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 2024

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 44 laboratories participating in PT1, 40 laboratories test for all the viruses included. 39 correctly identified all the pathogens they were investigating for in the designated ampoules.
43 laboratories participate in PT2. 37 out of 43 laboratories test for all the viruses included also including SAV and 36 laboratories succeeded in identifying all fish viral pathogens they were testing for.
The tests were sent from the EURL between 18th to 20th of September 2024 depending on courier.
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 EPC cells not infected, a coinfection of IPNV and IHNV (high titer), SVCV (medium titer), ECV (low titer) and VHSV (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 Commission Implementing Rregulation (EU) 2024/216.

PT1 addressed 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 SAV, KHV and two times ISAV 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 2024.
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/fish/proficiency-test) to be used for reporting results and to be submitted to the EURL electronically. Participants were asked to reply latest December 6th, 2024. The results of the inter-laboratory proficiency test for listed fish diseases 2024 and plans and idea for future inter-laboratory

tests will be presented at the 29th Annual Workshop of the NRLs for Fish Diseases on from May 26th to May 27th, 2025. The meeting will be in person and will take place at the premises of DTU Campus in Kgs. Lyngby. Furthermore, a specific online meeting in early spring 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 44 participants.
95% was delivered within the first week; 98% were delivered within the first two weeks and one parcel was delivered after 28 days due to internal customs clearance (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. One participant reported that one of the ampoules had small cracks. The EURL promptly submitted a new replicate of this sample while the cracked one was returned to the EURL. The EURL processed the ampoule for testing and the pathogen in question was recovered in a satisfactory titre.

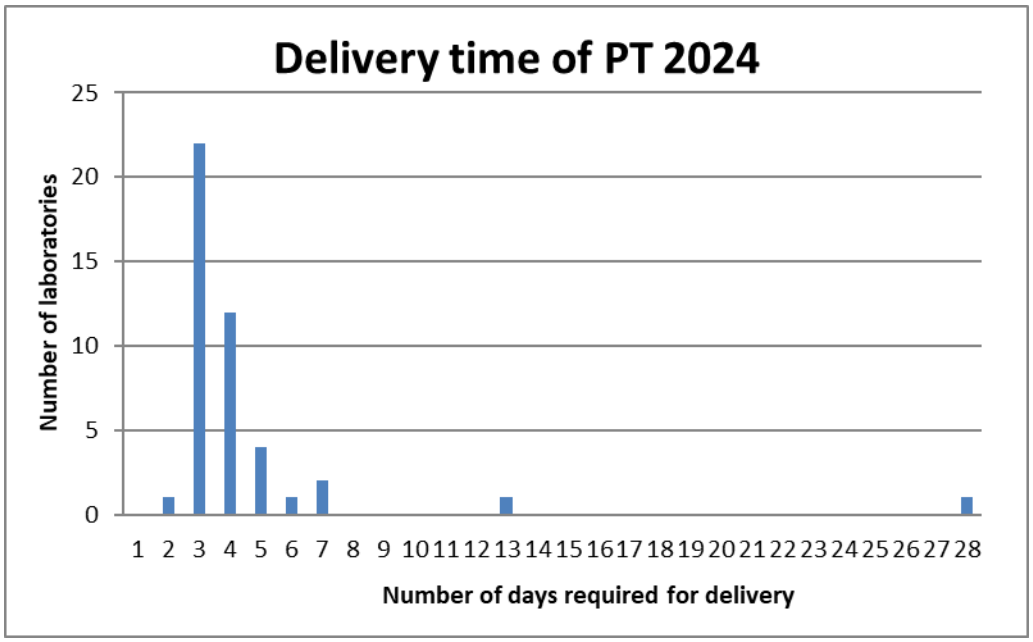

Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 44 laboratories received the annual proficiency test. 43 participants submitted the full spreadsheet within the deadline and one participant was granted 4 days delay.

Proficiency test 1, PT1

Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States including Denmark and likewise to the NRLs in Australia, Bosnia and Herzegovina, Canada, Faroe Islands, Iceland, Japan, New Zealand, Northern Ireland, Norway, Republic of North Macedonia, Serbia, Switzerland, Turkey, Tunesia, 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.

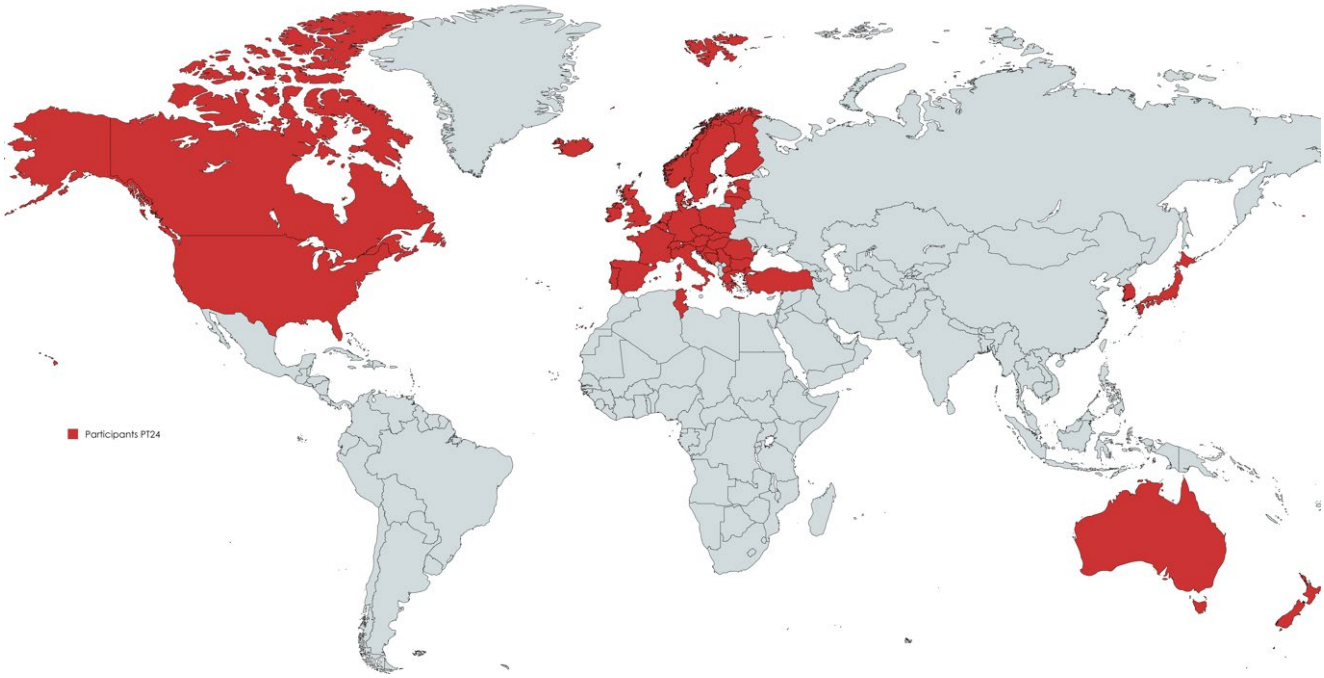

Figure 2. Worldwide distribution of the participants in the EURL proficiency test 2024

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: Blank	EPC cell supernatant.
Ampoule II: IHNV/IPNV	**IHNV isolate DK 21-4070-1** From farmed rainbow trout in Denmark **Genotype:** E **Received from:** National Institute of Aquatic Resources, DTU AQUA, Technical University of Denmark. **GenBank accession numbers:** The isolate is unpublished. **+** **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
Ampoule III: SVCV	**SVCV Isolate DK-203273** Spring Viraemia of Carp Virus isolated from Koi Carp in Denmark June 2003 **Genotype:** 1a **Received from:** National Veterinary Institute, Technical University of Denmark. **GenBank accession numbers:** MN094793.1

Code	Specifications/References
Ampoule IV: 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.
Ampoule V: VHSV	**VHSV strain DK-6137** The isolate originated from an outbreak of VHS with high mortality in sea water aquaculture in 1991. **Received from:** National Veterinary Institute, Technical University of Denmark. **Genotype:** Ia **GenBank accession number:** AY546593 **Reference on isolate:** Olesen NJ, Lorenzen N & Jørgensen PEV (1993). Serological differences among isolates of viral haemorrhagic septicaemia virus detected by neutralizing monoclonal and polyclonal antibodies. *Diseases of Aquatic Organisms* 16, 163-170. Olesen NJ, Lorenzen N & LaPatra S (1999). Production of neutralizing antisera against viral hemorrhagic septicaemia (VHS) virus by intravenous injections of rabbits. *Journal of Aquatic Animal Health* 11, 10-16. **Reference on sequence and genotype:** Einer-Jensen K, Ahrens P, Forsberg R & Lorenzen N (2004). Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal of General Virology* 85, 1167-1179.

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 (Report-2007.pdf) ; it can survive exposure to 30°C for 24 hours (Report-2010.pdf) and a temperature raise from 20 to 42°C over a period of 5 hours (Report-2011.pdf).

The identities of the viruses in all 5 ampoules (I-V) were checked and confirmed before lyophilisation by ELISA, PCR and/or qPCR and RT-PCR and/or RT-qPCR as well as the titter of the virus on cell cultures were measured. After lyophilisation and before shipment the homogeneity of the content in the ampoules were assessed in five random ampoules of each of the five ampoules (I-V) by titrating the virus on cell cultures and identifying it by ELISA and IFAT furthermore PCR based tests were performed. After handling in the result, the stability of the content was assessed in one random ampoule of each of the five ampoules (I-V) by titrating the virus on cell cultures and identifying by PCR based methods. This year small reductions of the titres after lyophilisation were observed in ampoule II, III and V, the reduction of the titre was ≤1 log in the same cell line except for ampoule III on BF-2 cells, where we had a low titter before lyophilisation due to difficulties determine CPE or not. The reductions of the titres after lyophilisation in ampoule IV was 1-2 log.
No significant reductions (≤1 log) were observed after long term storage, except for ampoule III on RTG-2 cells, where the reductions were between 1-2 log (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: Blank EPC cell supernatant	BF-2	< 1,9E+02	< 1,9E+02	< 1,9E+02
	RTG-2	< 1,9E+02	< 1,9E+02	< 1,9E+02
	EPC	< 1,9E+02	< 1,9E+02	< 1,9E+02
	FHM	< 1,9E+02	< 1,9E+02	< 1,9E+02
Ampoule II: IHNV DK 21-4070-1 + IPNV Strain Sp	BF-2	1.9E+07	4.0E+06 *(1,3E+06 → 5,9E+06)*	8.6E+05
	RTG-2	1.3E+07	4.0E+06 *(2,7E+06 → 5,9E+06)*	1.9E+06
	EPC	1.9E+07	8.6E+06 *(5,9E+06 → 1,3E+07)*	5.9E+06
	FHM	1.3E+07	4.0E+06 *(1,9E+06 → 5,9E+06)*	2.7E+06
Ampoule III: SVCV DK-203273	BF-2	5.9E+04	2.7E+06 *(2,7E+06 → 4,0E+06)*	8.6E+05
	RTG-2	8.6E+04	1.9E+05 *(5,9E+04 → 4,0E+05)*	2.7E+03
	EPC	8.6E+06	2.7E+06 *(1,3E+06 → 4,0E+06)*	1.3E+06
	FHM	5.9E+06	2.7E+06 *(1,3E+06 → 2,7E+06)*	2.7E+05
Ampoule IV: ECV 562/92	BF-2	8.6E+04	1.3E+03 *(1,3E+03 → 2,7E+03)*	1.9E+03
	RTG-2	1.3E+04	1.3E+03 *(8,6E+02 → 1,9E+03)*	2.7E+02
	EPC	5.9E+04	1.3E+03 *(1,3E+03 → 1,9E+03)*	1.3E+03
	FHM	5.9E+02	1.9E+02 *(<1,9E+02 → 1,9E+02)*	< 1,9E+02
Ampoule V: VHSV DK-6137	BF-2	1.9E+04	5.9E+04 *(4,0E+04 → 1,9E+05)*	2.7E+04
	RTG-2	1.3E+06	4.0E+05 *(1,3E+05 → 4,0E+05)*	8.6E+04
	EPC	1.3E+06	1.3E+05 *(1,3E+05 → 2,7E+05)*	1.3E+05
	FHM	8.6E+05	4.0E+05 *(1,9E+05 → 8,6E+05)*	4.0E+04

Table 2. PT1:

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

- Before lyophilisation, (stored at -80°C).
- After lyophilisation and before shipment (median titre of 5 replicates), (stored at 4°C). 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, 2024 - Virus identification and score obtained by participants.

Laboratory code number	Score	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
		Blank EPC cell supernatant	**IHNV DK 21-4070-1 + IPNV Strain Sp**	**SVCV DK-203273**	**ECV 562/92**	**VHSV DK-6137**
1	10/10	Blank	IHNV & IPNV	SVCV	Ranavirus-Not EHNV	VHSV
2	8/10	No virus isolated	IHNV and IPNV	SVCV	EHNV	VHSV
3	10/10	-	IPNV / IHNV	SVCV	Ranavirus - NOT EHNV	VHS
4	10/10	Negative/ Blank	IHNV +IPNV	SVCV	European Sheatfish virus	VHSV
5	10/10	Negative	IPNV & IHNV	SVCV	Ranavirus – NOT EHNV	VHSV
6	10/10	-	IHNV, IPNV	SVCV	Ranavirus was identified by conventional PCR and than REA was applied as given by WOAH manuel to identified ECV	VHSV
7[2+3+4]	6/6	0	IHNV	0	0	VHSV
8	10/10	Negative	IHNV and IPNV	SVCV	Ranavirus-Not EHNV	VHSV
9	10/10	No VHSV, no IHNV, no EHNV, no Ranavirus, no IPNV, no SVCV, no PRV	IHNV & IPNV	SVCV	Ranavirus – NOT EHNV	VHSV

10	10/10	0	IPNV IHNV	SVCV	Ranavirus	VHSV
11	10/10	NEG	IHNV + IPNV	SVCV	Ranavirus NOT EHNV	VHSV
12	8/10	0	IPNV, IHNV	SVCV	EHN	VHSV
13	10/10	Negative	IHNV, IPNV	SVCV	Ranavirus - NOT EHNV	VHSV
14	10/10	Negative/Not detected	IHNV, IPNV	SVCV	ECV (Ranavirus-NOT EHNV)	VHSV
15	10/10	0	IHNV, IPNV	SVCV	Ranavirus - Not EHNV	VHSV
16	10/10	0	IPNV IHNV	SVCV	Ranavirus not EHNV	VHSV
17	10/10	Negative	IHNV and IPNV	SVCV	Ranavirus – NOT EHNV	VHSV
18	10/10	Negative	IHNV, IPNV	SVCV	Ranavirus – NOT EHNV	VHSV
19[2;3]	8/8	Negative for IHNV, Ranavirus and VHSV	IHNV	Negative for IHNV, Ranavirus and VHSV	Ranavirus – Not EHNV	VHS
20	10/10	-	IHNV IPNV	SVC	Ranavirus – NOT EHNV	VHSV
21	10/10	No virus detected	IHNV and IPNV	SVCV	Ranavirus - NOT EHNV	VHSV
22	10/10	virus not detected	IPNV and IHNV	SVCV	Ranavirus - Not EHNV	VHSV
23[1]	9/10	NEGATIVE	IHNV and IPNV	SVCV	Ranavirus, not EHNV	VHSV
24	6/10	0	IHNV and IPNV	0	not-EHNV Ranavirus	0
25	10/10	0	IPNV and IHNV	SVCV	Ranavirus	VHSV
26	10/10	NEG	IHN+IPN	SVCV	ECV	VHS
27	10/10	Blank	IHNV and IPNV	SVCV	Ranavirus-not EHNV	VHSV
28	10/10	Not VHSV, not IPNV, not IHNV, not SVCV, not Ranavirus	IPNV and IHNV	SVCV	Ranavirus-NOT EHNV	VHSV
29	10/10	0	IHNV; IPNV	SVCV	European Catfish Virus	VHSV
30	10/10	NO VHSV,NO IHNV,NO EHNV,NO RANAVIRUS ,NO IPNV,NO SVCV	IPNV & IHNV	SVCV	Ranavirus -NOT EHNV	VHSV
31	10/10	negative	IHNV and IPNV	SVCV	ESV/ECV	VHSV
32	10/10	0	IHNV IPNV	SVCV	Ranavirus – NOT EHNV	VHSV
33	10/10	No virus	IHNV, IPNV	SVCV	Ranavirus - NOT EHNV	VHSV
34	10/10	No virus detected	IHNV and IPNV	SVCV	European Catfish Virus	VHSV
35	10/10	negative	IHNV and IPNV	SVCV	Ranavirus - NOT EHNV	VHSV

36	10/10	None of the following viruses detected: VHSV, IHNV, Ranavirus (incl. EHNV), IPNV or SVCV	IHNV and IPNV	SVCV	Ranavirus - NOT EHNV	VHSV
37	10/10	negative	IHNV, IPNV	SVCV	Ranavirus - Not EHNV	VHSV
38	10/10	Not VHSV, Not IHNV, Not EHNV, Not Ranavirus, Not IPNV, Not SVCV	IHNV, IPNV	SVCV	ECV	VHSV
39	10/10	Neg	IHNV + IPNV	SVCV	Ranavirus - NOT EHNV	VHSV
40[1]	9/10	no virus	IHNV IPNV	SVCV	Ranavirus	VHSV
41	10/10	NEGATIVE	IHNV + IPNV	SVCV	Ranavirus – NOT EHNV	VHSV
42[2+3]	8/8	-	IHNV	-	Ranavirus - NOT EHNV	VHSV
43	7/10	No virus	IHNV	SVCV and IPNV	EHNV	VHSV
44	10/10	Negative	IPNV and IHNV	SVCV	Ranavirus - NOT EHNV	VHSV

1. Did not corroborate the findings in ampoule IV 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
4. Do not test for Rana

Table 4. Inter-Laboratory Proficiency Test, PT1, 2024 – **Results of titration of ampoule I.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	Blank	<1,9E+02	<1,9E+02	N/A	<1,9E+02
2	No virus isolated	<1,9E+02	<1,9E+02	<1,9E+02	N/A
3	-	<1,9E+02	1.3E+03	N/A	N/A
4	Negative/Blank	N/A	N/A	N/A	N/A
5	Negative	N/A	N/A	N/A	N/A
6	-	<1,9E+02	<1,9E+02	N/A	N/A
7	0	<1,9E+02	<1,9E+02	N/A	N/A
8	Negative	<1,9E+02	N/A	N/A	<1,9E+02
9	No VHSV, no IHNV, no EHNV, no Ranavirus, no IPNV, no SVCV, no PRV	<1,9E+02	<1,9E+02	N/A	N/A
10	0	<1,9E+02	N/A	N/A	<1,9E+02
11	NEG	<1,9E+02	<1,9E+02	N/A	N/A
12	0	<1,9E+02	<1,9E+02	N/A	<1,9E+02
13	Negative	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
14	Negative/Not detected	<1,9E+02	<1,9E+02	N/A	N/A
15	0	<1,9E+02	<1,9E+02	N/A	<1,9E+02
16	0	<1,9E+02	<1,9E+02	N/A	N/A
17	Negative	<1,9E+02	<1,9E+02	N/A	N/A
18	Negative	N/A	N/A	N/A	N/A
19	Negative for IHNV, Ranavirus and VHSV	N/A	N/A	N/A	N/A
20	-	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
21	No virus detected	<1,9E+02	<1,9E+02	N/A	N/A
22	virus not detected	<1,9E+02	<1,9E+02	N/A	N/A
23	NEGATIVE	N/A	<1,9E+02	<1,9E+02	N/A
24	0	<1,9E+02	<1,9E+02	N/A	N/A
25	0	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
26	NEG	<1,9E+02	<1,9E+02	N/A	N/A
27	Blank	<1,9E+02	<1,9E+02	N/A	N/A
28	Not VHSV, not IPNV, not IHNV, not SVCV, not Ranavirus	<1,9E+02	<1,9E+02	N/A	N/A
29	0	N/A	N/A	N/A	N/A
30	NO VHSV,NO IHNV,NO EHNV,NO RANAVIRUS ,NO IPNV,NO SVCV	<1,9E+02	<1,9E+02	N/A	N/A
31	negative	<1,9E+02	<1,9E+02	<1,9E+02	N/A
32	0	<1,9E+02	<1,9E+02	<1,9E+02	N/A
33	No virus	<1,9E+02	<1,9E+02	N/A	N/A
34	No virus detected	N/A	N/A	N/A	N/A

35	negative	<1,9E+02	<1,9E+02	N/A	N/A
36	None of the following viruses detected: VHSV, IHNV, Ranavirus (incl. EHNV), IPNV or SVCV	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
37	negative	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
38	Not VHSV, Not IHNV, Not EHNV, Not Ranavirus, Not IPNV, Not SVCV	<1,9E+02	<1,9E+02	N/A	N/A
39	Neg	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
40	no virus	<1,9E+02	<1,9E+02	N/A	N/A
41	NEGATIVE	<1,9E+02	<1,9E+02	N/A	N/A
42	-	N/A	N/A	N/A	N/A
43	No virus	<1,9E+02	<1,9E+02	<1,9E+02	N/A
44	Negative	<1,9E+02	<1,9E+02	<1,9E+02	N/A

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

Blank (EPC cell supernatant)	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	31	30	9	10
Median titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
Maximum titre	<1,9E+02	1.3E+03	<1,9E+02	<1,9E+02
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
75% quartile titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02

Table 5. Inter-Laboratory Proficiency Test, PT1, 2024 – **Results of titration of ampoule II.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IHNV & IPNV	4.0E+05	1.3E+06	N/A	5.9E+05
2	IHNV and IPNV	8.6E+04	1.3E+06	4.0E+04	N/A
3	IPNV / IHNV	5.9E+05	5.9E+06	N/A	N/A
4	IHNV +IPNV	4.0E+05	N/A	2.7E+04	8.6E+04
5	IPNV & IHNV	2.7E+06	5.9E+07	N/A	N/A
6	IHNV, IPNV	1.9E+06	2.7E+06	N/A	N/A
7	IHNV	N/A	N/A	N/A	N/A
8	IHNV and IPNV	1.9E+06	N/A	N/A	2.7E+06
9	IHNV & IPNV	8.6E+06	2.7E+06	N/A	N/A
10	IPNV IHNV	2.7E+06	N/A	N/A	1.9E+05
11	IHNV + IPNV	5.9E+06	4.0E+06	N/A	N/A
12	IPNV, IHNV	1.9E+07	1.3E+04	N/A	2.7E+04
13	IHNV, IPNV	2.7E+05	1.9E+05	2.7E+04	1.9E+05
14	IHNV, IPNV	5.9E+09	5.9E+09	N/A	N/A
15	IHNV, IPNV	8.6E+05	1.9E+06	N/A	4.0E+04
16	IPNV IHNV	5.9E+04	4.0E+04	N/A	N/A
17	IHNV and IPNV	5.9E+07	2.7E+06	N/A	N/A
18	IHNV, IPNV	N/A	N/A	N/A	N/A
19	IHNV	N/A	N/A	N/A	N/A
20	IHNV IPNV	4.0E+06	1.9E+07	8.6E+06	1.9E+07
21	IHNV and IPNV	2.7E+05	5.9E+05	N/A	N/A
22	IPNV and IHNV	5.9E+06	2.7E+06	N/A	N/A
23	IHNV and IPNV	N/A	1.3E+05	2.7E+04	N/A
24	IHNV and IPNV	1.9E+04	2.7E+05	N/A	N/A
25	IPNV and IHNV	1.9E+07	4.0E+07	1.3E+06	2.7E+06
26	IHN+IPN	5.9E+06	8.6E+06	N/A	N/A
27	IHNV and IPNV	1.9E+07	2.7E+06	N/A	N/A
28	IPNV and IHNV	4.0E+06	4.0E+06	N/A	N/A
29	IHNV; IPNV	N/A	N/A	N/A	N/A
30	IPNV & IHNV	1.9E+06	4.0E+06	N/A	N/A
31	IHNV and IPNV	1.3E+07	2.7E+06	2.7E+06	N/A
32	IHNV IPNV	5.9E+06	5.9E+06	2.7E+06	N/A
33	IHNV, IPNV	4.0E+06	8.6E+06	N/A	N/A
34	IHNV and IPNV	N/A	N/A	N/A	N/A

35	IHNV and IPNV	1.3E+06	2.7E+05	N/A	N/A
36	IHNV and IPNV	5.9E+06	5.9E+06	5.9E+06	2.7E+06
37	IHNV, IPNV	1.9E+06	8.6E+06	4.0E+06	4.0E+06
38	IHNV, IPNV	8.6E+06	5.9E+05	N/A	N/A
39	IHNV + IPNV	2.7E+05	1.3E+06	4.0E+05	1.3E+06
40	IHNV IPNV	2.7E+06	2.7E+07	N/A	N/A
41	IHNV + IPNV	8.6E+04	4.0E+05	N/A	N/A
42	IHNV	N/A	N/A	N/A	N/A
43	IHNV	1.3E+06	5.9E+06	5.9E+06	N/A
44	IPNV and IHNV	1.3E+07	2.7E+07	5.9E+05	N/A

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

IHNV, DK 21-4070-1 + IPNV, Strain Sp	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	37	35	13	12
Median titre	2.7E+06	2.7E+06	1.3E+06	9.3E+05
Maximum titre	5.9E+09	5.9E+09	8.6E+06	1.9E+07
Minimum titre	1.9E+04	1.3E+04	2.7E+04	2.7E+04
25% quartile titre	5.9E+05	9.3E+05	4.0E+04	1.6E+05
75% quartile titre	5.9E+06	7.2E+06	4.0E+06	2.7E+06

Table 6. Inter-Laboratory Proficiency Test, PT1, 2024 – **Results of titration of ampoule III**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	SVCV	2.7E+03	4.0E+04	N/A	1.3E+04
2	SVCV	5.9E+03	5.9E+04	5.9E+02	N/A
3	SVCV	5.9E+04	2.7E+05	N/A	N/A
4	SVCV	N/A	8.62E+04	N/A	N/A
5	SVCV	1.3E+05	1.3E+06	N/A	N/A
6	SVCV	<1,9E+02	5.9E+04	N/A	N/A
7	0	N/A	N/A	N/A	N/A
8	SVCV	4.0E+03	N/A	N/A	1.9E+05
9	SVCV	5.9E+05	2.7E+05	N/A	N/A
10	SVCV	2.7E+03	N/A	N/A	4.0E+03
11	SVCV	2.7E+04	1.9E+05	N/A	N/A
12	SVCV	5.87E+03	1.86E+04	N/A	5.87E+04
13	SVCV	1.86E+04	1.00E+00	<1,9E+02	1.86E+04
14	SVCV	<1,9E+02	5.9E+09	N/A	N/A
15	SVCV	8.6E+02	1.3E+03	N/A	5.9E+02
16	SVCV	5.9E+04	4.0E+04	N/A	N/A
17	SVCV	4.0E+04	<1,9E+02	N/A	N/A
18	SVCV	N/A	N/A	N/A	N/A
19	Negative for IHNV, Ranavirus and VHSV	N/A	N/A	N/A	N/A
20	SVC	1.3E+06	2.7E+05	4.0E+03	8.6E+05
21	SVCV	5.9E+03	8.6E+03	N/A	N/A
22	SVCV	2.7E+03	4.0E+05	N/A	N/A
23	SVCV	N/A	1.9E+04	1.3E+03	N/A
24	0	<1,9E+02	<1,9E+02	N/A	N/A
25	SVCV	2.7E+04	2.7E+05	<1,9E+02	5.9E+05
26	SVCV	1.3E+05	2.7E+05	N/A	N/A
27	SVCV	5.9E+05	1.3E+05	N/A	N/A
28	SVCV	4.0E+05	1.9E+05	N/A	N/A
29	SVCV	N/A	N/A	N/A	N/A
30	SVCV	4.0E+05	1.3E+05	N/A	N/A
31	SVCV	4.0E+04	1.3E+05	5.9E+03	N/A
32	SVCV	1.9E+05	8.6E+05	<1,9E+02	N/A
33	SVCV	1.3E+04	5.9E+05	N/A	N/A
34	SVCV	N/A	N/A	N/A	N/A
35	SVCV	5.9E+05	4.0E+04	N/A	N/A

36	SVCV	1.9E+06	1.3E+06	1.3E+04	8.6E+05
37	SVCV	1.3E+04	4.0E+05	8.6E+04	1.9E+05
38	SVCV	5.9E+04	5.9E+04	N/A	N/A
39	SVCV	<1,9E+02	4.0E+05	<1,9E+02	1.3E+05
40	SVCV	1.9E+03	5.9E+03	N/A	N/A
41	SVCV	1.26E+03	4.00E+04	N/A	N/A
42	-	N/A	N/A	N/A	N/A
43	SVCV and IPNV	2.7E+03	8.6E+05	1.9E+05	N/A
44	SVCV	1.3E+05	1.9E+05	<1,9E+02	N/A

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

SVCV DK-203273	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	36	36	12	11
Median titre	2.3E+04	1.3E+05	9.3E+02	1.3E+05
Maximum titre	1.9E+06	5.9E+09	1.9E+05	8.6E+05
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	5.9E+02
25% quartile titre	2.7E+03	4.0E+04	<1,9E+02	1.6E+04
75% quartile titre	1.3E+05	3.0E+05	7.6E+03	3.9E+05

Table 7. Inter-Laboratory Proficiency Test, PT1, 2024 – **Results of titration of ampoule IV.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	Ranavirus-Not EHNV	1.9E+03	2.7E+03	N/A	1.3E+03
2	EHNV	5.9E+03	1.3E+03	2.7E+02	N/A
3	Ranavirus - NOT EHNV	5.9E+03	2.7E+03	N/A	N/A
4	European Sheatfish virus	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
5	Ranavirus – NOT EHNV	1.9E+04	5.9E+03	N/A	N/A
6	Ranavirus was identified by conventional PCR and than REA was applied as given by WOAH manuel to identified ECV	1.3E+03	1.9E+03	N/A	N/A
7	0	N/A	N/A	N/A	N/A
8	Ranavirus-Not EHNV	8.6E+03	N/A	N/A	<1,9E+02
9	Ranavirus – NOT EHNV	2.7E+03	<1,9E+02	N/A	N/A
10	Ranavirus	5.9E+03	N/A	N/A	<1,9E+02
11	Ranavirus NOT EHNV	4.0E+04	1.3E+04	N/A	N/A
12	EHN	2.7E+03	1.9E+04	N/A	5.9E+03
13	Ranavirus - NOT EHNV	1.3E+03	<1,9E+02	<1,9E+02	<1,9E+02
14	ECV (Ranavirus-NOT EHNV)	1.3E+05	5.9E+05	N/A	N/A
15	Ranavirus - Not EHNV	1.9E+03	1.3E+04	N/A	1.9E+03
16	Ranavirus not EHNV	2.7E+04	4.0E+04	N/A	N/A
17	Ranavirus – NOT EHNV	1.9E+04	<1,9E+02	N/A	N/A
18	Ranavirus – NOT EHNV	N/A	N/A	N/A	N/A
19	Ranavirus – Not EHNV	N/A	N/A	N/A	N/A
20	Ranavirus – NOT EHNV	5.9E+03	1.3E+03	2.7E+03	1.3E+03
21	Ranavirus - NOT EHNV	1.3E+03	1.9E+03	N/A	N/A
22	Ranavirus - Not EHNV	2.7E+03	<1,9E+02	N/A	N/A
23	Ranavirus, not EHNV	N/A	5.9E+02	2.7E+02	N/A
24	not-EHNV Ranavirus	<1,9E+02	<1,9E+02	N/A	N/A
25	Ranavirus	<1,9E+02	1.9E+04	<1,9E+02	1.3E+03
26	ECV	2.7E+03	5.9E+02	N/A	N/A
27	Ranavirus-not EHNV	4.0E+03	8.6E+02	N/A	N/A
28	Ranavirus-NOT EHNV	<1,9E+02	<1,9E+02	N/A	N/A
29	European Catfish Virus	N/A	N/A	N/A	N/A
30	Ranavirus -NOT EHNV	4.0E+03	1.9E+04	N/A	N/A
31	ESV/ECV	8.6E+02	1.9E+02	2.7E+03	N/A
32	Ranavirus – NOT EHNV	2.7E+04	1.9E+03	1.9E+02	N/A

33	Ranavirus - NOT EHNV	5.9E+03	2.7E+02	N/A	N/A
34	European Catfish Virus	N/A	N/A	N/A	N/A
35	Ranavirus - NOT EHNV	2.7E+04	4.0E+03	N/A	N/A
36	Ranavirus - NOT EHNV	5.9E+02	4.0E+02	5.9E+02	<1,9E+02
37	Ranavirus - Not EHNV	8.6E+03	2.7E+03	4.0E+04	1.3E+03
38	ECV	1.3E+04	1.3E+04	N/A	N/A
39	Ranavirus - NOT EHNV	1.9E+03	2.7E+02	<1,9E+02	<1,9E+02
40	Ranavirus	4.0E+03	5.9E+02	N/A	N/A
41	Ranavirus – NOT EHNV	1.3E+03	5.9E+03	N/A	N/A
42	Ranavirus - NOT EHNV	N/A	N/A	N/A	N/A
43	EHNV	<1,9E+02	4.0E+03	5.9E+03	N/A
44	Ranavirus - NOT EHNV	1.9E+04	4.0E+03	<1,9E+02	N/A

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

ECV, 562/92	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	37	36	13	12
Median titre	4.0E+03	1.9E+03	2.7E+02	6.3E+02
Maximum titre	1.3E+05	5.9E+05	4.0E+04	5.9E+03
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	1.3E+03	2.7E+02	<1,9E+02	<1,9E+02
75% quartile titre	8.6E+03	5.9E+03	2.7E+03	1.3E+03

Table 8. Inter-Laboratory Proficiency Test, PT1, 2024 – Results of titration of **ampoule V**.

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	VHSV	4.0E+02	8.6E+03	N/A	4.0E+04
2	VHSV	2.7E+02	1.3E+04	5.9E+02	N/A
3	VHS	1.3E+04	8.6E+04	N/A	N/A
4	VHSV	1.9E+04	8.6E+03	N/A	N/A
5	VHSV	1.9E+04	4.0E+05	N/A	N/A
6	VHSV	4.0E+03	1.9E+04	N/A	N/A
7	VHSV	N/A	N/A	N/A	N/A
8	VHSV	5.9E+03	N/A	N/A	2.7E+04
9	VHSV	1.9E+04	1.9E+04	N/A	N/A
10	VHSV	2.7E+03	N/A	N/A	4.0E+03
11	VHSV	1.3E+04	1.3E+05	N/A	N/A
12	VHSV	1.9E+03	8.6E+03	N/A	5.9E+03
13	VHSV	1.9E+03	4.0E+03	1.9E+02	1.3E+03
14	VHSV	1.3E+03	5.9E+09	N/A	N/A
15	VHSV	2.7E+03	4.0E+03	N/A	1.3E+03
16	VHSV	4.0E+04	5.9E+04	N/A	N/A
17	VHSV	1.3E+04	< 1,9E+02	N/A	N/A
18	VHSV	N/A	N/A	N/A	N/A
19	VHS	N/A	N/A	N/A	N/A
20	VHSV	1.9E+04	1.3E+05	4.0E+04	8.6E+04
21	VHSV	1.9E+03	8.6E+03	N/A	N/A
22	VHSV	2.7E+03	2.7E+04	N/A	N/A
23	VHSV	N/A	1.9E+04	1.9E+04	N/A
24	0	< 1,9E+02	< 1,9E+02	N/A	N/A
25	VHSV	8.6E+03	5.9E+04	4.0E+03	5.9E+04
26	VHS	8.6E+03	4.0E+04	N/A	N/A
27	VHSV	1.9E+04	2.7E+04	N/A	N/A
28	VHSV	1.3E+04	2.7E+04	N/A	N/A
29	VHSV	N/A	N/A	N/A	N/A
30	VHSV	5.9E+03	8.6E+04	N/A	N/A
31	VHSV	1.3E+04	1.9E+04	1.9E+04	N/A
32	VHSV	2.7E+04	5.9E+04	5.9E+03	N/A
33	VHSV	5.9E+03	1.9E+05	N/A	N/A
34	VHSV	N/A	N/A	N/A	N/A

35	VHSV	1.3E+06	1.9E+05	N/A	N/A
36	VHSV	5.9E+05	1.9E+05	2.7E+05	1.9E+05
37	VHSV	2.7E+04	5.9E+04	8.6E+04	1.3E+05
38	VHSV	1.3E+04	4.0E+03	N/A	N/A
39	VHSV	1.9E+04	1.3E+05	8.6E+03	1.9E+05
40	VHSV	1.9E+04	4.0E+04	N/A	N/A
41	VHSV	1.3E+03	5.9E+04	N/A	N/A
42	VHSV	N/A	N/A	N/A	N/A
43	VHSV	2.7E+02	8.6E+04	5.9E+03	N/A
44	VHSV	1.9E+04	2.7E+04	1.3E+03	N/A

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

VHSV, DK-6137	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	37	36	12	11
Median titre	1.3E+04	3.4E+04	7.2E+03	4.0E+04
Maximum titre	1.3E+06	5.9E+09	2.7E+05	1.9E+05
Minimum titre	<1,9E+02	<1,9E+02	1.9E+02	1.3E+03
25% quartile titre	2.7E+03	1.2E+04	3.3E+03	4.9E+03
75% quartile titre	1.9E+04	8.6E+04	2.4E+04	1.1E+05

Identification of content

- 41 laboratories out of 44 participants analysed for all viruses; 37 of these laboratories correctly identified all viruses in all ampoules.
- Three laboratories did not test for neither IPNV nor SVCV and one laboratory did not test for Rana.

Ampoule I – Blank (EPC cell supernatant)

- All 44 laboratories ruled out the presence of pathogens they were testing for, the answers varied from 'Not VHSV, Not IHNV, Not EHNV, Not Ranavirus, Not IPNV, Not SVCV' to leaving the field empty.

Ampoule II – IPNV (Sp) + IHNV (DK 21-4070-1)

- 40 laboratories correctly identified the two isolates as IPNV and IHNV in ampoule II.
- 1 laboratory correctly identified the IHNV but did not identify the IPNV.
- 3 laboratories do not test for IPNV.

Ampoule III – SVCV (DK-203273)

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

Ampoule IV – ECV (562/92)

- 37 laboratories correctly identified the isolate as Not EHNV or ECV in ampoule IV by sequencing or REA.
- 1 laboratory identified Ranavirus but did not sequence or analyse by REA.
- 2 laboratories answered Ranavirus but identified the isolate as Not EHNV or ECV by sequencing.
- 3 laboratories answered EHNV but the sequencing blast showed ECV
- 1 laboratory do not test for Rana

Ampoule V – VHSV (DK-6137)

- 43 laboratories correctly identified the isolate as VHSV in ampoule V.
- 1 laboratory did not find the present virus in the ampoule.

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**: finding no pathogen was given the score 2.
- **Ampoule II**: identification of both IPNV/IHNV was given the score 2.
  IHNV identification and no detection of IPNV was given the score 1.
  IHNV identification and not testing for IPNV was given the score 2
- **Ampoule III**: identification of SVCV was given the score 2.
  SVCV identification and detection of another (non-listed) virus was given the score 1.
  No identification of SVCV was given the score 0.

- **Ampoule IV**: identification of ECV or Not EHNV by sequencing was given the score 2.
  Answering Ranavirus but identifying ECV or Not EHNV by sequencing was given the score 2
  No identification of the Ranavirus by sequencing was given the score 1.
  Answering EHNV even though sequencing shows ECV was given the score 0.
- **Ampoule V**: identification of VHSV was given the score 2.
  No identification of VHSV was given the score 0.

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 44 laboratories participating in the PT 1 2024, 38 obtained a score on 100%.
The score 10/10 was assigned to 35 participants as they did test for all viruses.
The score 8/8 was assigned to two participants as they did not test for SVCV.
The score 6/6 was assigned to one participant as they did not test for SVCV and Rana.
Not testing for IPNV in the coinfected ampoule IV did not affect the total score

6 laboratories scored below 100% due to:

- finding a virus not present in the ampoule
- not finding the present virus/viruses
- wrong identification of the Ranavirus
- no identification by sequencing of the Ranavirus.

Cells applied for solving the test

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

- 37 laboratories used BF-2 cells
- 36 laboratories used EPC cells
- 13 laboratories used RTG-2 cells
- 12 laboratories used FHM cells
- 6 laboratories used CHSE-214
- 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 (with one exception) 4 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should consider exchanging 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. The EURL is aware that the titers for ampoule III and V on all four cell lines are over the 75% quartile, and we are investigating this further to determine the cause.

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, 2024 – Ct.-values.

Laboratory Code number	Ct. value Ampoule II (IPNV)	Ct. value Ampoule II (IHNV)	Ct. value Ampoule III (SVCV)	Ct. value Ampoule IV (ECV)	Ct. value Ampoule V (VHSV)
EURL	**24.15**	**20.09**	**23.30**	**21.57**	**26.40**
1	-	-	-	-	36.25
2	-	-	29.00	26.00	29.30
3	27.10	21.20	-	22.50	27.00
4	27.37	21.19	25.61	20.59	29.09
5	-	-	-	-	-
6	28.09	18.68	26.06	-	27.82
7	-	27.87	-	-	29.99
8	17.55	17.47	24.30	18.84	25.36
9	26.92	23.18	-	-	30.31
10	22.50	19.60	-	16.90	26.50
11	12.50	13.30	-	-	23.80
12	-	-	-	-	-
13	-	-	-	-	-
14	-	21.23	-	-	25.64
15	-	24.10	-	-	26.80
16	27.00	20.00	26.00	-	30.50
17	-	-	-	-	-
18	24.00	20.50	21.90	-	28.20
19	-	30.89	-	-	29.61
20	29.59	22.33	31.16	-	18.11
21	19.26	20.46	-	-	24.82
22	-	20.29	-	23.06	27.23
23	-	24.76	25.12	-	31.02
24	-	32.65	-	-	-
25	-	-	-	-	-
26	22.00	20.48	-	-	27.42
27	-	-	-	-	-
28	25.59	28.44	-	19.03	30.71
29	28.55	26.40	-	20.51	32.14
30	-	-	-	-	-
31	-	20.49	-	-	26.55
32	17.95	20.90	-	-	25.43
33	31.37	27.45	-	-	29.60

34	22.86	21.73	-	18.53	25.44
35	-	20.42	-	-	26.23
36	25.50	21.25	24.44	21.84	25.07
37	-	-	-	-	25.69
38	-	34.30	-	-	28.70
39	20.72	21.19	26.64	18.86	27.60
40	-	-	-	-	-
41	-	-	25.92	-	-
42	-	22.81	-	23.15	27.89
43	-	22.04	24.88	-	25.28
44	25.97	19.63	24.01	18.29	24.94

- 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, 2024 - Genotyping

Code number	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
	IPNV, Sp Genogroup 5 + IHNV DK21-4070-1 Genotype E	**SVCV DK-203273 Genotype Ia**	**ECV 562/92**	**VHSV DK6137 Genotype Ia**
1	IPNV: Genotype 5 IHNV: Genogroup E	Genogroup: Ia	0	Ia
2	IHNV-E & IPNV III	1a	EHNV	Ia
3	Genogroup 5 / E	Genogroup 1a	Not EHNV	Ia
4	IHN Genogroup E IPN Genogroup 5, serotype: Sp, serogroup: A	SVCV Genogroup 1a	European sheatfish virus	VHS Genogroup Ia
5	E (IHNV) 5 (IPNV)	1a	Not EHNV	1a
6	IHN Genogroup E	0	0	Genotype 1a
7[2+3+4]	0	0	0	0
8	0	0	Ranavirus-Not EHNV	0
9	IHNV: E IPNV: Genogroup Sp 5	Genogroup 1a	Not EHNV	1a
10	IPNV Genogroup 5 IHNV E	0	Not EHNV	Ia

11	IHNV - Genotype E IPNV - Genogroup 5	Genogroup Ia	Not EHNV	Genotype Ia
12	IHN E, IPN 5	1a	EHN	Ia
13	E (IHNV), Genogroup 5 (IPNV)	Genogroup 1a	Not EHNV	Ia
14	IHNV: genotype E; IPNV: serotype Sp	Genotype Ia	ECV	Genotype: Ia
15	IHNV - genotype E, IPNV - genotype 5	Ia	Not EHNV	Ia
16	0	0	0	I-a
17	M and Genogroup 5	Genogroup 1(a)	Not EHNV	0
18	E	0	Not EHNV	I-a
19[2+3]	E	0	Not EHNV	Ia
20	IHN: genogroup E, IPN Sp, genogroup 5	1a	Not EHNV	1a
21	E (IHNV) and 5 (IPNV)	0	Not EHNV	Ia
22	IPNV genogroup 5, IHNV genogroup E.	Genogroup 1a	Not EHNV	1a
23	0	0	0	0
24[1]	E group	0	not-EHNV	0
25	IPNV: Genogroup 5 IHNV: Genotype E	SVC Genotype Ia	European catfish virus or European sheatfish virus	VHSV Genotype Ia
26	IPN: genogroup V IHN:E	Ia	ECV	Ia1
27	IHN Genogroup E IPN Genogroup 5(sp)	1a	Not EHNV	Ic
28	IHNV: E IPNV: Genogroup 3	Genogroup 1 (a)	Not EHNV	I(a)
29	IHNV: E; IPNV: Genogroup 5	1a	European Catfish Virus	Ia
30	1) Genogroup 5 2) Genotype E	Genotype 1a	Not EHNV	Genotype Ia
31	IHNV: E; IPNV: 5	Ia	ESV/ECV	Ia
32	IHNV : E IPNV : Genogroup 5	Ia	Not EHNV	Genotype Ia
33	IHNV: E IPNV: Genogroup 5	0	Not EHNV	Ia
34	IHNV Genogroup E. IPNV: Serotype Sp; serogroup A, Genogroup 5	Genotype Ia, Serogroup III	0	Genotype Ia, Serogroup III
35	0	0	ECV/ESV	0
36	E (IHNV) and Genogroup 5 (IPNV)	Genogroup 1a	Not EHNV	Ia
37	IHNV: E; IPNV: genogroup 5	1a	not EHNV	Ia
38	IHNV: Genotype E; IPNV: Genogroup 5	Genogroup 1a	ECV	Genotype Ia

39	IHNV - E IPNV - 5	Ia	Not EHNV	Ia
40[1]	0	0	0	0
41	0	0	Not EHNV	0
42[2+3]	N/A	N/A	N/A	N/A
43	Infectious hematopoietic necrosis virus; genotype E	Spring viremia of carp virus; genotype Ia Infectious pancreatic necrosis virus; Italy; Genogroup 5	European catfish virus; Spain: Valdeolmos	Viral hemorrhagic septicemia virus; Genotype: Ia
44	Genogroup 5 ; E	Genogroup 1a	Not EHNV	Ia

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

86% of the participating laboratories obtained 100% success rate in PT1.

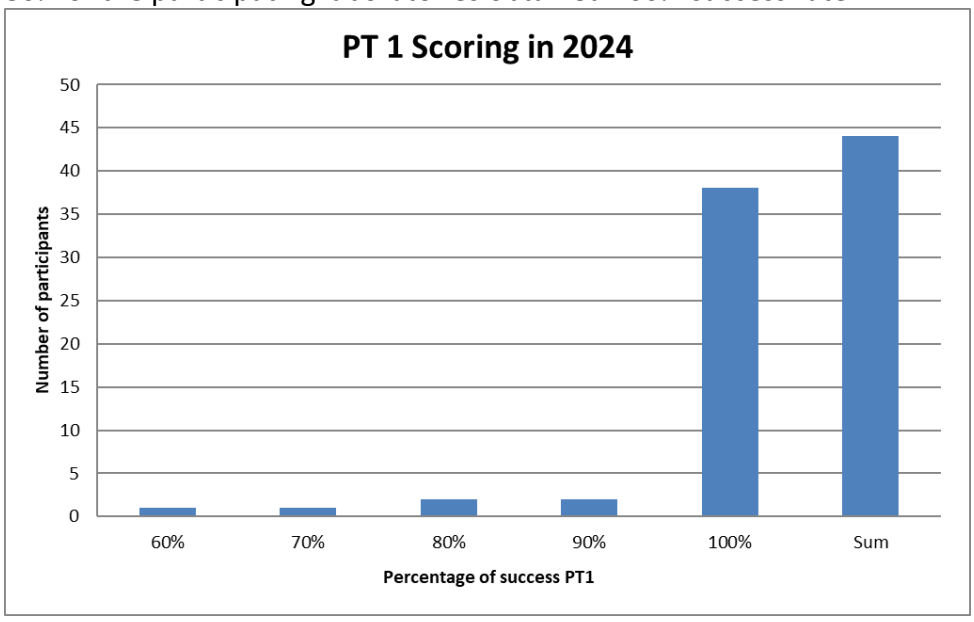

Figure 3 Success-rate of participating laboratories 2024 for PT1

Résumé and concluding remarks PT1

95% of the parcels were delivered by the shipping companies within one week and 100% was delivered within 28 days.
Overall, 38 out of 44 participants scored 100% success rate. Out of the 6 laboratories which underperformed two participants scored <100% for the sole reason that they did not back up their concluding results of ampoule IV (ECV) with sequencing. Three laboratories incorrectly answered EHNV in 'Concluding Result' on ampoule IV of these, one correctly answered ECV in the sequencing sheet. Two laboratories answered Rana in 'Concluding Result' on ampoule IV but correctly answered ECV in the sequencing sheet. one laboratory correctly answered "Not EHNV" in 'Concluding Result'

on ampoule IV but did not type any Genotype in the sequencing sheet. One laboratory identified IPNV in another ampoule than the designated one, suggesting a contamination. Two laboratories did not identify the present virus in all ampoules.
Suggestions to improve on underperformance will be provided individually to each laboratory.

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. With the exception of one outlier (ampoule III on EPC cells) the variations in titres between laboratories was up to 4 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should consider exchanging 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 online meeting in April.

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, 35 laboratories have sequenced VHSV, and 34 have correctly genotyped the isolate in ampoule V as Genotype Ia. 36 laboratories have sequenced IHNV in ampoule II, and all 36 have correctly genotyped the IHNV as Genogroup E or M (one 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 29th Annual Workshop of National Reference Laboratories for Fish Diseases to be held May 26th to May 27th, 2025.

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: SAV	**Salmonid alpha virus (SAV) 3, Pancreas Disease Virus (PD)** Norway – R-1_2007 **Received from**: Dr. Hilde Sindre, Norwegian Veterinary Institute, Norway **Reference on isolate:** Taksdal T, Jensen BB, Bockerman I, McLoughlin MF, Hjortaas MJ, Ramstad A, et al. Mortality and weight loss of Atlantic salmon, Salmon salar L., experimentally infected with salmonid alphavirus subtype 2 and subtype 3 isolates from Norway. J Fish Dis. (2015) 38:1047–61. doi: 10.1111/jfd.12312 **Gene Bank Ref**.: LT630447.1 **References on the sequences**: Hjortaas M.J., Bang Jensen B., Taksdal T., Olsen a B., Lillehaug a, Trettenes E. & Sindre H. (2016) Genetic characterization of salmonid alphavirus in Norway. *Journal of Fish Diseases* **39**, 249–257.

Code	Specifications/References
Ampoule VII: KHV	**KHV-TP 30 (syn: KHV-T (for Taiwan))** Isolated from koi in Taiwan and cloned for producing large plaques by Dr. Peiyu Lee, Institute of Medical Biotechnology, Central Taiwan University of Science and Technology, Dakeng, BeiTung District, TaiChung City 406, Taiwan in-2005. **Received from:** Dr. Sven M. Bergmann, Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Südufer 10, 17393 Greifswald-Insel Riems, Germany **Reference on isolate:** The TK gen is 100% identical to several isolates present in the Genbank e.g. KX609547.1
Ampoule VIII: ISAV	**Infectious Salmon Anaemia Virus. ISAV 2013-70-9227_Vir547** ISAV HPRΔ isolate from Atlantic salmon in Norway. Bonhammaren in 2013. **Received from** Norwegian Veterinary Institute. **Genbank accession number** MK216303 **Reference on isolate :** Spilsberg B, Leithaug M, Christiansen DH, Dahl MM, Petersen PE, Lagesen K, Fiskebeck EMLZ, Moldal T, Boye M. Development and application of a whole genome amplicon sequencing method for infectious salmon anemia virus (ISAV). Front Microbiol. 2024 May 30;15:1392607. doi: 10.3389/fmicb.2024.1392607. PMID: 38873156; PMCID: PMC11169708.
Ampoule IX: ISAV	**Infectious Salmon Anaemia Virus. ISAV 2016-60-1004_Vir1664** ISAV HPRΔ isolate from Atlantic salmon in Norway. Ørnøya in 2016. **Received from** Norwegian Veterinary Institute. **Genbank accession number** MK216306

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 contents 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	**SAV**	a	21.54	29.74	30.44
		b		29.79	
		c		29.67	
		d		29.63	
		e		29.58	
			21.54	**29.68**	**30.44**
Ampoule VII	**KHV**	a	25.35	28.40	28.91
		b		28.66	
		c		29.15	
		d		28.37	
		e		28.06	
			25.35	**28.53**	**28.91**
Ampoule VIII	**ISAV**	a	25.57	30.18	29.85
		b		30.45	
		c		30.20	
		d		30.27	
		e		30.17	
			25.57	**30.25**	**29.85**
Ampoule IX	**ISAV**	a	23.1	28.00	27.7
		b		28.05	
		c		27.76	
		d		28.14	
		e		27.98	
			23.10	**27.99**	**27.70**

The lyophilisation procedure caused a significant virus reduction in all the ampoules (3-8 Ct. values), especially in the ampoules containing SAV as detected by real-time PCR or real-time RT-PCR.
For each ampoule no other pathogens than the expected were detected.

Pathogen identification

In PT2, participants were asked to identify any of the fish viruses ISAV and KHV 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, 2024 - Virus identification.

Laboratory code number	Score	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
		SAV	KHV	ISAV	ISAV
1	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
2	8/8	SAV	KHV (CyHV3)	HPR-deleted ISAV	HPR-deleted ISAV
3	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
4	8/8	SAV3	KHV - CyHV3	ISAV HPR deleted	ISAV HPR deleted
5	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
6	6/8	SAV	KHV	HPR0 ISAV	HPR Deleted ISAV
7		0	0	0	0
8	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
9	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
10	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
11	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
12	6/8	SAV	KHV	ISAV	ISAV
13	8/8	SAV	KHV	ISAV	ISAV
14	8/8	SAV	KHV	ISAV	ISAV
15	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
16	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
17	7/8	SAV	KHV	HPR-deleted ISAV	KHV and HPR-deleted ISAV
18	8/8	SAV	KHV	HPR- deleted ISAV	HPR- deleted ISAV
19[1]	6/6	Negative for ISAV and KHV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
20	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV

21	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
22	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
23	8/8	SAV	KHV	ISAV	ISAV
24[1]	6/6	0	KHV	ISAV	ISAV
25	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
26	8/8	SAV3	KHV	HPR-deleted ISAV	HPR-deleted ISAV
27	6/8	SAV	KHV	HPR-DELETED ISAV	HPRO ISAV
28	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
29[1]	4/6	0	KHV	HPR0 ISAV	HPR-deleted ISAV
30[1]	4/6	NO KHV, NO ISAV	KHV	HPRO ISAV	HPR-deleted ISAV
31	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
32	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
33	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
34	8/8	SPDV/SAV	KHV	ISAV-HPRdeleted	ISAV-HPRdeleted
35[1]	6/6	0	KHV	HPR-deleted ISAV	HPR-deleted ISAV
36	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
37	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
38	8/8	SAV	KHV	HRP-deleted ISAV	HRP-deleted ISAV
39	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
40[2]	3/8	no virus	KHV	no virus	ISAV
41	8/8	SAV	KHV	HPR-deleted ISAV	HPR-deleted ISAV
42[1]	6/6	-	KHV	HPR-deleted ISAV	HPR-deleted ISAV
43	8/8	SAV	KHV	ISAV	ISAV
44	8/8	SAV	KHV	HPR-deleted ISAV	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 (SAV, KHV, ISAV, ISAV)
- All 43 laboratories tested for the two listed pathogens (KHV, ISAV)
- 37 laboratories tested for SAV.

Ampoule VI – SAV

- 36 (out of 37) laboratories correctly identified SAV.
- 6 laboratories did not participate for SAV and answered '0', 'No virus' '-' or 'no ISAV; no KHV'

Ampoule VII – KHV

- All 43 laboratories correctly identified KHV.

Ampoule VIII – ISAV

- 42 laboratories correctly identified ISAV but hereof three laboratories wrongly answered ISAV HPR0.
- One laboratory did not find the pathogen.

Ampoule IX – ISAV

- All 43 laboratories correctly identified ISAV but hereof two laboratories wrongly answered ISAV HPR0 and one laboratory did not sequence and one laboratory also found KHV in the ampoule.

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 VIII and 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 36 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.

- **Ampoule VI**: SAV identification was given the score 2 if testing for SAV
  No identification of SAV was given the score 0 if testing for SAV.
- **Ampoule VII**: KHV identification was given the score 2.
- **Ampoule VIII and IX**: ISAV HPRΔ identification by sequencing was given the score 2.
  Answering ISAV but identifying HPRΔ ISAV by sequencing was given the score 2
  No discrimination between the listed HPRΔ ISAV and non-listed HPR0 ISAV by sequencing was giving the score 1
  HPRΔ ISAV identification and detection of another virus was given the score 1.
  Wrong identification of HPR type on ISAV was given the score 0.
  Not finding ISAV in the ampoule was given the score 0.

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, 2024 – Ct.-values.

Laboratory Code number	Ct. value Ampoule VI (SAV)	Ct. value Ampoule VII (KHV)	Ct. value Ampoule VIII (ISAV)	Ct. value Ampoule IX (ISAV)
EURL	**29.68**	**28.53**	**30.25**	**27.99**
1	35.00	33.91	30.63	28.57
2	32.45	31.45	30.20	28.30
3	33.80	32.50	31.10	29.10
4	33.60	29.40	34.37	31.43
5	-	-	-	-
6	34.04	29.31	34.57	28.80
7	No participation			
8	29.84	28.45	28.30	26.91
9	-	31.31	32.69	31.94
10	33.40	27.80	30.20	28.10
11	-	25.60	21.30	18.40
12	-	-	-	-
13	-	-	-	-
14	-	27.79	-	-
15	-	31.80	28.90	26.50
16	-	30.00	-	-
17	-	-	-	-
18	-	26.00	-	-
19	Not testing	28.37	32.37	29.97
20	32.00	26.79	34.80	30.20
21	31.80	27.36	27.95	25.94
22	33.68	29.41	36.91	34.46
23	35.70	26.73	30.53	28.56
24	Not testing	25.77	25.60	26.83
25	-	-	-	-
26	32.92	25.60	32.09	31.70
27	32.29	31.93	32.83	30.56
28	34.20	-	33.78	32.79
29	Not testing	31.23	34.30	32.25
30	Not testing	-	-	-
31	30.53	28.30	30.36	28.71
32	33.93	28.53	30.60	28.11
33	38.24	36.44	35.36	32.73
34	30.08	-	28.32	26.24
35	Not testing	29.50	32.43	30.69
36	29.90	29.95	29.64	28.02
37	-	28.81	32.66	31.07
38	-	26.93	-	-
39	34.71	26.51	32.34	28.96
40	-	-	-	-
41	-	28.32	-	-

42	Not testing	29.82	29.91	29.68
43	29.91	29.63	28.90	27.05
44	36.41	27.70	30.01	27.74

- No Ct-value given by the participating laboratory.

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. One laboratory did not sequence the ISAV isolate. Three laboratories wrongly answered HPR0 ISAV in ampul VIII and two laboratories wrongly answered HPR0 ISAV in ampul IX. The identification of KHV in ampoule VII didn't pose particular issues. Finally, regarding sequencing of SAV isolate in ampoule VI, 29 out of 30 laboratories which performed genotyping of SAV, assigned the correct genotype and one 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, 2024 – Genotyping

Code number	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
	SAV	KHV	ISAV	ISAV
1	0	0	0	0
2	SAV 3	CyHV-3	HPR- deleted/European	HPR- deleted/European
3	3	CyHV 3	HPR-deleted	HPR-deleted
4	SAV subtype 3	CyHV 3	ISA HPR deleted	ISA HPR deleted
5	3	CyHV-3	HPR-deleted	HPR-deleted
6	0	0	0	0
7	0	0	0	0
8	0	0	HRP-deleted	HRP-deleted
9	3	CyHV (3)	HPR-deleted	HPR-deleted
10	3	0	HPR-deleted	HPR-deleted
11	SAV3	CyHV-3	HPR-deleted	HPR-deleted
12	3	CyHV-3	HPR-deleted	HPR0
13	3	CyHV 3	HPR-deleted	HPR-deleted
14	Salmon pancreas disease virus/Salmonid alphavirus (SAV) subtype 3	CyHV3	ISAV -HPRΔ (Genotype 2)	ISAV HPRΔ
15	3	CyHV3	HPR-deleted	HPR-deleted
16	3	0	0	0
17	3	CyHV3	HPR-deleted	CyHV3 and HPR-deleted
18	III	CyHV 3	HPR-deleted	HPR-deleted

19	0	CyHV-3	HPR-deleted	HPR-deleted
20	SAV3	0	HPR-deleted	HPR-deleted
21	SAV3	CyHV3	HPR-deleted	HPR-deleted
22	3	CyHV 3	HPR-deleted	HPR-deleted
23	0	0	Genotype- PR3	Genotype- PR14
24	0	CyHV3	HPR9	HPR7b
25	SAV Genotype 3	KHV-I	ISAV HPRΔ	ISAV HPRΔ
26	SAV3	CyHV-3	HPR-deleted	HPR-deleted
27	3	CyHV (3)	HPR-deleted	HPR0
28	0	CyHV(3)	HPR-deleted	HPR-deleted
29	0	CyHV 3	HPR0	HPR-deleted ISAV
30	0	CyHV-3	HPR0	ISA-HPRΔ
31	3	CyHV-3	HPR1	HPR14
32	3	CyHV3	HPR-deleted	HPR-deleted
33	0	CyHV-3	HPR-deleted	HPR-deleted
34	SAV3	0	Clade = CIIIa	Clade = CIIIa
35	0	0	HPR3	HPR15
36	3	CyHV 3	HPR-deleted	HPR-deleted
37	3	CyHV3	HPR-deleted	HPR-deleted
38	Genotype 3	CyHV 3	HPR-deleted	HPR-deleted
39	3	CyHV-3	HPR-deleted	HPR-deleted
40	0	0	0	0
41	0	0	HPR-deleted	HPR-deleted
42	N/A	N/A	N/A	N/A
43	Salmonid alphavirus subtype 3; SAV3	Cyprinid herpesvirus 3; genotype: 3	ISAV; HPR-deleted	ISAV; HPR-deleted
44	3	CyHV3	HPR-deleted	HPR-deleted

84% of the participating laboratories obtained 100% success rate in PT2.

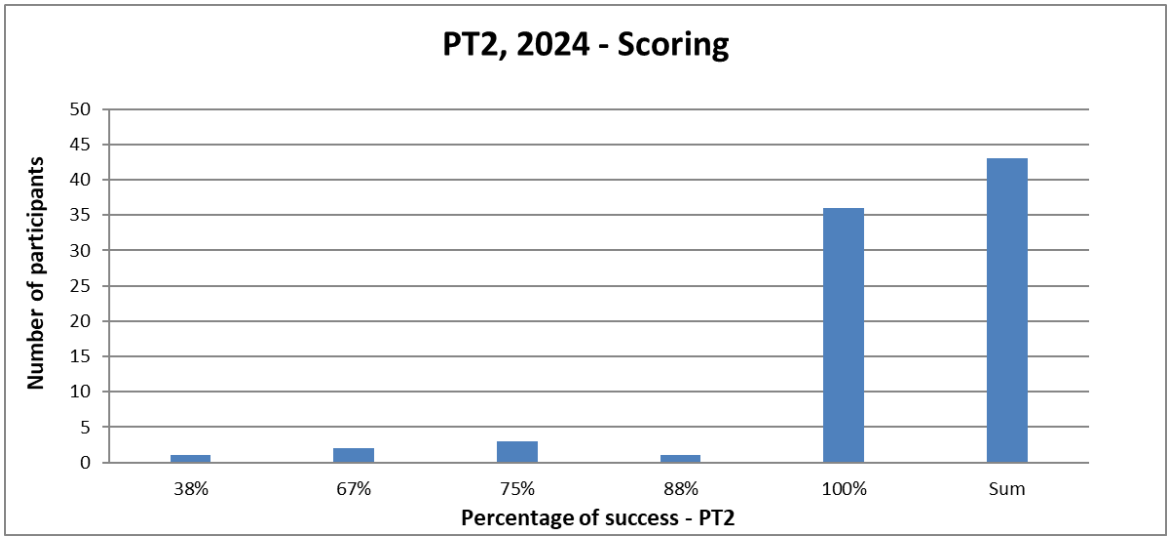

Figure 4 Success-rate of participating laboratories 2024 for PT2

Concluding remarks PT2

43 laboratories participated in PT2, 36 obtained 100% success rate.

37 laboratories tested for SAV and 36 correctly identified the virus in Ampoule VI, 6 laboratories did not test for SAV and one laboratory who tested for SAV did not find the SAV in this ampoule.

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

42 laboratories correctly identified the ISA virus in ampoule VIII and all 43 laboratories correctly identified the ISA virus in ampoule IX. One laboratory did not sequence, one laboratory did not find the ISAV in ampoule VIII, five laboratories gave the wrong HPR-type for the ISAV isolate in ampoule VIII or IX and six laboratories answered ISAV in 'Concluding Result' on ampoule VIII and IX but correctly identified the isolates as HPR-deleted in the sequencing sheet.

One laboratory identified KHV in another ampoule than the designated one, suggesting a contamination.
One laboratory did not identify the present virus in all ampoules.

This point will be addressed directly with the participants that has underperformed.

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 29th Annual Workshop of National Reference Laboratories for Fish Diseases to be held May 26th -28th, 2025.

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March 2025.

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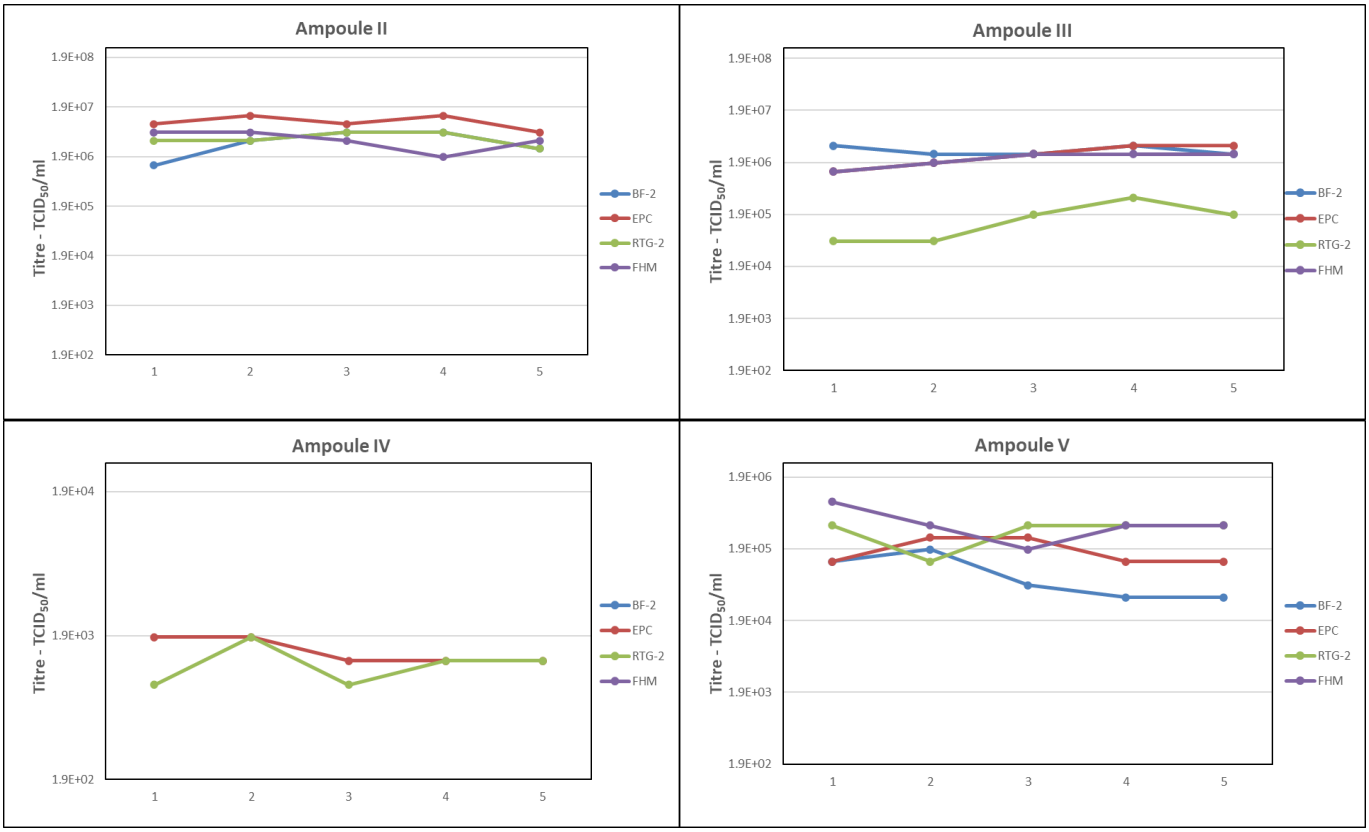
Appendix

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

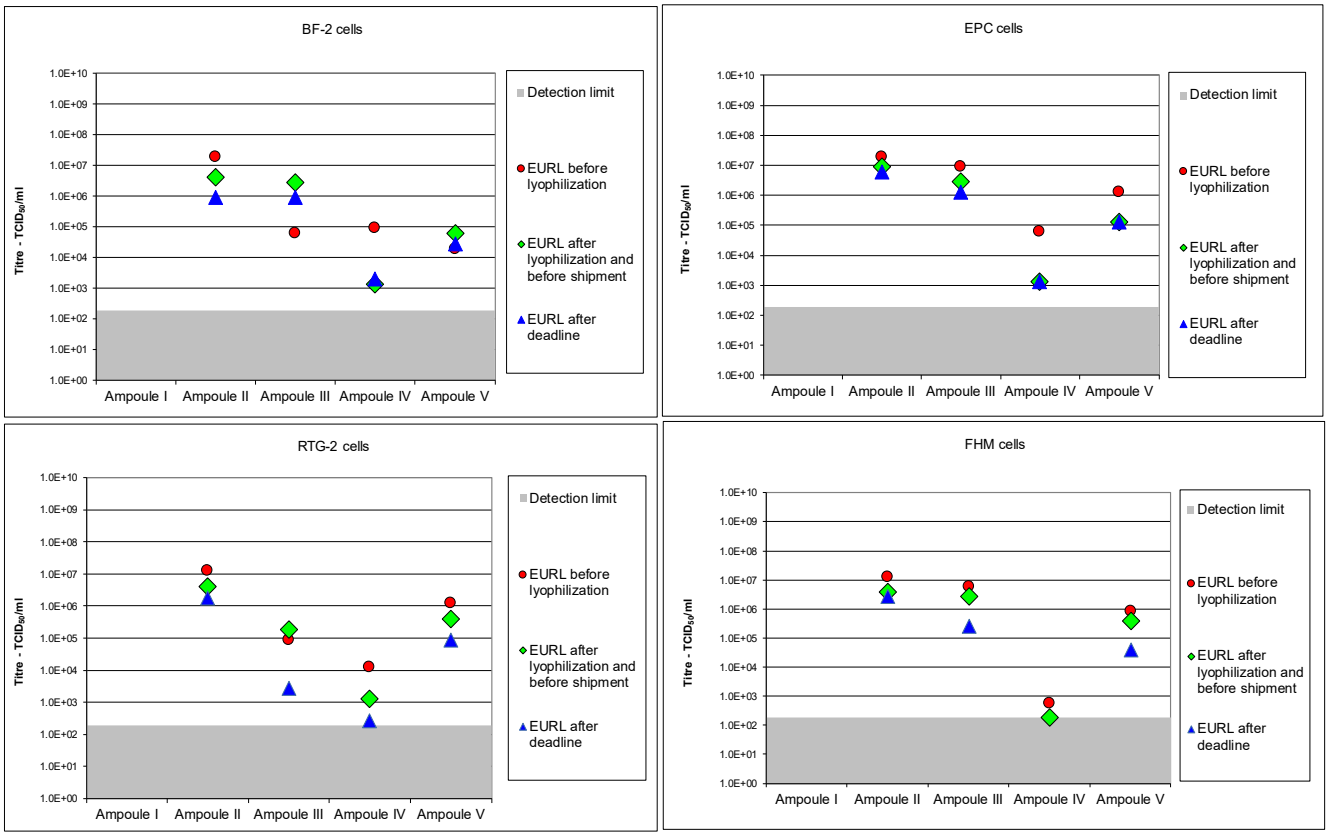
After Lyophilization the stability of the content in the ampoules were assessed by titrating the virus in five ampoules on cell cultures. For all the ampoules, the reduction of the titre was below 1 log in the same cell line.

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 1-2 log in the same cell line. No significant reductions were observed after long term storage ≤1log except from ampoule III on RTG-2 cells where the reduction was approx. 2 log.

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



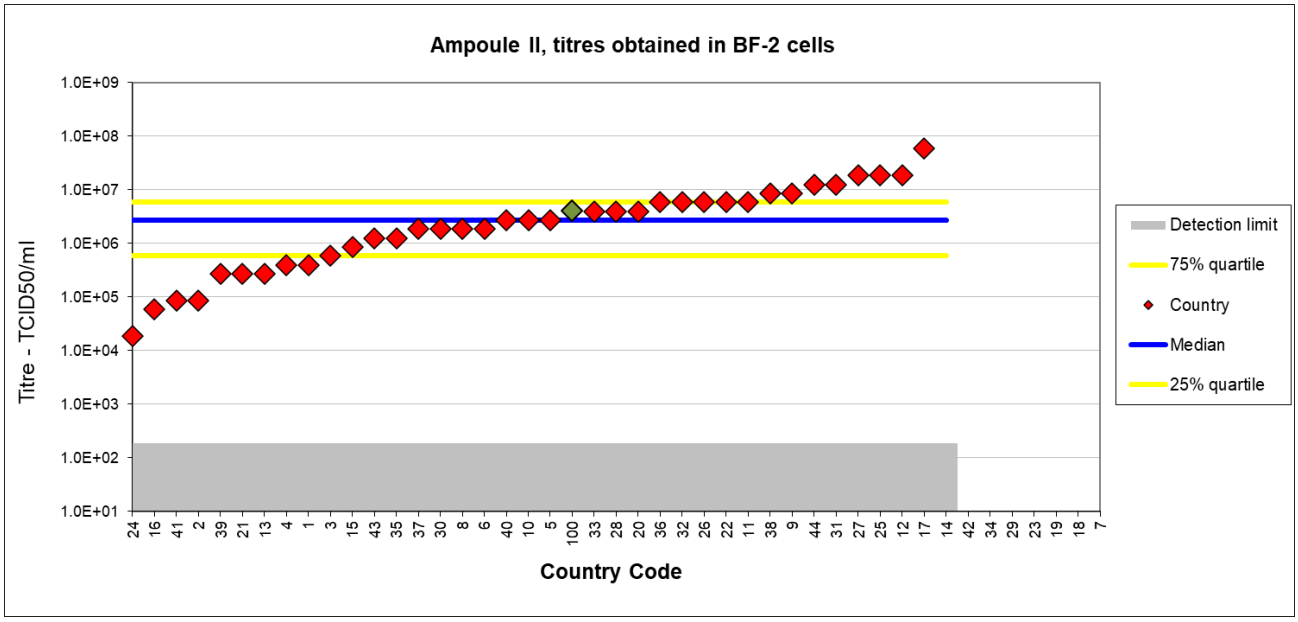
Annex 2: The titres obtained from each participating laboratory represented graphically.

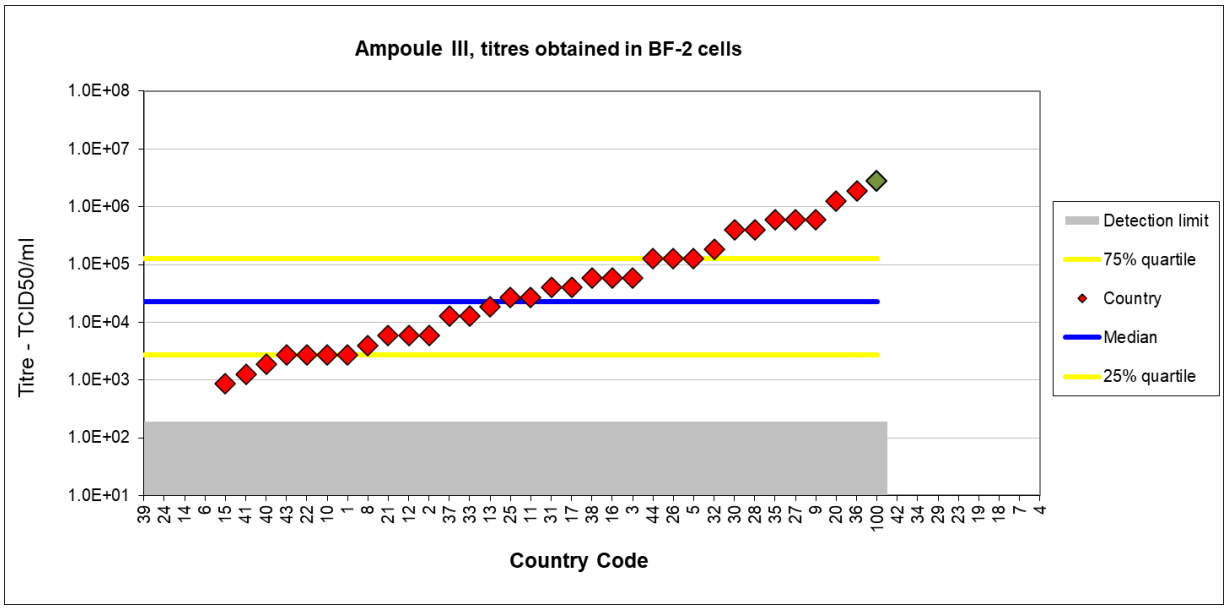
The titres (red diamonds) obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories as well as the titre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

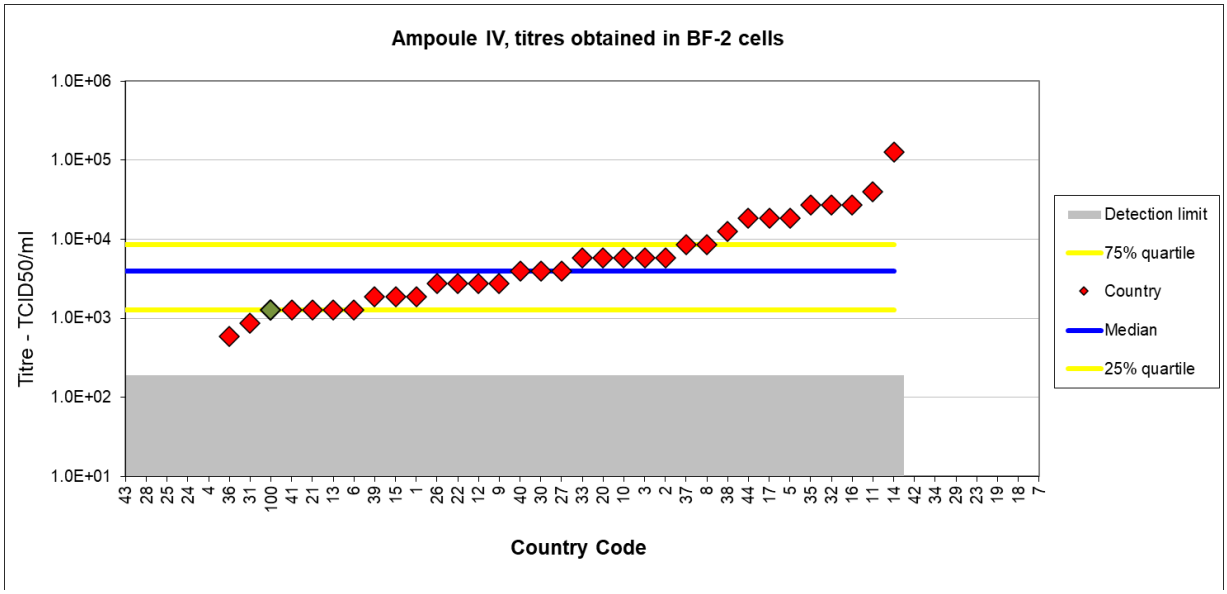
On these figures, the detection limit (grey shadow), the median titre (blue line), the 25% quartile (lower yellow line) and 75% quartile (upper yellow line) are plotted on all graphs.
Participants failing to obtain any titre are listed on the x axis under the grey zone, but no red diamond is plotted; 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.

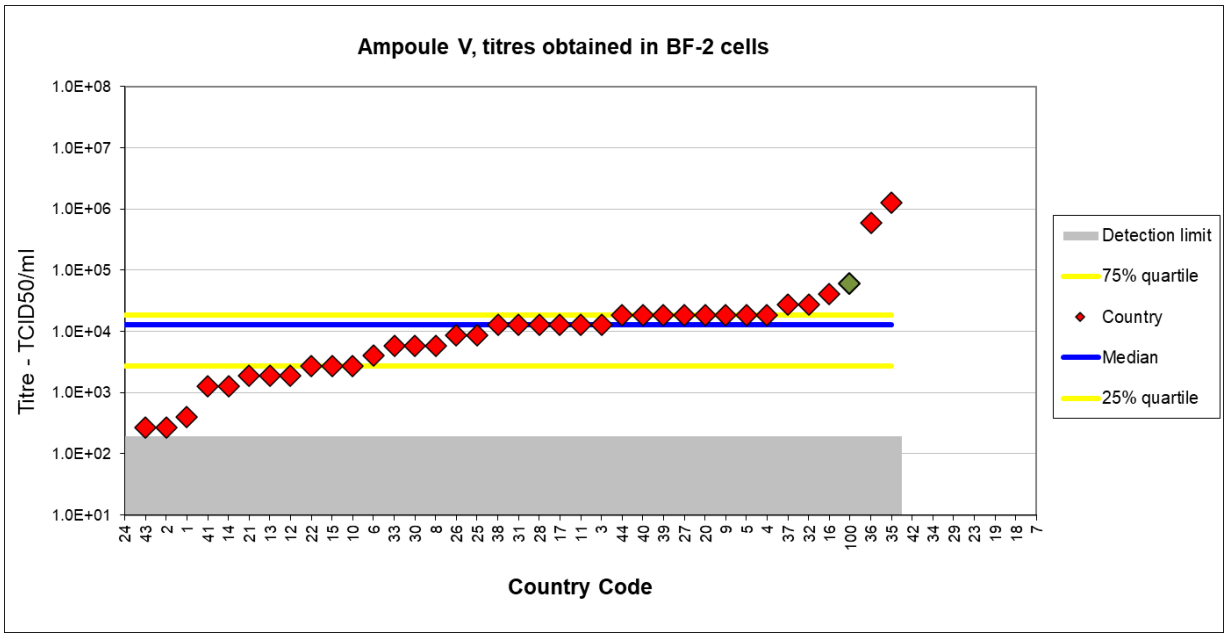
The optimal titre will be within the 25% and 75% inter-quartile range. A low titre, below 25% quartile may be indicator of low sensitivity of the cell culture in use; conversely a very high titre, beyond 75% quartile may indicate errors in assessing CPE.

Virus titres obtained in BF-2 cells. Ampoule I is not represented graphically due to no titre obtained by the participants

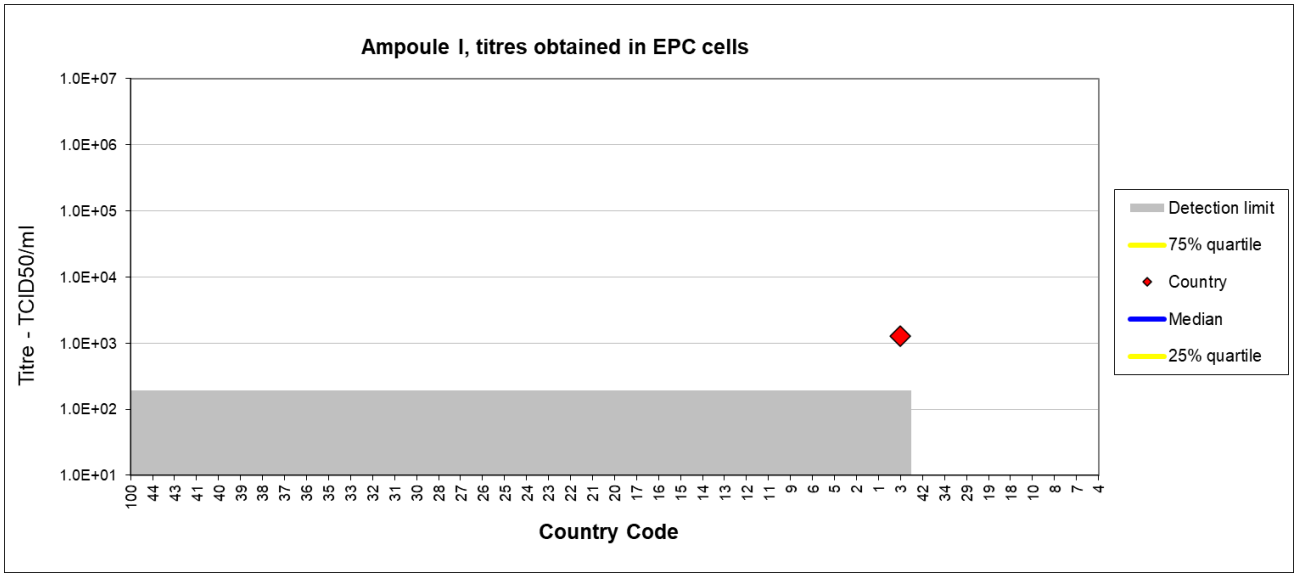


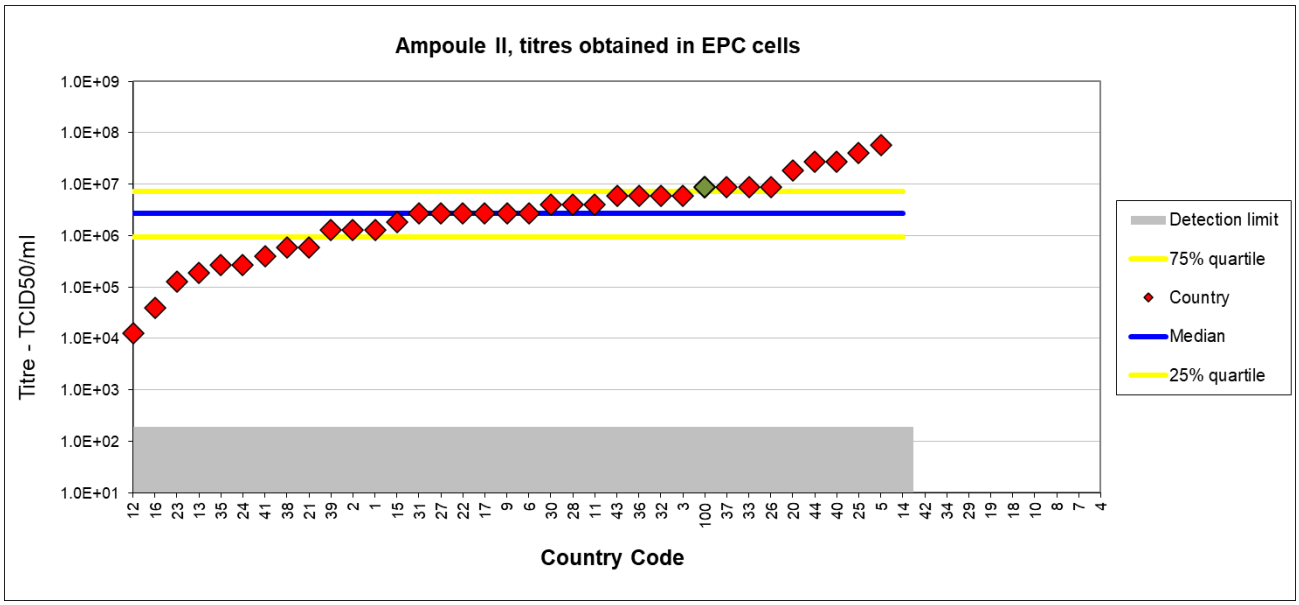

Ampoule III, titres obtained in BF-2 cells
Titre - TCID50/ml
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
39 24 14 6 15 41 40 43 22 10 1 8 21 12 2 37 33 13 25 11 31 17 38 16 3 44 26 5 32 30 28 35 27 9 20 36 100 42 34 29 23 19 18 7 4
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

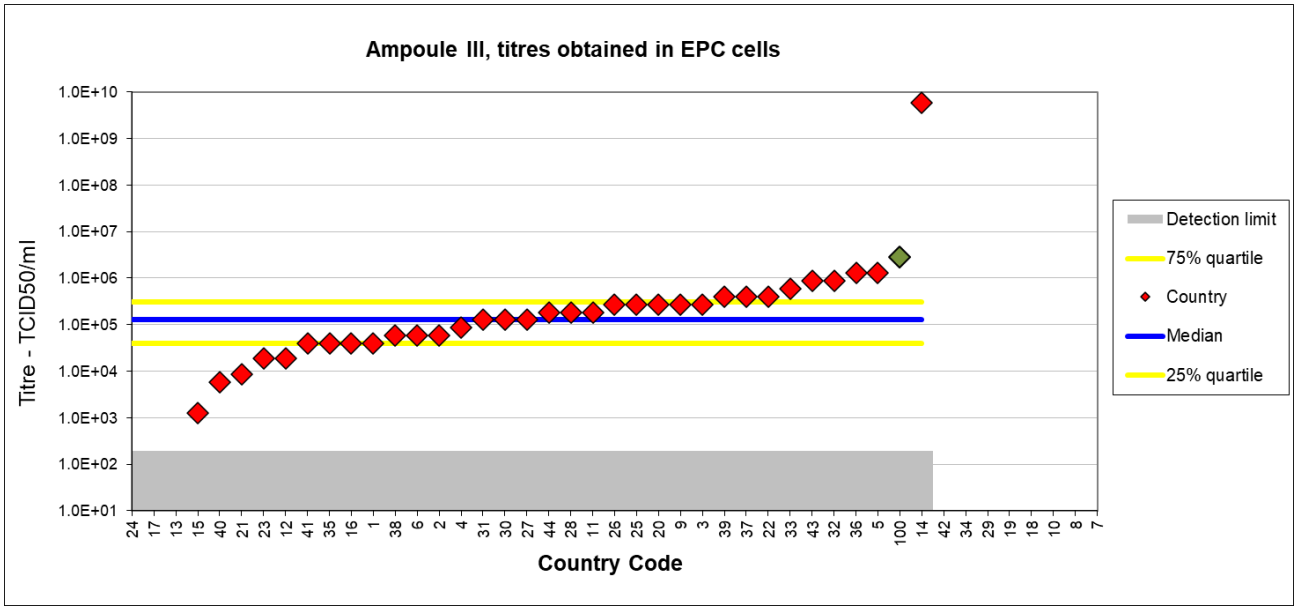

Ampoule IV, titres obtained in BF-2 cells
Titre - TCID50/ml
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
43 28 25 24 4 36 31 100 41 21 13 6 39 15 1 26 22 12 9 40 30 27 33 20 10 3 2 37 8 38 44 17 5 35 32 16 11 14 42 34 29 23 19 18 7
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

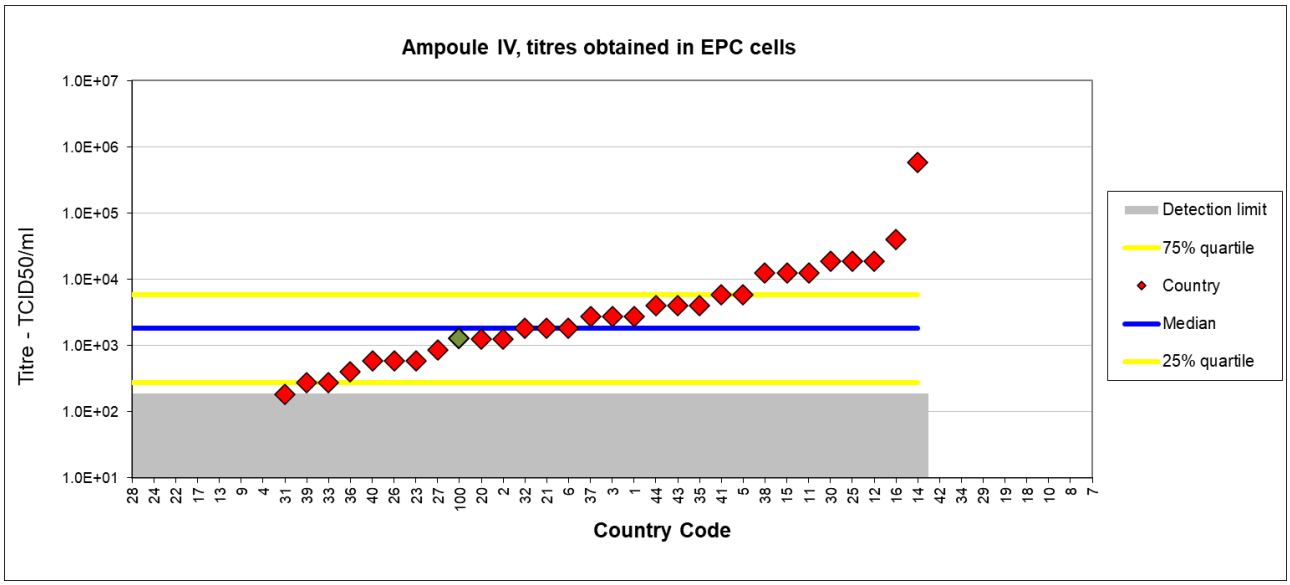

Ampoule V, titres obtained in BF-2 cells
Titre - TCID50/ml
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
24 43 2 1 41 14 21 13 12 22 15 10 6 33 30 8 26 25 38 31 28 17 11 3 44 40 39 27 20 9 5 4 37 32 16 100 36 35 42 34 29 23 19 18 7
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

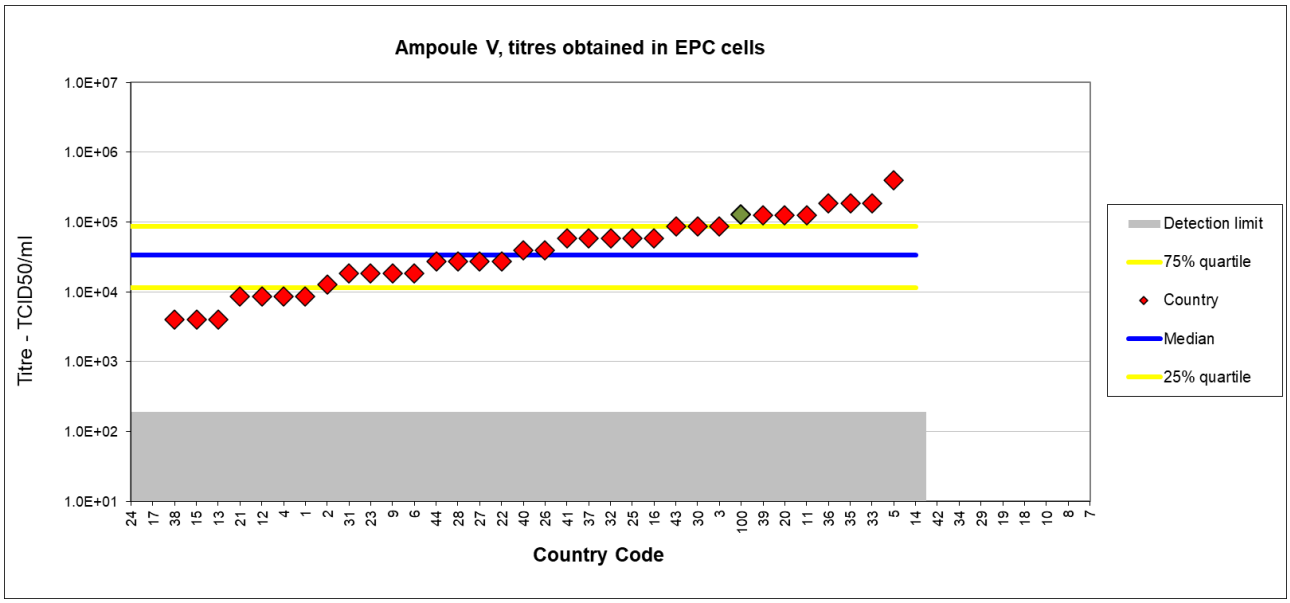
Virus titres obtained in EPC cells.



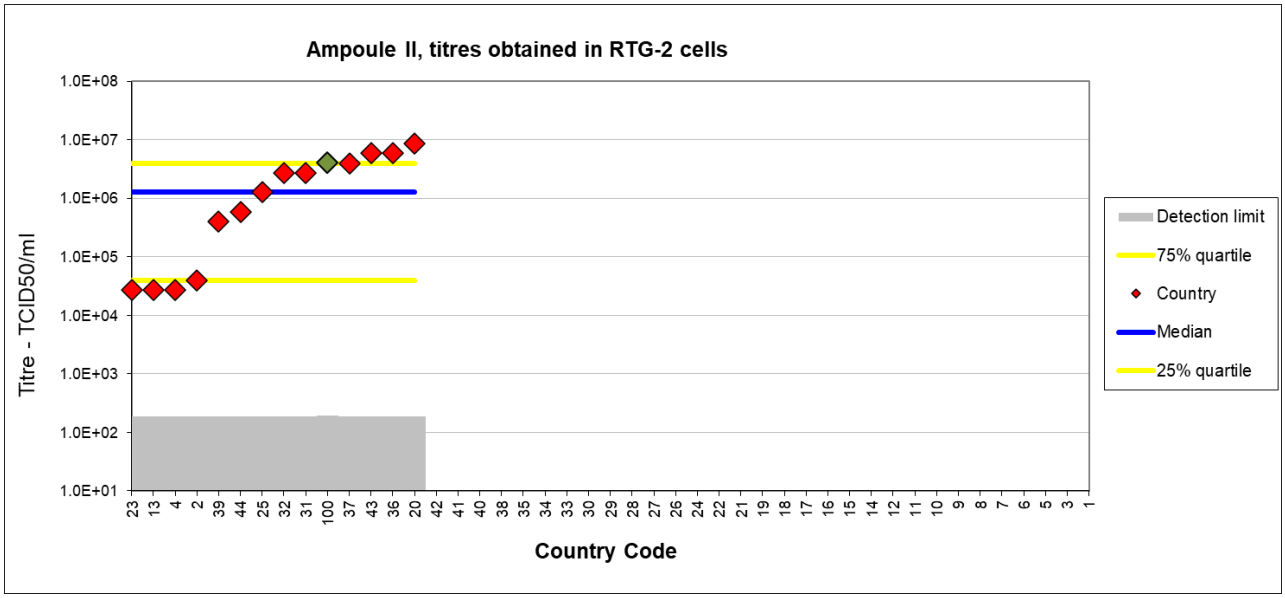


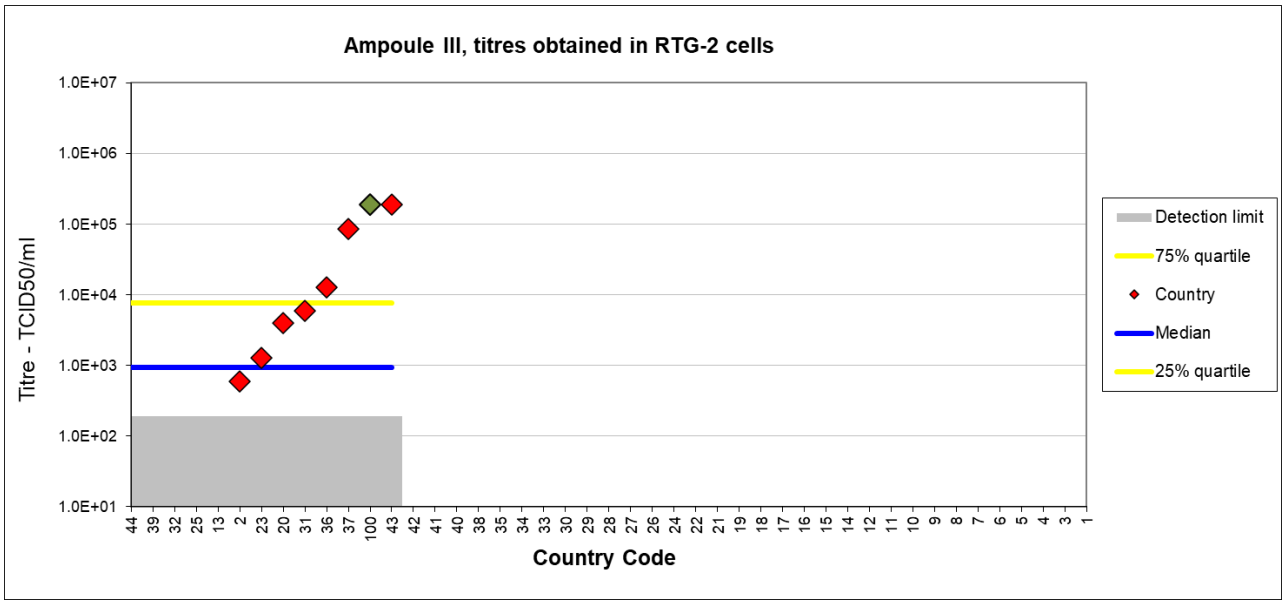


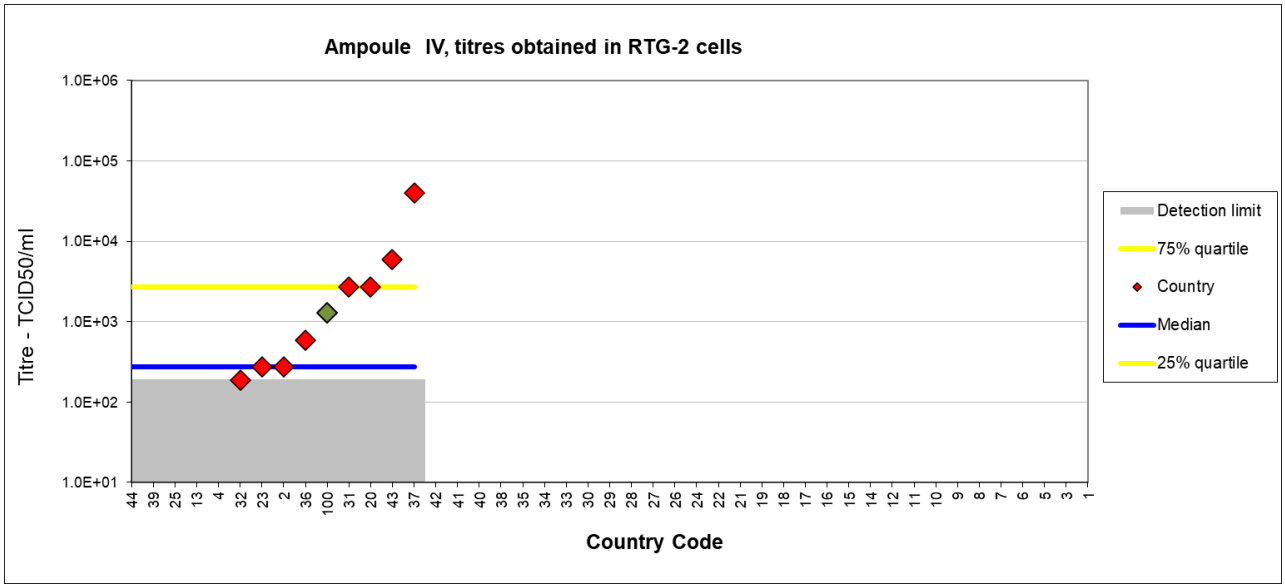


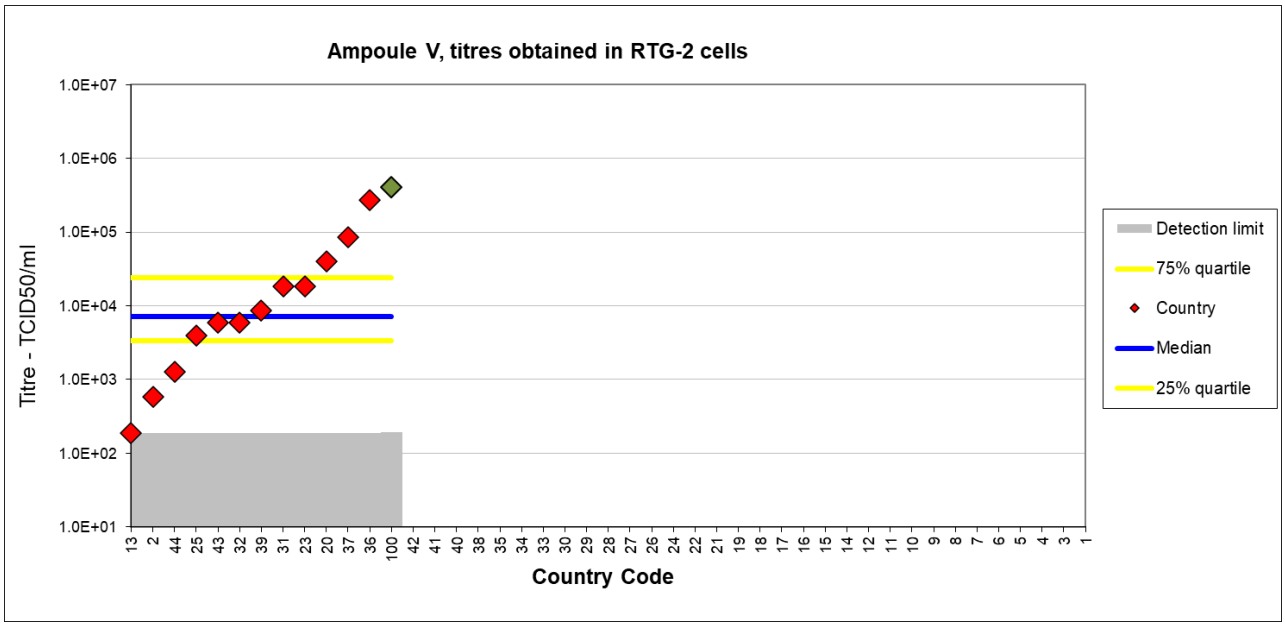


Virus titre obtained in RTG-2 cells. Ampoule I is not represented graphically due to no titre obtained by the participants

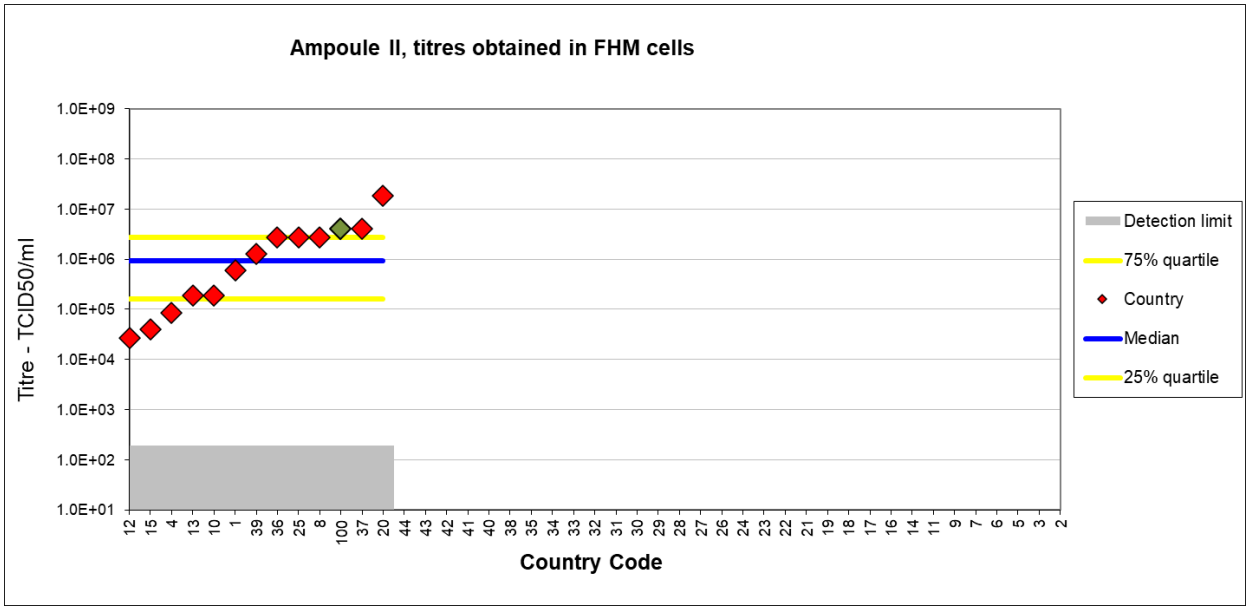


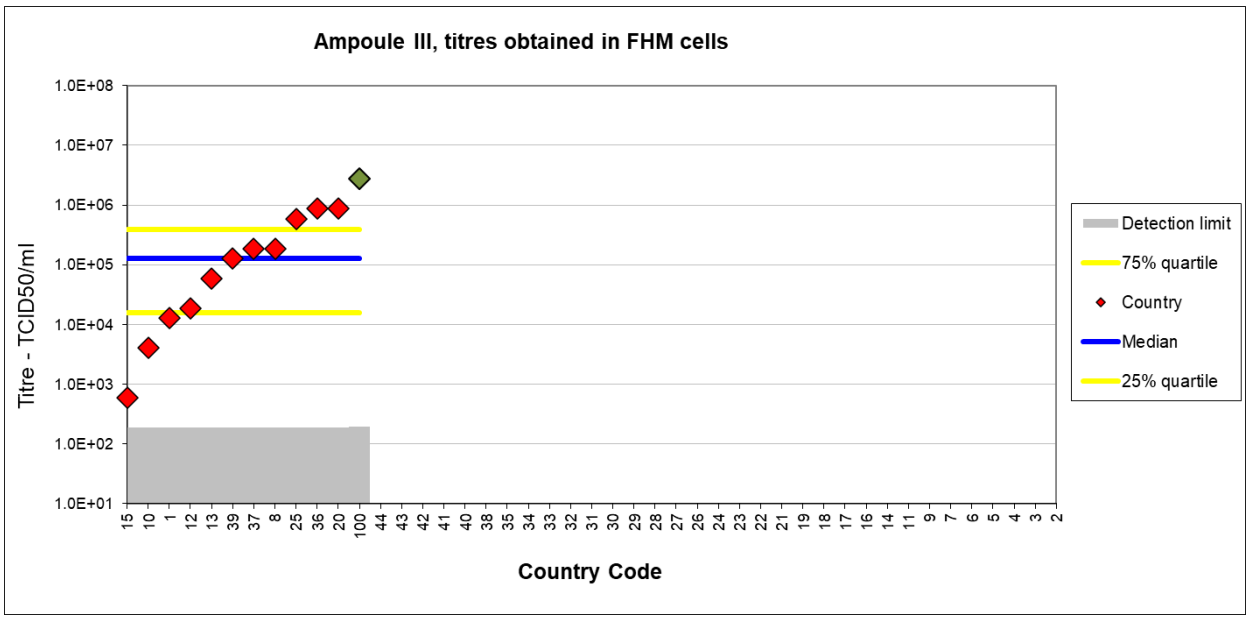

Ampoule III, titres obtained in RTG-2 cells
Titre - TCID50/ml
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
44 39 32 25 13 2 23 20 31 36 37 100 43 42 41 40 38 35 34 33 30 29 28 27 26 24 22 21 19 18 17 16 15 14 12 11 10 9 8 7 6 5 4 3 1
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

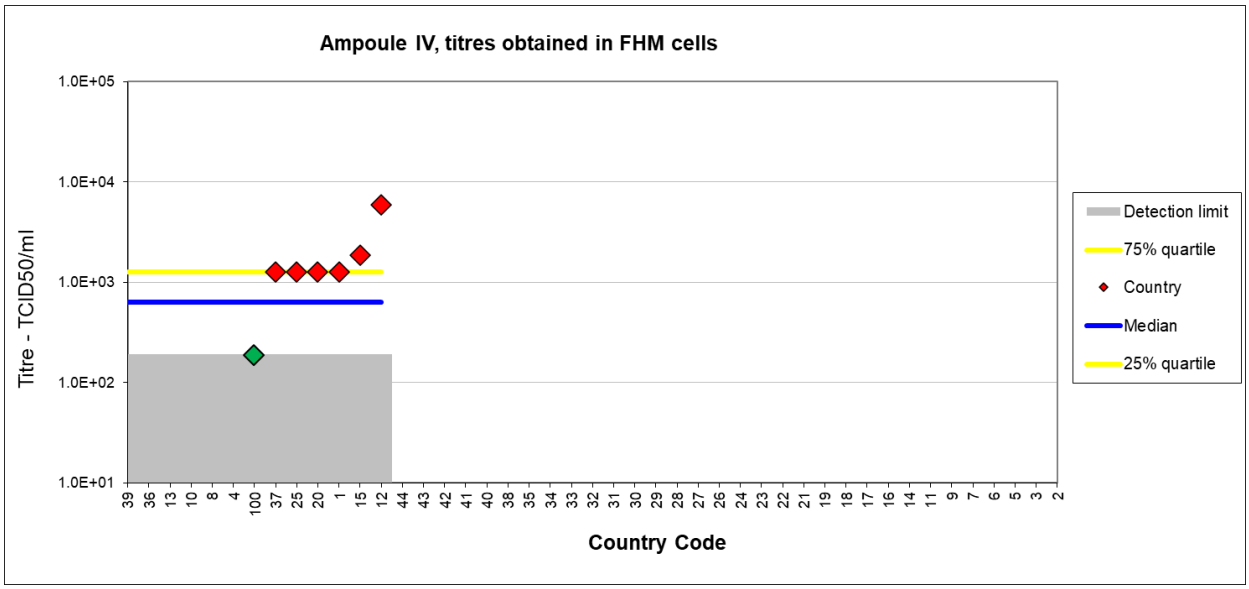

Ampoule IV, titres obtained in RTG-2 cells
Titre - TCID50/ml
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
44 39 25 13 4 32 23 2 36 100 31 20 43 37 42 41 40 38 35 34 33 30 29 28 27 26 24 22 21 19 18 17 16 15 14 12 11 10 9 8 7 6 5 3 1
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

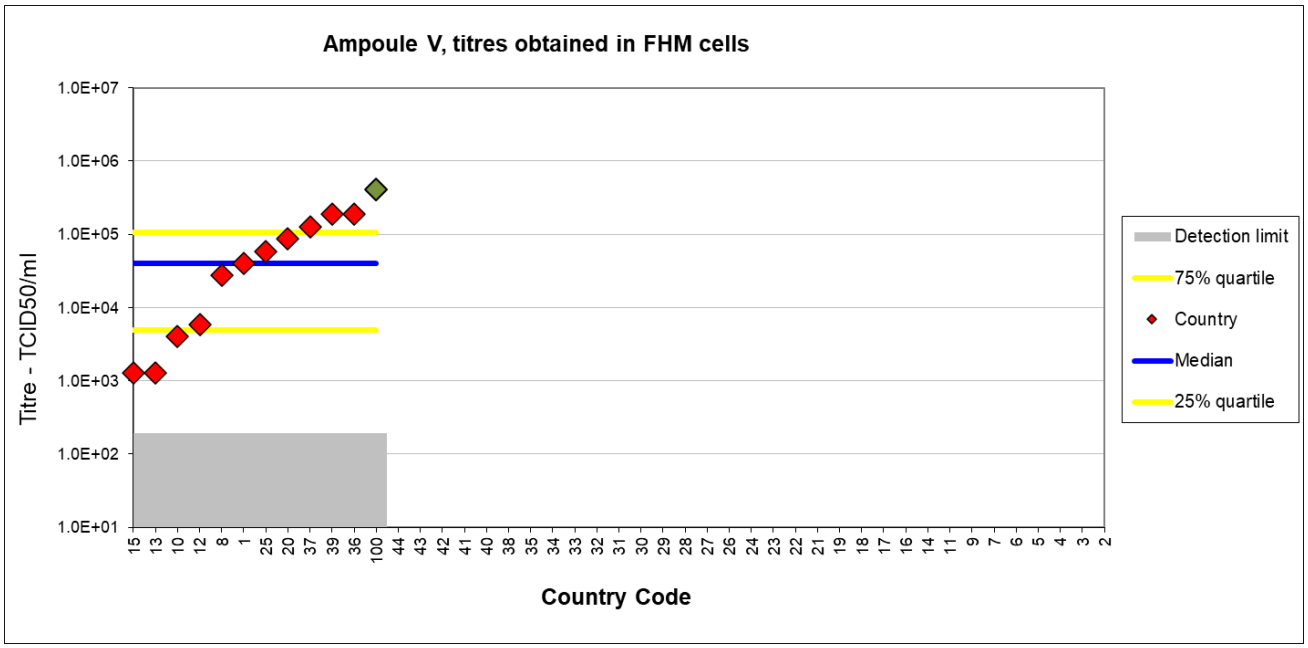

Ampoule V, titres obtained in RTG-2 cells
Titre - TCID50/ml
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
13 2 44 25 43 32 39 31 23 20 37 36 100 42 41 40 38 35 34 33 30 29 28 27 26 24 22 21 19 18 17 16 15 14 12 11 10 9 8 7 6 5 4 3 1
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

Virus titres obtained in FHM cells. Ampoule I is not represented graphically due to no titre obtained by the participants

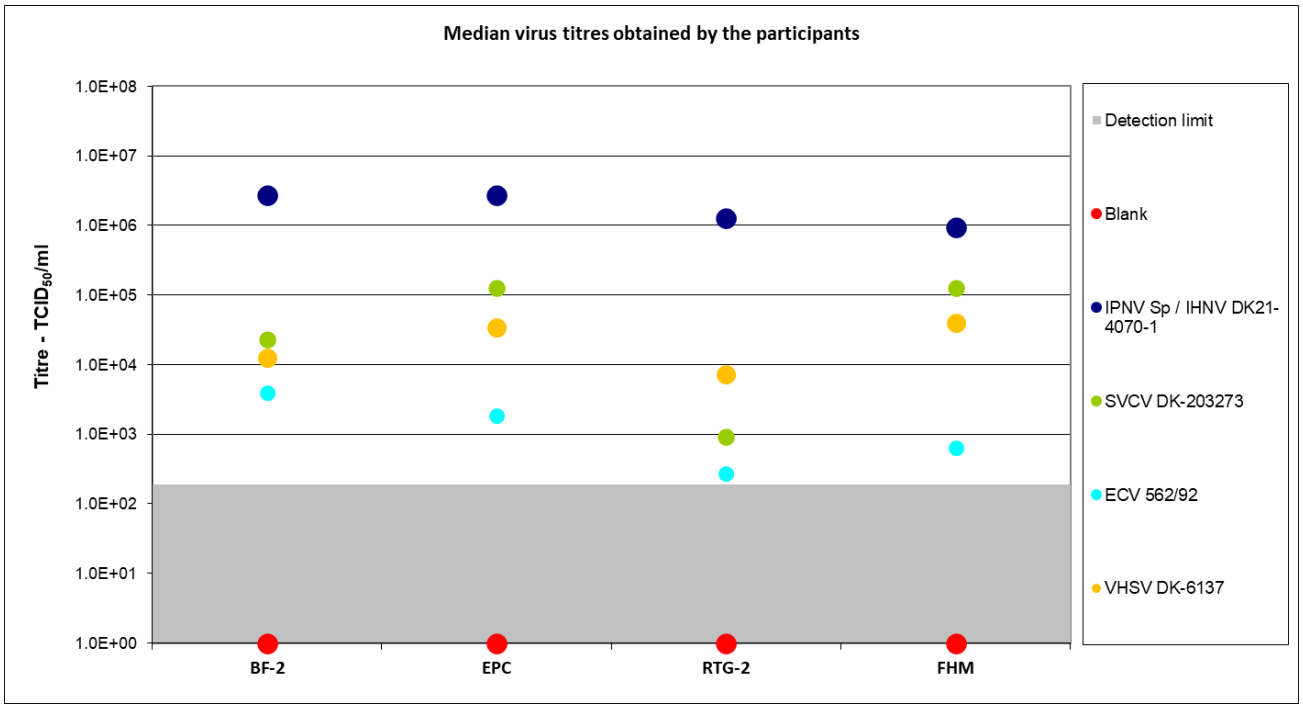








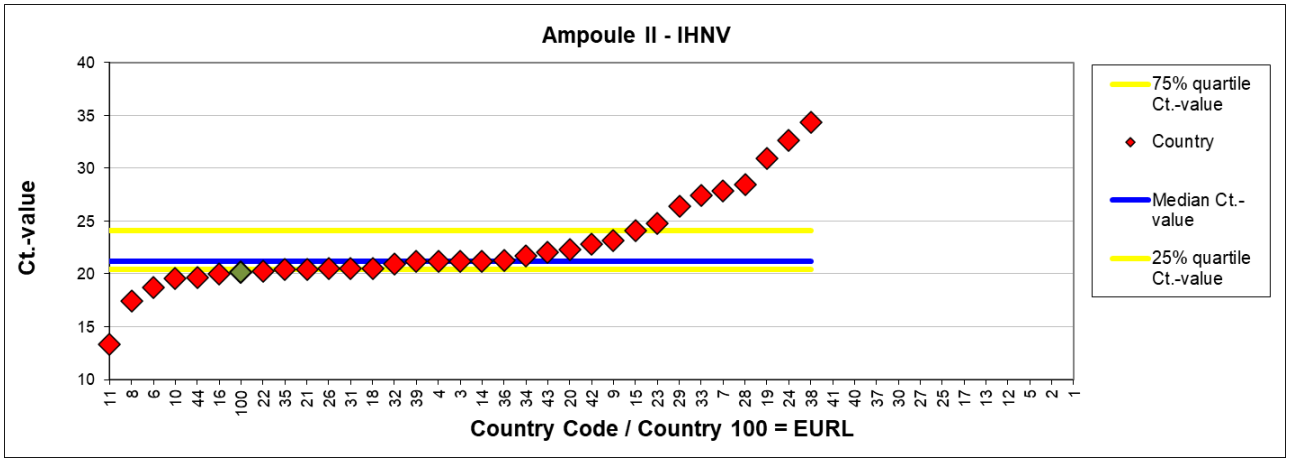
Median virus titres obtained by the participants in 4 different cell lines



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.

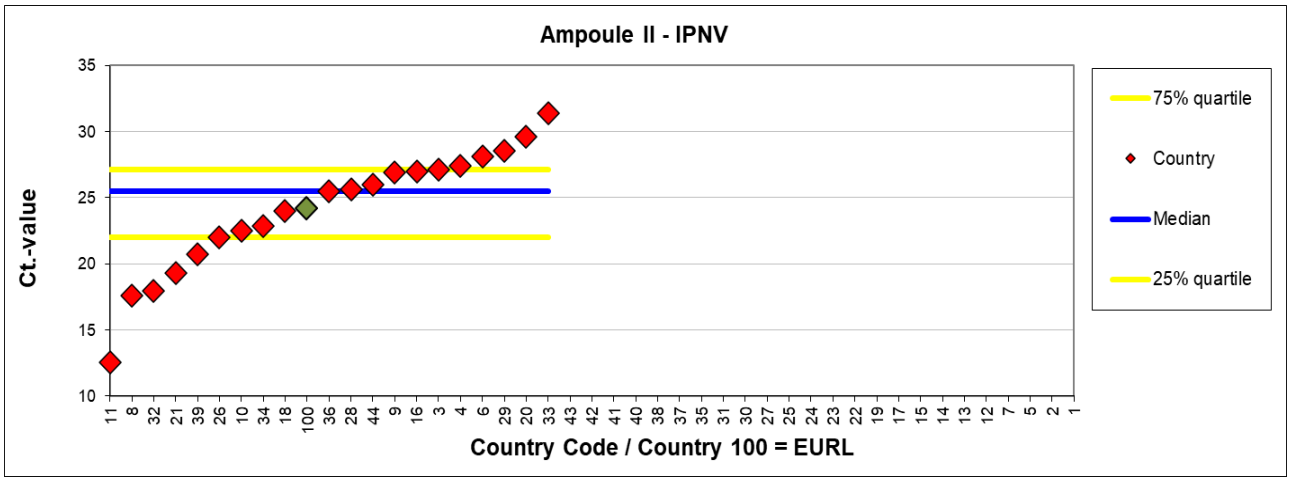
All Ct values submitted by the participants for each ampoule, are compared to each other, No red diamond = No Ct. value given. On these figures, the median values and the 25% and 75% inter-quartile range is displayed, the optimal value will be within these quartiles. A low Ct, below 25% quartile may 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.

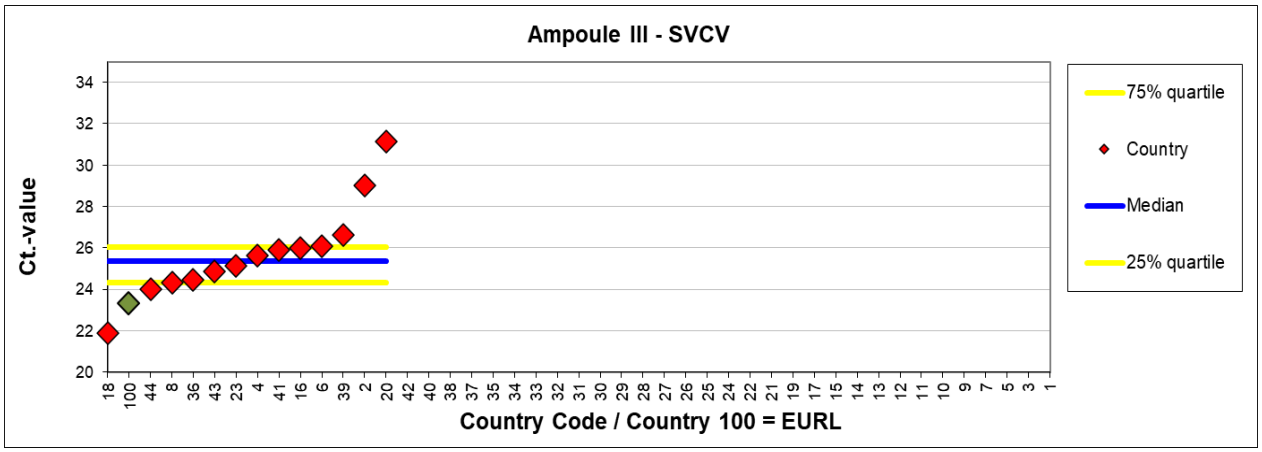


Number of laboratories	33
Median Ct.-value	21.2
Maximum Ct.-value	34.3
Minimum Ct.-value	13.3
25% quartile Ct.-value	20.4
75% quartile Ct.-value	24.1

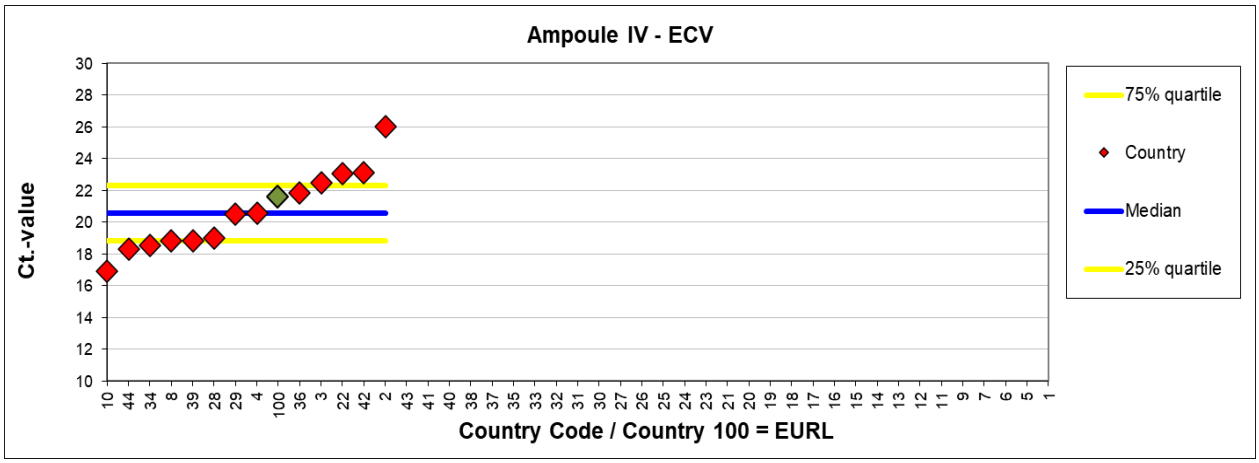
← IHNV IPNV →

Number of laboratories	21
Median Ct.-value	25.5
Maximum Ct.-value	31.4
Minimum Ct.-value	12.5
25% quartile Ct.-value	22.0
75% quartile Ct.-value	27.1

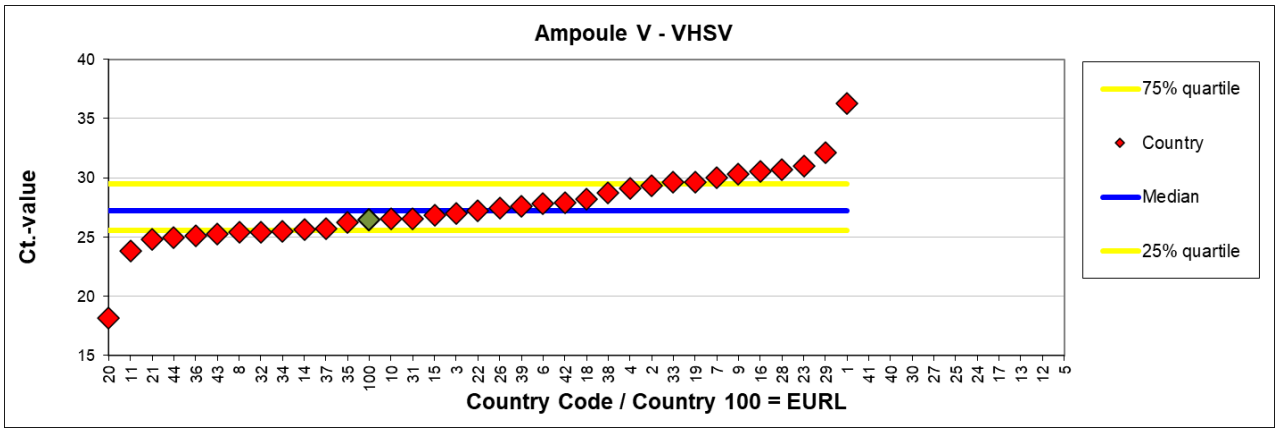




Number of laboratories	14
Median Ct.-value	25.4
Maximum Ct.-value	31.2
Minimum Ct.-value	21.9
25% quartile Ct.-value	24.3
75% quartile Ct.-value	26.0



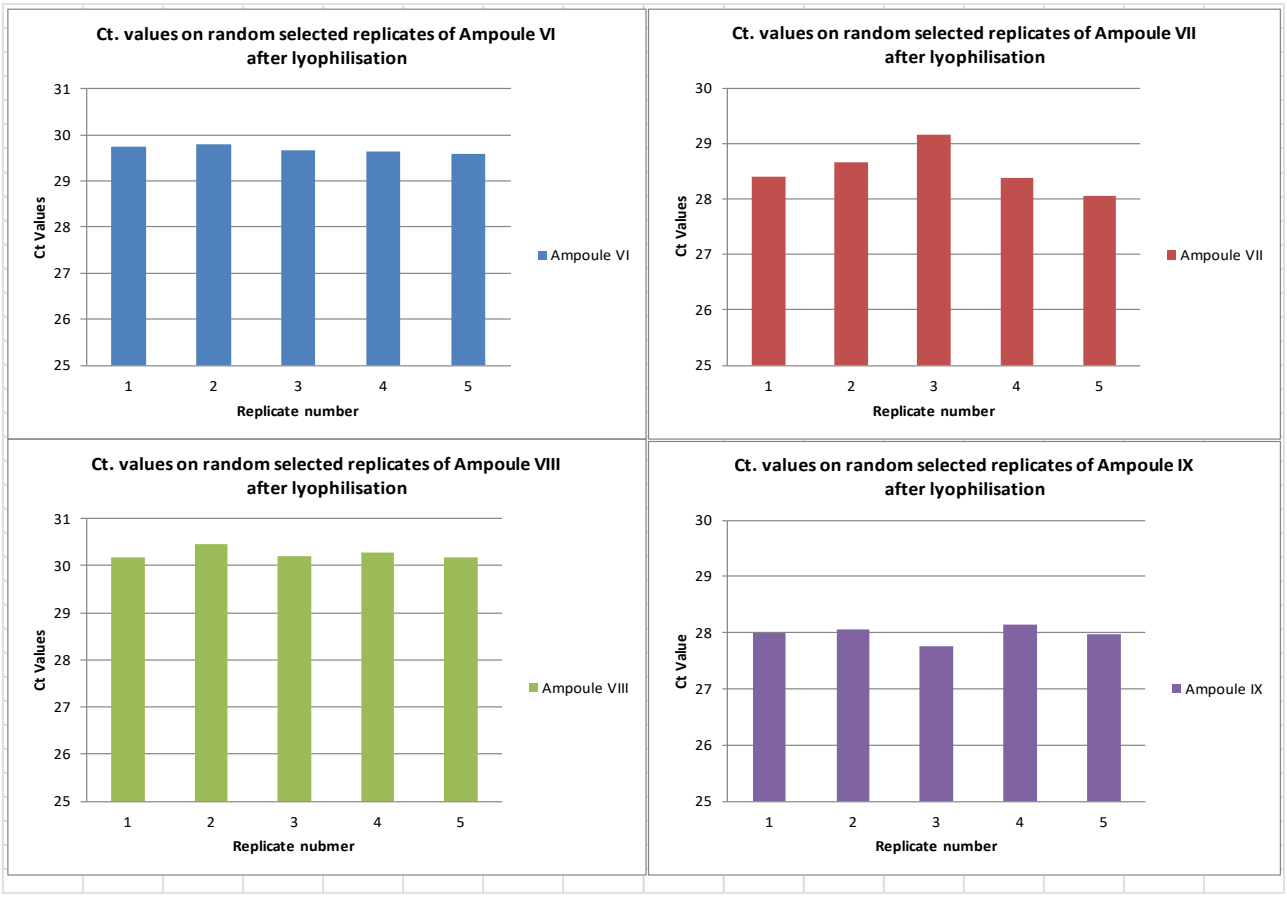
Number of laboratories	14
Median Ct.-value	20.6
Maximum Ct.-value	26.0
Minimum Ct.-value	16.9
25% quartile Ct.-value	18.8
75% quartile Ct.-value	22.3



Number of laboratories	35
Median Ct.-value	27.2
Maximum Ct.-value	36.3
Minimum Ct.-value	18.1
25% quartile Ct.-value	25.5
75% quartile Ct.-value	29.5

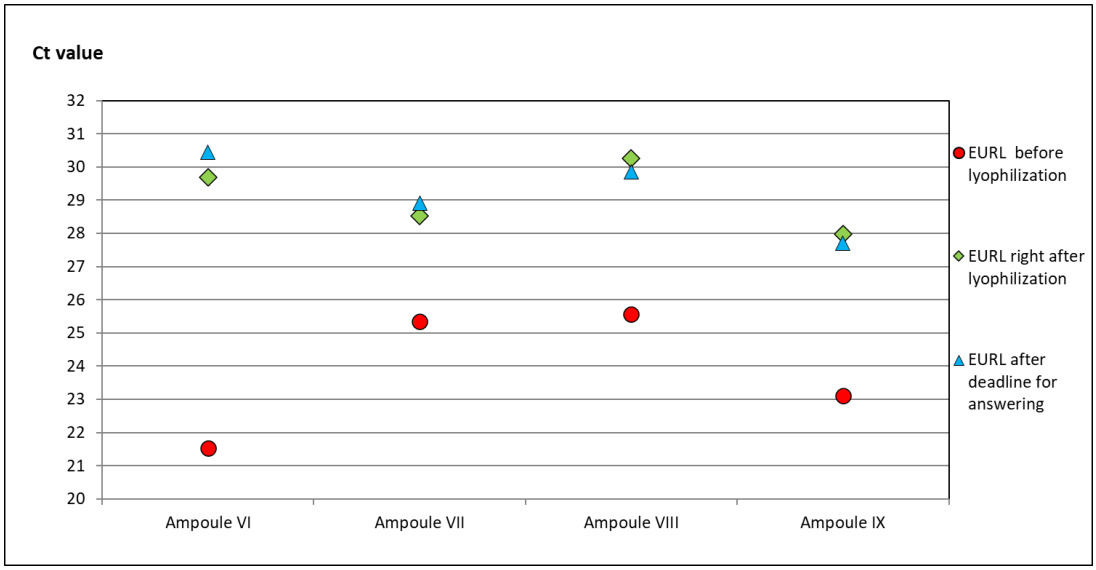
Annex 4: Stability and homogeneity of the content in the ampoules (PT2)

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

The reduction in Ct. values before and after freeze-drying was between 3-8 log. No significant reductions were observed after long term storage.



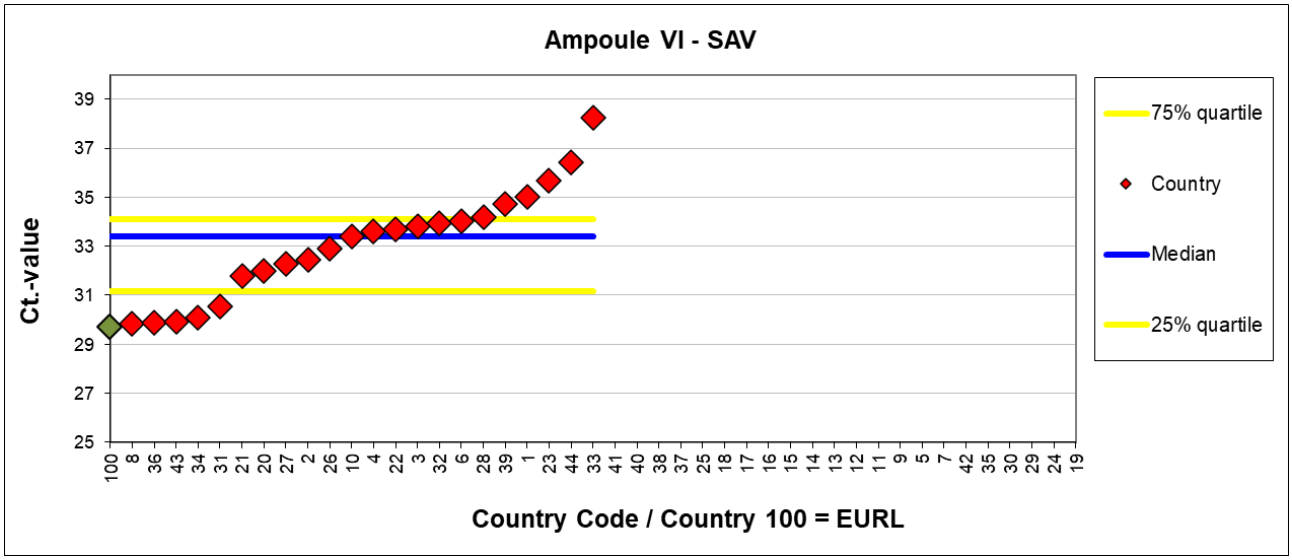
Ampoule VI, VII, VIII and IX tested before and after lyophilisation and after deadline for handling in results.

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

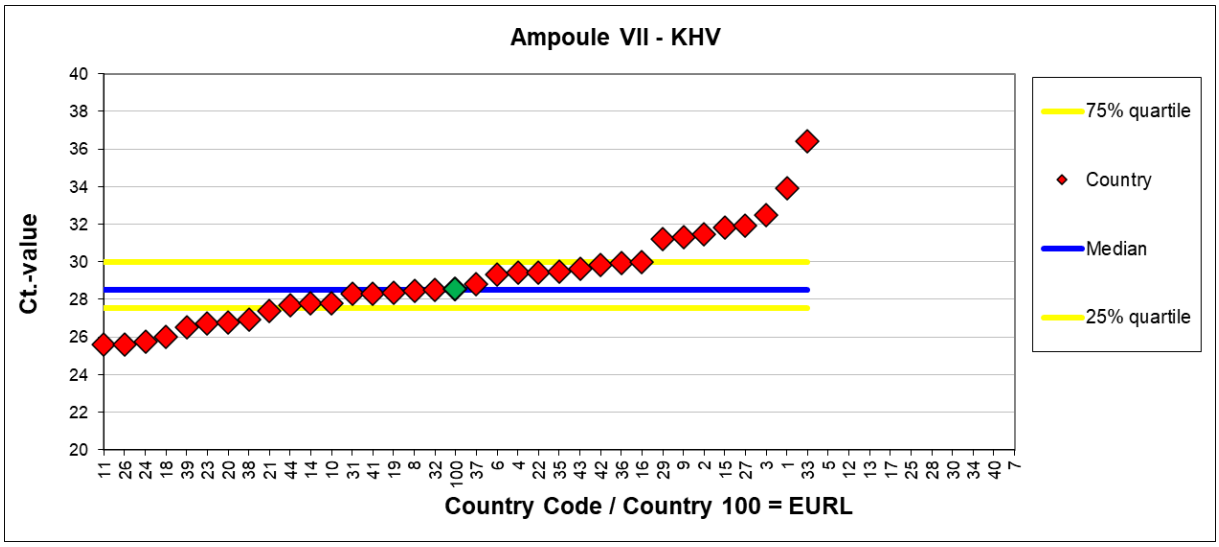
The Ct. values (red diamonds) obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare their Ct. values with the other participating laboratories as well as the Ct. value obtained by the EURL (green diamond and 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.

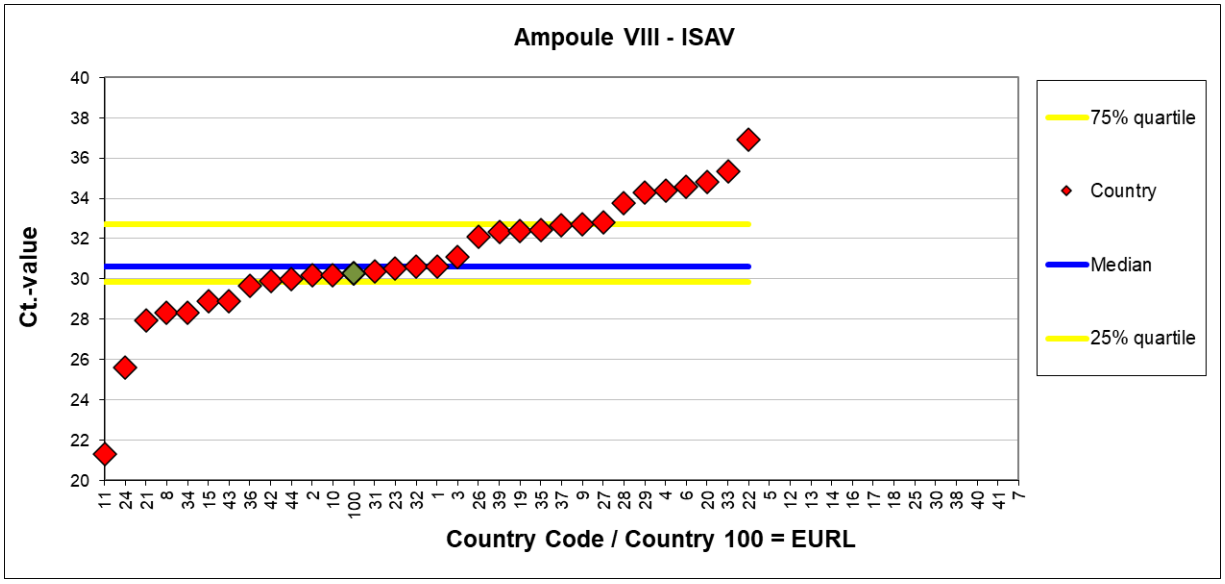
Ct. values obtained in PT2 by participants. No red diamond = No Ct. value given.



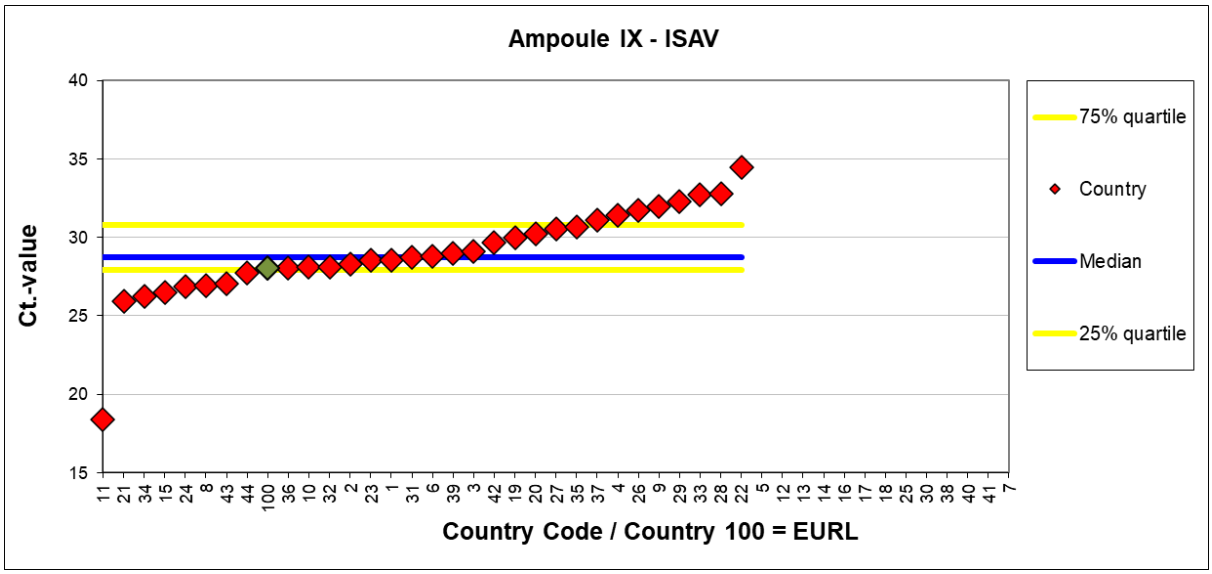
Number of laboratories	23
Median Ct.-value	33.4
Maximum Ct.-value	38.2
Minimum Ct.-value	29.7
25% quartile Ct.-value	31.2
75% quartile Ct.-value	34.1



Number of laboratories	35
Median Ct.-value	28.5
Maximum Ct.-value	36.4
Minimum Ct.-value	25.6
25% quartile Ct.-value	27.5
75% quartile Ct.-value	30.0



Number of laboratories	32
Median Ct.-value	30.6
Maximum Ct.-value	36.9
Minimum Ct.-value	21.3
25% quartile Ct.-value	29.8
75% quartile Ct.-value	32.7



Number of laboratories	32
Median Ct.-value	28.8
Maximum Ct.-value	34.5
Minimum Ct.-value	18.4
25% quartile Ct.-value	27.9
75% quartile Ct.-value	30.8