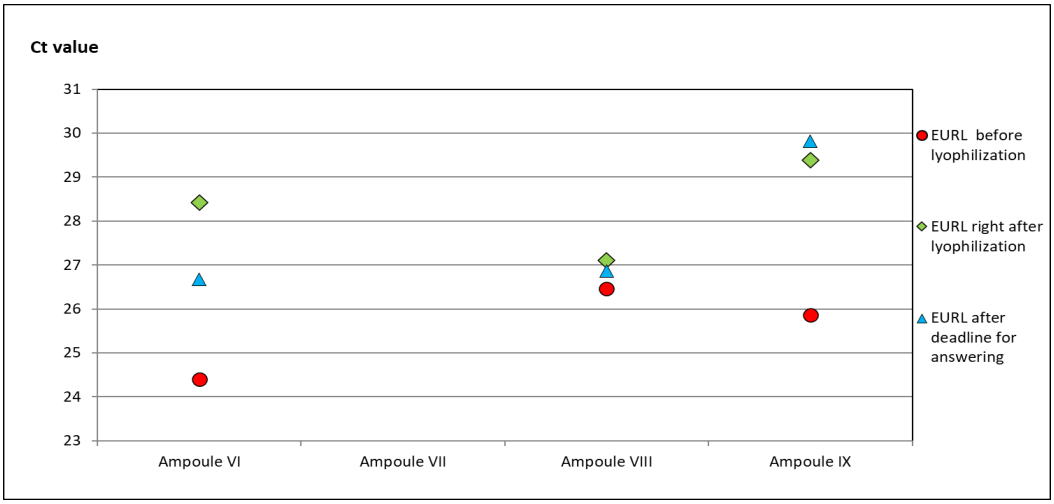
Annex 4: Stability and homogeneity of the content in the ampoules (PT2)

After Lyophilization the stability of the content in the ampoules were assessed by (RT)-qPCR in five random ampoules of VI to IX. For all of the ampoules, the difference in Ct. values was belove one.



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

The reduction in Ct. values before and after freeze-drying was between 1-4 log. No significant reductions were observed after long term storage.



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

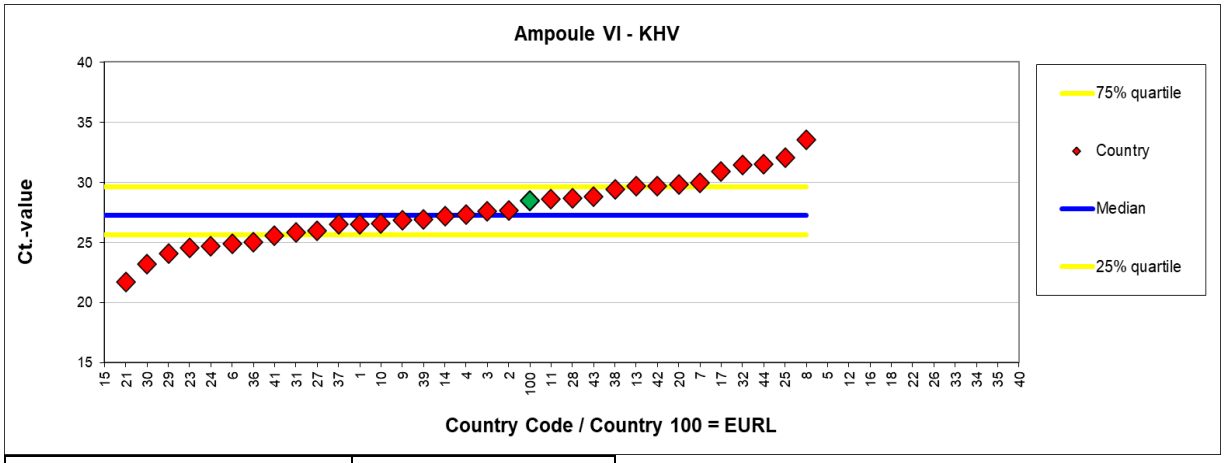
Annex 5: The Ct. values graphically (PT2) obtained from each participating laboratory.

The Ct. values (red diamonds) obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare their Ct. values with the other participating laboratories as well as the Ct. value obtained by the EURL (green diamond and code No. 100), to keep in mind that the Ct values cannot be directly compared due to the use of different methods, reagents and equipment.

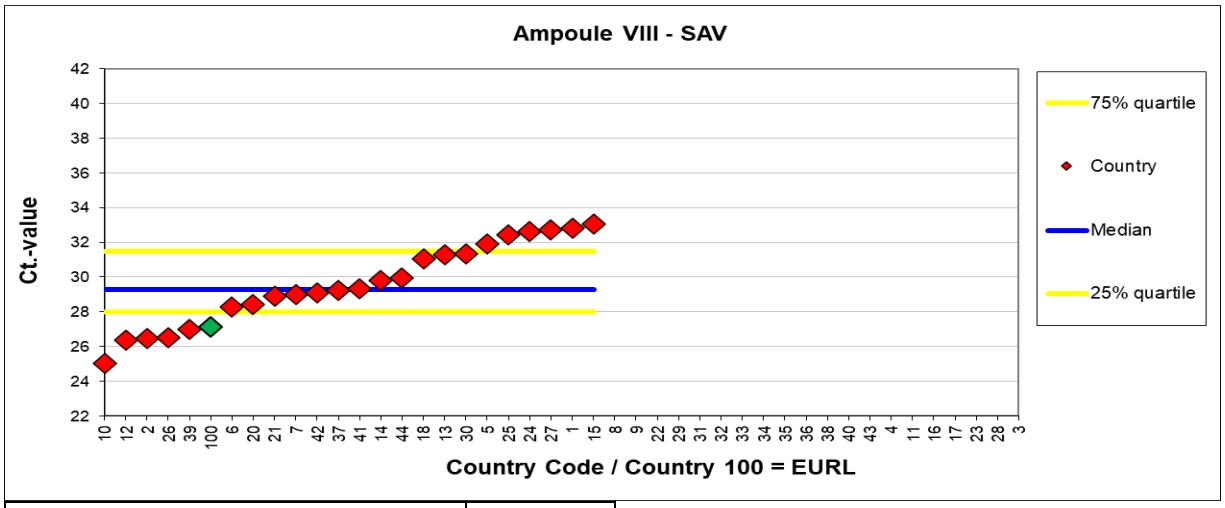
All Ct values submitted by the participants for each ampoule, are compared to each other. On these figures, the median values and the 25% and 75% inter-quartile range is displayed, the optimal value will be within these quartiles. Exceeding the values defined by the quartiles could suggest the laboratories to assess the laboratory procedures or the assay in use.

NB: Ampoule VII was Blank and therefore not represented graphically.

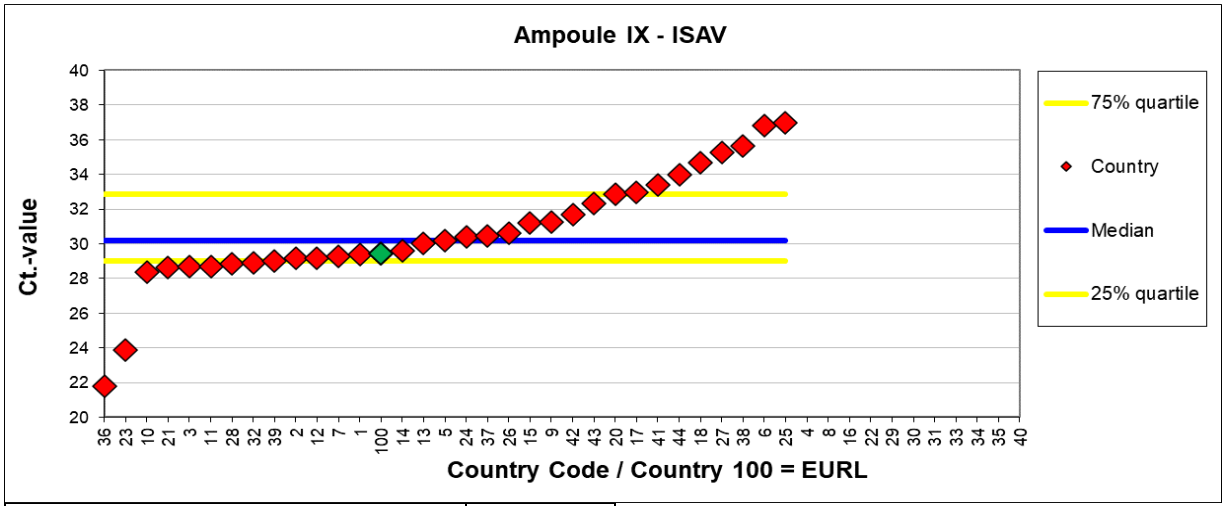
Ct. values obtained in PT2 by participants. No red diamond = No Ct. value given.



Number of laboratories	34
Median Ct.-value	27.2
Maximum Ct.-value	33.6
Minimum Ct.-value	13.0
25% quartile Ct.-value	25.6
75% quartile Ct.-value	29.6



Number of laboratories	24
Median Ct.-value	29.3
Maximum Ct.-value	33.0
Minimum Ct.-value	25.0
25% quartile Ct.-value	28.0
75% quartile Ct.-value	31.5



Number of laboratories	33
Median Ct.-value	30.2
Maximum Ct.-value	37.0
Minimum Ct.-value	21.8
25% quartile Ct.-value	29.0
75% quartile Ct.-value	32.9