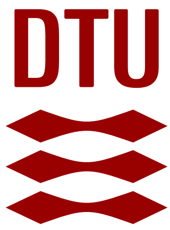

European Union Reference Laboratory for Fish and Crustacean Diseases
NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2025

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
European Union Reference Laboratory for Fish and Crustacean Diseases,
National Institute of Aquatic Resources, Technical University of Denmark,
Kgs. Lyngby, Denmark
Version 2, 11-03-2026 coded report to be provided to all participants

Disclaimer: participants to the ILPT and other users are welcome to use this report or part of it, however it shall be properly quoted as "Klinge T., and Vendramin N., Report of the Inter-Laboratory Proficiency Test 2025 for identification and titration of VHSV, IHNV, EHNV (fish ranaviruses), SVCV and IPNV (PT1) and identification of CyHV-3 (KHV), SAV and ISAV (PT2)"

Table of contents

Major changes from the previous version 02-03-2026 1

Introduction 2

Distribution of the test 5

Shipment and handling 5

Participation 6

Proficiency test 1, PT1 6

Content of ampoules 7

Testing of the PT1 test 10

Virus identification and titration 12

Identification of content 25

Scores 25

Cells applied for solving the test 26

Ct. values comparison 26

Genotyping and sequencing 28

Résumé and concluding remarks PT1 30

Proficiency test 2, PT2 32

Content of ampoules 32

Testing of the PT2 test 34

Pathogen identification 35

Identification of content 37

Scores 37

Ct. values comparison 38

Genotyping and sequencing 39

Concluding remarks PT2 41

References 43

Appendix 44

Annex 1: Stability and homogeneity of the content in the ampoules (PT1) 44

Annex 2: The titres obtained from each participating laboratory represented graphically. 46

Annex 3: The Ct. values graphically (PT1) obtained from each participating laboratory. 51

Annex 4: Stability and homogeneity of the content in the ampoules (PT2) 54

Annex 5: The Ct. values graphically (PT2) obtained from each participating laboratory. 55

Major changes from the previous version 02-03-2026

In paragraph ”scores” at page 25, the ampoule content of ampoule IV and ampoule V were incorrect. These has been updated and paragraph reads “

- Ampoule IV: identification of IHNV was given the score 2.
- Ampoule V: identification of SVCV was given the score 2.
  No identification of SVCV was given the score 0.

Notably, this only a discrepancy in the text, and has no consequences on the scores assigned to the participants

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 46 laboratories participating in PT1, 43 laboratories test for all the viruses included. 43 correctly identified all the pathogens they were investigating for in the designated ampoules.
44 laboratories participate in PT2, 38 laboratories test for all the viruses included also including SAV. 41 laboratories succeeded in identifying all fish viral pathogens they were testing for.
The tests were sent from the EURL between 22nd to 24th of September 2025 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 EHNV (medium titter), VHSV genotype IIIb (medium/high titer), VHSV genotype Ib (high titer), IHNV (medium titer) and SVCV (high 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 **[2]** .

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.

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, ISAV, SAV cell supernatant and one ampoule contained not infected BF-2 cells, 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, 38 of 44 laboratories tested for SAV in 2025.
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 8th, 2025, due to an initial delay of shipment occurred at the courier facility, the deadline for submitting results were extended to December 12th, 2025.

The results of the inter-laboratory proficiency test for listed fish diseases 2025 and plans and idea for future inter-laboratory tests will be presented at the 30th Annual Workshop of the NRLs for Fish Diseases on from May 26th to May 27th, 2026. The meeting will be held online; the EURL team will be hosting the meeting from the premises of DTU Campus in Kgs. Lyngby. Furthermore, a specific online meeting in 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 shipped to 47 participants.
As soon as the shipment process initiated from the courier facility 100% of the packages reached the destination country eight days after dispatch. One participant out of EU received the package after 22 days due to internal delays within the country (Figure 1). One participant never received the package due to customs issues in the recipient country, this package was returned to the EURL, after which the ampoules were reassigned for use in post-deadline confirmatory testing conducted in accordance with the laboratory's established quality assurance and verification procedures.

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.

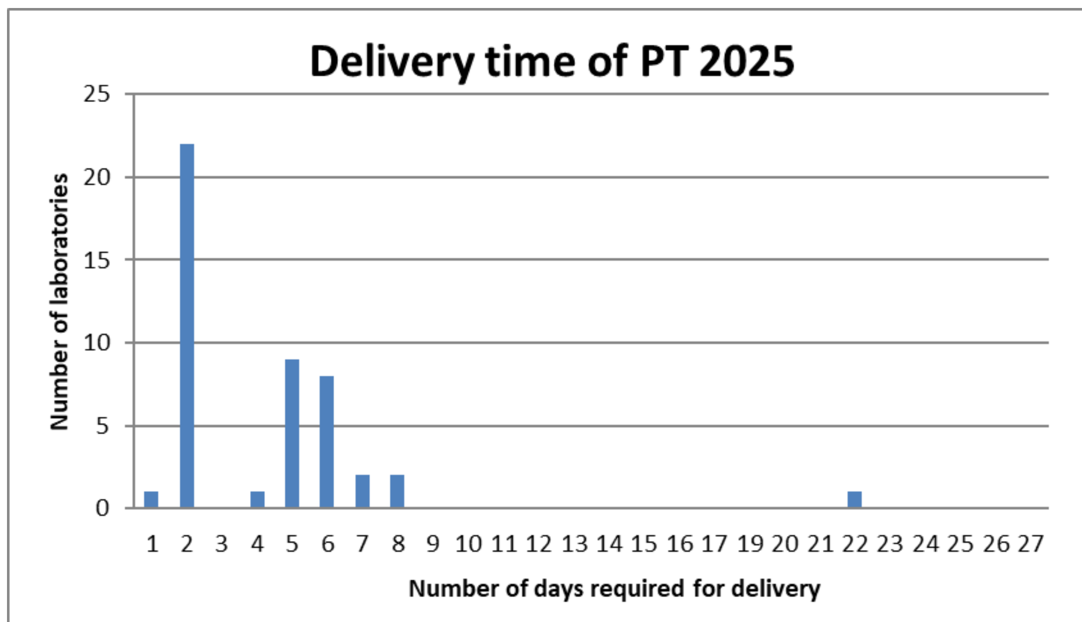

Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 46 laboratories received the annual proficiency test. All 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, Hong Kong - China, 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 2025

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 Isolate 86/8774** Australian freshwater isolate of epizootic haematopoietic necrosis virus from rainbow trout from Adaminaby Trout Farm, NSW obtained in 1986 by Jeremy Langdon. **Received from:** Prof. Whittington, The OIE reference laboratory for EHN, University of Sidney, Australia. **GenBank accession numbers:** FJ433873, AY187045, AF157667 **Reference on isolate:** Langdon JS, Humphrey JD & Williams LM (1988). Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, *Salmo gairdneri* Richardson, in Australia. *Journal of Fish Diseases* 11, 93-96. **References on sequences:** Hyatt AD, Gould AR, Zupanovic Z, Cunningham AA, Hengstberger S, Whittington RJ, Kattenbelt J & Coupar BEH (2000). Comparative studies of piscine and amphibian iridoviruses. *Archives of Virology* 145, 301-331. Jancovich JK, Bremont M, Touchman JW & Jacobs BL (2010). Evidence for multiple recent host species shifts among the ranaviruses (family Iridoviridae). *Journal of Virology* 84, 2636-2647. Marsh IB, Whittington RJ, O'Rourke B, Hyatt AD & Chisholm O (2002) Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Molecular and Cellular Probes* 16, 137-151. 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).

Code	Specifications/References
Ampoule II	**VHSV NO-2007-50-385** VHSV isolate from sea farmed Rainbow trout in Norway **Received from** Norwegian Veterinary Institute **Genotype:** IIIb **Ref on isolate:** Dale OB, Ørpetveit I, Lyngstad TM, Kahns S, Skall HF, Olesen NJ, Dannevig BH (2009) Outbreak of viral haemorrhagic septicaemia (VHS) in seawater-farmed rainbow trout in Norway caused by VHS virus Genotype III. Dis Aquat Org 85:93-103. **GenBank accession numbers:** G gene: EU547740 ; Full genome: MT162436.1 **Reference on sequence:** Ito, T., Kurita, J., Mori, Ki. et al. Virulence of viral haemorrhagic septicaemia virus (VHSV) genotype III in rainbow trout. Vet Res 47, 4 (2016). https://doi.org/10.1186/s13567-015-0303-z
Ampoule III	**VHSV 1p8** Marine isolate (1996) from herring (*Clupea harengus*) caught in the Baltic Sea. (Mortensen et al. 1999). **Genotype** Ib. **GenBank accession numbers**: AY546573 (G-gene) and GQ325430, AY356652 (N-gene) **Reference on isolate:** Mortensen HF, Heuer OE, Lorenzen N, Otte L and Olesen NJ (1999). Isolation of viralhaemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea,Kattegat, Skagerrak and the North Sea. *Virus Research* 63, 97-108. **References on sequences:** Campbell S., Collet B., Einer-Jensen K., Secombes C.J. & Snow M. (2009) Identifying potential virulence determinants in viral haemorrhagic septicaemia virus (VHSV) for rainbow trout. Diseases of Aquatic Organisms 86, 205-212. Einer-Jensen K, Ahrens P, Forsberg R and Lorenzen N (2004). Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal of General Virology* 85, 1167-1179. Snow M, Bain N, Black J, Taupin V, Cunningham CO, King JA, Skall HF and Raynard RS (2004). Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diseases of Aquatic Organisms* 61, 11-21.

<table>
<tr><th>Code</th><th>Specifications/References</th></tr>
<tr><td>Ampoule IV</td><td>IHNV isolate 224-2/ITT18
Received from: Dr. Anna Toffan – IZSVe Italy

Genogroup: E

GenBank accession numbers: MN914165

Reference on isolate:
A. Marsella, A. Buratin, F. Pascoli, M. Abbadi, M. Toson, A. Cuenca, A. Toffan, N. Vendramin, Temperature impact on replication and virulence of European infectious hematopoietic necrosis viruses, Aquaculture, Volume 609, 2025, 742786, ISSN 0044-8486.

References on sequences:
Miriam Abbadi, Michele Gastaldelli, Francesco Pascoli, Gianpiero Zamperin, Alessandra Buratin, Giulia Bedendo, Anna Toffan, Valentina Panzarin, Increased virulence of Italian infectious hematopoietic necrosis virus (IHNV) associated with the emergence of new strains, Virus Evolution, Volume 7, Issue 2, December 2021, veab056</td></tr>
<tr><td>Ampoule V</td><td>SVCV strain 56/70
Spring viraemia of carp virus isolate from carp.

The isolate is most likely identical to the Yugoslavian S30 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: Id3

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.</td></tr>
</table>

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, the stability analysis after deadline was performed on the ampoules returned from the country in which the shipment failed, therefore the analysis was conducted on ampoules that had been subjected to the same storage and transport conditions as those received by the participants.
This year small reductions of the titres after lyophilisation were observed.
In ampoule I and III the reduction of the titre was ≤1 log on BF-2 and RTG-2 cells, and the reduction of the titre was between 1 - 2 log on EPC and FHM. In ampoule II the reduction of the titre was ≤1 log when compared with the titre in the same cell line obtained before shipment. In ampoule IV and V, the reduction of the titre was ≤1 log when compared with the titre in the same cell line obtained before shipment and for ampoule IV on EPC and ampoule V on RTG-2 cells the reduction was between 1-2 log.
No significant reductions (≤1 log) were observed after long term storage (Table 2 and annex 1).

Table 2. Titers of ampoules in PT1:

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	4.0E+06	1.3E+06 *(5,9E+05 → 1,3E+06)*	4.0E+05
	RTG-2	8.6E+05	8.6E+04 *(8,6E+04 → 1,3E+05)*	1.3E+04
	EPC	1.3E+06	8.6E+04 *(5,4E+04 → 1,9E+05)*	8.6E+04
	FHM	8.6E+04	5.9E+03 *(5,9E+03 → 1,4E+04)*	1.9E+03
Ampoule II: VHSV No-2007-50-385	BF-2	1.9E+07	1.9E+07 *(8,6E+06 → 1,9E+07)*	2.7E+06
	RTG-2	1.9E+08	1.9E+07 *(1,3E+07 → 2.7E+07)*	2.7E+06
	EPC	1.9E+08	5.9E+07 *(4,0E+07 → 8,6E+07)*	1.3E+07
	FHM	2.7E+08	4.0E+07 *(1,9E+07 → 8,6E+07)*	5.9E+06
Ampoule III: VHSV 1p8	BF-2	2.7E+07	8.6E+06 *(5,9E+06 → 1,9E+07)*	1.9E+06
	RTG-2	5.9E+02	1.9E+02 *(<1,9E+02 → 1,9E+02)*	< 1,9E+02
	EPC	1.3E+05	5.9E+03 *(2,7E+03 → 5,9E+03)*	5.9E+03
	FHM	1.9E+08	1.3E+07 *(5,9E+06 → 1,9E+07)*	2.7E+06
Ampoule IV: IHNV 224-2/ITT18	BF-2	< 1,9E+02	< 1,9E+02	< 1,9E+02
	RTG-2	2.7E+05	8.6E+04 *(5,9E+04 → 1,3E+05)*	2.7E+04
	EPC	1.3E+06	4.0E+04 *(1,9E+04 → 8,6E+04)*	4.0E+03
	FHM	2.7E+06	4.0E+05 *(1,3E+05 → 4,0E+05)*	5.9E+04
Ampoule V: SVCV 56/70	BF-2	8.6E+07	1.9E+07 *(1,3E+07 → 4,0E+07)*	4.0E+06
	RTG-2	2.7E+07	1.3E+06 *(8,6E+05 → 2,7E+06)*	1.9E+05
	EPC	5.9E+07	1.3E+07 *(8,6E+06 → 2,7E+07)*	5.9E+06
	FHM	1.3E+08	1.9E+07 *(1,9E+07 → 4,0E+07)*	2.7E+06

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 [8], 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). EURL is designated as code 100 in the tables, and the reported value was obtained by titration directly from the ampoules that were resuspended and titrated after the official submission deadline.
The titration results obtained by the participating laboratories and EURL after deadline are summarised in tables 4 to 8.
The titres obtained from each participating laboratory and the EURL are also represented graphically. This year, we have elected to include only titration graphs generated from BF-2 and EPC cells (Annex 2). Titration graphs based on RTG-2 and FHM cells can be provided upon request.

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, 2025 - Virus identification and score obtained by participants.

Laboratory code number	Score	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
		EHNV 86/8774	VHSV NO-2007-50-385	VHSV 1p8	IHNV 224-2/ITT18	SVCV 56/70
1	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
2[2]	8/8	Rhana, EHNV	VHSV	VHSV	IHNV	-
3[1]	7/10	Ranavirus	VHSV	VHSV	IHNV	no virus
4	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
5	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
6	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
7	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
8	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
9	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
10	8/10	Ranavirus -NOT EHNV	VHSV	VHSV	IHNV	SVCV
11	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
12	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
13	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
14	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
15	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
16	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
17[2]	8/8	EHNV	VHSV	VHSV	IHNV	Negative for IHNV, Ranavirus and VHSV
18	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
19	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
20	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
21	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
22	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
23	10/10	EHNV	VHSV	VHSV	IHNV	SVSV
24	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
25	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
26	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
27	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
28	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
29	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
30	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
31	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
32	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
33	10/10	EHN	VHS	VHS	IHN	SVCV

34	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
35	10/10	Ranavirus was identified by conventional PCR and than Sequencing was applied to identified EHN	VHSV	VHSV	IHNV	SVCV
36	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
37	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
38	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
39	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
40	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
41	10/10	EHN	VHSV	VHSV	IHNV	SVC
42	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
43	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
44	10/10	EHNV	VHSV	VHSV	IHNV	SVCV
45[2, 3]	6/6	0	VHSV	VHSV	IHNV	0
46	8/10	EHNV	VHSV	-	IHNV	SVCV

1. Did not corroborate the findings in ampoule I by sequencing or REA.
2. Do not test for SVCV
3. Do not test for Rana

Table 4. Inter-Laboratory Proficiency Test, PT1, 2025 – **Results of titration of ampoule I.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
100	**EHNV**	**4.0E+05**	**1,3E+04**	**8,6E+04**	**1,9E+03**
1	EHNV	4.0E+05	4.0E+04	4.0E+05	N/A
2	Rhana, EHNV	N/A	N/A	N/A	N/A
3	Ranavirus	5.9E+05	1.3E+03	N/A	N/A
4	EHNV	4.0E+03	4.0E+04	8.6E+03	4.0E+04
5	EHNV	8.6E+05	1.3E+05	1.9E+04	4.0E+03
6	EHNV	2.7E+04	1.3E+04	N/A	N/A
7	EHNV	1.9E+06	4.0E+05	N/A	N/A
8	EHNV	5.9E+06	8.6E+04	< 1,9E+02	N/A
9	EHNV	4.0E+04	4.0E+03	1.9E+03	N/A
10	Ranavirus -NOT EHNV	1.9E+05	1.9E+05	N/A	N/A
11	EHNV	N/A	N/A	N/A	N/A
12	EHNV	1.3E+06	4.0E+04	N/A	N/A
13	EHNV	1.9E+06	4.0E+05	N/A	N/A
14	EHNV	5.9E+03	5.9E+02	N/A	N/A
15	EHNV	N/A	N/A	N/A	N/A
16	EHNV	4.0E+04	2.7E+04	1.3E+03	1.3E+03
17	EHNV	N/A	N/A	N/A	N/A
18	EHNV	1.9E+05	1.3E+03	N/A	N/A
19	EHNV	4.0E+03	8.6E+02	5.9E+02	2.7E+03
20	EHNV	5.9E+09	2.7E+09	N/A	N/A
21	EHNV	1.3E+06	5.9E+05	1.3E+06	1.3E+05
22	EHNV	4.0E+04	N/A	N/A	1.9E+03
23	EHNV	1.9E+05	N/A	N/A	4.0E+03
24	EHNV	8.6E+05	1.9E+05	N/A	N/A
25	EHNV	8.6E+04	5.9E+05	N/A	N/A
26	EHNV	4.0E+05	4.0E+05	1.9E+04	N/A
27	EHNV	8.6E+04	2.7E+04	< 1,9E+02	2.7E+03
28	EHNV	2.7E+05	1.3E+05	N/A	N/A
29	EHNV	1.9E+05	2.7E+04	N/A	N/A
30	EHNV	1.9E+03	2.7E+03	N/A	N/A
31	EHNV	1.9E+06	1.3E+06	1.9E+05	1.9E+05
32	EHNV	1.9E+05	2.7E+04	N/A	N/A
33	EHN	5.9E+07	5.9E+07	N/A	N/A
34	EHNV	1.3E+04	1.9E+04	N/A	N/A

35	Ranavirus was identified by conventional PCR and than Sequencing was applied to identified EHN	1.9E+04	4.0E+03	N/A	N/A
36	EHNV	1.9E+06	4.0E+04	2.7E+05	N/A
37	EHNV	1.3E+06	1.9E+05	N/A	1.3E+05
38	EHNV	8.6E+03	2.7E+02	N/A	N/A
39	EHNV	8.6E+04	1.9E+04	N/A	N/A
40	EHNV	1.9E+04	1.3E+03	1.3E+03	1.3E+03
41	EHN	8.6E+05	2.7E+06	N/A	1.9E+05
42	EHNV	4.0E+05	5.9E+04	N/A	N/A
43	EHNV	8.6E+05	4.0E+05	8.6E+04	N/A
44	EHNV	N/A	N/A	N/A	N/A
45	0	N/A	N/A	N/A	N/A
46	EHNV	1.9E+05	2.7E+06	N/A	N/A

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

EHNV 86/8774	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	40	38	14	12
Median titre	1.9E+05	4.0E+04	1.4E+04	4.0E+03
Maximum titre	5.9E+09	2.7E+09	1.3E+06	1.9E+05
Minimum titre	1.9E+03	2.7E+02	<1,9E+02	1.3E+03
25% quartile titre	4.0E+04	1.4E+04	1.3E+03	2.5E+03
75% quartile titre	9.6E+05	4.0E+05	1.6E+05	1.3E+05

Table 5. Inter-Laboratory Proficiency Test, PT1, 2025 – **Results of titration of ampoule II.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	**EPC**	**RTG-2**	**FHM**
100	**VHSV**	**2,7E+06**	**2,7E+06**	**1,3E+07**	**5,9E+06**
1	VHSV	1.9E+06	8.6E+06	5.9E+06	N/A
2	VHSV	N/A	N/A	N/A	N/A
3	VHSV	1.9E+04	2.7E+03	N/A	N/A
4	VHSV	8.6E+04	4.0E+07	1.9E+06	1.9E+07
5	VHSV	2.7E+05	4.0E+07	2.7E+07	8.6E+07
6	VHSV	2.7E+03	1.3E+06	N/A	N/A
7	VHSV	4.0E+05	1.3E+07	N/A	N/A
8	VHSV	2.7E+06	1.3E+07	1.9E+06	N/A
9	VHSV	1.9E+06	5.9E+06	5.9E+06	N/A
10	VHSV	< 1,9E+02	< 1,9E+02	N/A	N/A
11	VHSV	N/A	N/A	N/A	N/A
12	VHSV	1.3E+07	5.9E+07	N/A	N/A
13	VHSV	2.7E+06	1.9E+07	N/A	N/A
14	VHSV	< 1,9E+02	2.7E+03	N/A	N/A
15	VHSV	N/A	N/A	N/A	N/A
16	VHSV	8.6E+05	5.9E+06	1.3E+07	1.9E+07
17	VHSV	N/A	N/A	N/A	N/A
18	VHSV	8.6E+05	1.3E+04	N/A	N/A
19	VHSV	1.3E+03	1.9E+03	8.6E+02	4.0E+02
20	VHSV	5.9E+04	5.9E+09	N/A	N/A
21	VHSV	2.7E+06	8.6E+06	1.9E+06	8.6E+07
22	VHSV	5.9E+05	N/A	N/A	1.9E+05
23	VHSV	1.3E+07	N/A	N/A	4.0E+06
24	VHSV	2.7E+07	8.6E+08	N/A	N/A
25	VHSV	5.9E+03	5.9E+06	N/A	N/A
26	VHSV	2.7E+06	8.6E+06	4.0E+05	N/A
27	VHSV	1.9E+06	5.9E+06	8.6E+06	4.0E+06
28	VHSV	1.9E+06	2.7E+06	N/A	N/A
29	VHSV	2.7E+05	5.9E+06	N/A	N/A
30	VHSV	1.9E+06	1.9E+07	N/A	N/A
31	VHSV	4.0E+02	1.3E+06	1.3E+05	1.9E+06
32	VHSV	1.3E+05	1.9E+06	N/A	N/A
33	VHS	8.6E+05	2.7E+06	N/A	N/A
34	VHSV	1.3E+06	1.9E+07	N/A	N/A

35	VHSV	1.3E+05	8.6E+06	N/A	N/A
36	VHSV	5.9E+04	2.7E+06	8.6E+04	N/A
37	VHSV	1.3E+05	4.0E+06	N/A	8.6E+06
38	VHSV	8.6E+04	1.9E+06	N/A	N/A
39	VHSV	5.87E+06	5.87E+06	N/A	N/A
40	VHSV	4.00E+04	2.73E+05	4.00E+04	2.73E+04
41	VHSV	5.87E+05	1.86E+06	N/A	5.9E+04
42	VHSV	8.6E+06	8.6E+06	N/A	N/A
43	VHSV	4.0E+06	2.7E+07	2.7E+07	N/A
44	VHSV	N/A	N/A	N/A	N/A
45	VHSV	N/A	N/A	N/A	N/A
46	VHSV	8.6E+06	1.3E+07	N/A	N/A

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

VHSV, NO-2007-50-385	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	40	38	14	12
Median titre	7.2E+05	5.9E+06	1.9E+06	4.0E+06
Maximum titre	2.7E+07	5.9E+09	2.7E+07	8.6E+07
Minimum titre	<1,9E+02	<1,9E+02	8.6E+02	4.0E+02
25% quartile titre	7.9E+04	1.9E+06	1.9E+05	1.5E+05
75% quartile titre	2.7E+06	1.3E+07	7.9E+06	1.9E+07

Table 6. Inter-Laboratory Proficiency Test, PT1, 2025 – **Results of titration of ampoule III**

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

35	VHSV	4.0E+04	5.9E+03	N/A	N/A
36	VHSV	5.9E+04	2.7E+05	< 1,9E+02	N/A
37	VHSV	8.6E+03	1.9E+04	N/A	1.9E+04
38	VHSV	4.0E+05	< 1,9E+02	N/A	N/A
39	VHSV	8.62E+05	< 1,9E+02	N/A	N/A
40	VHSV	1.86E+04	5.87E+04	< 1,9E+02	< 1,9E+02
41	VHSV	4.00E+04	1.86E+05	N/A	4.00E+04
42	VHSV	8.62E+05	5.87E+03	N/A	N/A
43	VHSV	4.0E+06	1.9E+05	< 1,9E+02	N/A
44	VHSV	N/A	N/A	N/A	N/A
45	VHSV	N/A	N/A	N/A	N/A
46	-	< 1,9E+02	< 1,9E+02	N/A	N/A

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

VHSV, 1p8	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	40	38	14	12
Median titre	2.7E+05	5.9E+03	<1,9E+02	2.9E+04
Maximum titre	4.0E+06	2.7E+07	1.3E+06	2.7E+07
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	3.7E+04	8.6E+02	<1,9E+02	2.1E+03
75% quartile titre	8.6E+05	2.5E+04	1.3E+03	1.9E+05

Table 7. Inter-Laboratory Proficiency Test, PT1, 2025 – **Results of titration of ampoule IV.**

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
100	**IHNV**	**<1,9E+02**	**2,7E+04**	**4,0E+03**	**5,9E+04**
1	IHNV	4.0E+03	4.0E+04	1.9E+04	N/A
2	IHNV	N/A	N/A	N/A	N/A
3	IHNV	2.7E+03	5.9E+04	N/A	N/A
4	IHNV	8.6E+04	5.9E+04	8.6E+03	2.7E+05
5	IHNV	< 1,9E+02	2.7E+05	5.9E+05	5.9E+05
6	IHNV	8.6E+02	8.6E+03	N/A	N/A
7	IHNV	8.6E+02	5.9E+05	N/A	N/A
8	IHNV	2.7E+03	4.0E+02	4.0E+04	N/A
9	IHNV	1.9E+03	1.9E+04	4.0E+04	N/A
10	IHNV	< 1,9E+02	< 1,9E+02	N/A	N/A
11	IHNV	N/A	N/A	N/A	N/A
12	IHNV	8.6E+02	1.9E+04	N/A	N/A
13	IHNV	2.7E+03	4.0E+03	N/A	N/A
14	IHNV	< 1,9E+02	1.9E+04	N/A	N/A
15	IHNV	N/A	N/A	N/A	N/A
16	IHNV	2.7E+03	2.7E+03	1.3E+04	5.9E+03
17	IHNV	N/A	N/A	N/A	N/A
18	IHNV	2.7E+04	< 1,9E+02	N/A	N/A
19	IHNV	8.6E+02	8.6E+02	5.9E+02	8.6E+02
20	IHNV	1.9E+04	1.3E+08	N/A	N/A
21	IHNV	2.7E+03	4.0E+04	5.9E+02	8.6E+04
22	IHNV	< 1,9E+02	N/A	N/A	1.9E+03
23	IHNV	< 1,9E+02	N/A	N/A	1.9E+05
24	IHNV	1.3E+03	1.9E+05	N/A	N/A
25	IHNV	< 1,9E+02	8.6E+04	N/A	N/A
26	IHNV	1.3E+04	1.9E+04	2.7E+04	N/A
27	IHNV	< 1,9E+02	1.9E+04	1.9E+04	4.0E+03
28	IHNV	5.9E+02	1.3E+04	N/A	N/A
29	IHNV	1.3E+03	4.0E+04	N/A	N/A
30	IHNV	< 1,9E+02	5.9E+03	N/A	N/A
31	IHNV	< 1,9E+02	8.6E+03	1.9E+04	2.7E+03
32	IHNV	1.3E+03	2.7E+03	N/A	N/A
33	IHN	1.9E+06	1.3E+06	N/A	N/A
34	IHNV	< 1,9E+02	8.6E+04	N/A	N/A

35	IHNV	< 1,9E+02	5.9E+03	N/A	N/A
36	IHNV	1.3E+03	1.9E+04	4.0E+02	N/A
37	IHNV	8.6E+02	1.9E+03	N/A	< 1,9E+02
38	IHNV	1.3E+03	1.9E+02	N/A	N/A
39	IHNV	1.3E+03	8.62E+03	N/A	N/A
40	IHNV	1.3E+03	2.73E+03	2.7E+03	< 1,9E+02
41	IHNV	1.86E+04	4.00E+04	N/A	1.3E+04
42	IHNV	4.0E+04	2.7E+04	N/A	N/A
43	IHNV	< 1,9E+02	8.6E+04	1.3E+05	N/A
44	IHNV	N/A	N/A	N/A	N/A
45	IHNV	N/A	N/A	N/A	N/A
46	IHNV	8.6E+04	5.9E+04	N/A	N/A

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

IHNV, 224-2/ITT18	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	40	38	14	12
Median titre	1.3E+03	1.9E+04	1.9E+04	4.9E+03
Maximum titre	1.9E+06	1.3E+08	5.9E+05	5.9E+05
Minimum titre	<1,9E+02	<1,9E+02	4.0E+02	<1,9E+02
25% quartile titre	<1,9E+02	4.5E+03	4.2E+03	1.6E+03
75% quartile titre	2.7E+03	5.9E+04	3.7E+04	1.1E+05

Table 8. Inter-Laboratory Proficiency Test, PT1, 2025 – Results of titration of **ampoule V**.

Laboratory Code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
100	SVCV	4,0E+06	1,9E+05	5,9E+06	2,7E+06
1	SVCV	4.0E+05	5.9E+06	1.9E+06	N/A
2	-	N/A	N/A	N/A	N/A
3	no virus	< 1,9E+02	< 1,9E+02	N/A	N/A
4	SVCV	2.7E+04	1.9E+06	4.0E+05	2.7E+06
5	SVCV	2.7E+06	8.6E+06	1.9E+06	1.3E+07
6	SVCV	8.6E+03	4.0E+05	N/A	N/A
7	SVCV	2.7E+06	1.3E+07	N/A	N/A
8	SVCV	4.0E+06	5.9E+06	8.6E+02	N/A
9	SVCV	5.9E+05	2.7E+06	< 1,9E+02	N/A
10	SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
11	SVCV	N/A	N/A	N/A	N/A
12	SVCV	1.3E+07	8.6E+06	N/A	N/A
13	SVCV	5.9E+06	5.9E+06	N/A	N/A
14	SVCV	< 1,9E+02	1.3E+04	N/A	N/A
15	SVCV	N/A	N/A	N/A	N/A
16	SVCV	1.3E+07	1.3E+06	2.7E+06	2.7E+06
17	Negative for IHNV, Ranavirus and VHSV	N/A	N/A	N/A	N/A
18	SVCV	1.3E+06	1.3E+03	N/A	N/A
19	SVCV	5.9E+02	1.3E+03	5.9E+02	8.6E+02
20	SVCV	1.9E+04	5.9E+08	N/A	N/A
21	SVCV	1.3E+06	5.9E+06	4.0E+06	4.0E+06
22	SVCV	8.6E+04	N/A	N/A	8.6E+04
23	SVSV	8.6E+06	N/A	N/A	4.0E+06
24	SVCV	1.3E+06	1.3E+07	N/A	N/A
25	SVCV	< 1,9E+02	5.9E+06	N/A	N/A
26	SVCV	8.6E+06	1.3E+06	5.9E+04	N/A
27	SVCV	2.7E+06	5.9E+06	2.7E+06	5.9E+06
28	SVCV	1.9E+07	1.3E+06	N/A	N/A
29	SVCV	1.9E+04	2.7E+05	N/A	N/A
30	SVCV	8.6E+06	1.3E+07	N/A	N/A
31	SVCV	2.7E+06	2.7E+06	1.3E+06	1.9E+06
32	SVCV	2.7E+06	2.7E+06	N/A	N/A
33	SVCV	1.3E+06	2.7E+06	N/A	N/A
34	SVCV	1.3E+05	1.9E+07	N/A	N/A

35	SVCV	1.3E+03	5.9E+05	N/A	N/A
36	SVCV	1.3E+06	1.3E+06	1.9E+04	N/A
37	SVCV	5.9E+04	4.0E+06	N/A	1.9E+05
38	SVCV	1.9E+05	2.7E+06	N/A	N/A
39	SVCV	1.9E+05	8.6E+05	N/A	N/A
40	SVCV	2.7E+04	2.7E+04	4.0E+04	4.0E+04
41	SVC	5.9E+05	4.0E+07	N/A	1.9E+06
42	SVCV	1.3E+06	2.7E+06	N/A	N/A
43	SVCV	2.7E+06	4.0E+06	< 1,9E+02	N/A
44	SVCV	N/A	N/A	N/A	N/A
45	0	N/A	N/A	N/A	N/A
46	SVCV	1.3E+06	4.0E+06	N/A	N/A

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	35	33	12	10
Median titre	1.3E+06	2.7E+06	8.3E+05	2.7E+06
Maximum titre	1.9E+07	5.9E+08	4.0E+06	1.3E+07
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	8.6E+02
25% quartile titre	2.3E+04	8.6E+05	1.4E+04	6.0E+05
75% quartile titre	2.7E+06	5.9E+06	2.1E+06	4.0E+06

Identification of content

- 43 laboratories out of 46 participants analysed for all viruses; 41 of these laboratories correctly identified all viruses in all ampoules.
- Three laboratories did not test for SVCV and one laboratory did not test for Rana.

Ampoule I – EHNV (86/8774)

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

Ampoule II – VHSV (No-2007-50-385)

- All 46 laboratories correctly identified the isolate as VHSV in ampoule II.

Ampoule III – VHSV (1p8)

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

Ampoule IV – IHNV (224-2/ITT18)

- All 46 laboratories correctly identified the isolate as IHNV in ampoule IV.

Ampoule V – SVCV (56/70)

- 42 laboratories correctly identified the isolate as SVCV in ampoule V.
- 1 laboratory did not find the present virus in the ampoule.
- 3 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**: identification of EHNV by sequencing was given the score 2.
  No identification of the Ranavirus by sequencing was given the score 1.
  Answering Not EHNV even though sequencing shows EHNV was given the score 0.
- **Ampoule II**: identification of VHSV was given the score 2.
- **Ampoule III**: identification of VHSV was given the score 2.
  No identification of VHSV was given the score 0.
- **Ampoule IV**: identification of IHNV was given the score 2.
- **Ampoule V**: identification of SVCV was given the score 2.
  No identification of SVCV 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 46 laboratories participating in the PT 1 2025, 43 obtained a score on 100%.
The score 10/10 was assigned to 40 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.

3 laboratories scored below 100% due to:

- not finding the present virus
- 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:

- 43 laboratories used BF-2 cells
- 41 laboratories used EPC cells
- 30 laboratories used RTG-2 cells
- 32 laboratories used FHM cells
- 7 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 [8] 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 the obtained titres between laboratories was quite high with few exceptions the differences from the lowest to the most sensitive performances was between 5 to 8 log. Participants whose results are higher or lower than 1 log from the median should look carefully into their results and procedures . The participants obtaining a titer more than 1 log below the median should consider exchanging their cell lines with more sensitive ones or assess if the performance of their cells could be improved, and those above the median for more than 1 log 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 and the available data indicate that all participants adhered to this instruction.

Table 9. Inter-Laboratory Proficiency Test, PT1, 2025 – Ct.-values. No Ct-value given by the participating laboratory: **-**

Laboratory Code number	Ct. value Ampoule II (EHNV)	Ct. value Ampoule II (VHSV)	Ct. value Ampoule III (VHSV)	Ct. value Ampoule IV (IHNV)	Ct. value Ampoule V (SVCV)
100	21.73	16.63	19.19	21.16	18.34
1	22.22	17.53	21.07	23.84	20.41
2	22.91	18.76	21.72	23.36	-
3	-	-	-	-	-
4	-	18.87	20.97	22.42	-
5	21.10	16.30	18.80	20.50	17.70
6	-	18.80	21.58	21.21	-
7	-	23.24	25.20	25.39	-
8	-	18.68	21.54	20.71	-
9	-	18.61	21.37	24.79	-
10	-	-	-	-	-
11	22.74	20.03	23.21	25.44	-
12	-	-	-	-	-
13	-	23.29	26.16	25.11	-
14	-	29.41	18.93	22.08	-
15	21.62	18.79	21.71	23.64	19.25
16	-	21.26	22.99	19.94	16.36
17	-	18.34	20.80	19.89	-
18	-	-	-	-	-
19	-	19.60	23.10	24.00	-
20	-	18.65	20.68	21.40	-
21	-	16.10	18.20	16.70	-
22	19.52	19.91	22.54	21.64	-
23	19.42	20.92	20.96	19.91	18.77
24	-	-	-	-	-
25	21.00	22.40	25.20	26.90	-
26	18.29	16.90	19.81	20.32	17.93
27	17.73	20.08	20.51	18.72	21.88
28	-	20.16	22.45	26.97	-
29	20.72	19.44	22.33	17.09	-
30	19.06	18.40	21.42	21.93	-
31	-	-	-	-	-
32	-	17.93	21.72	24.14	-
33	-	23.00	22.00	20.00	-
34	-	22.02	25.02	25.19	-
35		18.49	21.98	24.76	18.40
36	21.90	20.50	23.30	25.80	-
37	-	27.57	30.73	-	-
38	25.97	23.56	21.29	22.46	-
39	-	-	-	-	21.90
40	-	-	-	-	-
41	-	-	-	-	-
42	20.43	27.90	23.00	19.21	19.90
43	22.96	21.33	23.89	30.79	20.92
44	-	16.50	19.40	19.10	17.20
45	Do not test	25.07	28.63	30.12	Do not test
46	20.85	-	-	-	19.43

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. 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, 2025 - Genotyping

Laboratory Code number	Ampoule II	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
	EHNV 86/8774	VHSV NO-2007-50-385 Genotype IIIb	VHSV 1p8 Genotype Ib	IHNV 224-2/ITT18 Genotype E	SVCV 56/70 Genotype Id3
1	EHNV	III	Ib	E	1d
2[2]	N/A	N/A	N/A	N/A	N/A
3[1]	0	0	0	0	0
4	EHNV	III	Ib	E	Genogroup 1d
5	EHNV	III	I b	E	Genogroup 1d
6	EHNV	0	0	0	0
7	EHNV	III	Ib	E	0
8	EHNV	Genotype III	Genotype Ib	Genogroup E	Genogroup Id
9	EHNV	III	Ib	E	Id
10	0	0	0	0	0
11	EHNV	III	Ib	E	Id
12	EHNV	III	Ie	E	1d
13	EHNV	III	Ib	E	Id
14	EHNV	III	1b	E	1d
15	0	0	0	0	0
16	EHNV	Genotype III	Genotype Ib	Genogroup E	0
17[2]	EHNV	III	Ib	E	0
18	EHNV	III	I(b)	E	Genogroup 1(d)
19	EHNV	III	Ib	E	Id
20	EHNV	Genotype III	Genotype Ib	Genotype E	Genotype Id
21	EHNV	Genotype III	Genotype Ib	Genotype E	Genogroup Id
22	EHNV	III	Ib	E	0
23	EHNV	0	0	0	0
24	EHNV	III	Ib	E	1d

25	EHNV	III	Ib	E	1d
26	EHNV	III	Ib	E	1d
27	EHNV	III	Ib	E	Id
28	EHNV	Genotype III	Genotype Ib	Genotype E	Genogroup 1d
29	0	Genotype III	Genotype Ib	Genogroup E, non A	0
30	EHNV	III	Ib	E	1d
31	Epizootic haematopoietic necrosis virus	Genotype III	Genotype Ib	Group A in Genogroup M, E	Genotype Id
32	EHNV	III	Ib	E	0
33	EHN	Ib	III	E	/
34	EHNV	III	I (b)	E	Genogroup 1 (d)
35	0	IIIb	Ib	E	0
36	EHNV	III	Ib	E	Id
37	0	Genotype III	Genotype Ib	Genotype E	Genogroup Id
38	EHNV	Genotype III	Genotype Ib	Genotype E	Genogroup 1d
39	EHNV	0	0	0	0
40	EHNV	Genotype Ⅲa	Genotype Ib	Genogroup E	Genogroup Id
41	EHNV	Ⅲ	Ⅰb	E	Ⅰd
42	EHNV	Genogroup III	Genogroup Ib	Genoproup E	Genogroup Id
43	0	Genotype III	Genotype Ib	Genogroup E	0
44	EHNV	III	I-b	E	0
45[2, 3]	0	0	0	0	0
46	0	0	0	0	0

[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 Ranavirus

93% of the participating laboratories obtained 100% success rate in PT1.

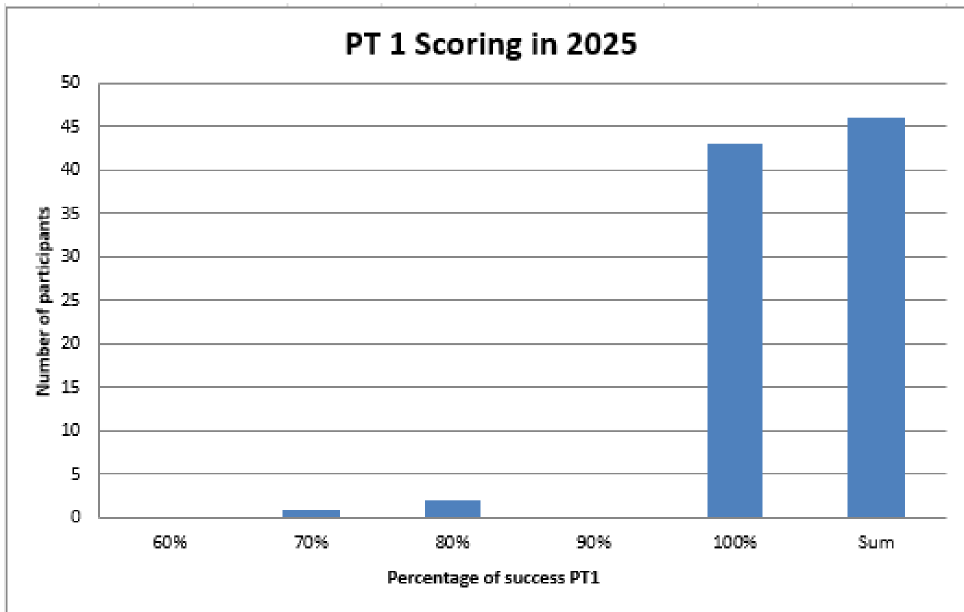

Figure 3 Success-rate of participating laboratories 2025 for PT1

Résumé and concluding remarks PT1

100% of the packages reached the destination country eight days after dispatch. One participant received the package after 22 days due to internal delays and one country never received the parcels due to customs issues.
Overall, 43 out of 46 participants scored 100% success rate. Out of the 3 laboratories which underperformed one participant scored <100% for the sole reason that they did not back up their concluding results of ampoule I (EHNV) with sequencing. One laboratory incorrectly answered NOT EHNV in 'Concluding Result' on ampoule I. Two laboratories did not identify the virus included 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. The variations in titres between laboratories was quite high, up to 7 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 March.

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 in ampoule II and III, and 35 have correctly genotyped the isolate in ampoule II as Genotype III and 35 have correctly genotyped the isolate in ampoule III as Genotype Ib. 37 laboratories have sequenced IHNV in ampoule IV, and all 37 have correctly genotyped the IHNV as Genogroup E.

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 30th Annual Workshop of National Reference Laboratories for Fish Diseases to be held May 26th to May 27th, 2026.

Proficiency test 2, PT2

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

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) [9], 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) [11] .
Each viral stock was further identified by PCR and sequencing. For KHV according to the method described by Bercovier et *al*. (2005) [12] , for SAV according to the conventional PCR targeting segment E2 described by Fringuelli et al. (2008) [13] and for ISAV with conventional RT-PCR protocol described by Mjaaland et *al*. (2002) [14].
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 isolate 1287** Koi Herpesvirus **Received from**: Dr. Kei Yuasa, National Research Institute of Aquaculture, Japan Isolate from Common Carp (Cyprinus Carpio), from river in the Okayama region, Japan in 2012.
Ampoule VII:	**ISAV 2018-50-542_Vir369** Infectious Salmon Anaemia Virus. ISAV HPRΔ isolate from Atlantic salmon in Norway. Kviksholmen in 2018. **Received from** Norwegian Veterinary Institute. **Genbank accession number MK216321**
Ampoule VIII:	**SAV6** Salmonid alpha virus 6, Pancreas Disease Virus (PD) Ireland F104596 **GenBank accession numbers**: EF675499 (nsp3 gene); EF675547 (E2 gene) **Reference on isolate:** Phylogenetic analyses and molecular epidemiology of European salmonid alphaviruses (SAV) based on partial E2and nsP3 gene nucleotide sequences. E Fringuelli, H M Rowley, J C Wilson, R Hunter, H Rodger and D A Graham Journal of Fish Diseases 2008, 31, 811–823 doi:10.1111/j.1365-2761.2008. 00944.x
Ampoule IX:	**Blank** Uninfected BF-2 cell supernatant US grade.

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))[9] for KHV, by real-time RT-PCR (Snow et *al*. (2006)) [11]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, but this year, the stability analysis after deadline was performed on the ampoules returned from the country in which the shipment failed to clear customs hereby the analysis was conducted on ampoules that had been subjected to the same storage and transport conditions as those received by the participants. (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	22.69	27.53	27.25
		b		27.55	
		c		27.76	
		d		27.55	
		e		27.73	
			22.69	**27.62**	**27.25**
Ampoule VII	**ISAV**	a	20.1	25.05	25.35
		b		25.15	
		c		25.16	
		d		25.13	
		e		25.01	
			20.10	**25.10**	**25.35**
Ampoule VIII	**SAV**	a	22.14	26.55	27.85
		b		26.71	
		c		26.70	
		d		26.76	
		e		26.85	
			22.14	**26.71**	**27.85**
Ampoule IX	**Blank**	a	No Ct	No Ct	No Ct
		b		No Ct	
		c		No Ct	
		d		No Ct	
		e		No Ct	
			No Ct.	**No Ct.**	**No Ct.**

The lyophilisation procedure caused a significant virus reduction in all the ampoules I to VIII (5 Ct. values).
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 [8]. 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.

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

Table 13. Inter-Laboratory Proficiency Test, PT2, 2025 - Virus identification.

Laboratory code number	Score	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
		KHV	ISAV	SAV	Blank
1	8/8	KHV	HPR-deleted ISAV	SAV	-
2[1]	6/6	KHV	HPR-deleted ISAV	-	-
3[2]	7/8	KHV	ISAV	SAV	no virus
4	8/8	KHV	HPR-deleted ISAV	SAV	negative
5	8/8	KHV	HPR-deleted ISAV	SAV	None of the following viruses detected: ISAV, KHV or SAV
6[1]	6/6	KHV	HPR-deleted ISAV	no KHV no ISA	no KHV no ISA
7	8/8	KHV	HPR-deleted ISAV	SAV	No virus
8	8/8	KHV	HPR-deleted ISAV	SAV	0
9	8/8	KHV	ISAV	SAV	negativ
10[1]	6/6	KHV	HPR-deleted ISAV	NO KHV, NO ISAV	NO KHV, NO ISAV
11[1]	6/6	KHV	ISAV	0	0
12	8/8	KHV	HPR-DELETED ISAV	SAV	Blank
13	8/8	KHV	HPR-deleted ISAV	SAV6	NEG
14[1]	6/6	KHV	ISAV	0	0
15	8/8	KHV	ISAV	SAV	NEGATIVE

16	6/8	KHV	HPR0 ISAV	SAV	-
17[1]	6/6	KHV	ISAV	Negative for ISAV and KHV	Negative for ISAV and KHV
18	8/8	KHV	HPR-deleted ISAV	SAV	Negative
19	6/8	KHV	HPR0 ISAV	SAV	0
20	8/8	KHV	ISAV	SAV	Negative/Not detected
21	8/8	KHV	HPR-deleted ISAV	SAV	Negative
22	8/8	KHV	HPR-deleted ISAV	SAV	Negative
23	8/8	KHV	ISAV	SAV	negative
24	8/8	KHV	HPR-deleted ISAV	SAV	NEGATIVE
25	8/8	KHV	HPR-deleted ISAV	SAV	-
26	8/8	KHV	HPR-deleted ISAV	SAV	N/A
27	8/8	KHV	HPR-deleted ISAV	SAV	Neg
28	8/8	KHV	ISAV	SAV	Not KHV, Not ISAV, Not SAV
29	8/8	KHV	ISAV	SAV	No virus detected
30	8/8	KHV	HPR-deleted ISAV	SAV	Not KHV, Not ISAV, Not SAV
31	8/8	KHV	HPR-deleted ISAV	SAV	0
32	8/8	KHV	HPR-deleted ISAV	SAV	No virus detected
33	8/8	KHV	ISA	SAV	-
34	8/8	KHV	HRP-deleted ISAV	SAV	No ISAV, no KHV, no SAV, no VHSV, no IHNV, no EHNV, no Ranavirus, no IPNV, no SVCV, no PRV
35	8/8	KHV	HPR Deleted ISAV	SAV	-
36	8/8	KHV (CyHV3)	HPR-deleted ISAV	SAV	not ISAV, KHV, or SAV
37	8/8	KHV	HPR-deleted ISAV	SAV	Blank
38	8/8	KHV	HPR-deleted ISAV	SAV	Virus not detected by PCR
39	8/8	KHV	HPR-deleted ISAV	SAV	BLANK/NEGATIVE
40	8/8	KHV	HPR-deleted ISAV	SAV	Negative
41	8/8	KHV	ISA	SAV	0
42	8/8	KHV CyHV3	HPR-deleted ISAV	SAV6	NEGATIVE/BLANK
43	8/8	KHV	HPR-deleted ISAV	SAV	No virus detected
44	8/8	KHV	HPR-deleted ISAV	SAV	Not KHV, Not SAV, Not ISAV
45[3]		0	0	0	0
46[3]		0	0	0	0

1) Did not test for SAV
2) Did not sequence ISAV
3) Did not participate in PT2

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

Identification of content

- 44 laboratories submitted results.
- 38 laboratories correctly identified all four ampoules (KHV, ISAV, SAV, No virus)
- All 44 laboratories tested for the two listed pathogens (KHV, ISAV)
- 38 laboratories tested for SAV.

Ampoule VI – KHV

- All 44 laboratories correctly identified KHV.

Ampoule VIII – ISAV

- 44 laboratories correctly identified ISAV but hereof two laboratories uncorrectly answered ISAV HPR0 due to wrong interpretation of correct sequence and one laboratory did not sequence.

Ampoule VIII – SAV

- All 38 laboratories testing for SAV correctly identified SAV.
- 6 laboratories did not participate for SAV and answered ‘0’, ‘Negativ for ISAV and KHV’ or ‘no ISAV; no KHV’

Ampoule IX – Blank

- All 44 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 (N/A).

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 VII, 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 44 laboratories submitting results 41 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**: KHV identification was given the score 2.
- **Ampoule VII**: 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.
  Wrong identification of HPR type on ISAV was given the score 0.
- **Ampoule VIII**: SAV identification was given the score 2 if testing for SAV.
- **Ampoule IX:** finding no pathogen was given the score 2.

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 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, 2025 – Ct.-values.

Laboratory Code number	Ct. value Ampoule VI (KHV)	Ct. value Ampoule VII (ISAV)	Ct. value Ampoule VIII (SAV)	Ct. value Ampoule IX (No virus)
100	**27.62**	**25.10**	**26.71**	**No Ct.**
1	27.57	26.08	30.19	No Ct.
2	28.31	25.13	-	No Ct.
3	-	33.98	34.09	No Ct.
4	24.52	27.66	-	No Ct.
5	26.90	25.10	26.90	No Ct.
6	24.25	28.27	-	No Ct.
7	32.29	29.24	32.28	No Ct.
8	26.72	25.17	29.18	No Ct.
9	25.58	25.07	28.16	No Ct.
10	-	-	-	No Ct.
11	31.32	27.90	-	No Ct.
12	29.81	29.13	29.73	No Ct.
13	21.87	24.15	31.95	No Ct.
14	24.52	23.63	-	No Ct.
15	26.69	28.17	30.87	No Ct.
16	27.71	31.02	32.29	No Ct.
17	25.99	23.42	-	No Ct.
18	-	-	-	
19	29.30	24.30	-	No Ct.
20	24.96	-	-	No Ct.
21	22.70	16.80	-	No Ct.
22	26.08	25.46	-	No Ct.
23	26.18	24.49	26.31	No Ct.
24	29.88	29.04	36.96	No Ct.
25	30.30	29.60	32.50	No Ct.
26	25.89	25.39	33.46	No Ct.
27	24.50	26.56	30.36	No Ct.
28	26.50	-	-	No Ct.
29	-	25.40	29.46	No Ct.
30	-	28.50	33.60	No Ct.
31	-	-	-	-
32	25.17	25.63	28.31	No Ct.
33	26.00	-	-	No Ct.
34	26.10	28.42	-	No Ct.
35	27.18	26.19	27.90	No Ct.

36	28.40	26.70	30.10	No Ct.
37	34.25	30.74	35.00	No Ct.
38	28.54	31.89*	33.49	No Ct.
39	26.57	-	-	No Ct.
40	-	-	-	-
41	-	-	-	-
42	27.15	28.50	31.19	No Ct.
43	29.76	27.46	35.64	No Ct.
44	25.10	-	-	No Ct.
45[1]	-	-	-	-
46[1]	-	-	-	-

- No Ct-value given by the participating laboratory.

[1)] Did not participate in PT2

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. Two laboratories wrongly answered HPR0 ISAV in ampul VII. The identification of KHV in ampoule VI didn't pose any issues. Regarding sequencing of SAV isolate in ampoule VIII, 27 out of 29 laboratories who sequenced the SAV isolate performed correct genotyping and two did not give any genotype.
A number of participants did not enter the genotype in the designated field in the Excel sheet *'Sequencing Results'* but instead recorded it only in the *'Pathogen Identification'* field. Consequently, Table 15 contains entries reported as N/A or 0, even though these participants did perform sequencing and genotyping.
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, 2025 – Genotyping

Code number	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
	KHV	ISAV	SAV	Blank
1	CyHV-3	HPR-deleted	SAV6	0
2[2]	N/A	N/A	N/A	N/A
3[1]	0	0	0	0
4	CyHV3	HPR-deleted	6	-
5	CyHV 3	HPR-deleted	6	0
6[2]	0	HRP3	0	0
7	CyHV-3	HPR-deleted	0	0
8	CyHV3	HPR-deleted	Subtype VI	0
9	CyHV-3	HPRdel	VI	0
10[2]	CyHV-3	ISA-HPRΔ	0	0
11[2]	CyHV 3	HPR-deleted	0	0

12	CyHV (3)	HPR-deleted	6	0
13	CyHV-3	HPR-deleted	SAV6	NEG
14[2]	CyHV3	HPR3	0	0
15	0	Genotype- PR3	0	0
16	0	HPR0	0	0
17[2]	CyHV (3)	HPR-deleted	0	0
18	CyHV3	HPR-deleted	6	0
19	CyHV 3	HPR0	6	0
20	Cyprinid herpesvirus 3 (CyHV3)	ISAV -HPRΔ (Genotype 2)	Salmon pancreas disease virus isolate (SAV6)	0
21	CyHV3	HPR-deleted	SAV6	0
22	0	HPR-deleted ISAV	0	0
23	0	HRP-deleted	0	0
24	CyHV-3	HPR-deleted	6	0
25	CyHV 3	HPR-deleted	6	0
26	CyHV3	HPR-deleted	6	N/A
27	CyHV-3	HPR-deleted	6	0
28	CyHV-3	HPR-deleted	Genotype 6	-
29	0	ISAV-HPRdeleted; Clade IV	Genotype SAV6	0
30	CyHV3	HPR-deleted	6	0
31	KHV-I	ISAV HPRΔ (EUG3)	SAV Genotype 6	0
32	CyHV-3	HPR-deleted ISAV	0	0
33	0	HPR deleted	0	0
34	CyHV (3)	HRP-deleated	6	N/A
35	0	0	0	0
36	0	EU-HPR deleted	SAV6	0
37	0	0	0	0
38	CyHV-3	HPR-deleted	6	n/a
39	0	HPR-deleted	0	0
40	Cyprinid herpesvirus 3	HPR-deleted	SAV6	0
41	3	HPR-deleted	6	0
42	CyHV3	HPR-deleted ISAV	SAV subtype 6	0
43	0	HPR 3	SAV 6	0
44	CyHV 3	HPR-deleted	6	0
45[3]	0	0	0	0
46[3]	0	0	0	0

[1)] This laboratory has not provided corroborating data to support the finding of ISAV in ampoule VII
[2)] This laboratory doesn't test for SAV
[3)] Did not participate in PT2

93% of the participating laboratories obtained 100% success rate in PT2.

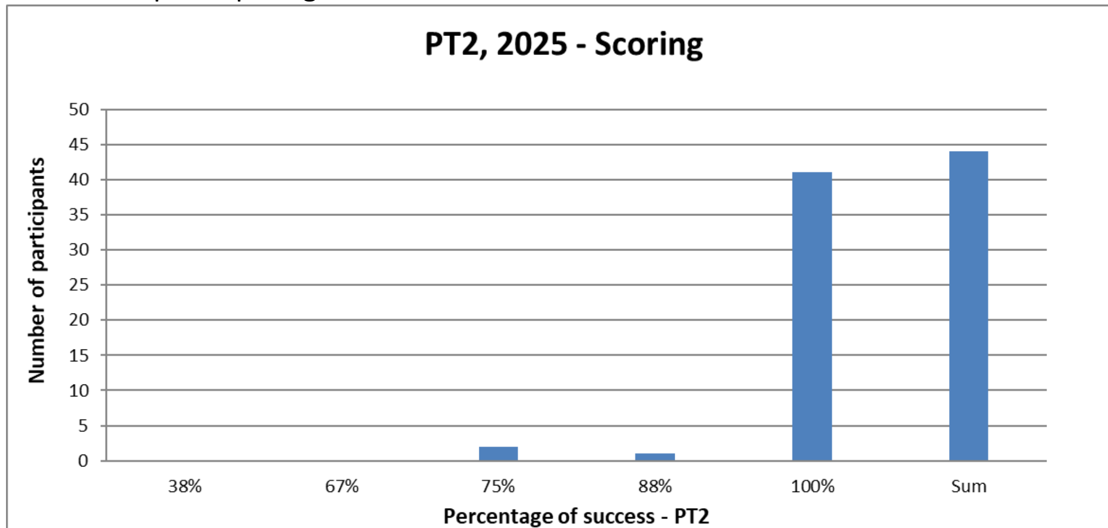

Figure 4 Success-rate of participating laboratories 2025 for PT2

Concluding remarks PT2

44 laboratories participated in PT2, 41 obtained 100% success rate.

- All 44 laboratories correctly identified the CyHV-3 (KHV) in ampoule VI.
- All 44 laboratories correctly identified the ISA virus in ampoule VII. One laboratory did not sequence the isolate, two laboratories gave the incorrect HPR-type due to wrong interpretation of correct sequence. Finally, eleven laboratories answered ISAV in 'Concluding Result' but correctly identified the isolates as HPR-deleted in the sequencing sheet.
- 38 laboratories tested for SAV and all 38 correctly identified the virus in Ampoule VIII, 6 laboratories did not test for SAV.

Underperformances related to sequence analysis of HPR of ISAV 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 30th Annual Workshop of National Reference Laboratories for Fish Diseases to be held May 26th -28th, 2026.

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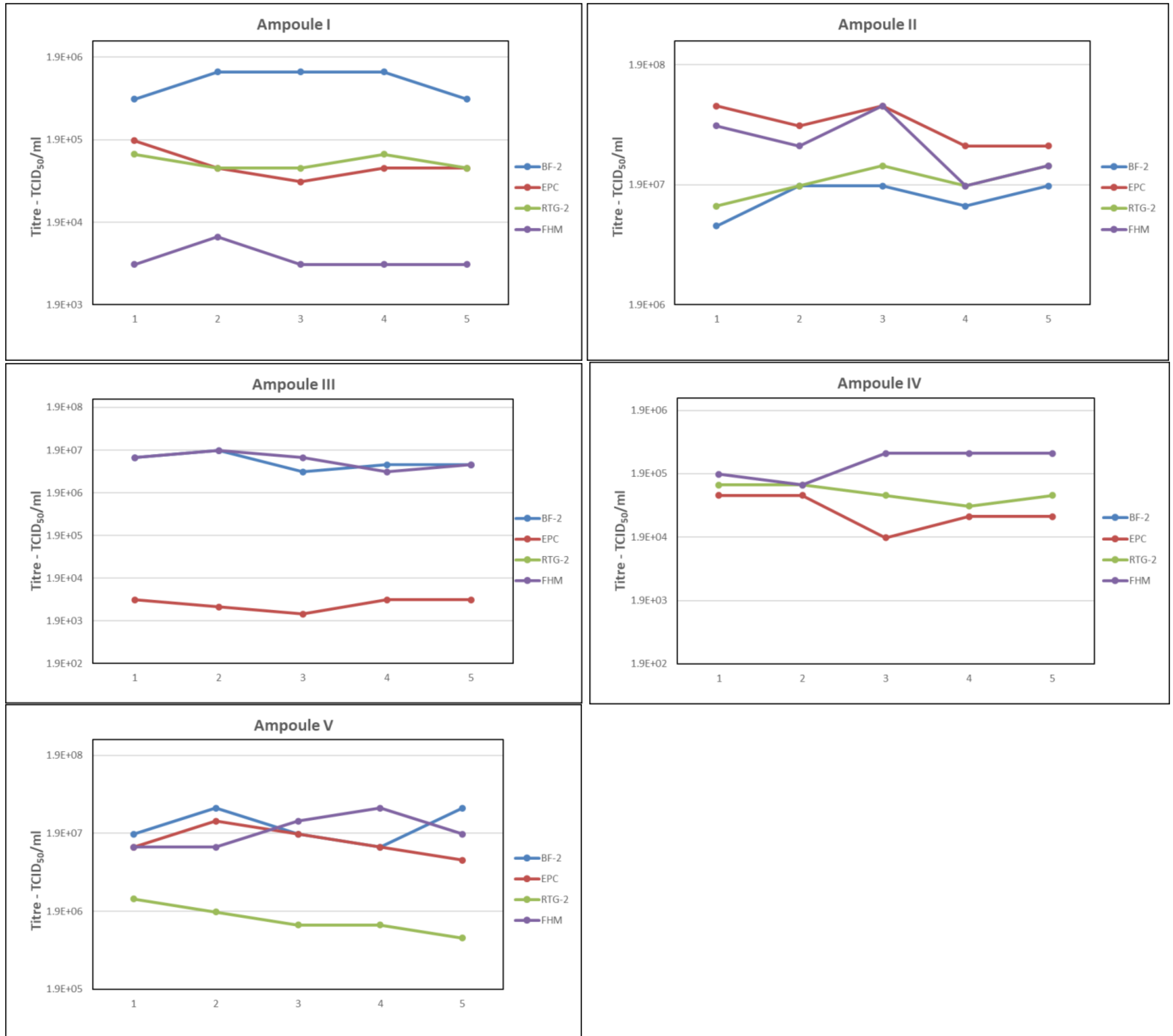
Appendix

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

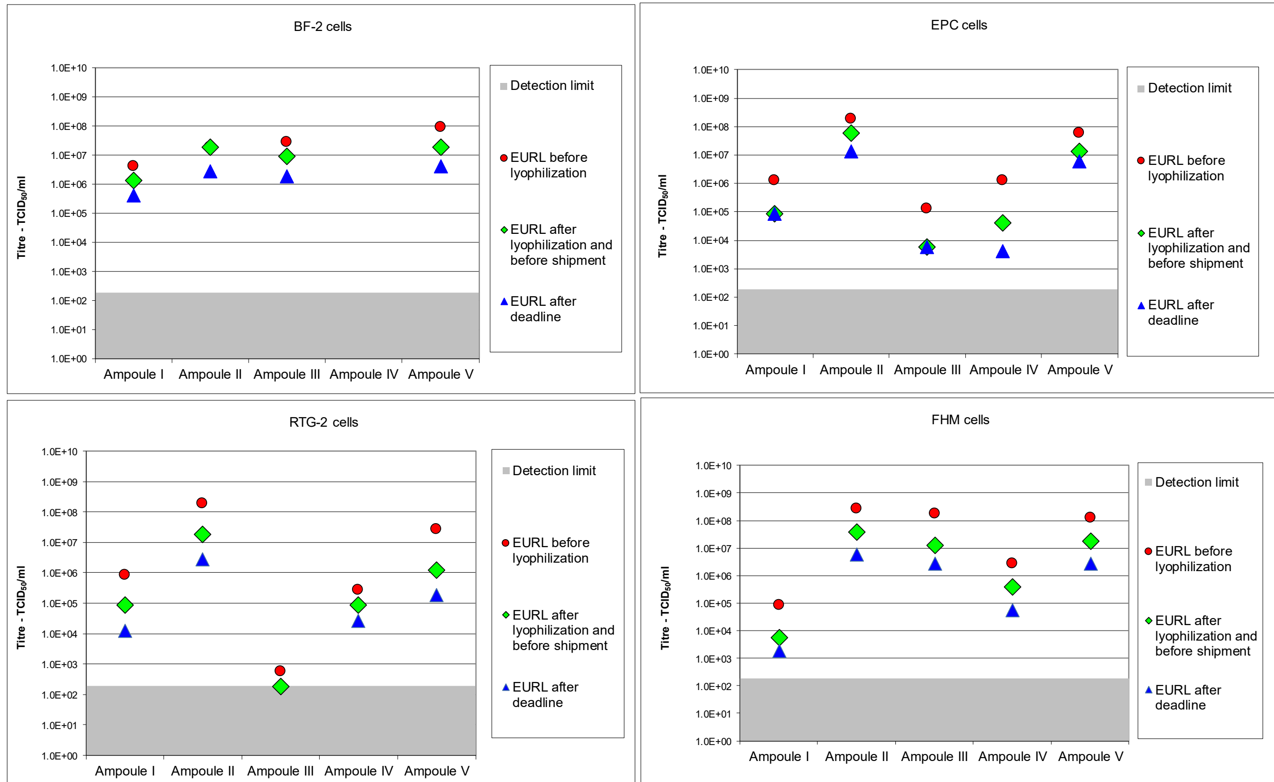
After Lyophilization the stability of the content in the ampoules were assessed by titrating the virus in five ampoules on cell cultures. For all the ampoules, the reduction of the titre was below 1 log in the same cell line.

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 ≤1 log.

Virus titres in different cell lines: before lyophilisation, before shipment and after deadline for handling in results.



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

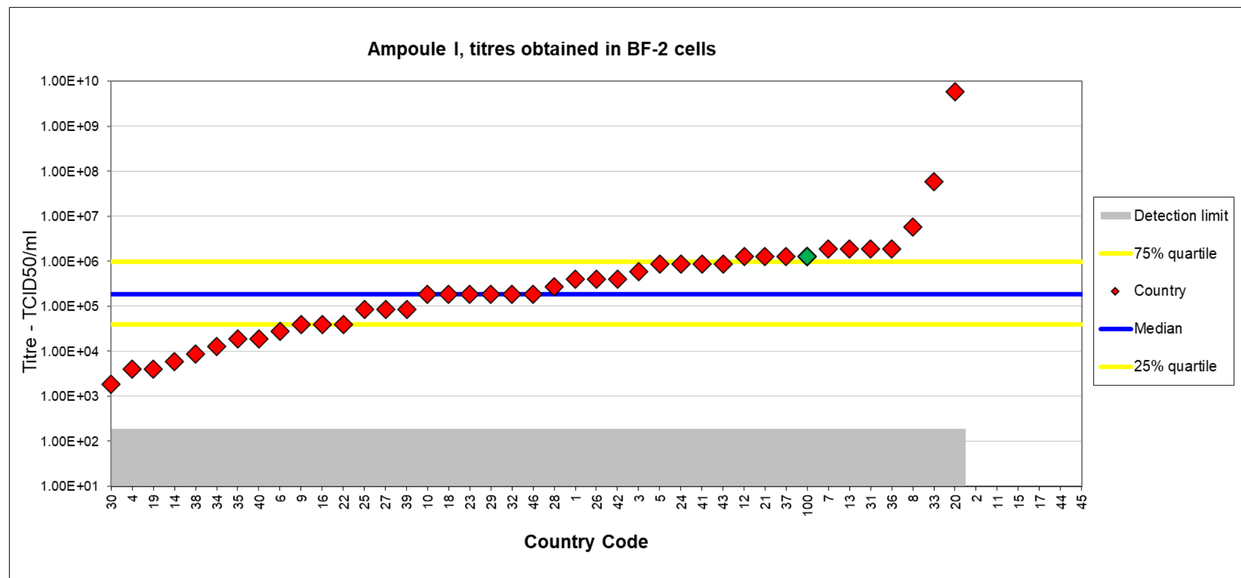
The titres (red diamonds) obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories as well as the titre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

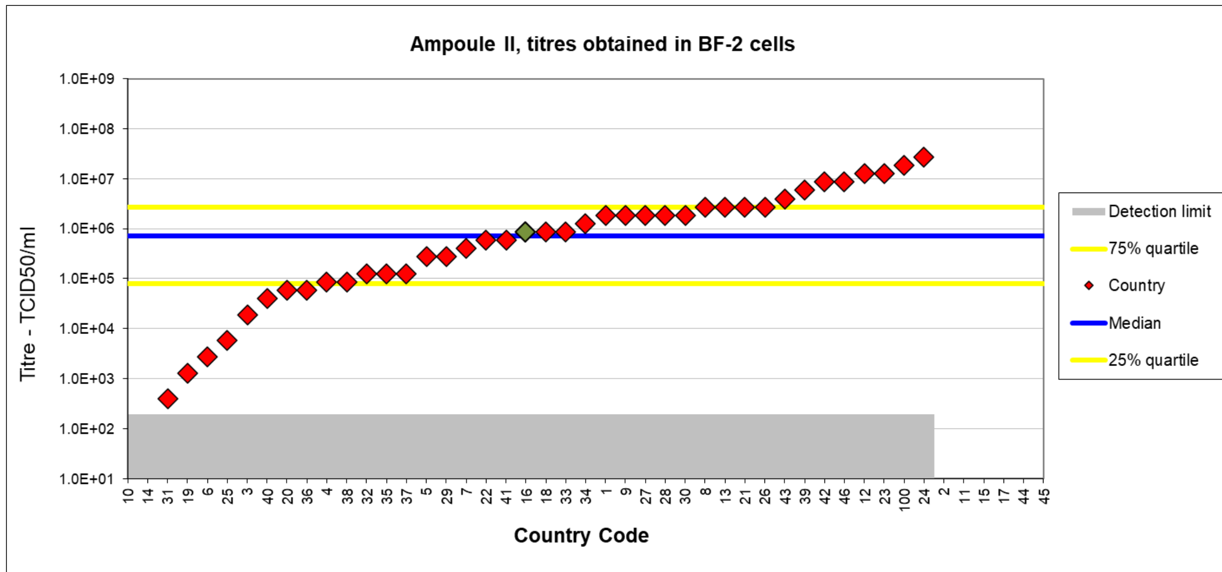
This year, we have elected to include only titration graphs generated from BF-2 and EPC cells. Titration graphs based on RTG-2 and FHM cells can be provided upon request.

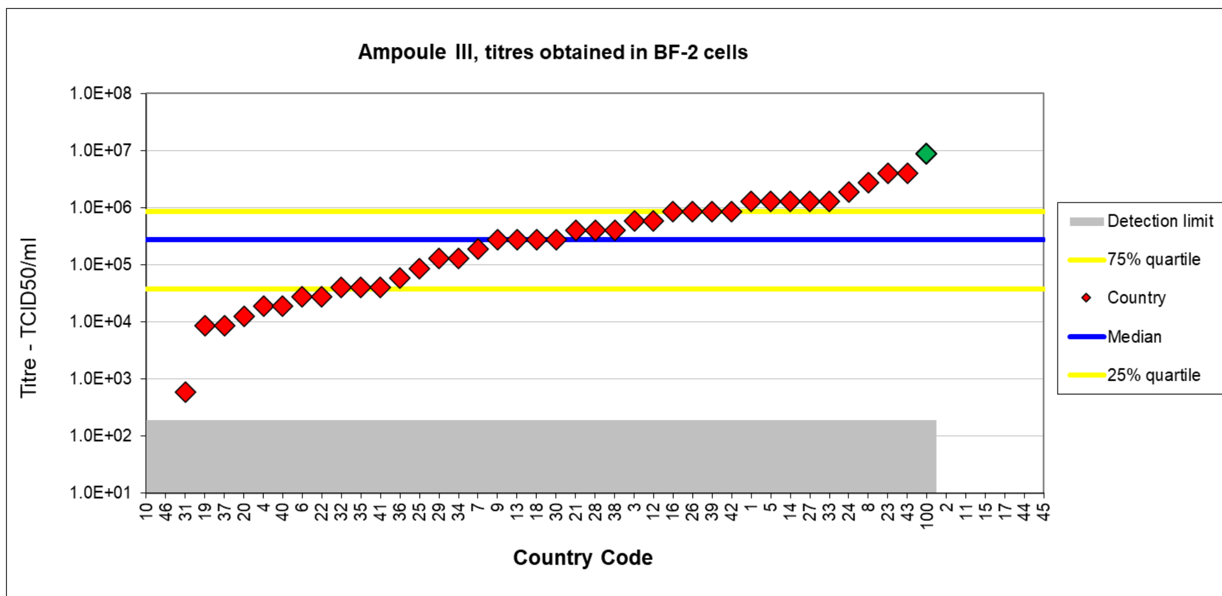
On these figures, the detection limit (grey shadow), the median titre (blue line), the 25% quartile (lower yellow line) and 75% quartile (upper yellow line) are plotted on all graphs.
Participants failing to obtain any titre are listed on the x axis under the grey zone, but no red diamond is plotted;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

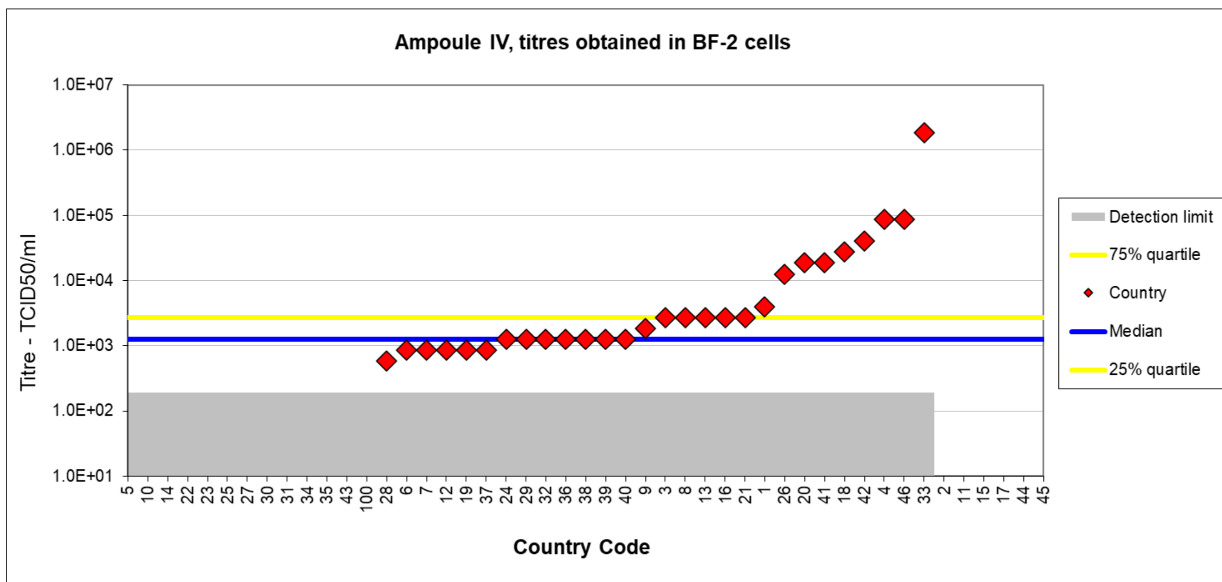
The optimal titre will be within the 25% and 75% inter-quartile range. A low titre, below 25% quartile may be indicator of low sensitivity of the cell culture in use; conversely a very high titre, beyond 75% quartile may indicate errors in assessing CPE.

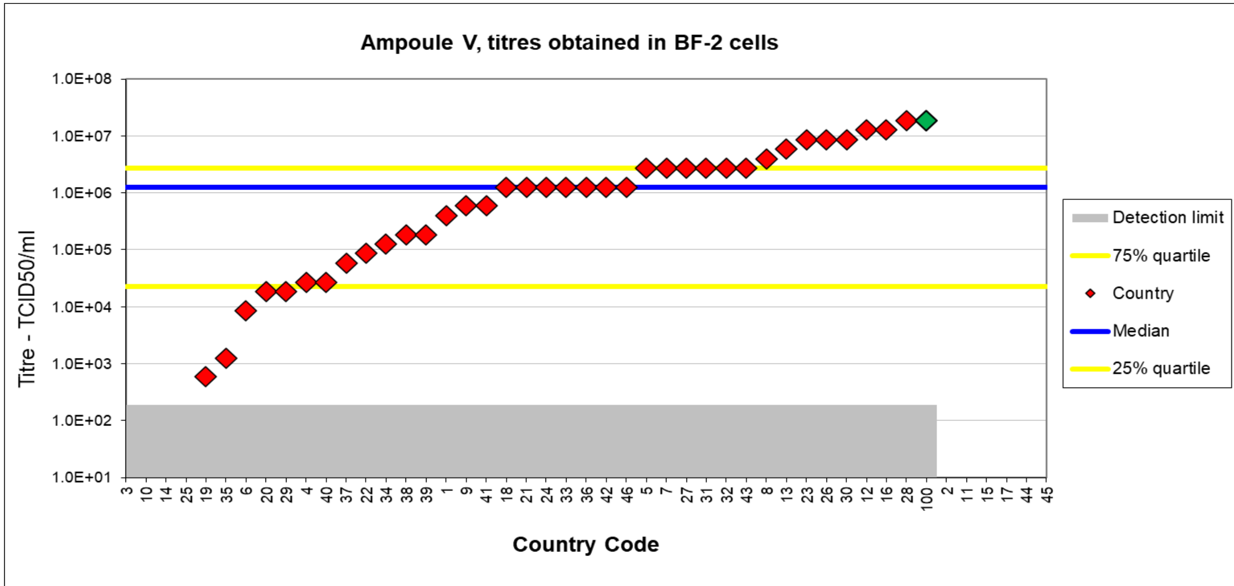
Virus titres obtained in BF-2 cells.



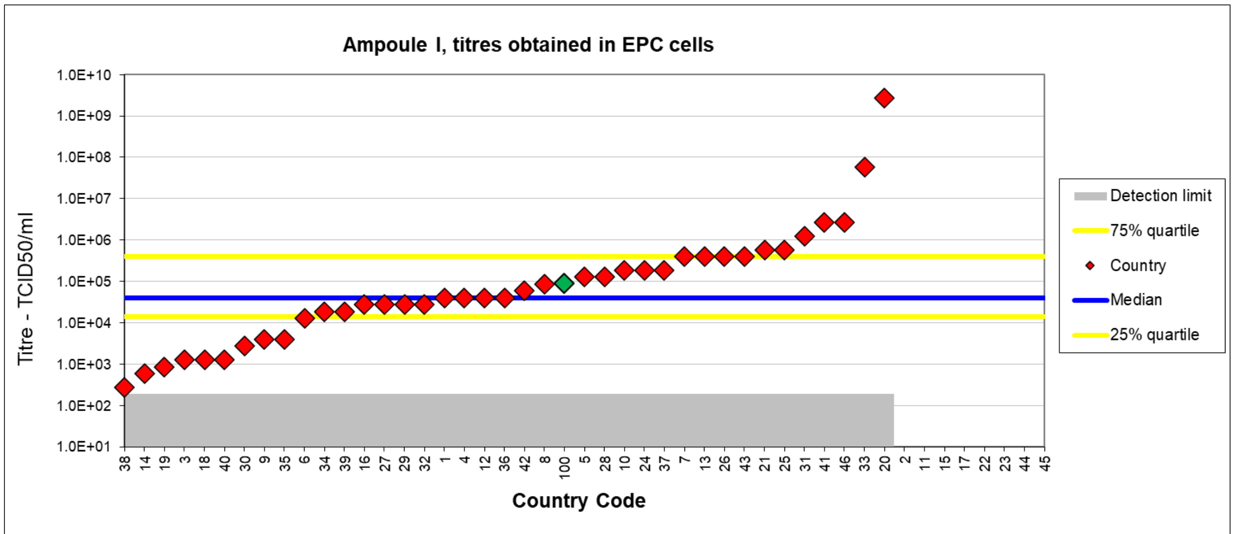

Ampoule II, titres obtained in BF-2 cells
Titre - TCID50/ml
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

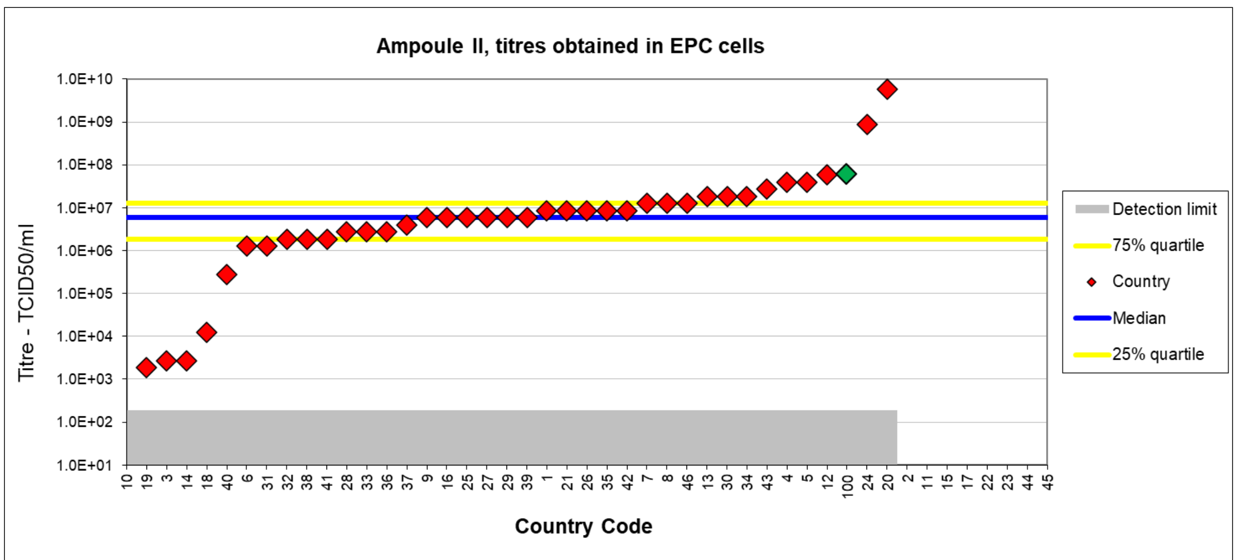

Ampoule III, titres obtained in BF-2 cells
Titre - TCID50/ml
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

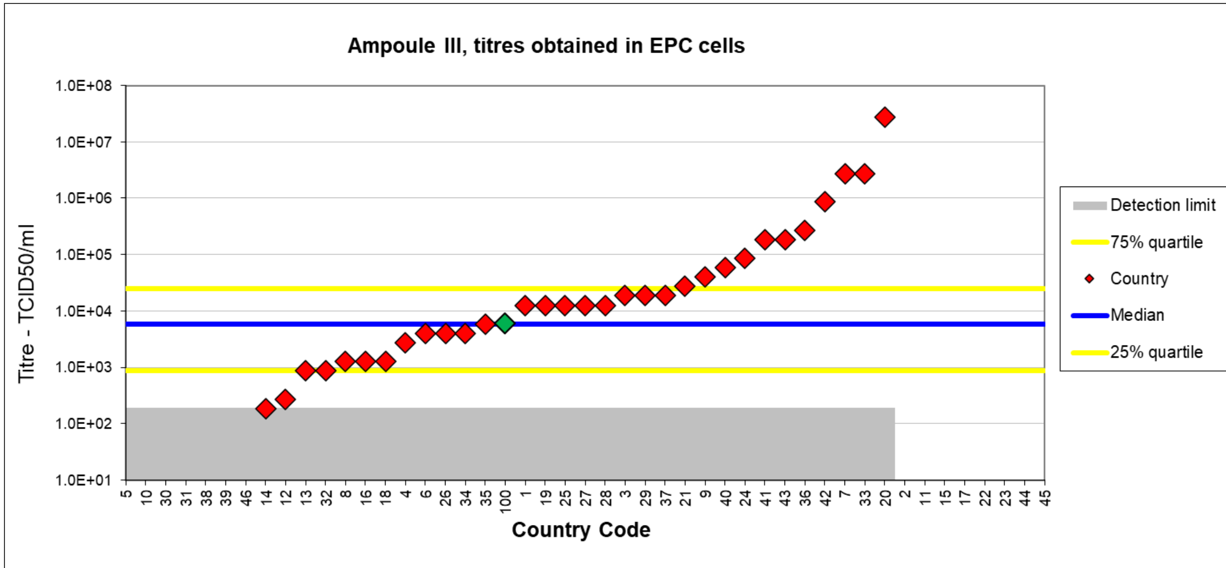

Ampoule IV, titres obtained in BF-2 cells
Titre - TCID50/ml
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

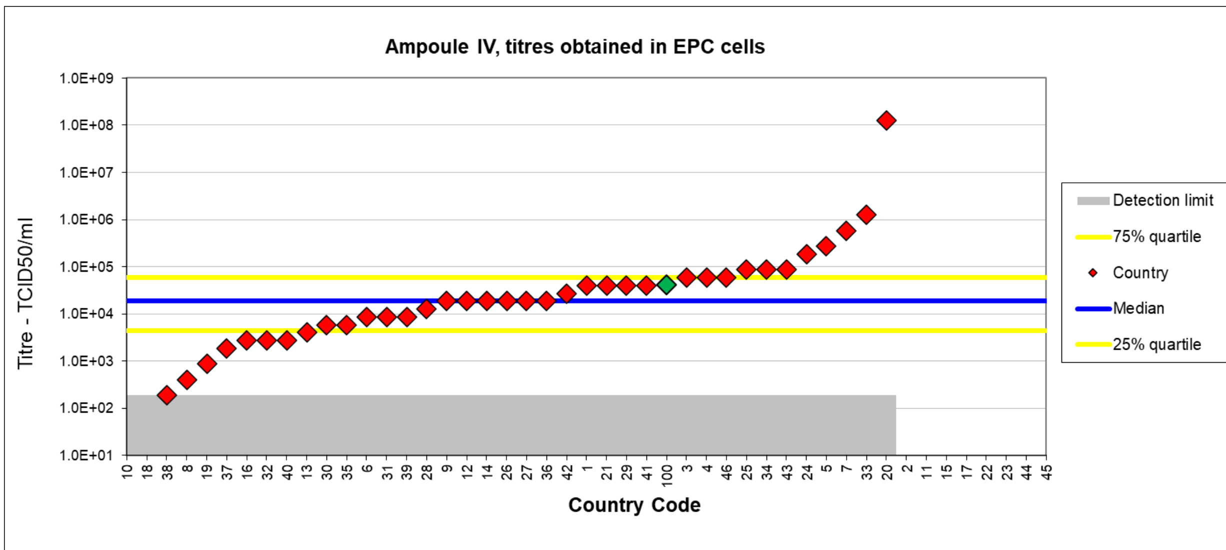


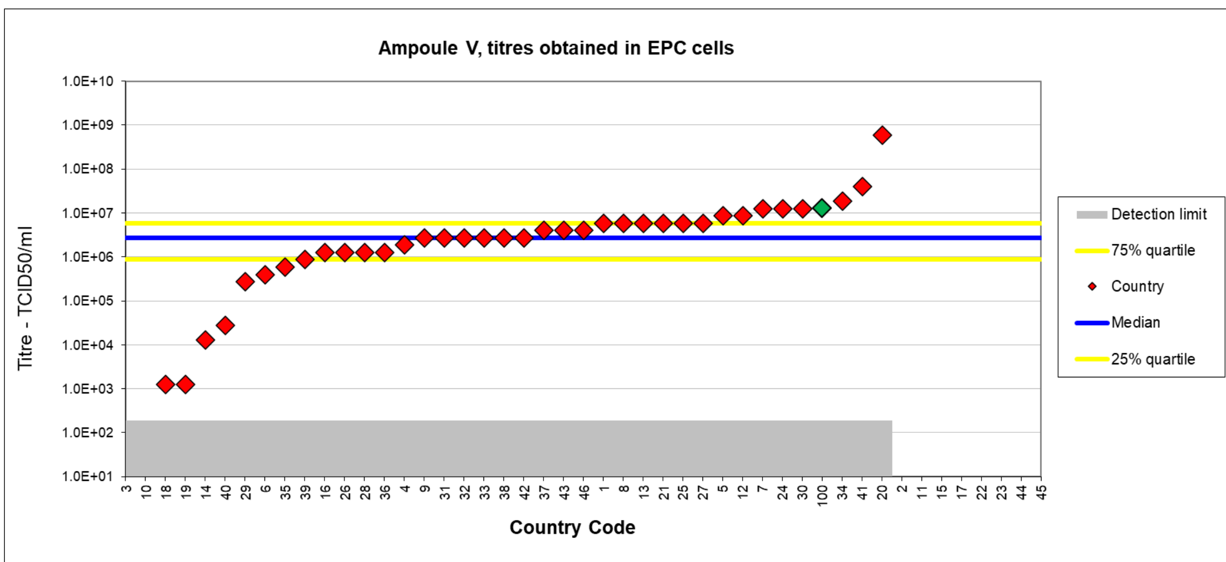
Virus titres obtained in EPC cells.



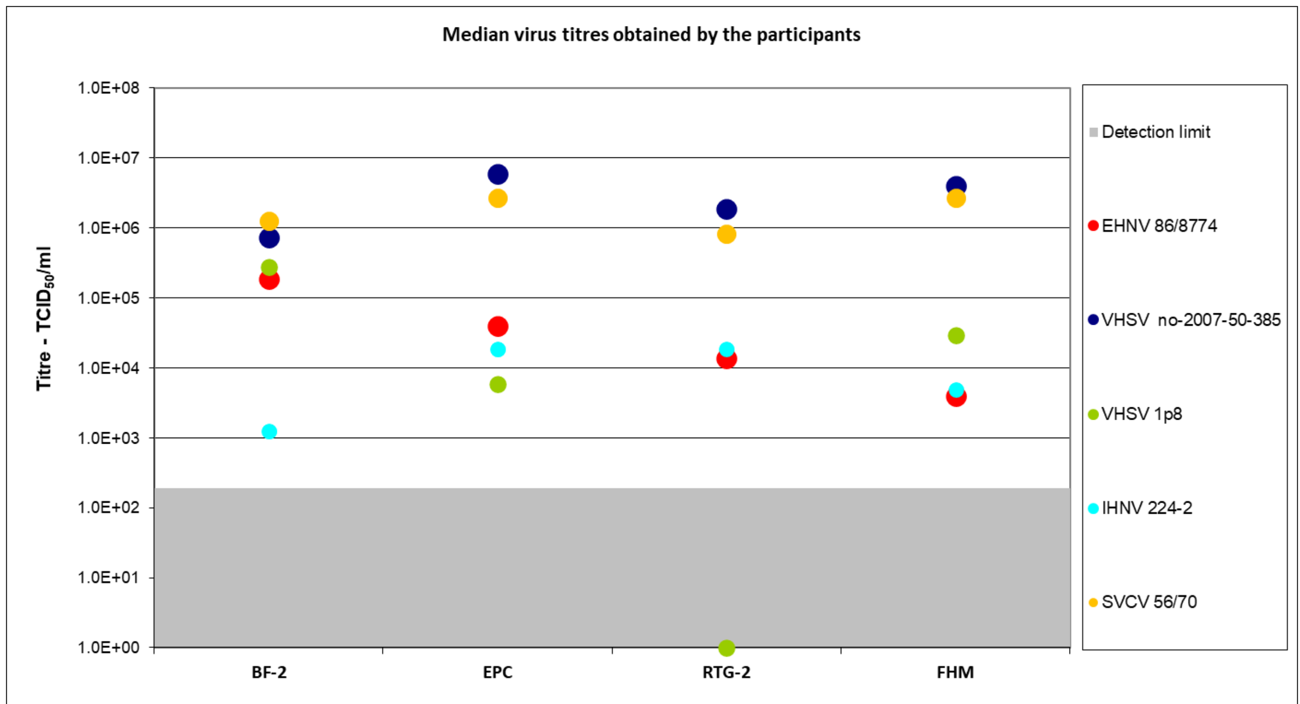



Ampoule III, titres obtained in EPC cells
Titre - TCID50/ml
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
5 10 30 31 38 39 46 14 12 13 32 8 16 18 4 6 26 34 35 100 1 19 25 27 28 3 29 37 21 9 40 24 41 43 36 42 7 33 20 2 11 15 17 22 23 44 45
Country Code
Detection limit
75% quartile
Country
Median
25% quartile


Ampoule IV, titres obtained in EPC 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
10 18 38 8 19 37 16 32 40 13 30 35 6 31 39 28 9 12 14 26 27 36 42 1 21 29 41 100 3 4 46 25 34 43 24 5 7 33 20 2 11 15 17 22 23 44 45
Country Code
Detection limit
75% quartile
Country
Median
25% quartile


Ampoule V, titres obtained in EPC 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
3 10 18 19 14 40 29 6 35 39 16 26 28 36 4 9 31 32 33 38 42 37 43 46 1 8 13 21 25 27 5 12 7 24 30 100 34 41 20 2 11 15 17 22 23 44 45
Country Code
Detection limit
75% quartile
Country
Median
25% quartile

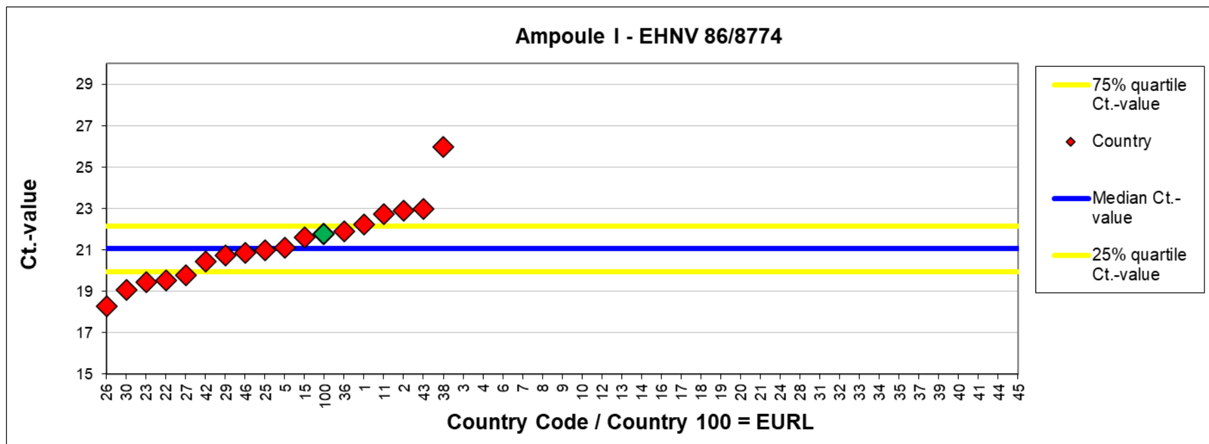
Median virus titres obtained by the participants in 4 different cell lines



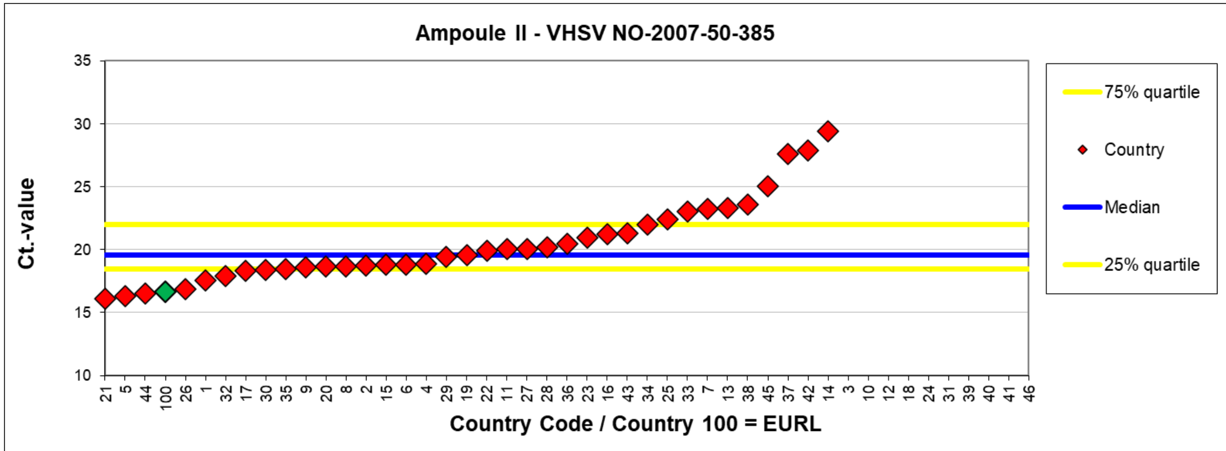
Annex 3: The Ct. values graphically (PT1) obtained from each participating laboratory.

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.

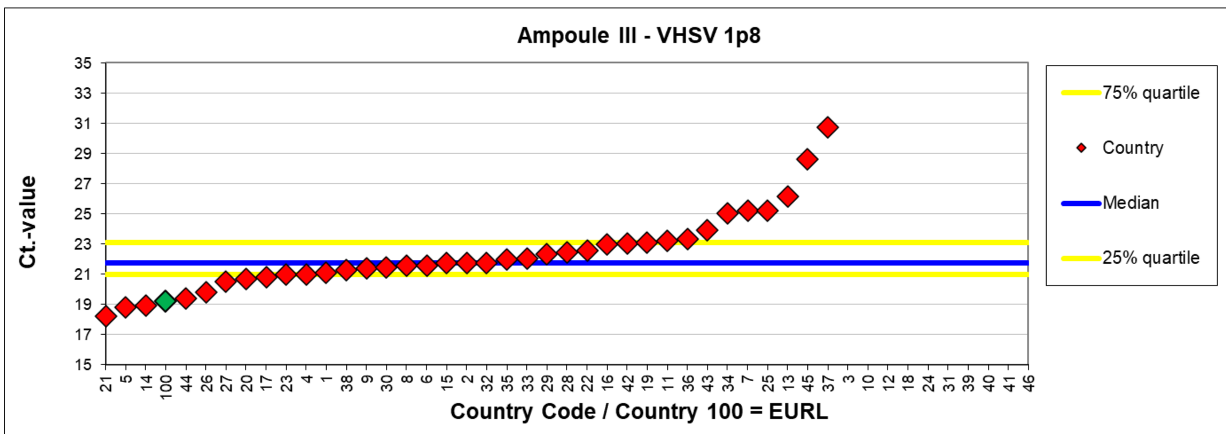
All Ct values submitted by the participants for each ampoule, are compared to each other, no red diamond = No Ct. value given. On these figures, the median values and the 25% and 75% inter-quartile range is displayed, the optimal value will be within these quartiles. A low Ct, below 25% quartile may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



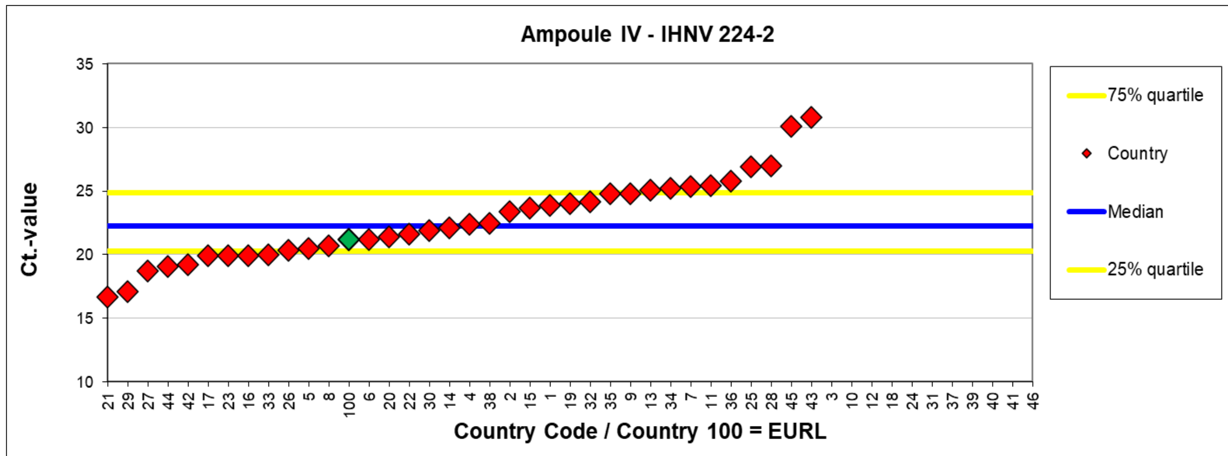
Number of laboratories	18
Median Ct.-value	21.1
Maximum Ct.-value	26.0
Minimum Ct.-value	18.3
25% quartile Ct.-value	19.9
75% quartile Ct.-value	22.1



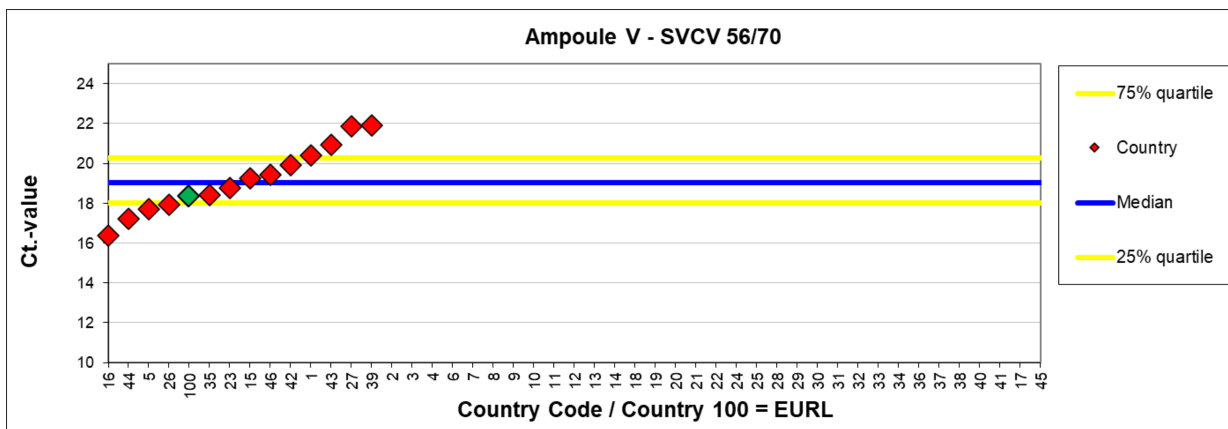
Number of laboratories	37
Median Ct.-value	19.6
Maximum Ct.-value	29.4
Minimum Ct.-value	16.1
25% quartile Ct.-value	18.5
75% quartile Ct.-value	22.0



Number of laboratories	37
Median Ct.-value	21.7
Maximum Ct.-value	30.7
Minimum Ct.-value	18.2
25% quartile Ct.-value	21.0
75% quartile Ct.-value	23.1



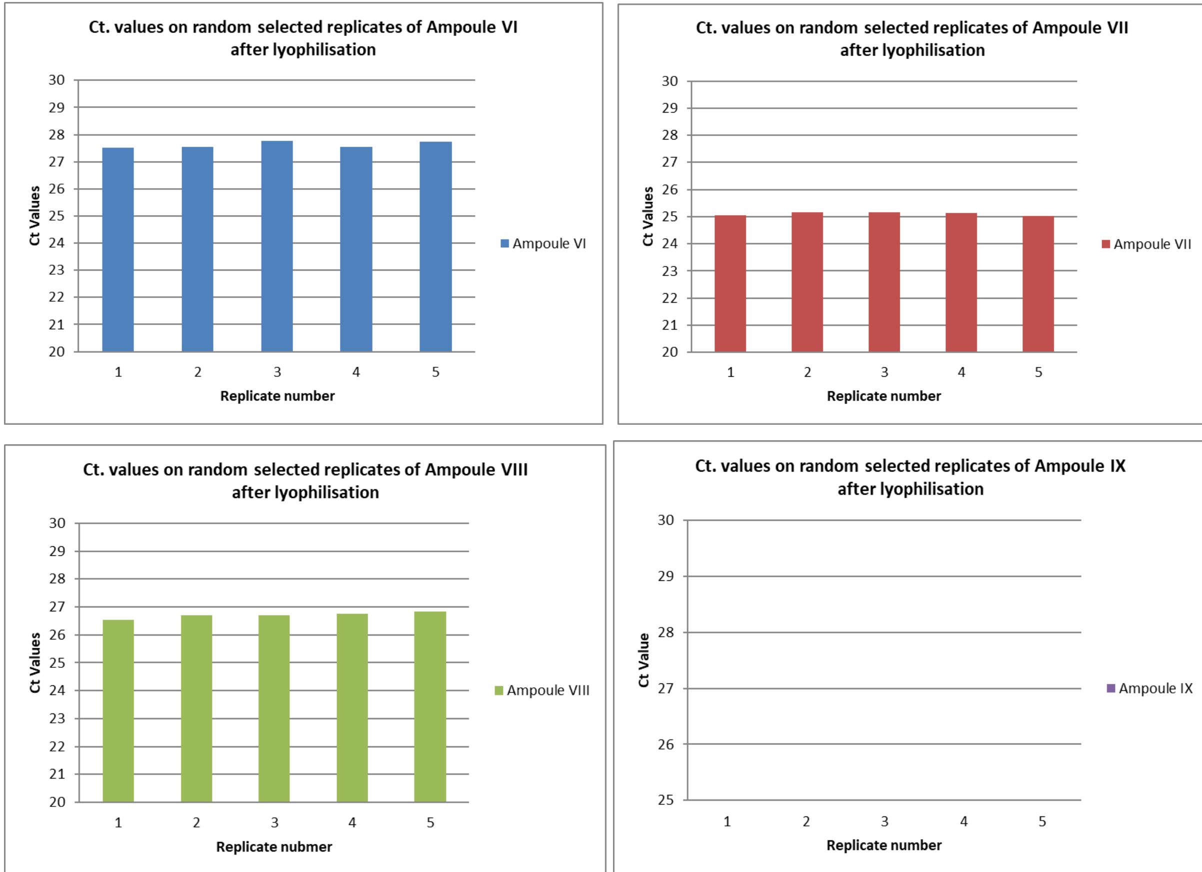
Number of laboratories	36
Median Ct.-value	22.3
Maximum Ct.-value	30.8
Minimum Ct.-value	16.7
25% quartile Ct.-value	20.2
75% quartile Ct.-value	24.9



Number of laboratories	14
Median Ct.-value	19.0
Maximum Ct.-value	21.9
Minimum Ct.-value	16.4
25% quartile Ct.-value	18.0
75% quartile Ct.-value	20.3

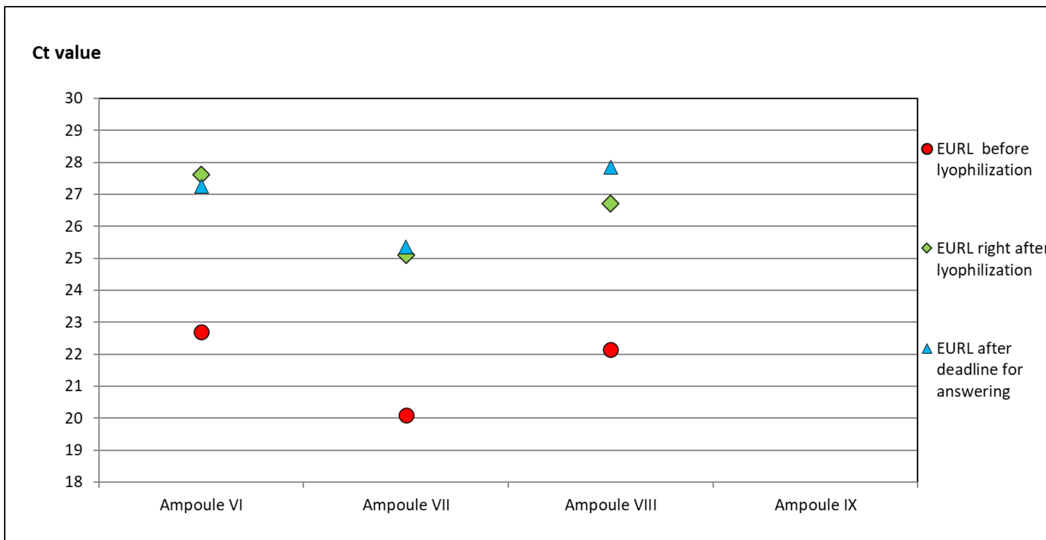
Annex 4: Stability and homogeneity of the content in the ampoules (PT2)

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 the ampoules, the difference in Ct. values was belove one.



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

The reduction in Ct. values before and after freeze-drying was approximately 5 log.
No significant reductions were observed after long term storage.



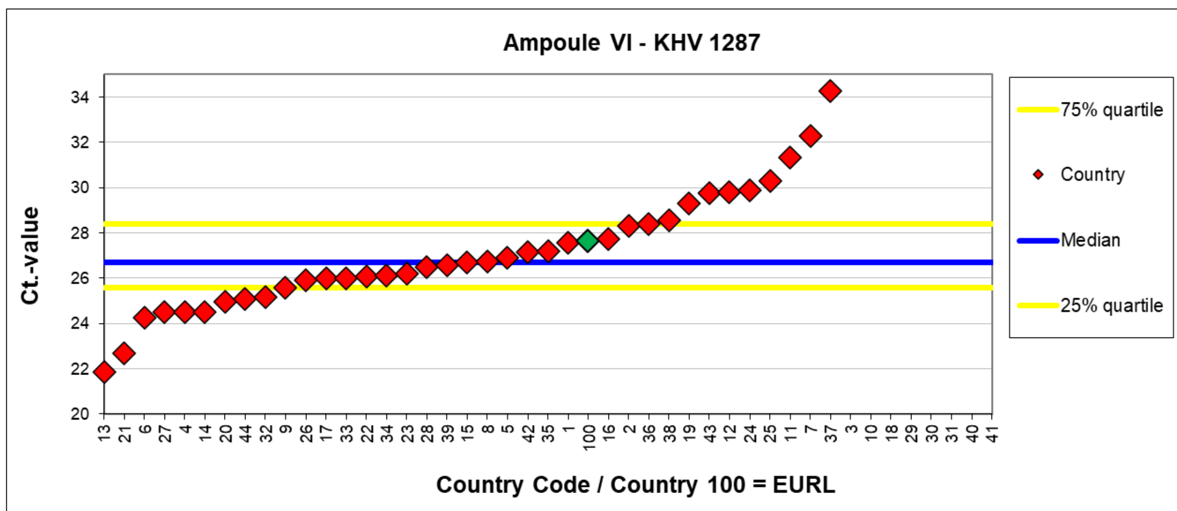
Ampoule VI, VII, VIII and IX tested before and after lyophilisation and after deadline for handling in results.

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

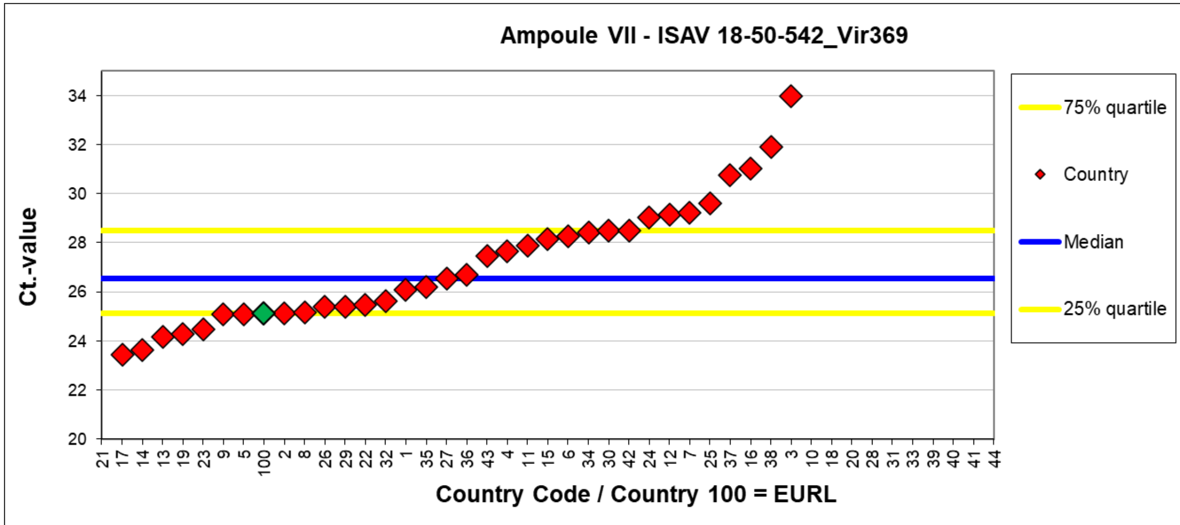
The Ct. values (red diamonds) obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare their Ct. values with the other participating laboratories as well as the Ct. value obtained by the EURL (green diamond and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

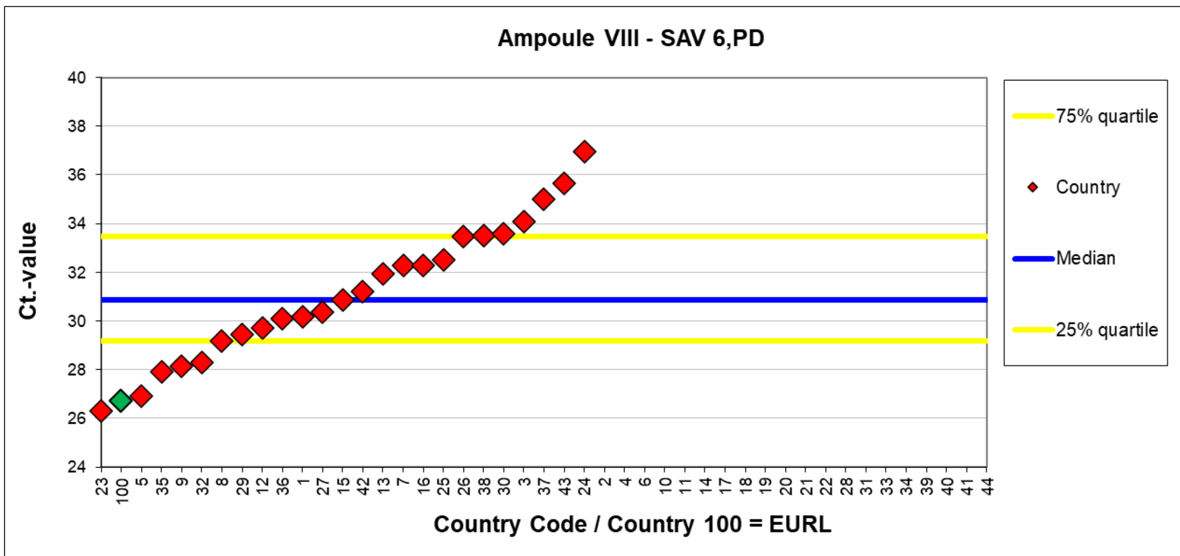
Ct. values obtained in PT2 by participants. No red diamond = No Ct. value given.



Number of laboratories	37
Median Ct.-value	26.7
Maximum Ct.-value	34.3
Minimum Ct.-value	21.9
25% quartile Ct.-value	25.6
75% quartile Ct.-value	28.4



Number of laboratories	37
Median Ct.-value	26.6
Maximum Ct.-value	34.0
Minimum Ct.-value	16.8
25% quartile Ct.-value	25.1
75% quartile Ct.-value	28.5



Number of laboratories	27
Median Ct.-value	30.9
Maximum Ct.-value	37.0
Minimum Ct.-value	26.3
25% quartile Ct.-value	29.2
75% quartile Ct.-value	33.5

Ampoule IX was confirmed by all participants to be free of pathogenic material and is therefore not included in annex 5.