EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2017

for identification and titration of

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

and identification of

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

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PT Reg. no.: 515

DANAK

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Introduction

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
PT1 was designed to primarily assess the identification of the fish viruses causing notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and in addition other fish pathogenic viruses as pike fry rhabdovirus (PFR), spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) by cell culture based methods.
PT2 was designed for assessing the ability of participating laboratories to identify the fish viruses: infectious salmon anaemia virus (ISAV), salmonid alphavirus (SAV) and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as koi herpes virus – KHV) by biomolecular methods (PCR based).
45 laboratories participated in PT1 while 44 participated in PT2.
Regarding PT1 and PT2, 42 and 38 laboratories respectively participated in identifying all viruses included.
The tests were sent from the EURL mid of September 2017.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.
This report covers both the results of PT1 and PT2.

PT1 consisted of five coded ampoules (I-V). These ampoules contained VHSV (2 isolates in 2 different ampoules), IHNV, European catfish ranavirus (ECV) and sterile cell culture supernatant from BF-2 cells (see table 1). The proficiency test was designed to primarily assess the ability of participating laboratories to identify any of the fish viruses VHSV, IHNV and to be able to discriminate between the exotic listed EHNV from other ranaviruses (Council Directive 2006/88/EC Annex IV part II and Commission Implementing Directive 2014/22/EU of 13 February 2014). Furthermore the inter-laboratory proficiency test is also suitable for maintaining accreditation for identification of SVCV, and IPNV; participants have to consider that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). In addition the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Implementing Decision (EU) 2015/1554 and by using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.

If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2015. Laboratories were encouraged to identify VHSV and IHNV isolates by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al*. (2004) for VHSV and either method as mentioned in the IHN chapter of the 2013 version of the OIE manual on Aquatic Animal Diseases (Emmenegger et al. (2000)) or in Kurath et *al*. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of four coded ampoules (VI-IX). One ampoule contained CyHV-3 (KHV), one contained SAV, one contained ISAV and one contained sterile cell culture supernatant from BF-2 cells, see table 9. The test was designed to primarily assess the ability of participating laboratories to identify

the notifiable fish pathogens ISAV and KHV (listed inCouncil Directive 2006/88/EC, Annex IV and Commission Implementing Directive 2014/22/EU) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test for SAV or not. The EURL team would then take care of calculating the score accordingly, overall 38 of 44 laboratories tested for SAV in 2017, which was an increase of one laboratory compared to 2016.
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 OIE manual Chapter 2.3.5b. — Infection with salmonid alphavirus . It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might had been possible to replicate them in cell cultures.

During the preparation of the current report the EURL has acknowledged the big effort that many participants are putting in sequencing and genotyping the isolates of the PT panel. For this reason, the EURL proposes to provide a separate scoring system for the genotyping results, which will be attached to the annexes (Table 9 and 13) which display the genotyping results provided by all participants. This topic will be explained and discussed at the next Annual Workshop 30-31st May 2018 in Denmark.

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

Participants were asked to download an excel sheet from the EURL web site (http://www.eurl-fish.eu/) to be used for reporting results and to be submitted to the EURL electronically. Additionally, participants were requested to answer a questionnaire regarding the accreditation status of their laboratory. Collected accreditation data will not be presented in this report but will be presented at the 22th Annual Workshop of the NRLs for Fish Diseases week 22, 2018 in Kgs. Lyngby. Participants were asked to reply latest November 20th 2017.

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 tests parcels were delivered by courier and when possible participants were provided with a tracking number so they were able follow the shipment.

Shipment and handling
Within one day, the tests were delivered to 18 participants; 18 more tests were delivered within the first week; 5 more within the first two weeks; 1 further within four weeks; due to delivery problems in the receiving countries 3 tests were 7 – 9 weeks in transit (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 the PT 2012 report.

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

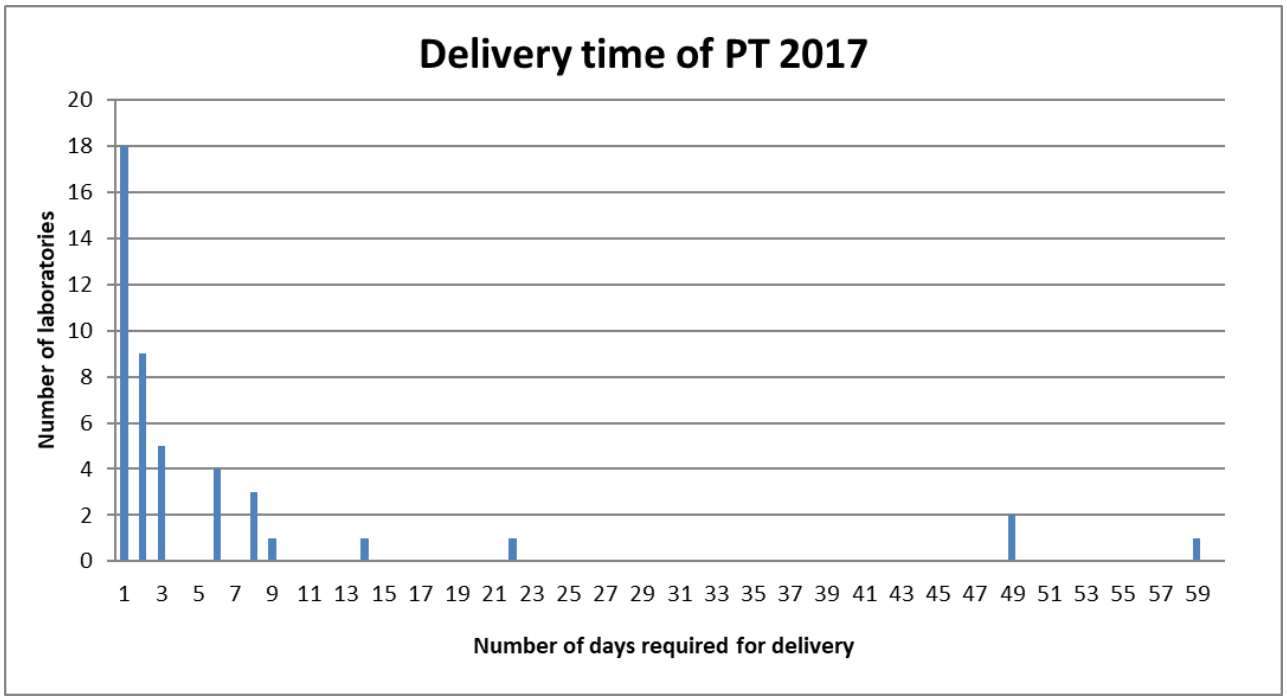


Figure 1. Transport time for the parcels to reach the participants.

Participation

PT1 and PT2: 45 laboratories received the annual proficiency test. The initial deadline was extended due to delivery problems to three participants. 39 participants submitted the full spreadsheet within the original deadline; 2 additional participants delivered the results for ampoule content within the deadline but provided sequencing results within the extended deadline; all laboratories provided the full spreadsheet within the extended deadline. Figure 2 show how many laboratories that participated in the proficiency test from 1996 to 2017.

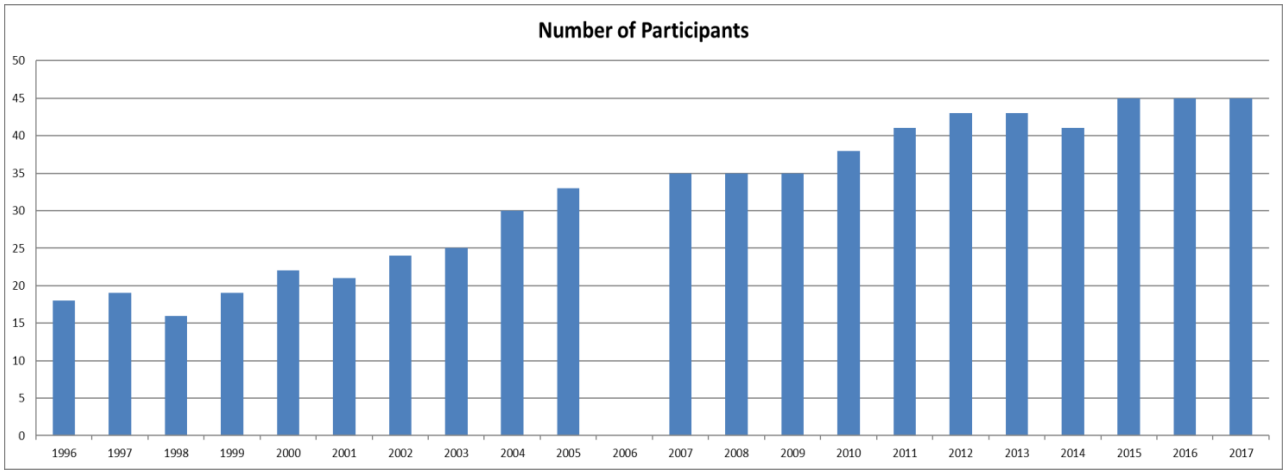


Figure 2. Participants in the EURL proficiency test over the years.

Proficiency test 1, PT1

Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States, including Denmark, and likewise to the NRLs in Australia, Bosnia and Herzegovina, Canada, Faroe Islands, Iceland, Iran, Japan, New Zealand, Norway, Serbia, Switzerland, Turkey and to two laboratories in P.R. China, South Korea and USA, respectively.
The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy, Cyprus and Malta for identification of all listed diseases. Figure 3 shows the worldwide distribution of the participating NRLs. It is foreseen that from 2018 laboratories from south American and African countries will participate in the PTs as well.

Figure 3. Worldwide distribution of the participants in the EURL proficiency test 2017.

Content of ampoules

The viruses were propagated on each of their preferred cell line, and when total cytopathic effect (CPE) was observed, the supernatants were collected and filtrated through a 45 µm filter, mixed with equal volumes of 2% w/v lactalbumin hydrolysate solution and lyophilized in glass ampoules. The ampoules were sealed by melting. The details of the virus isolates used in the proficiency test are outlined in table 1.

Table 1. Content of each ampoule with reference to culture conditions and major publications of the included viruses.

Code	Specifications/References
Ampoule I: VHSV	**VHSV strain DK-6137** Genotype: Ia Received from: National Veterinary Institute, Technical University of Denmark. The isolate originated from an outbreak of VHS with high mortality in sea water aquaculture in 1991. 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.
Ampoule II: IHNV	**IHNV 217/A** Genotype: M Received from: Received from Dr. G. Bovo, ISZ-Ve, Padova, Italy. First Italian IHNV isolate from rainbow trout. . Isolated in 1987. GenBank accession numbers**:** FJ265716.1 or KU878277.1 Reference on isolate: Bovo G, Giorgetti G, Jørgensen PEV and Olesen (1987). Infectious haematopoietic necrosis: first detection in Italy. *Bulletin of the European Association of Fish Pathologists* 7, 124. References on sequence and genotype: Johansson T, Einer-Jensen K, Batts W, Ahrens P, Björkblom C, Kurath G, Björklund H & Lorenzen N (2009). Genetic and serological typing of European infectious haematopoietic necrosis virus (IHNV) isolates. *Diseases of Aquatic Organisms* 86, 213-221.
Ampoule III: VHSV	**VHSV - Isolate TR-WS13G (= TR-SW13G)** Genotype Ie. Received from Dr. Mamoru Yoshimizu. Turkish isolate (Trabzon coastal area) from turbot (*Psetta maxima*). DTU Vet protocol: 207005-1, recieved as VHS SW 13G – P3 050707. GenBank accession number: AB231160 or LN877207.1 or KM97680.1 References: Nishizawa T, Savas H, Isidan H, Üstündag C, Iwamoto H & Yoshimizu M (2006). Genotyping and pathogenicity of viral hemorrhagic septicemia virus from free-living turbot (*Psetta maxima*) in a Turkish coastal area of the Black Sea. *Applied and Environmental Microbiology* 72, 2373-2378.
Ampoule IV: ECV	**Ranavirus ECV: European catfish virus isolate 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

Code	Specifications/References
	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: Blank	Sterile cell culture supernatant from BF-2 cells

Testing of the PT1 test

The PT1 test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043. Prior to distribution the EURL tested 5 ampoules of each virus preparation by titration in 4 cell lines (BF-2, EPC, RTG-2 and FHM), to ascertain a satisfactory titre in the preferred cell line and homogeneity of content of ampoules (Table 2 and Figure 4).

The lyophilisation procedure is known to determine some reduction especially for VHSV. Previous experience reported during the past Proficiency tests demonstrated a rather high stability for SVCV, EHNV and IPNV serotype Sp. We have previously shown that lyophilised virus kept in glass sealed ampoules is stable for more than half a year when kept at room temperature (Inter-Laboratory Proficiency Test report 2007).
We have furthermore shown that lyophilised virus in glass sealed ampoules is stable after exposure to 30°C for 24 hours (Inter-Laboratory Proficiency Test report 2010)
In 2011 we have shown that lyophilised virus in glass sealed ampoules is stable when temperature raised from 20-42°C over a period of 5 hours (Inter-Laboratory Proficiency Test 2011)

The identities of the viruses in all 5 ampoules were checked and confirmed before shipment by ELISA, IFAT, serum neutralisation tests (SNT), RT-PCR and/or RT-qPCR. After shipment the stability of the content in the ampoules were assessed by titrating the virus on cell cultures, and identifying it by ELISA, furthermore PCR based tests were performed on the original content of all the ampoules. This year limited reductions of titres (never exceeding 2 logs) were observed following lyophilisation and no reduction were observed after long term storage (Table 2 and figure 4)

Ampoul No.	Cell line	Titre before Lyophilisation	Titre after Lyophilisation and before shipment	Titre after deadline for handling in results (storage 4°C in the dark)
		$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I: VHSV DK-6137	BF-2	5,9E+08	5,9E+06	2,7E+06
	EPC	5,9E+08	8,6E+06	1,3E+07
	RTG-2	2,7E+07	1,3E+07	1,9E+07
	FHM	1,9E+07	1,9E+07	1,9E+07
Ampoule II: IHNV 217/A	BF-2	4,0E+05	2,7E+02	1,9E+03
	EPC	4,0E+07	5,9E+05	5,9E+05
	RTG-2	5,9E+04	2,7E+04	1,3E+05
	FHM	5,9E+04	1,9E+05	5,9E+04
Ampoule III: VHSV TR-WS13G	BF-2	4,0E+08	8,6E+06	8,6E+06
	EPC	4,0E+07	2,7E+05	1,3E+05
	RTG-2	8,6E+02	1,3E+03	1,9E+03
	FHM	1,9E+06	8,6E+06	2,7E+07
Ampoule IV: ECV 562/92	BF-2	1,3E+07	1,3E+06	2,7E+07
	EPC	1,9E+07	1,3E+06	1,3E+06
	RTG-2	8,6E+04	2,7E+05	4,0E+06
	FHM	2,7E+03	1,9E+03	1,3E+04
Ampoule V: Sterile cell culture supernatant from BF-2 cells	BF-2	< 1,9E+02	< 1,9E+02	< 1,9E+02
	EPC	< 1,9E+02	< 1,9E+02	< 1,9E+02
	RTG-2	< 1,9E+02	< 1,9E+02	< 1,9E+02
	FHM	< 1,9E+02	< 1,9E+02	< 1,9E+02

Table 2. PT1:

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

- Before lyophilisation, (stored at -80°C).
- After lyophilisation and before shipment (median titre of 5 replicates), (stored at 4°C), the variation of the titre of the 5 replicates was within 1 log in the same cell line.
- After deadline for handling in results (1 ampoule), (stored at 4°C).

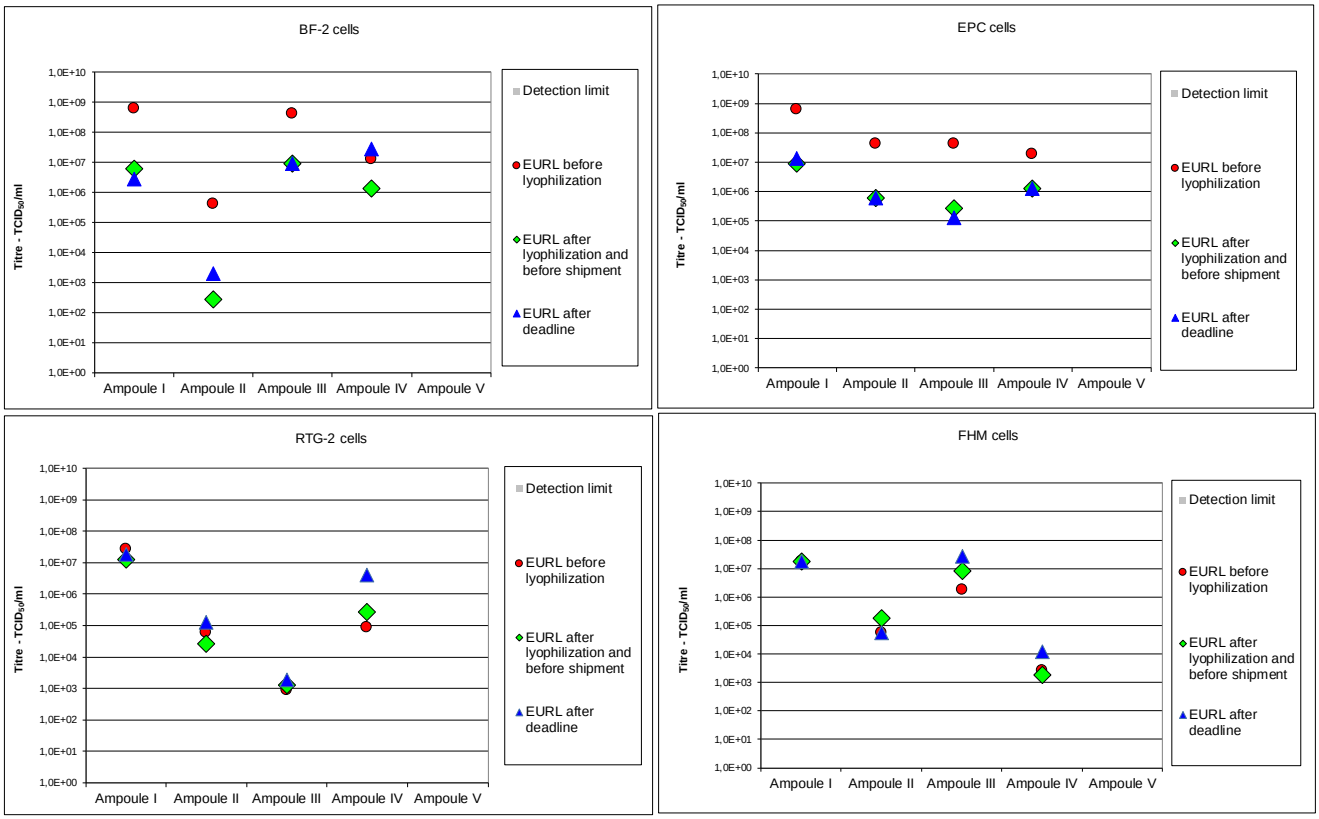


Figure 4. Virus titers in different cell lines:
Before lyophilisation, before shipment and after deadline for handling in results (storage 4°C in the dark).

Virus identification and titration

Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in Commission Decision 2015-1554, i.e. by cell culture followed by ELISA, IFAT, neutralisation test and/or RT-PCR/RT-qPCR. The results of the content in the 5 ampoules as reported by the participating laboratories are summarised in table 3.

Participants were also asked to assess the viral load in the ampoules by conducting titrations. The titration procedures were described in the instructions enclosed with the test. All titres were calculated by the EURL based on the crude data submitted by each participant and given as Tissue Culture Infective Dose 50% per ml ($TCID_{50}$/ml). The titre of the re-dissolved virus was multiplied by a factor of 10 to compensate for the dilution of the original volume of virus in the ampoules (200 µl virus + 200 µl lactalbumin in vials re-dissolved in a total of 2.0 ml cell culture medium). The titration results obtained by the participating laboratories are summarised in tables 4 to 8. The titres obtained from each participating laboratory are represented graphically. In Figures 5-8, all titres submitted by the participants for each cell line and ampoule, respectively are compared to each other. On these figures, the median titre and the 25% and 75% inter-quartile range is displayed. In this way, the titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories. CHSE-214 cells are not displayed graphically or commented on in this report as only 6 laboratories used these cells.

Laboratories with the required facilities were strongly encouraged to identify the genotype of the virus isolates. It was not mandatory to perform these analyses for VHSV and IHNV. However, for ranaviruses it is mandatory to perform a sequence or restriction endonuclease analysis of the isolate in order to determine if the isolate is EHNV.

Table 3. Inter-Laboratory Proficiency Test, PT1, 2017 - Virus identification and score obtained by participants.

Laboratory code number	Score	Answer received at EURL	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
			VHSV DK-6137	IHNV 217/A	VHSV TR-WS13G	ECV 562/92	Blank BF-2 cell supernatant
1	**10/10**	21-11-2017	VHSV	IHNV	VHSV	Ranavirus, not EHNV	Not VHSV, not IHNV, and not ranavirus
2[1]	**8/8**	13-11-2017	VHSV	IHNV	VHSV	no IHNV , VHSV, IPNV, SVCV	negative
3	**9/10**	16-11-2017	VHSV	IHNV	VHSV + Ranavirus	Ranavirus	No CPE
4	**10/10**	16-11-2017	VHSV genotype Ia	IHNV genogroup M	VHSV genotype Ie	ECV	No virus
5	**10/10**	07-11-2017	VHSv	IHNv	VHSv	ECV	negative for VHSV, IHNV, EHNV, Ranavirus, IPNV and SVCV
6	**10/10**	28-11-2017	VHSV	IHNV	VHSV	Ranavirus (ECV/ESV)	negative
7	**10/10**	19-11-2017	VHSV	IHNV	VHSV	RANAVIRUS (ECV)	NO VHSV,NO IHNV,NO EHNV,NO RANAVIRUS,NO IPNV,NO SVC
8	**10/10**	17-11-2017	VHSV	IHNV	VHSV	Ranavirus	NEGATIVE
9	**10/10**	17-11-2017	VHSV	IHNV	VHSV	ECV	No virus detected
10[1]	**8/8**	05-12-2017	VHSV	IHNV	VHSV	ND	ND
11	**10/10**	13-11-2017	VHSV	IHNV	VHSV	ECV	Negative
12	**10/10**	07-11-2017	VHSV	IHNV	VHSV	ECV	NEG
13	**10/10**	17-11-2017	VHSV	IHNV	VHSV	Ranavirus	-
14	**9/10**	17-11-2017	VHSV	IHNV	VHSV	EHNV	no VHSV/ no IHNV/no EHNV/ no IPNV/ no SVCV
15	**9/10**	17-11-2017	VHSV	IHNV	VHSV	EHNV	NEGATIVE
16	**10/10**	20-11-2017	VHSV	IHNV	VHSV	Ranavirus	No virus was detected in this ampoule
17	**10/10**	15-11-2017	VHSV viable virus	INHV viable virus	VHSV viable virus	ECV (rana like) viable virus	Negative
18	**10/10**	10-11-2017	VHSV	IHNV	VHSV	Ranavirus	No virus detected
19	**10/10**	08-12-2017	VHSV	IHNV	VHSV	Ranavirus (ECV)	BLANK
20	**9/10**	18-12-2017	VHSV & SVCV	IHNV	VHSV	ECV	-
21	**10/10**	20-11-2017	VHSV	IHNV	VHSV	ECV	-
22	**10/10**	20-11-2017	VHSV	IHNV	VHSV	Ranavirus (Non-EHNV)	Negative for: VHSV; IHNV;EHNV; Ranavirus; IPNV;SVCV

23	**10/10**	20-11-2017	VHSV	IHNV	VHSV	ECV/ESV	Negative
24	**8/10**	16-11-2017	VHSV	IHNV	VHSV	EHNV	Negative
25	**10/10**	13-11-2017	VHSV	IHNV	VHSV	Ranavirus (ECV)	no VHSV, no IHNV, no IPNV, no ECV, no EHNV, no SVCV, no PFRV
26	**10/10**	16-11-2017	VHSV	IHNV	VHSV	ECV/ESV	NEGATIVE
27	**10/10**	20-11-2017	VHSV	IHNV	VHSV	Ranavirus(European catfish virus)	Negative
28	**10/10**	20-11-2017	VHSV	IHNV	VHSV	ECV	Negative
29	**10/10**	14-11-2017	VHSV	IHNV	VHSV	Ranavirus ECV	NO VIRUS
30	**10/10**	20-11-2017	VHSV	IHNV	VHSV	ECV/ESV	Negative (no virus)
31	**10/10**	15-11-2017	VHSV	IHNV	VHSV	ECV	none
32	**10/10**	20-11-2017	VHSV	IHNV	VHSV	ECV	STERILE
33	**10/10**	20-11-2017	VHSV	IHNV	VHSV	Ranavirus was identified by conventional RT-PCR and than REA was applied as given by OIE manuel to identified ECV	Virus was not detected
34	**10/10**	17-11-2017	VHSV	IHNV	VHSV	Catfish/ sheetfish virus	Negative
35	**10/10**	14-11-2017	VHSV	IHNV	VHSV	ECV/ESV	Negative
36	**10/10**	17-11-2017	VHSV	IHNV	VHSV	Ranavirus (see sequencing result)	Negative
37	**10/10**	20-11-2017	VHSV	IHNV	VHSV	European sheatfish virus	not VHSV, IHNV, EHNV, IPNV, or SVCV
38	**10/10**	19-11-2017	VHSV	IHNV	VHSV	Ranavirus (ECV/ESV)	Negative
39	**10/10**	14-11-2017	VHSV	IHNV	VHSV	ECV	no virus detected
40	**10/10**	15-11-2017	VHS	IHN	VHS	Ranavirus ECV/ESV	No virus growth
41[1]	**8/8**	20-11-2017	VHSV	IHNV	VHSV	NO IHNV,VHSV, SVCV,IPNV	NO CPE
42[2]	**9/10**	20-11-2017	VHSV	IHNV	VHSV	RANA VIRUS	no virus
43	**10/10**	24-11-2017	VHSV	IHNV	VHSV	ECV	Neg
44	**10/10**	16-11-2017	VHSV	IHNV	VHSV	ECV/ESV	Negative for VHSV, IHNV, EHNV, Ranavirus, IPNV and SVCV
45	**10/10**	16-11-2017	VHSV	IHNV	VHSV	ECV	

1) Do not test for Ranavirus

2) Did not corroborate the findings in ampoule IV by sequencing or REA

Table 4. Inter-Laboratory Proficiency Test, PT1, 2017 – Results of titration of **ampoule I**.

VHSV DK-6137					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	VHSV	2,7E+06	2,7E+07	2,7E+07	1,9E+07
2	VHSV	1,3E+05	1,3E+07	N/A	N/A
3	VHSV	1,3E+06	1,9E+07	N/A	N/A
4	VHSV genotype Ia	5,9E+05	1,9E+07	N/A	N/A
5	VHSv	4,0E+07	2,7E+07	5,9E+06	N/A
6	VHSV	5,9E+05	1,3E+07	4,0E+05	N/A
7	VHSV	2,7E+05	5,9E+06	N/A	N/A
8	VHSV	8,6E+04	1,9E+06	N/A	N/A
9	VHSV	4,0E+05	2,7E+06	N/A	N/A
10	VHSV	N/A	N/A	N/A	N/A
11	VHSV	1,9E+06	5,9E+06	N/A	N/A
12	VHSV	2,7E+06	8,6E+06	N/A	N/A
13	VHSV	4,0E+05	5,9E+05	1,3E+06	2,7E+04
14	VHSV	1,3E+06	1,9E+06	N/A	N/A
15	VHSV	N/A	8,6E+06	8,6E+06	N/A
16	VHSV	1,9E+06	8,6E+06	N/A	N/A
17	VHSV viable virus	8,6E+05	1,3E+07	N/A	N/A
18	VHSV	1,3E+06	1,3E+07	N/A	N/A
19	VHSV	1,3E+07	1,3E+04	N/A	2,7E+06
20	VHSV & SVCV	N/A	5,9E+09	1,3E+03	N/A
21	VHSV	1,9E+06	2,7E+06	1,3E+06	4,0E+06
22	VHSV	1,9E+03	1,9E+06	N/A	N/A
23	VHSV	1,9E+04	1,9E+03	N/A	N/A
24	VHSV	2,7E+07	8,6E+07	N/A	N/A
25	VHSV	2,7E+04	5,9E+04	2,7E+04	1,3E+03
26	VHSV	4,0E+04	2,7E+06	N/A	N/A
27	VHSV	1,9E+06	5,9E+06	1,3E+06	1,9E+06
28	VHSV	N/A	4,0E+04	N/A	8,6E+03
29	VHSV	8,6E+05	2,7E+07	2,7E+06	1,9E+07
30	VHSV	1,3E+06	N/A	N/A	4,0E+06
31	VHSV	1,3E+06	2,7E+07	N/A	N/A
32	VHSV	5,9E+06	N/A	N/A	2,7E+07
33	VHSV	4,0E+06	4,0E+07	N/A	N/A
34	VHSV	5,9E+06	5,9E+07	N/A	N/A

35	VHSV	8,6E+05	N/A	N/A	8,6E+07
36	VHSV	8,6E+04	1,3E+06	N/A	5,9E+06
37	VHSV	2,7E+04	1,3E+07	4,0E+05	< 1,9E+02
38	VHSV	2,7E+06	5,9E+06	4,0E+06	N/A
39	VHSV	4,0E+05	2,7E+06	N/A	N/A
40	VHS	8,6E+04	8,6E+06	< 1,9E+02	N/A
41	VHSV	1,9E+05	1,9E+06	N/A	N/A
42	VHSV	N/A	5,9E+05	N/A	N/A
43	VHSV	1,9E+06	4,0E+06	8,6E+05	2,7E+06
44	VHSV	8,6E+04	4,0E+04	N/A	N/A
45	VHSV	N/A	2,7E+06	2,7E+06	8,6E+05

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	39	41	15	15
Median titre	8,6E+05	5,9E+06	1,3E+06	2,7E+06
Maximum titre	4,0E+07	5,9E+09	2,7E+07	8,6E+07
Minimum titre	1,9E+03	1,9E+03	<1,9E+02	<1,9E+02
25% quartile titre	1,6E+05	1,9E+06	4,0E+05	4,4E+05
75% quartile titre	1,9E+06	1,3E+07	3,4E+06	1,2E+07

Table 5. Inter-Laboratory Proficiency Test, PT1, 2017 – Results of titration of **ampoule II**

IHNV 217/A					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	IHNV	< 1,9E+02	5,9E+05	1,9E+05	1,3E+05
2	IHNV	1,9E+04	8,6E+05	N/A	N/A
3	IHNV	4,0E+03	5,9E+05	N/A	N/A
4	IHNV genogroup M	2,7E+03	4,0E+06	N/A	N/A
5	IHNv	1,9E+03	4,0E+06	8,6E+04	N/A
6	IHNV	< 1,9E+02	1,9E+06	8,6E+03	N/A
7	IHNV	< 1,9E+02	4,0E+06	N/A	N/A
8	IHNV	< 1,9E+02	2,7E+02	N/A	N/A
9	IHNV	8,6E+02	8,6E+04	N/A	N/A
10	IHNV	N/A	N/A	N/A	N/A
11	IHNV	4,0E+03	2,7E+06	N/A	N/A
12	IHNV	2,7E+03	1,3E+06	N/A	N/A
13	IHNV	< 1,9E+02	1,3E+06	4,0E+05	5,9E+03
14	IHNV	< 1,9E+02	4,0E+05	N/A	N/A
15	IHNV	N/A	1,3E+05	1,9E+06	N/A
16	IHNV	1,3E+05	1,3E+06	N/A	N/A
17	INHV viable virus	N/A	8,6E+05	N/A	5,9E+05
18	IHNV	4,0E+04	4,0E+06	N/A	N/A
19	IHNV	5,9E+05	< 1,9E+02	N/A	4,0E+02
20	IHNV	N/A	1,3E+06	< 1,9E+02	N/A
21	IHNV	1,3E+03	1,3E+05	8,6E+05	1,3E+06
22	IHNV	< 1,9E+02	1,9E+06	N/A	N/A
23	IHNV	4,0E+03	1,3E+03	N/A	N/A
24	IHNV	8,6E+05	4,0E+06	N/A	N/A
25	IHNV	1,3E+04	1,3E+04	1,3E+03	1,3E+03
26	IHNV	< 1,9E+02	4,0E+04	N/A	N/A
27	IHNV	2,73E+04	8,62E+04	5,87E+04	8,62E+04
28	IHNV	N/A	1,86E+04	N/A	4,00E+03
29	IHNV	1,3E+04	1,9E+06	1,3E+05	2,7E+06
30	IHNV	< 1,9E+02	N/A	N/A	2,7E+05
31	IHNV	2,7E+06	1,3E+07	N/A	N/A
32	IHNV	2,7E+03	N/A	N/A	8,6E+04
33	IHNV	< 1,9E+02	1,3E+07	N/A	N/A
34	IHNV	2,7E+04	5,9E+06	N/A	N/A
35	IHNV	1,3E+05	N/A	N/A	4,0E+06

36	IHNV	1,3E+03	4,0E+05	N/A	5,9E+05
37	IHNV	< 1,9E+02	8,6E+05	1,9E+06	< 1,9E+02
38	IHNV	4,0E+04	8,6E+05	2,7E+05	N/A
39	IHNV	1,9E+03	1,3E+06	N/A	N/A
40	IHN	1,3E+03	1,9E+05	< 1,9E+02	N/A
41	IHNV	< 1,9E+02	8,62E+04	N/A	N/A
42	IHNV	N/A	1,3E+03	N/A	N/A
43	IHNV	< 1,9E+02	1,9E+05	4,0E+04	8,6E+04
44	IHNV	1,3E+03	4,0E+04	N/A	N/A
45	IHNV	N/A	2,7E+05	5,9E+04	8,6E+04

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

IHNV 217/A	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	38	41	15	16
Median titre	1,9E+03	8,6E+05	8,6E+04	8,6E+04
Maximum titre	2,7E+06	1,3E+07	1,9E+06	4,0E+06
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	8,6E+04	2,4E+04	5,4E+03
75% quartile titre	1,7E+04	1,9E+06	3,4E+05	5,9E+05

Table 6. Inter-Laboratory Proficiency Test, PT1, 2017 – Results of titration of **ampoule III**.

VHSV TR-WS13G					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	VHSV	1,3E+06	1,3E+05	1,9E+03	8,6E+06
2	VHSV	5,9E+05	4,0E+06	N/A	N/A
3	VHSV + Ranavirus	2,7E+06	1,9E+06	N/A	N/A
4	VHSV genotype Ie	1,9E+06	1,3E+05	N/A	N/A
5	VHSv	4,0E+07	2,7E+05	< 1,9E+02	N/A
6	VHSV	5,9E+05	1,3E+06	< 1,9E+02	N/A
7	VHSV	2,7E+05	4,0E+05	N/A	N/A
8	VHSV	4,0E+05	5,9E+05	N/A	N/A
9	VHSV	1,3E+05	2,7E+05	N/A	N/A
10	VHSV	N/A	N/A	N/A	N/A
11	VHSV	4,0E+06	1,9E+06	N/A	N/A
12	VHSV	5,9E+06	2,7E+05	N/A	N/A
13	VHSV	4,0E+05	2,7E+04	1,9E+02	4,0E+04
14	VHSV	8,6E+06	4,0E+05	N/A	N/A
15	VHSV	N/A	1,3E+05	2,7E+04	N/A
16	VHSV	5,9E+06	2,7E+05	N/A	N/A
17	VHSV viable virus	1,86E+06	8,62E+05	N/A	N/A
18	VHSV	1,3E+06	5,9E+05	N/A	N/A
19	VHSV	8,6E+03	4,0E+02	N/A	8,6E+04
20	VHSV	N/A	1,3E+05	< 1,9E+02	N/A
21	VHSV	4,0E+06	1,9E+06	1,9E+06	1,3E+06
22	VHSV	2,7E+03	1,3E+06	N/A	N/A
23	VHSV	2,7E+05	1,9E+03	N/A	N/A
24	VHSV	1,9E+06	4,0E+06	N/A	N/A
25	VHSV	5,9E+04	1,3E+05	1,3E+04	5,9E+04
26	VHSV	4,0E+04	5,9E+03	N/A	N/A
27	VHSV	1,26E+04	1,86E+04	< 1,9E+02	2,73E+03
28	VHSV	N/A	2,73E+04	N/A	1,86E+03
29	VHSV	4,0E+06	8,6E+05	4,0E+06	1,3E+05
30	VHSV	1,9E+06	N/A	N/A	1,9E+06
31	VHSV	8,6E+06	1,9E+06	N/A	N/A
32	VHSV	1,3E+07	N/A	N/A	5,9E+06
33	VHSV	2,7E+06	2,7E+05	N/A	N/A

34	VHSV	2,7E+06	1,3E+06	N/A	N/A
35	VHSV	1,9E+06	N/A	N/A	4,0E+06
36	VHSV	2,7E+05	2,7E+04	N/A	8,6E+04
37	VHSV	8,6E+04	4,0E+04	1,3E+03	< 1,9E+02
38	VHSV	2,7E+06	1,9E+05	8,6E+02	N/A
39	VHSV	1,3E+06	1,3E+05	N/A	N/A
40	VHS	1,9E+04	4,0E+04	< 1,9E+02	N/A
41	VHSV	4,00E+06	1,26E+04	N/A	N/A
42	VHSV	N/A	5,9E+04	N/A	N/A
43	VHSV	1,9E+06	2,7E+05	< 1,9E+02	2,7E+05
44	VHSV	5,9E+05	5,9E+04	N/A	N/A
45	VHSV	N/A	1,9E+05	4,0E+04	5,9E+04

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

VHSV TR-WS13G	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	39	41	15	15
Median titre	1,9E+06	2,7E+05	8,6E+02	8,6E+04
Maximum titre	4,0E+07	4,0E+06	4,0E+06	8,6E+06
Minimum titre	2,7E+03	4,0E+02	<1,9E+02	<1,9E+02
25% quartile titre	2,7E+05	5,9E+04	<1,9E+02	4,9E+04
75% quartile titre	3,4E+06	8,6E+05	2,0E+04	1,6E+06

Table 7. Inter-Laboratory Proficiency Test, PT1, 2017 – Results of titration of **ampoule IV**.

ECV 562/92					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	Ranavirus, not EHNV	5,9E+05	1,3E+06	2,7E+05	1,3E+03
2	no IHNV , VHSV, IPNV, SVCV	5,9E+07	1,3E+05	N/A	N/A
3	Ranavirus	1,3E+06	2,7E+06	N/A	N/A
4	ECV	2,7E+06	2,7E+06	N/A	N/A
5	ECV	4,0E+07	2,7E+05	1,9E+05	N/A
6	Ranavirus (ECV/ESV)	1,3E+07	2,7E+06	1,3E+03	N/A
7	RANAVIRUS (ECV)	1,3E+05	4,0E+06	N/A	N/A
8	Ranavirus	4,0E+06	1,9E+06	N/A	N/A
9	ECV	8,6E+03	1,3E+03	N/A	N/A
10	ND	N/A	N/A	N/A	N/A
11	ECV	2,7E+07	4,0E+05	N/A	N/A
12	ECV	4,0E+06	4,0E+06	N/A	N/A
13	Ranavirus	5,9E+06	8,6E+05	8,6E+03	1,9E+05
14	EHNV	4,0E+05	8,6E+04	N/A	N/A
15	EHNV	N/A	2,7E+04	4,0E+03	N/A
16	Ranavirus	1,9E+07	1,9E+06	N/A	N/A
17	ECV (rana like) viable virus	8,6E+05	1,9E+05	N/A	N/A
18	Ranavirus	1,3E+07	1,9E+06	N/A	N/A
19	Ranavirus(ECV)	< 1,9E+02	8,6E+04	N/A	< 1,9E+02
20	ECV	N/A	5,9E+09	< 1,9E+02	N/A
21	ECV	4,0E+04	8,6E+06	1,9E+06	1,3E+08
22	Ranavirus (Non-EHNV)	2,7E+06	4,0E+06	N/A	N/A
23	ECV/ESV	1,9E+05	5,9E+03	N/A	N/A
24	EHNV	4,0E+06	1,9E+07	N/A	N/A
25	Ranavirus (ECV)	1,3E+06	2,7E+05	5,9E+04	1,3E+04
26	ECV/ESV	2,7E+06	5,9E+06	N/A	N/A
27	Ranavirus(European catfish virus)	1,3E+05	2,73E+03	1,3E+03	1,3E+03
28	ECV	N/A	5,87E+03	N/A	1,9E+03
29	Ranavirus ECV	1,9E+07	1,3E+07	2,7E+07	4,0E+06
30	ECV/ESV	1,9E+07	N/A	N/A	1,3E+03
31	ECV	4,0E+08	1,9E+08	N/A	N/A
32	ECV	1,3E+06	N/A	N/A	8,6E+03
33	Ranavirus was identified	4,0E+07	2,7E+07	N/A	N/A

	by conventional RT-PCR and than REA was applied as given by OIE manuel to identified ECV				
34	Catfish/sheetfish virus	2,7E+08	5,9E+07	N/A	N/A
35	ECV/ESV	4,0E+05	N/A	N/A	8,6E+04
36	Ranavirus (see sequencing result)	1,3E+05	5,9E+05	N/A	1,9E+05
37	European sheatfish virus	5,9E+06	1,3E+07	2,7E+06	< 1,9E+02
38	Ranavirus (ECV/ESV)	5,9E+06	1,9E+07	1,3E+04	N/A
39	ECV	5,9E+05	1,3E+06	N/A	N/A
40	Ranavirus ECV/ESV	1,9E+07	4,0E+05	< 1,9E+02	N/A
41	NO IHNV,VHSV,SVCV,IPNV	1,3E+07	1,26E+06	N/A	N/A
42	RANA VIRUS	N/A	5,9E+04	N/A	N/A
43	ECV	1,3E+07	1,3E+06	< 1,9E+02	1,3E+04
44	ECV/ESV	2,7E+07	5,9E+05	N/A	N/A
45	ECV	N/A	4,0E+04	1,3E+05	8,6E+04

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	39	41	15	15
Median titre	4,0E+06	1,3E+06	1,3E+04	1,3E+04
Maximum titre	4,0E+08	5,9E+09	2,7E+07	1,3E+08
Minimum titre	<1,9E+02	1,3E+03	<1,9E+02	<1,9E+02
25% quartile titre	5,9E+05	1,9E+05	1,3E+03	1,3E+03
75% quartile titre	1,9E+07	4,0E+06	2,3E+05	1,4E+05

Table 8. Inter-Laboratory Proficiency Test, PT1, 2017 – Results of titration of **ampoule V**.

Blank, BF-2 cell supernatant					
Laboratory Code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
1	Not VHSV, not IHNV, and not ranavirus (see comment)	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
2	negative	< 1,9E+02	< 1,9E+02	N/A	N/A
3	No CPE	< 1,9E+02	< 1,9E+02	N/A	N/A
4	No virus	< 1,9E+02	< 1,9E+02	N/A	N/A
5	negative for VHSV, IHNV, EHNV, Ranavirus, IPNV and SVCV	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
6	negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
7	NO VHSV,NO IHNV,NO EHNV,NO RANAVIRUS,NO IPNV,NO SVC	< 1,9E+02	< 1,9E+02	N/A	N/A
8	NEGATIVE	< 1,9E+02	< 1,9E+02	N/A	N/A
9	No virus detected	< 1,9E+02	< 1,9E+02	N/A	N/A
10	ND	N/A	N/A	N/A	N/A
11	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
12	NEG	< 1,9E+02	< 1,9E+02	N/A	N/A
13	-	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
14	no VHSV/ no IHNV/no EHNV/ no IPNV/ no SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
15	NEGATIVE	N/A	< 1,9E+02	< 1,9E+02	N/A
16	No virus was detected in this ampoule	< 1,9E+02	< 1,9E+02	N/A	N/A
17	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
18	No virus detected	< 1,9E+02	< 1,9E+02	N/A	N/A
19	BLANK	< 1,9E+02	< 1,9E+02	N/A	< 1,9E+02
20	-	N/A	< 1,9E+02	< 1,9E+02	N/A
21	-	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
22	Negative for: VHSV; IHNV;EHNV; Ranavirus;IPNV;SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
23	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
24	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
25	no VHSV, no IHNV, no IPNV, no ECV, no EHNV, no SVCV, no PFRV	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
26	NEGATIVE	< 1,9E+02	< 1,9E+02	N/A	N/A
27	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
28	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02

29	NO VIRUS	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
30	Negative (no virus)	< 1,9E+02	N/A	N/A	< 1,9E+02
31	none	< 1,9E+02	< 1,9E+02	N/A	N/A
32	STERILE	< 1,9E+02	N/A	N/A	< 1,9E+02
33	Visus was not detected	< 1,9E+02	< 1,9E+02	N/A	N/A
34	Negative	< 1,9E+02	< 1,9E+02	N/A	N/A
35	Negative	< 1,9E+02	N/A	N/A	< 1,9E+02
36	Negative	< 1,9E+02	< 1,9E+02	N/A	< 1,9E+02
37	not VHSV, IHNV, EHNV, IPNV, or SVCV	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
38	Negative	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
39	no virus detected	< 1,9E+02	< 1,9E+02	N/A	N/A
40	No virus growth	< 1,9E+02	< 1,9E+02	< 1,9E+02	N/A
41	NO CPE	< 1,9E+02	< 1,9E+02	N/A	N/A
42	no virus	N/A	< 1,9E+02	N/A	N/A
43	Neg	< 1,9E+02	< 1,9E+02	< 1,9E+02	< 1,9E+02
44	Negative for VHSV, IHNV, EHNV, Ranavirus, IPNV and SVCV	< 1,9E+02	< 1,9E+02	N/A	N/A
45		N/A	< 1,9E+02	< 1,9E+02	< 1,9E+02

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

Blank, BF-2 cell supernatant	**BF-2**	**EPC**	**RTG-2**	**FHM**
Number of laboratories	40	41	16	15
Median titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
Maximum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
Minimum titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
75% quartile titre	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02

Figure 5. Virus titres obtained in BF-2 cells. The titre (red diamond) of each participating laboratory (country code) using BF-2 cells illustrated for ampoule I, II, III, IV and V. The detection level (grey shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line) are plotted on all graphs. For participants failing to obtain any titre no red diamond is shown.

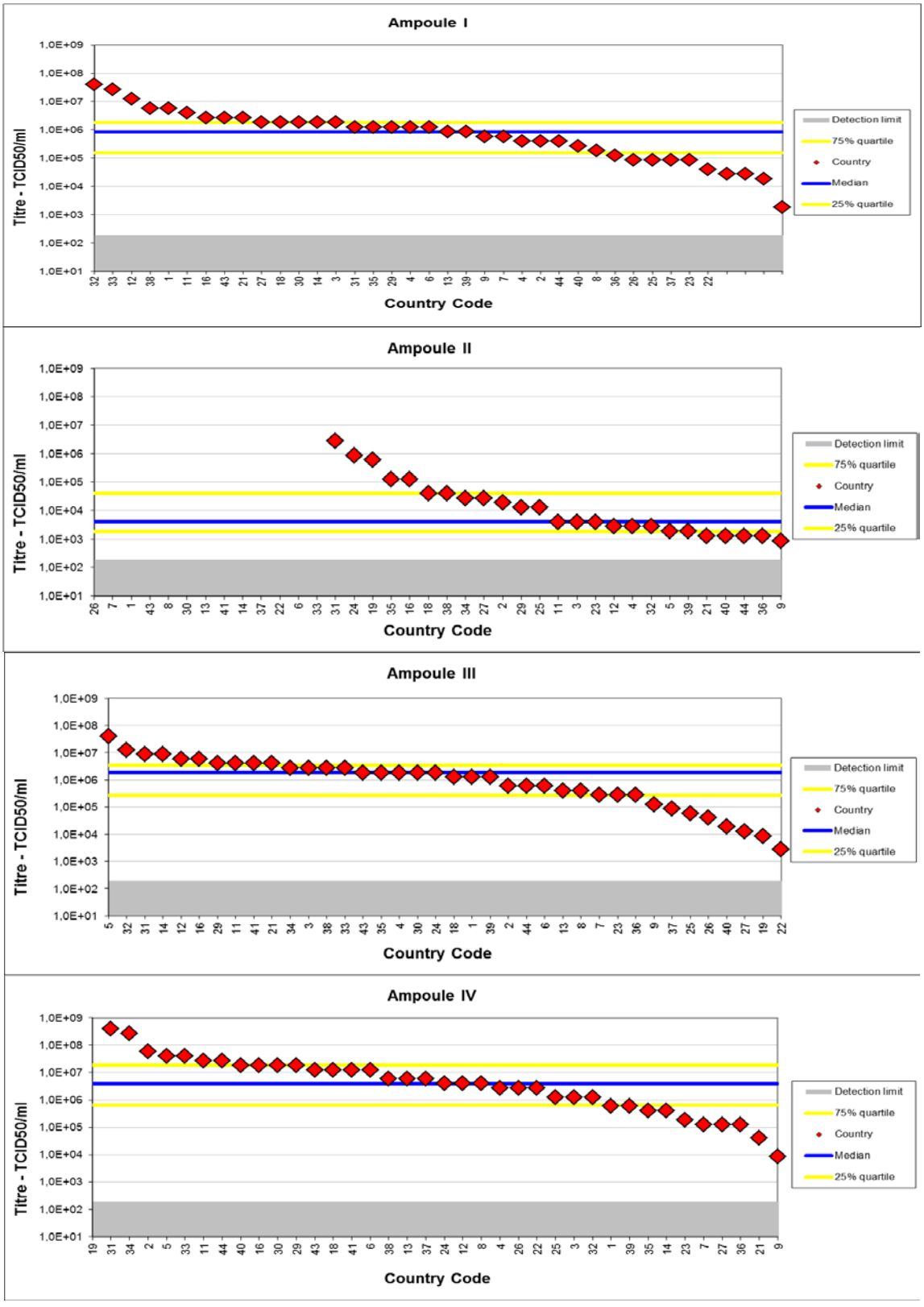


Figure 6. Virus titres obtained in EPC cells. For further details see description at Figure 5

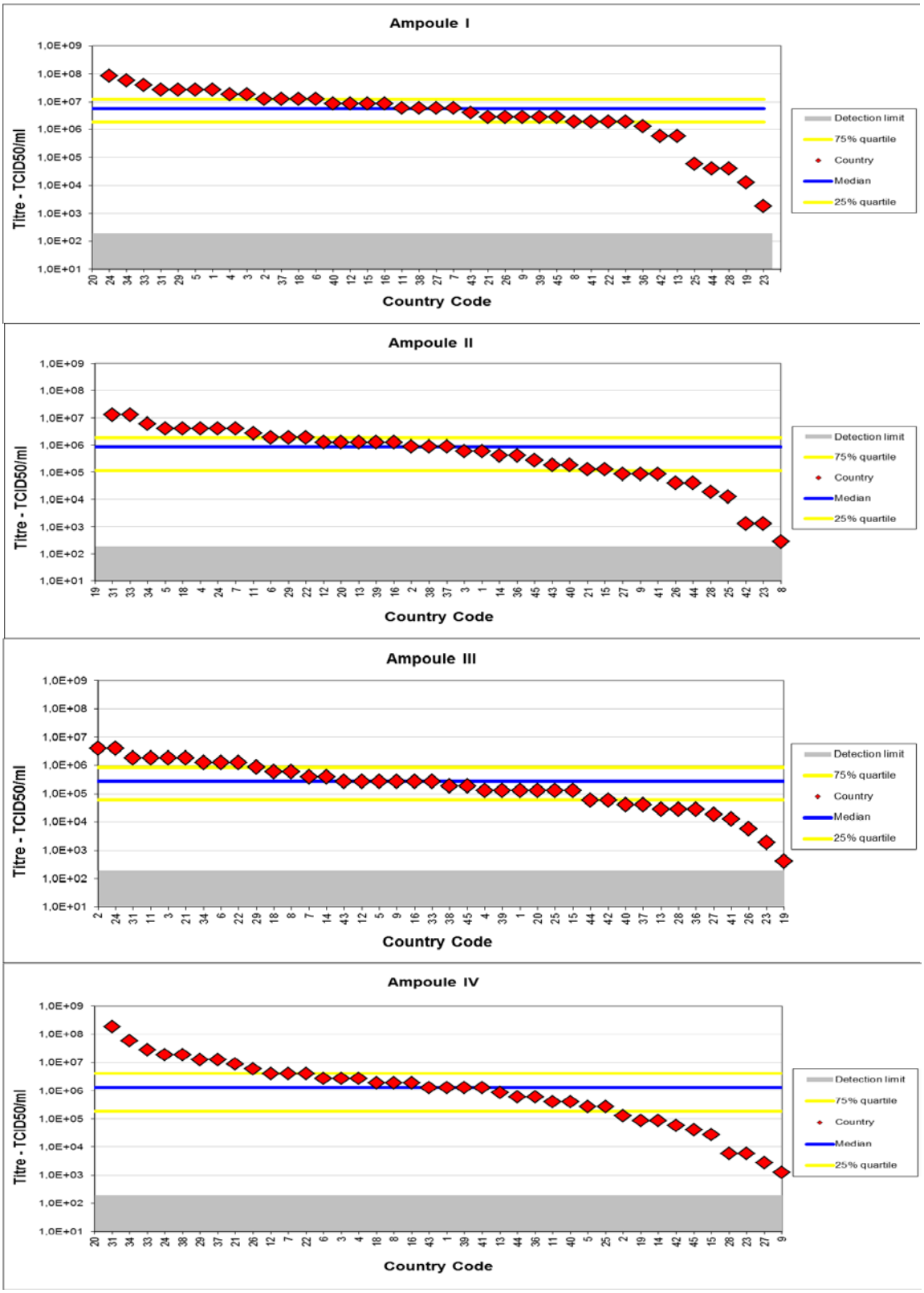


Figure 7. Virus titre obtained in RTG-2 cells. For further details see description at Figure 5

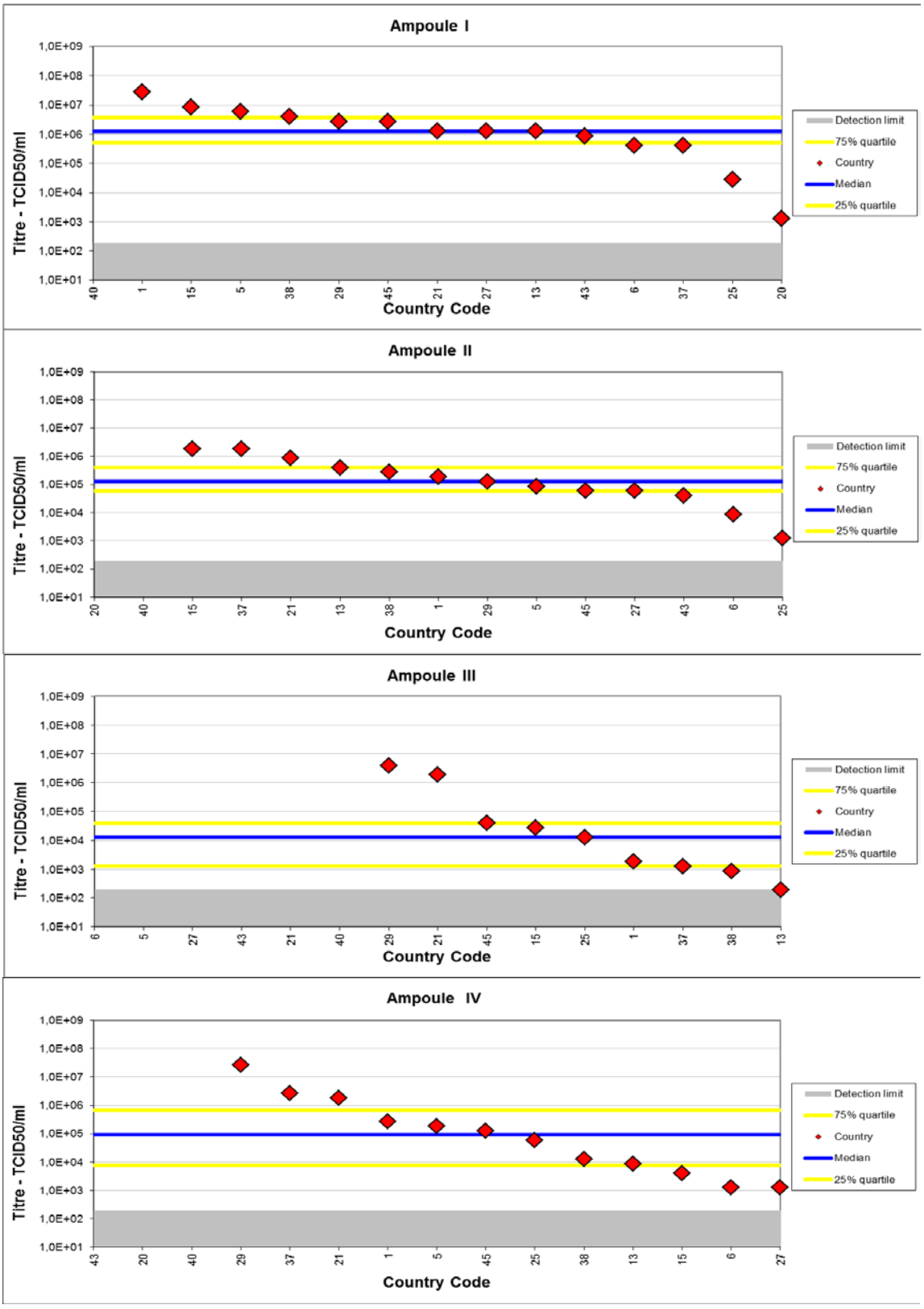
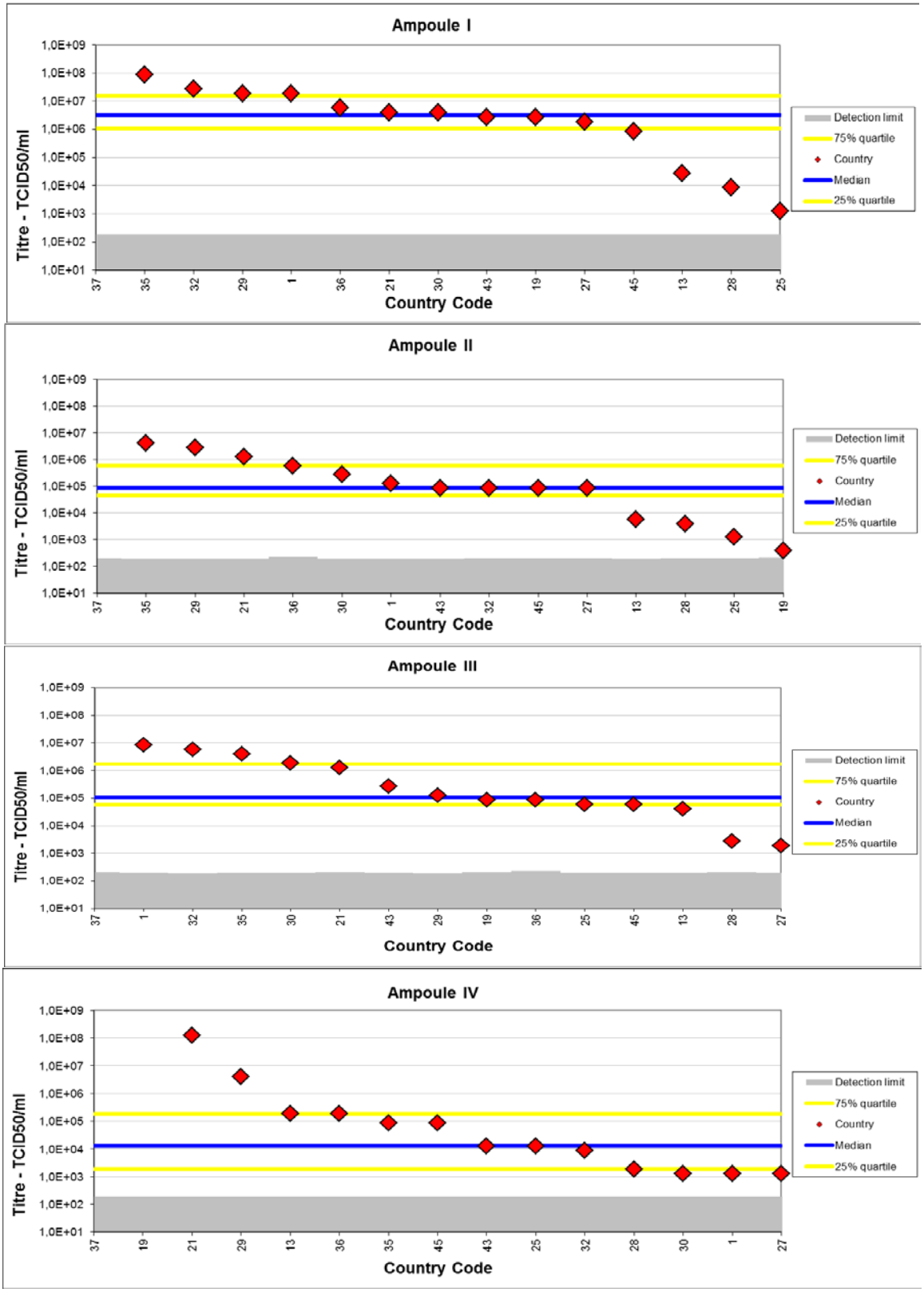


Figure 8. Virus titres obtained in FHM cells. For further details see description at Figure 5



Identification of content

- 42 laboratories analysed for all viruses; 36 of these laboratories correctly identified all viruses in all ampoules
- 39 participants submitted the spreadsheet within the deadline, 2 participants delivered the results for ampoule content within the deadline but needed a small extension to provide sequencing results. 3 participant needed 2-3 weeks extension due to delivery problems.

Ampoule I – VHSV Ia (DK-6137)

- 44 laboratories correctly identified VHSV
- 1 laboratory correctly identified VHSV in Ampoule I but contaminated the ampoule with an SVCV which was not included in the PT 2017 panel.

Ampoule II – IHNV E (217/A)

- All 45 laboratories correctly identified IHNV

Ampoule III – VHSV Ie (TR-WS13G)

- 44 laboratories correctly identified VHSV
- 1 laboratory correctly identified VHSV in Ampoule III but contaminated the ampoule with another virus (Ranavirus) which was included in the PT 2017 panel.

Ampoule IV – ECV (562/92)

- 38 laboratories correctly identified the isolate as ranavirus and not as the listed EHNV by sequencing or REA (restriction enzyme analysis)
- 1 laboratory identified Ranavirus but did not specify if the isolate was the listed EHNV or not by sequencing or REA
- 1 laboratory identified EHNV
- 2 laboratories answered EHNV even though the sequence showed ECV
- 3 laboratories do not test for Ranavirus.

Ampoule V – Blank (Sterile cell supernatant from BF-2 cells)

- All 45 laboratories correctly did not isolated any virus in ampoule V

Scores

Starting with proficiency test 2003 we have provided a scoring system for the identification part of the proficiency tests. We have assigned a score of 2 for each correct answer/identification, giving the possibility for obtaining a maximum score of 10 (Table 3).

Ampoule I: VHSV identification was given the score 2. Incorrectly finding of “no virus” or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule II: IHNV identification was given the score 2. Incorrectly finding of “no virus” or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule III: VHSV identification was given the score 2. Incorrectly finding of “no virus” or other type of viruses than the one included in the ampoule scored 0. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule IV: ECV identification was given the score 2. The answer Ranavirus with no identification was given the score 1. Incorrectly finding of "no virus", EHNV or other type of viruses than the one included in the ampoule scored 0. The answering EHNV with sequencing showing ECV was given the score 1. Finding of additional type of viruses scored 0 if the contamination was with a listed pathogen, and 1 with a non-listed one.

Ampoule V: Identification "no virus", "Blank", "Not IHNV, not VHSV, not IPNV, not SVCV, not EHNV" or similar answer was given the score 2. Incorrectly findings of virus in the ampoule was given the score 0.

Out of 45 laboratories participating in the PT 1 2017, 36 obtained score 10/10, 3 obtained a score of 8/8 due to no testing for Ranavirus, 2 obtained 9/10 due to answering EHNV even though the sequence showed ECV, 1 obtained 9/10 due to answering Ranavirus without sequencing, 2 obtained 9/10 due to contamination with a non-listed virus and 1 obtained 8/10 due to answering EHNV without sequencing.

Cells applied for solving the test

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

- 39 laboratories used BF-2 cells
- 41 laboratories used EPC cells
- 15 laboratories used RTG-2 cells
- 15 laboratories used FHM cells
- 6 laboratories used CHSE-214 cells

- 1 laboratory used five cell lines: BF-2, EPC, RTG-2, FHM and CHSE-214

- 10 laboratories used four cell lines:
  - 7 laboratories used BF-2, EPC, RTG-2 and FHM
  - 3 laboratory used BF-2, EPC, RTG-2 and CHSE-214

- 3 laboratories used tree cell lines:
  - 2 laboratories used BF-2 cells in combination with EPC cells and FHM cells
  - 1 laboratory used RTG-2 cells in combination with EPC cells and FHM cells

- 29 laboratories used two cell lines:
  - 23 laboratories used BF-2 cells in combination with EPC cells
  - 2 laboratories used RTG-2 cells in combination with EPC cells
  - 3 laboratories used BF-2 cells in combination with FHM cells
  - 1 laboratory used EPC cells in combination with FHM cells

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

The combination of EPC and FHM cells or BF-2 and RTG 2 is not valid according to Commission Decision 2015-1554. The laboratories using these combinations are encouraged to include the use of BF-2 cells and EPC or FHM.

The median titre calculated from the submitted results of each ampoule and in each cell line is illustrated in Figure 9.
It appears that:
Ampoule I (VHSV, DK-6137) replicates equally well on all four cell lines (EPC, BF-2, FHM and RTG-2).
Ampoule II (IHNV, 217/A) replicate on all four cell lines, however it grows best on EPC cells, a little less efficiently on FHM and RTG-2 and poorly on BF-2.
Ampoule III (VHSV, TR-WS13G) replicates on all four cell lines, however it grows best on BF-2 cells and poorly on RTG-2.
Ampoule IV (ECV) replicates well on BF-2 and EPC and a little less efficiently on FHM and RTG-2.
Ampoule V (Blank) did not replicate on any of the cell lines.
As it appears from Table 3-8 the variations in titres between laboratories was very high – with more than 8 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved.

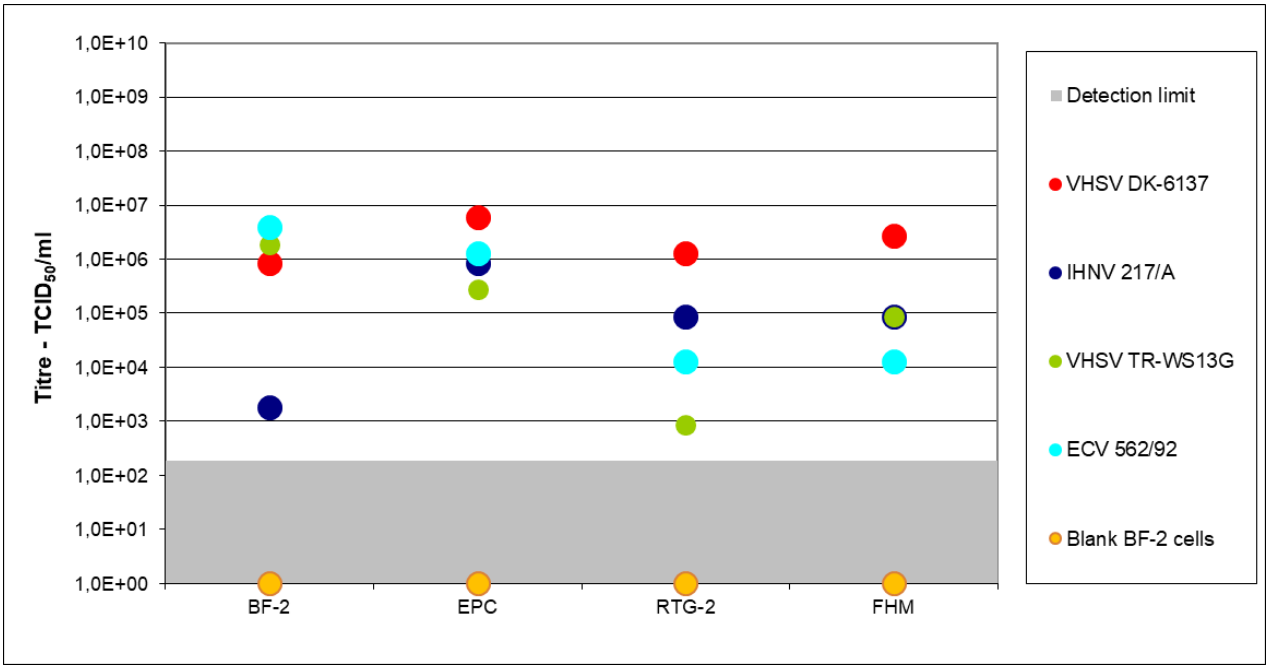


Figure 9. Median virus titres obtained by the participants in 4 different cell lines.

Genotyping and sequencing

In previous proficiency tests provided by the EURL, we have encouraged participants to genotype the identified viruses though it has not been a mandatory task. As ranaviruses are included in the test, it is mandatory to do sequence or REA analysis in order to discriminate EHNV from the non-listed ranaviruses. For IHNV and VHSV we still encouraged participants to genotype isolates according to the notification described in Einer-Jensen et *al.* (2004) for VHSV and in Kurath et *al.* (2003) and Emmenegger et *al.*, 2000 for IHNV.

An Overview of the genotyping results obtained for PT1 by all participants is displayed in the following table 9

Tabel 9. The genotyping results obtained for PT1 by all participants

Laboratory code number	Ampoule I	Ampoule II	Ampoule III	Ampoule IV
	VHSV DK-6137 Genotype Ia	IHNV 217/A Genotype M	VHSV TR-WS13G Genotype Ie	ECV 562/92
1	1a	*	1e	*
2	No sequence provided	No sequence provided	No sequence provided	Do not test for Ranavirus
3	1a	E	1e	*
4	Ia	M	Ie	*
5	Ia	E	Ie	*
6	Europe Ia	Europe E	Europe Ie	*
7	No sequence provided	No sequence provided	No sequence provided	*
8	*		*	*
9	No sequence provided	BLAST analysis of the sequence obtained showed highest sequence identity with accession number: FJ265710.1	No sequence provided	BLAST analysis of the sequence obtained showed highest sequence identity with accession number: KT989884.1
10	No sequence provided	No sequence provided	No sequence provided	Do not test for Ranavirus
11	1e	M	1a	*
12	Ia1	E	Ie	ECV
13	Ia	*	Ie	ECV, ESV
14	*	*	*	*
15	*	*	*	*
16	1a	M	1e	Not EHNV
17	1a	M	3	ECV
18	Ia	E (M)	Ie	Cod iridovirus, ECV or ESV
19	*	*	*	*
20	VHSV:Ia SVCV:Ia	M	Ib	ECV
21	*	*	*	*
22	No sequence provided	No sequence provided	No sequence provided	*
23	No sequence provided	No sequence provided	No sequence provided	*
24	No sequence provided	No sequence provided	No sequence provided	No sequence provided
25	genotype Ia	genogroup M	genotype Ie	*
26	*	*	*	*
27	Ia	European isolates (E–1–I–1)	Ie	*
28	Genotype Ia	Genotype E	Genotype Ie	*

		(Europe)		
29	Ia genotype	M genogroup	Ie genotype	*
30	Genotype 1a	Genotype J	Genotype 1e	*
31	Ia	M	Ie	*
32	No sequence provided	No sequence provided	No sequence provided	*
33	No sequence provided	No sequence provided	No sequence provided	*
34	Ia	*	Genotype Ie	*
35	1a	M	1e	Not EHNV
36	No sequence provided	*	No sequence provided	European catfish virus (same virus as European sheatfish virus)
37	I	*	I	*
38	VHSV Genotype Ia	IHNV Genotype M	VHSV Genotype Ie	ECV/ESV
39	Ia	M	Ie	European, Italy, Hungary
40	No sequence provided	No sequence provided	No sequence provided	ECV/ESV
41	No sequence provided	No sequence provided	No sequence provided	Do not test for Ranavirus
42	No sequence provided	No sequence provided	No sequence provided	No sequence provided
43	Ia	E	Ie	*
44	*	*	Ie	*
45	I - Ia	M	I - Ie	*
No. of sequence preformed	30	33	33	38
No. of correct genotyping	22	20	22	36 (38)

*Sequence provided but no genotyping assigned under Genotype in the spreadsheet for Sequencing results – Proficiency Test 1

AMPOULE I – VHSV DK-6137
30 laboratories sequenced the isolate in ampoule I.
22 laboratories correctly genotyped the isolate in ampoule I as genotype Ia, 1 laboratory identified the isolate as genotype 1, 6 laboratories did not indicate the genotype and 1 indicate the wrong genotype.

AMPOULE II- IHNV 217/A
33 laboratories sequenced IHNV isolate in Ampoule II; 20 correctly identified the isolate as belonging to genogroup M/E, 1 laboratory identified the isolate as genotype J, 12 laboratories sequenced but did not indicate a genotype.

AMPOULE III- VHSV TR-WS13G
30 laboratories sequenced the isolate in ampoule III. Out of these 26 indicated a genotype.
22 correctly identified the isolate as genotype Ie, 1 indicated generically genotype I, 1 indicated genotype Ib, 1 laboratory indicated genotype Ia, and one indicated the isolate as belonging to genotype III.

AMPOULE IV – ECV 562/92
38 laboratories sequenced ECV in ampoule IV. 36 laboratories correctly identified the isolate as ranavirus - NOT EHNV, or ECV/ESV. 2 laboratories identified the isolate as EHNV, despite they provided the correct sequence.

AMPOULE V – Blank.
No sequence

Résumé and concluding remarks PT1

40% of parcels were delivered by the shipping companies within 1 day after submission, 80% was delivered within 1 week and 91% was delivered within 2 week. The remaining four parcels took longer for delivery primarily due to border controls, the maximum time of shipment was 59 days.

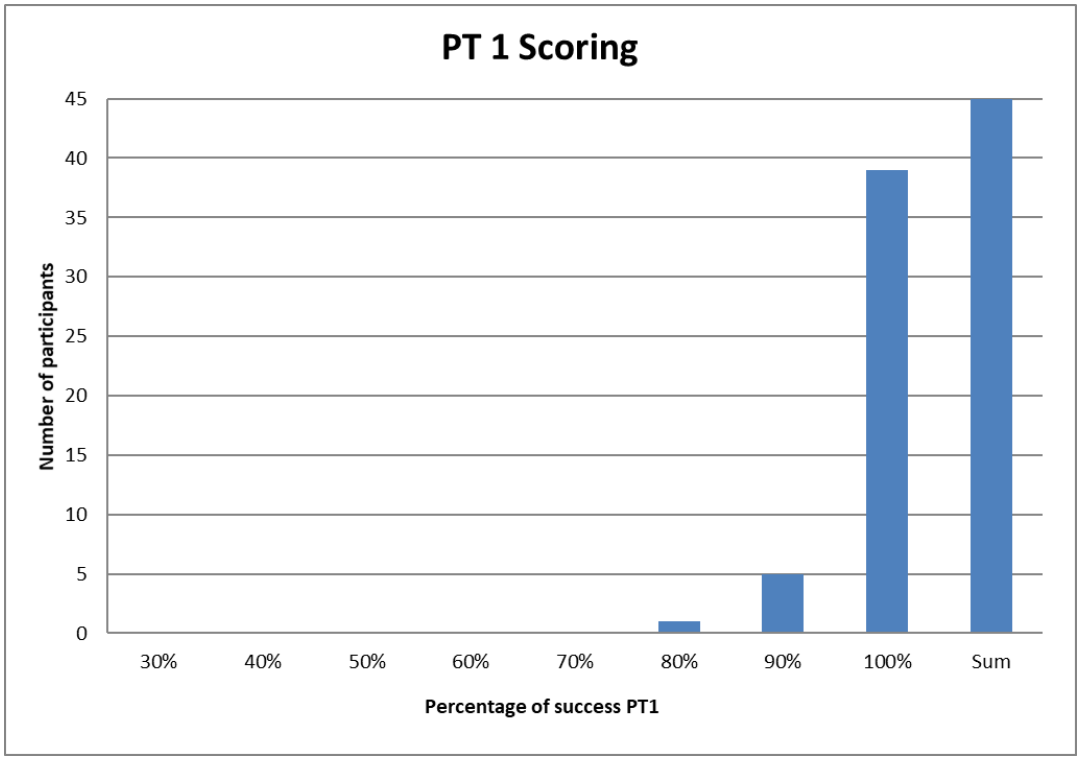
This year ECV was included in the Proficiency test. 3 out of the 45 countries do not test for Ranavirus. 38 participants provided the correct identification, 2 laboratory identified correctly the isolate as ECV by sequencing but submitted the result as EHNV and to laboratories did no sequencing.

In this report (Figures 5-8), all the viral titres submitted by participants are compared to each other. In this way, the titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory is able to compare the sensitivity of its cell lines to the sensitivity of those used by the other participants. We recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose, especially as it appears that the variations in titres between laboratories was very high – with more than 8 log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We will also take the opportunity to provide a comment to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

Overall 39 out of 45 participants scored 100% success rate and 5 participants scored 90% due to sequencing of the content in ampoule IV (ECV) or contamination of ampoule contents. These points will be assessed directly with the single participants that has underperformed.

The results presented in this report will be further presented and discussed at the 22th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 30th and 31st of May, 2018 in Kgs. Lyngby, Denmark.



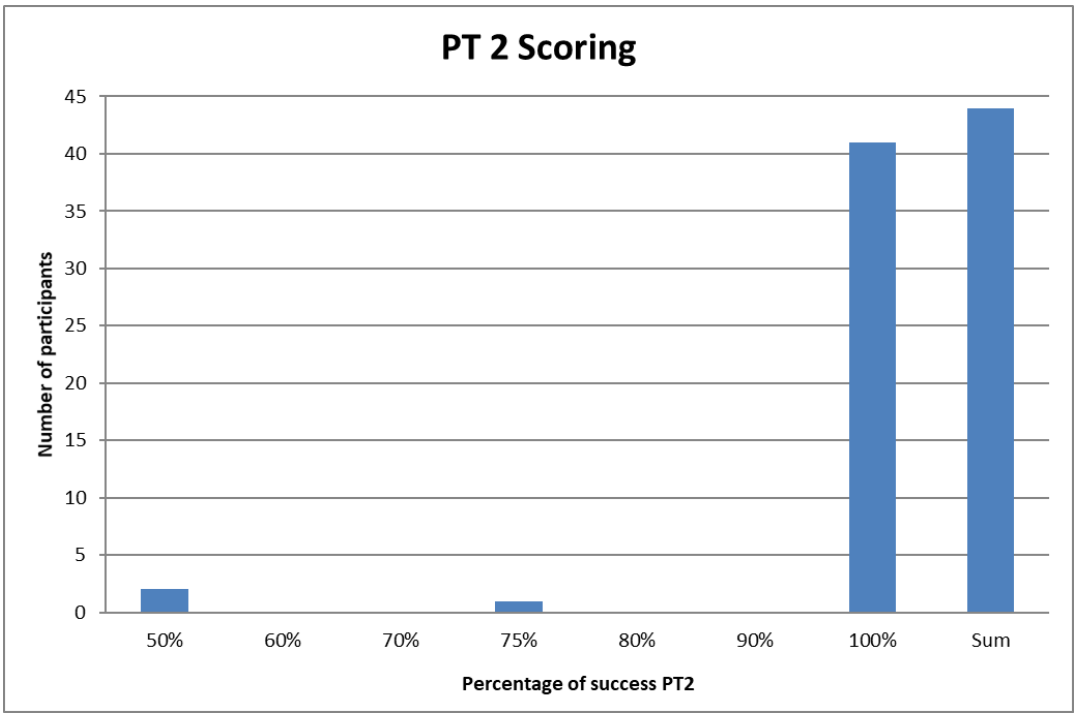


Figure 10 "a" and "b" Success-rate of participating laboratories 2017

Proficiency test 2, PT2

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

Content of ampoules

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

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

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

Code	Specifications/References
Ampoule VI: SAV	**Salmonid alpha virus (SAV) 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 VII: ISAV	**ISAV Glesvaer/2/90** **HPR Genotype:** 2 **Received from:** Dr. B. Dannevig, OIE Reference Laboratory for ISA, Oslo, Norway **GenBank accession numbers:** HQ259676, or AF220607.1 or DQ785248.1 **References on isolate:** Dannevig BH, Falk K & Namork E (1995). Isolation of the causal virus of infectious salmon anaemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *Journal of General Virology* 76, 1353–1359. Falk K, Namork E, Rimstad E, Mjaaland S & Dannevig BH (1997). Characterization of infectious salmon anemia virus, an orthomyxo-like virus isolated from Atlantic salmon (Salmo salar L.) *Journal of Virology* 71, 9016-9023. **References on sequence:** Mérour E, LeBerre M, Lamoureux A, Bernard J, Brémont M & Biacchesi S (2011). Completion of the full-length genome sequence of the infectious salmon anemia virus, an aquatic orthomyxovirus-like, and characterization of mAbs. *Journal of General Virology* 92, 528-533. **References on genotype:** Table 15. Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety 26.01.07. Which risk factors relating to spread of Infectious Salmon Anaemia (ISA) require development of management strategies? Dok.nr.06/804, 68 pages.
Ampoule VIII: Blank	BF-2 NON infected cell culture supernatant
Ampoule IX : KHV	**Cyprinid herpes virus 3 CyHV-3 – isolate KHV-TP 30 (syn: KHV-T (for Taiwan))** **Received from Dr. Sven Begmann** KHV-TP 30 was isolated from koi in Taiwan and cloned for producing large plaques by Dr. Peiyu Lee, Taiwan in-2005. **References:** The TK gen is 100% identical to serval isolates present in the Genbank e.g. KX609547.1

Testing of the test

The PT2 test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043. Prior to the distribution we tested 5 ampoules of each virus preparation, by real-time PCR (Gilad et *al*. (2004)) for KHV, by RT-PCR (Mjaaland et *al*. (1997)) and real-time RT-PCR (Snow et *al*. (2006)) for ISAV and by real-time RT PCR (Hodneland et al. (2006)) for SAV, to ascertain identity and homogeneity of the content in the ampoules (Figure 11). As a result all the standard deviations were below 1 Ct. value. Furthermore, after deadline for handling in results and minimum 3 months after lyophilisation and storage in the dark at 4°C, the content of the ampoules were tested to assess their stability (Table 11 and Figure 12).
Conventional PCR/RT-PCR fragments were sequenced and so was the HPR region in segment 6 of the ISAV isolate.

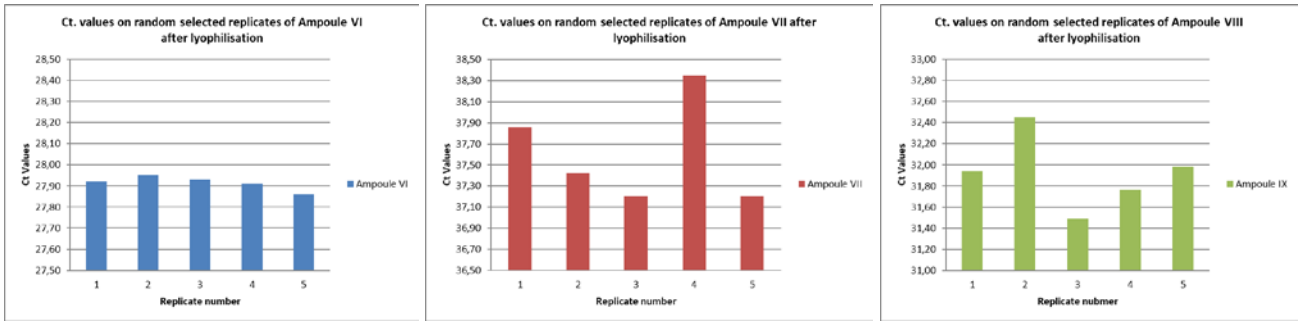


Figure 11, Ampule VI (SAV), VII (ISAV) and IX (CyHV-3) tested shortly after lyophilisation to assess homogeneity of the content.

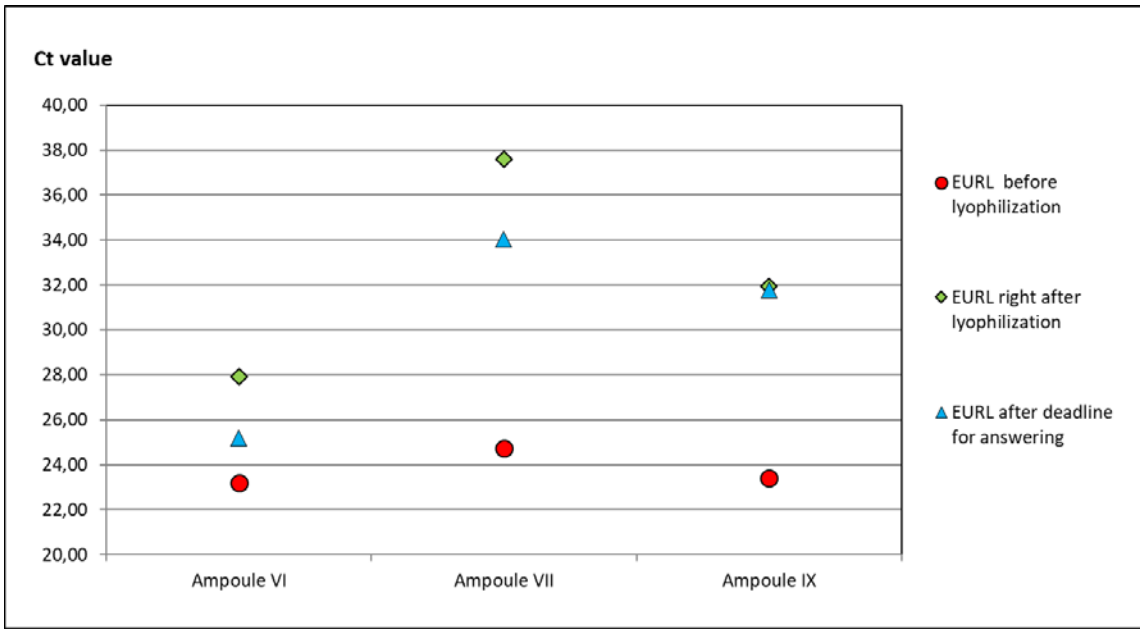


Figure 12, Ampoule VI, VII and IX tested before and after lyophilisation and after deadline for handling in results.

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

Ampoule	Content	Cell line	EURL before lyophilization	EURL right after lyophilization	EURL after deadline for answering
Ampoule VI	SAV	a	23,2	27,92	25,19
		b		27,95	
		c		27,93	
		d		27,91	
		e		27,86	
	Median Value		**23,20**	**27,91**	**25,19**
Ampoule VII	ISAV	a	24,72	37,86	34,03
		b		37,42	
		c		37,20	
		d		38,35	
		e		37,20	
	Median Value		**24,72**	**37,61**	**34,03**
Ampoule VIII	BF-2 cells	a	No Ct.	No Ct	No Ct
		b		No Ct	
		c		No Ct	
		d		No Ct	
		e		No Ct	
	Median Value		**No Ct**	**No Ct**	**No Ct**
Ampoule IX	KHV	a	23,41	31,94	31,78
		b		32,45	
		c		31,49	
		d		31,76	
		e		31,98	
	Median Value		**23,41**	**31,92**	**31,78**

The lyophilisation procedure caused a significant virus reduction (mainly in ampule VII with ISAV and IX with KHV) as detected by real-time PCR or real-time RT-PCR.

For each ampoule no other pathogens than the expected were detected.

Pathogen identification

In PT2, participants were asked to identify any of the fish viruses ISAV and KHV (both listed in Council Directive 2006/88/EC) according to diagnostic procedures described in Council implementing directive 2015-1554. Bearing in mind that the test ampoules also could contain other viruses. Participants were expected to use their normal PCR or real-time PCR methods for detection of KHV and their normal RT-PCR or real-time RT-PCR methods for detection of ISAV.
It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses had not been inactivated and should thus be viable.
Also this year, the panel of pathogens to be investigated included SAV – salmonid alpha virus. Since this is not a listed disease in the European legislation the participation was voluntary and therefore the participants were asked to declare if the ampoules were tested for SAV or not. Regarding methods for detection of SAV the participants were notified that they could refer to the OIE manual chapter 2.3.5b – Infection with salmonid alpha virus.
In order to obtain uniform answers, participants were requested to download a spreadsheet available from the http://www.eurl-fish.eu, insert results in this and return by email.
The results from participating laboratories are shown in table 12.

Table 12. Inter-Laboratory Proficiency Test, PT2, 2017 - Virus identification.

Laboratory code number	Score	Answer received at EURL	Ampoule VI	Ampoule VII	Ampoule VIII	Ampoule IX
			SAV 6, (PD)	ISAV Glesvaer/2/90	Blank Non infected BF-2 cell culture	KHV (CyHV-3) TP 30
1	8/8	21-11-2017	SAV	ISAV	Not ISAV, not SAV, not KHV	KHV
2[1]	4/8	13-11-2017	ISAV	No KHV and no ISAV	No KHV and no ISAV	KHV
3	8/8	16-11-2017	SAV	ISAV	No virus detected	KHV
4	8/8	16-11-2017	SAV	ISAV HPR2	No virus	KHV
5	8/8	07-11-2017	SAV	ISAV (HPR deleted)	negative for ISAV, KHV and SAV	KHV Japanese lineage
6	8/8	28-11-2017	SDV	ISAV HPRdel	negative	KHV
7	8/8	19-11-2017	SAV	ISAV	NO KHV,NO ISAV, NO SAV	KHV
8	4/8	17-11-2017	SAV	NEGATIVE	ISAV	KHV
9[3]	6/6	17-11-2017	SAV	ISAV	No virus detected	No virus detected
10[4]	4/4	05-12-2017	ND	ND	ND	KHV
11	8/8	13-11-2017	SAV	ISAV	Negative	KHV
12	8/8	07-11-2017	SAV	ISAV	Negative	KHV
13	8/8	17-11-2017	SAV	ISAV	-	KHV
14[1]	8/8	17-11-2017	Not KHV, not ISAV	ISAV	Not KHV, not ISAV	KHV
15[1]	8/8	17-11-2017	0	ISAV	0	KHV
16	8/8	20-11-2017	SAV	ISAV	No virus was detected in this ampoule	KHV
17	8/8	15-11-2017	SAV viable virus	ISAV	Negative	KHV
18	8/8	10-11-2017	SAV	ISAV	No virus detected	KHV
19	8/8	18-12-2017	SAV	ISAV	0	KHV
20	8/8	18-11-2017	SAV	ISAV	-	KHV
21	8/8	20-11-2017	SAV	ISAV	-	KHV
22[1]	8/8	20-11-2017	Not ISA Not KHV	ISAV	Not ISA Not KHV	KHV
23	8/8	20-11-2017	SAV	ISAV	Negative	KHV
24	6/8	16-11-2017	Negative	ISAV	Negative	KHV
25	8/8	13-11-2017	SAV	ISAV	no SAV, no ISAV, no KHV (CyHV-3)	KHV (CyHV-3)
26	8/8	16-11-2017	SAV	ISAV	NEGATIVE	KHV
27	8/8	20-11-2017	SAV	ISAV	Negative	KHV
28	8/8	20-11-2017	SAV	ISAV	Negative	KHV
29	8/8	14-11-2017	SAV	ISAV	No virus detected	KHV
30	8/8	20-11-2017	SAV	ISAV	Negative (no virus)	KHV
31	8/8	15-11-2017	SPDV	ISAV	Not ISAV, not KHV, Not SAV	KHV
32	8/8	20-11-2017	SAV	ISAV	STERILE	KHV
33	8/8	20-11-2017	SAV	ISAV	Virus was not detected	KHV

34	8/8	17-11-2017	SAV Positive	ISAV Positive	Negative	KHV (CyHV-3) Positive
35	8/8	14-11-2017	SAV	ISAV	Negative	CyHV-3
36	8/8	17-11-2017	SAV	ISAV	Negative	KHV
37	8/8	20-11-2017	SAV	ISAV	not ISAV, KHV, or SAV	KHV
38	8/8	19-11-2017	SAV	ISAV	Negative	KHV
39	8/8	14-11-2017	SAV	ISA	No virus detected	KHV
40	8/8	15-11-2017	SAV	ISAV	no pathogen found	KHV
41[2]		20-11-2017	0	0	0	0
42	8/8	20-11-2017	SAV	ISAV	no virus	KHV
43	8/8	24-11-2017	SAV	ISAV	Neg	KHV
44[1]	8/8	16-11-2017	Negative for ISAV and KHV	ISAV	Negative for ISAV and KHV	KHV
45	8/8	16-11-2017	SAV	ISAV	0	KHV

[1)] Did not test for SAV, [2)] Did not participate in PT2, [3)] Did not test for KHV, [4)] Did only test for KHV

All laboratories were asked to sequence the HPR region of ISAV isolates on a voluntary basis. However, since ISAV HPR0 have been delisted in Council Directive 2006/88/EC Annex IV , this will be a mandatory task in future, and from 2018 an annex including genotyping scoring will be included in the report.

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
- 35 laboratories correctly identified all four ampoules (SAV, ISAV, Blank, KHV)
- 42 laboratories tested for the two listed pathogens
- 43 laboratories tested for ISAV
- 43 laboratories tested for KHV
- 39 laboratories tested for SAV
- 1 laboratory that did participate in PT 1 did not participate in PT2

Ampoule VI – SAV

- 37 laboratories correctly identified SAV
- 5 laboratories did not participated in identifying SAV and four correctly ruled out the other 2 listed pathogens (KHV and ISAV) in this ampoule.
- 1 laboratory identified ISAV and 1 laboratory answered 'Negative'.

Ampoule VII – ISAV

- 41 laboratories correctly identified ISAV
- 2 laboratory answered 'Negative' or 'No KHV and no ISAV'
- 1 laboratory did not participate for ISAV and answered 'ND'

Ampoule VIII – Blank

- 43 laboratories ruled out the presence of pathogens they were testing for
- 1 laboratory identified ISAV

Ampoule IX – KHV

- 43 laboratories correctly identified KHV
- 1 laboratory did not participate for KHV but correctly ruled out the presence of the SAV and ISAV from this ampoule

Scores

We have assigned a score of 2 for each correct answer (Table 12), giving the possibility for obtaining a maximum score of 8.
Incorrectly finding of pathogens not present in the ampoules gives the score 0.

Of the 44 laboratories submitting results 41 laboratories obtained maximum score. The maximum score was calculated according to the number of pathogen tested by the laboratory.
A laboratory could obtain a maximum score of 8 if tested for all three pathogens included (ISAV;KHV and SAV) or the two listed pathogens (ISAV and KHV) .

If a laboratory did not test for KHV or ISAV but tested for SAV the maximum score was 6 points.

Methods applied
The following methods were used by the participants:

KHV detection
- 26 laboratories used Real Time PCR protocols for KHV detection.
- 34 laboratories used KHV PCR.

ISAV detection
- 28 laboratories used ISAV real-time RT-PCR.
- 34 laboratories used conventional RT-PCR.

SAV detection
- 23 laboratories used SAV real-time RT-PCR.
- 26 laboratories used SAV RT-PCR.

Genotypning and sequencing
Participants were encouraged to sequence the HPR region of possible ISAV isolates though it was not a mandatory task this year
- 24 laboratories performed sequencing for KHV
- 27 laboratories performed sequencing for SAV
- 29 laboratories performed sequencing for ISAV

An Overview of the genotyping results obtained for PT2 by all participants is displayed in the following table 13

Tabel 13. The genotyping results obtained for PT2 by all participants

Laboratory code number	Ampoule VI	Ampoule VII	Ampoule IX
	SAV SAV-6, PD	**ISAV Glesvaer/2/90 HPR Genotype: 2**	**KHV CyHV-3**
1	*	HPR2	*
2	Did not test for SAV	N/A	N/A
3	SAV6	EU-G2	N/A
4	*	HPR2	*
5	N/A	Genotype HPR deleted	N/A
6	SAV 2 FW (SD) (according to OIE)	2	*
7	N/A	N/A	N/A
8	N/A	N/A	N/A
9	N/A	N/A	Did not test for KHV
10	Did not test for SAV	Did not test for ISAV	N/A
11	SAV6	HPR deleted, genotype 2	CyHV-3
12	type VI	N/A	CyHV-3
13	VI	PR4	N/A
14	Did not test for SAV	*	*
15	Did not test for SAV	N/A	N/A
16	N/A	HPR Genotype 2	N/A
17	subtype 6	G2 (HPR/deleted)	N/A
18	Subtype 6	HPR deleted	CyHV3
19	*	*	*
20	*	HPR deleted	*
21	SAV6	*	N/A
22	Did not test for SAV	Genotype 2	N/A
23	N/A	N/A	N/A
24	N/A	N/A	N/A
25	SAV6	ISAV4	*
26	*	*	*
27	*	HPR-2	*
28	*	HPR deleted(HPR2)	*
29	SAV 6 (PD)	ISAV (HPR2)	CyHV 3
30	Subtype VI	HPRdeleted	N/A
31	*	*	N/A
32	N/A	N/A	N/A
33	N/A	N/A	N/A
34	*	*	KHV
35	Type 6	HPR Type 2	CyHV-3
36	N/A	European, HPR2	N/A
37	*	*	*
38	SAV Genotype VI	ISAV HPR2	KHV
39	SAV6	HPR Genotype: 2	Asian
40	N/A	N/A	N/A
41	Did not participate in PT2		
42	N/A	N/A	N/A
43	VI	HPR2	A1
44	Did not test for SAV	*	*
45	PD	*	*
No. of sequence preformed	26	31	22
No. of correct genotyping	16	20	-

*Sequence provided but no genotyping assigned under Genotype in the spreadsheet for Sequencing results – Proficiency Test 1
N/A: No sequence provided

AMPOULE VI SAV-6:

- 26 laboratories sequenced the SAV isolate included in Ampoule VI, which was included in PT2 2017 on a volunteer basis.
- 16 participants correctly genotyped the isolated as SAV Genotype 6 – PD.
- 10 participants did not provide a genotype.

AMPOULE VII ISAV Glesvaer/2/90 HPR Genotype: 2:

- 31 laboratories sequenced the HPR region of the ISAV isolate in ampoule VII
- 22 laboratories provided a genotype
  - 20 participants correctly provide the genotype as HPR 2
  - 2 participants provide the genotype as HPR4.
  - 9 participants did not provide a genotype

AMPOULE IX KHV CyHV-3:

- 21 laboratories sequenced the KHV isolate included in Ampoule IX
  - 9 laboratories provided a genotype for the isolate in ampoule IX

Concluding remarks PT2

The EURL have decided to include SAV in the panel of viruses included in PT2 since this was regarded as a proper initiative that strengthen the diagnostic capacities of the NRLs in detecting emerging pathogens.
44 laboratories participated in PT2.
39 laboratories tested for SAV and 37 correctly identified the virus in Ampoule VI, 1 laboratory seems to have switch two of the ampoules and 1 laboratory answered 'negative'.
41 out of the 44 laboratories correctly identified the ISA virus in ampoule VII. 1 did not test for ISAV and 2 laboratories seems to have switch two of the ampoules.
43 laboratories correctly identified the KHV in ampoule IX and 1 did not test for KHV.

It has been a concern that two laboratories has identified the correct virus but not in the right ampoule, meaning that some mistake in traceability of the ampoules during the working flow procedure has occurred. These points will be assessed directly with the single participants that has underperformed.

It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis, as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains, especially after the delisting of ISAV HPR0 (Commission Implementing Directive 2014/22/EU).

From 2018 more focus and acknowledgement of the sequencing work conducted by the participants will be given. The EURL proposes to provide a separate scoring system for the genotyping results, which will be attached to the annexes (Table 9 and 13) which display the genotyping results provided by all participants. This topic will be explained and discussed at the next Annual Workshop 30-31st May 2018 Kgs. Lyngby.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they fare in relation to the other participants. It is up to the

individual laboratory to assess if they perform according to their own expectations and standards. We take the opportunity to provide comments to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

The results given in this report will be further presented and discussed at the 22th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 30th-31st of May 2018 in Kgs. Lyngby, Denmark.

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European Union Reference laboratory for Fish diseases
National Institute of Aquatic Resources, Technical University of Denmark, March 2018.

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