EURL for Fish Diseases

Report of the Inter-Laboratory Proficiency Test 2011

for identification of

VHSV, IHNV and EHNV (PT1)

and identification of

KHV, ISAV and *Aphanomyces invadans* (PT2)

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DANAK
PT Reg. no.: 515

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Introduction

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into two proficiency tests, proficiency test 1 (PT1) and proficiency test 2 (PT2). PT1 was designed as the proficiency tests provided by the EURL in previous years to primarily assess the identification of the fish viruses viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV) and epizootic haematopoietic necrosis virus (EHNV) by cell culture based methods. PT2 was included for the second time with the aim of assessing the ability of participating laboratories to identify the fish pathogens infectious salmon anaemia virus (ISAV), koi herpes virus (KHV) and *Aphanomyces invadans* by PCR based methods. It is the first time *Aphanomyces invadans* has been included in the proficiency test. The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 41.
The tests were sent from the EURL in the middle of October 2011.
Both PT1 and PT2 are accredited by DANAK under registration number 515 to 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). The ampoules contained VHSV, EHNV, European catfish virus (ECV), IHNV+IPNV and IPNV, respectively, see table 1. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the fish viruses VHSV, IHNV and ENHV (all listed in Council Directive 2006/88/EC) if present in the ampoules, bearing in mind 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 Decision 2001/183/EC using fish cell cultures followed by e.g. neutralisation test, immunofluorescence, ELISA or PCR. If ranavirus was present in any of the ampoules, it was mandatory to perform a 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 2009. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible 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 in Kurath et al. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of five coded ampoules (VI-X). The ampoules contained ISAV and KHV. Furthermore, one ampoule contained *Aphanomyces invadans*, see table 11. It was decided at the 15th Annual Meeting of the NRLs for Fish Diseases in Aarhus 26-27 May 2011, that testing for *A. invadans* for the first time should be included in the yearly proficiency test provided by the EURL. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV, KHV and *A. invadans* (listed in Council Directive 2006/88/EC) if present in the ampoules, bearing in mind that the test ampoules could also contain other viruses. Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and

they should thus be possible to amplify in cell cultures. If present, only **inactivated** *A. invadans* was included in the ampoules.

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 providers of the proficiency test have included comments to the participants if relevant. An uncoded version of the report is sent to the Commission.

The participants were requested to download an excel sheet for filling in results and submit the filled out sheet electronically. Additionally, participants were asked 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 16th Annual Meeting of the NRLs for Fish Diseases in Aarhus 30-31 May 2012. Participants were asked to reply latest 16 December 2011.

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

Thermo-loggers were included in 10 of the parcels. The thermo-loggers were returned immediately upon receipt of the proficiency tests and a computer programme translated the data into a graph, showing the temperature inside the parcel for every 30 minutes during transportation. The loggers were programmed to mark if the temperature had exceeded 30°C at some point during transportation. Inclusion of loggers should display if the temperature encountered during transport had been detrimental to the viability of the virus in the test.

Shipment and handling

Within four days, the tests were delivered to 32 participants; 6 tests were delivered within 7 days and 2 test within 2 weeks and 1 test within three weeks (figure 1). All the parcels were sent without cooling elements. The average temperature was 16°C for the transports to the 8 countries were the temperature did not exceed 30°C. The temperature exceeded 30°C for one transport for half an hour upon arrival and for one transport the temperature was 38-42°C for 2 hours.

To test the influence of temperature the ampoules were subjected for a period of 5 hours to temperatures from 20°C to 42°C (figure 2). Then ampoules I-V were titrated on cell cultures and ampoules VI-X were tested by PCR. No significant decrease in the titres was observed for any of the tested ampoules and it was still possible to identify the pathogens by PCR methods. As the ampoules followed by a logger at no point exceeded such extreme temperatures during shipment, it is considered that the temperature variation that the ampoules experienced during shipment did not influence considerably on the pathogens.

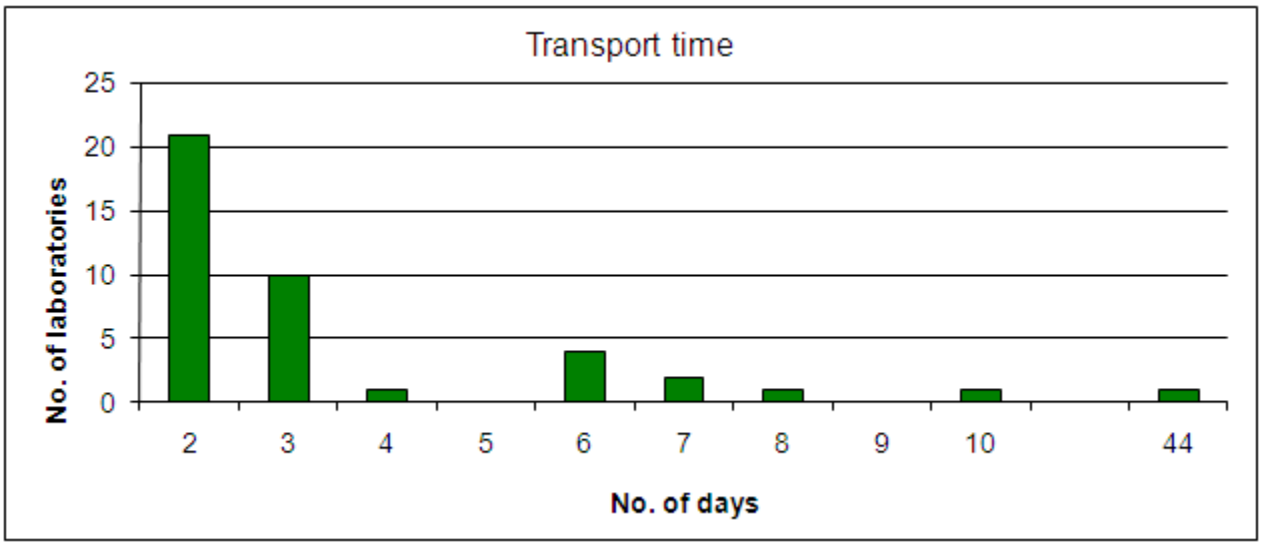

Figure 1. Transport time for the parcels to reach the participants.

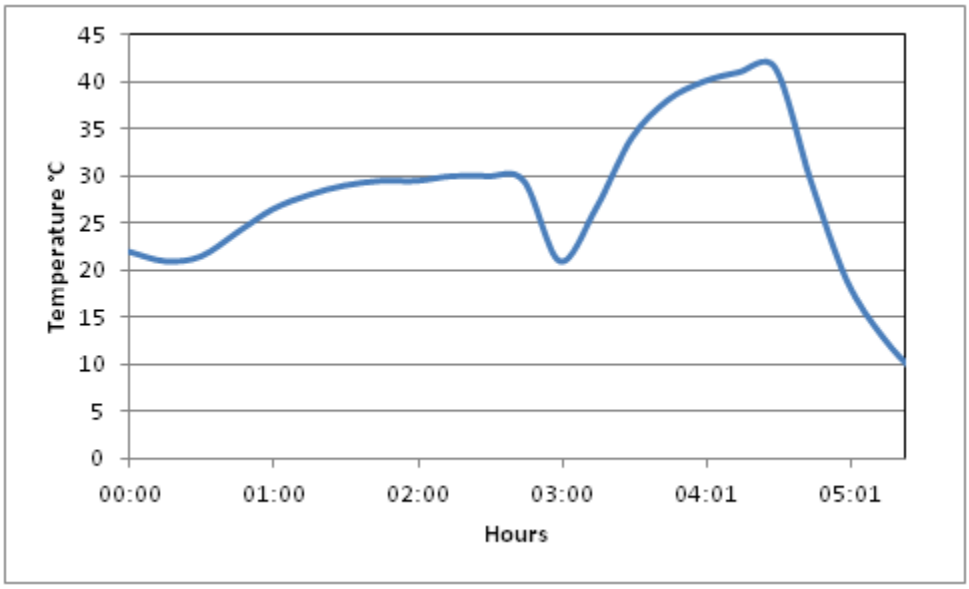

Figure 2. The ampoules were subjected to temperatures of 20-42°C for a period of 5 hours and then tested.

Participation

PT1: 41 laboratories received the annual proficiency test. Of these, 39 participants submitted results within the deadline. One participant submitted sequencing results 5 days after deadline but before the content of the ampoules was made public available. Two participants did not submit results.

PT2: 41 laboratories received the annual proficiency test. Of these, 38 participants submitted results within the deadline. One participant submitted the results for *A. invadans* 7 days after deadline but before the content of the ampoules was made public available. Three participants did not submit results.

Figure 3 show how many laboratories that participated in the proficiency test from 1996 to 2011.

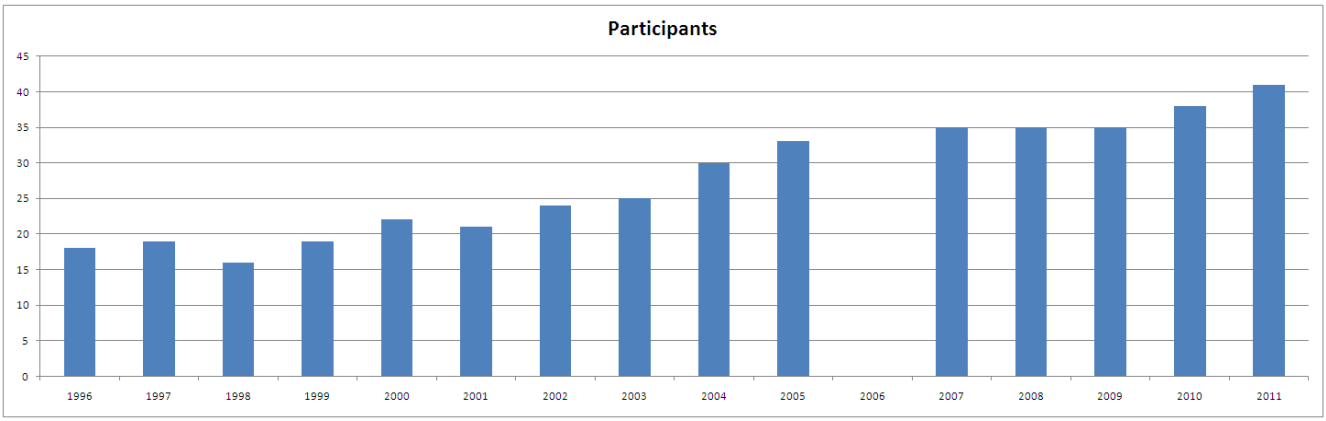

Figure 3. Participants in the EURL proficiency test over the years.

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, Croatia, Faroe Islands, Iceland, Israel, Iran, Japan, Norway, P.R. China, Serbia, Switzerland, Turkey and 2 from USA. The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy, Cyprus and Malta for identification of all listed diseases. Figure 4 shows the worldwide distribution of the participating NRLs.

Figure 4. Worldwide distribution of the participants in the EURL proficiency test 2011.

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 20% 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.

PT1	
Code	**Specifications**
Ampoule I: VHSV Isolate 1p8	**VHSV 1p8** Marine isolate from herring (*Clupea harengus*) caught in the Baltic Sea in 1996. Cell culture passage number: 7 Genotype Ib. GenBank accession numbers: AY546573 (G-gene) and GQ325430, AY356652 (N-gene) www.fishpathogens.eu ID number: 2251 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.
Ampoule II: EHNV Isolate 86/8774	**EHNV Isolate 86/8774** Received from the EHNV OIE reference laboratory (EURL file number 202213). Australian freshwater isolate from rainbow trout from Adaminaby Trout Farm, NSW obtained in 1986 by Jeremy Langdon. Cell culture passage number: 8 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 A.D., Gould A.R., Zupanovic Z., Cunningham A.A., Hengstberger S., Whittington R.J., Kattenbelt J. & Coupar B.E.H. (2000) Comparative studies of piscine and amphibian iridoviruses. Archives of Virology 145, 301-331. Jancovich J.K., Bremont M., Touchman J.W. & Jacobs B.L. (2010) Evidence for multiple recent host species shifts among the ranaviruses (family Iridoviridae). Journal of Virology 84, 2636-2647. Marsh I.B., Whittington R.J., O'Rourke B., Hyatt A.D. & 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.
Ampoule III: European Catfish virus (ECV) Isolate 562/92 Low titre	**European catfish virus 562/92.** Italian isolate from catfish suffering high mortality. Received from Dr. G. Bovo, ISZ-Ve, Padova, Italy. Cell culture passage number: 7 GenBank accession number: FJ358608 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.

PT1	
Code	**Specifications**
Ampoule IV: IHNV Isolate 32/87 + IPNV Strain Sp	**IHNV 32/87.** First French isolate (April 1987) from rainbow trout. Cell culture passage number: 9 GenBank accession numbers: J265717 and AY524121 (G-gene), FJ265711 (N-gene). Reference on isolate: Baudin Laurencin F**.** (1987) IHN in France. *Bulletin of the European Association of Fish Pathologists* **7**, 104. Reference on sequence: Kolodziejek J., Schachner O., Dürrwald R., Latif M. & Nowotny N. (2008) "Mid-G" region sequences of the glycoprotein gene of Austrian infectious hematopoietic necrosis virus isolates form two lineages within European isolates and are distinct from American and Asian lineages. *Journal of Clinical Microbiology* **46**, 22-30. Johansson T., Einer-Jensen K., Batts W., Ahrens P., Björkblom C., Kurath G., Björklund H. & Lorenzen N. (2009) Genetic and serological typing of European infectious haematopoietic necrosis virus (IHNV) isolates. *Diseases of Aquatic Organisms* **86**, 213-221. + **Type strain Sp (Spjarup) of IPN virus**. Cell culture passage number in BF-2: 17 Reference on isolate: Jørgensen P.E.V. & Bregnballe F. (1969) Infectious pancreatic necrosis in rainbow trout in Denmark. *Nordisk Veterinærmedicin* **21**, 142-148. Jørgensen P.E.V. & Grauballe P.C. (1971) Problems in the serological typing of IPN virus. *Acta Veterinaria Scandinavica* **12**, 145-147.
Ampoule V: IPNV Strain Sp	**Type strain Sp (Spjarup) of IPN virus**. Cell culture passage number in BF-2: 17 Reference on isolate: Jørgensen P.E.V. & Bregnballe F. (1969) Infectious pancreatic necrosis in rainbow trout in Denmark. *Nordisk Veterinærmedicin* **21**, 142-148. Jørgensen P.E.V. & Grauballe P.C. (1971) Problems in the serological typing of IPN virus. *Acta Veterinaria Scandinavica* **12**, 145-147.

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).

The lyophilisation procedure caused a significant titre reduction, especially for VHSV on BF-2, EPC and RTG-2 cells where a 2-3 log reduction was observed. For the other cell lines a reduction between 1 - 2 log for IHNV/IPNV was observed. For EHNV and ECV a minimal titre reduction of 0 - 1 log was observed. For IPNV the titre reduction was 1-2 log (table 2 and figure 5). However, all titres of the lyophilised viruses were above detection level, except for VHSV on RTG-2 cells. As participants are expected to use two different cell lines, VHSV should be detected on the other cell line.
Lyophilised viruses are very stable at storing. 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 (Report of the Inter-Laboratory Proficiency Test for National Reference Laboratories for Fish Diseases 2007).
We have furthermore shown that lyophilised virus in glass sealed ampoules is stable has after exposed to 30°C for 24 hours (Report of the Inter-Laboratory Proficiency Test for National Reference Laboratories for Fish Diseases 2010).

The identities of the viruses in all 5 ampoules were checked and confirmed by ELISA, IFAT, serum neutralisation tests, RT-PCR and sequencing for VHSV, IHNV and IPNV (RT-PCR/sequencing not performed for this virus) and by PCR, sequencing and IFAT for EHNV and ECV. For each ampoule, presence of viruses other than the expected was not observed.
We tested the titre of each virus preparation (ampoule) after 3 months storage in the dark at 4°C and observed no significant decrease compared to right after lyophilization.

Table 2. Titre of representative ampoules of no. I to V tested at the EURL in four cell lines before lyophilisation, immediately after lyophilisation (median titre of 5 replicates), and after 3 months of storage in the dark at 4°C and after being held at a temperature raised from 20-42°C over a period of 5 hours (1 replicate).

Ampoule No.	Cell line	Titre before lyophilisation	Median titre right after lyophilisation	Titre 3 months after lyophilisation (4°C, dark conditions)	Titre 3 months after lyophilisation (20-42°C, dark conditions)
		$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I: VHSV Isolate 1p8	BF-2	$2,7*10^8$	$5,9*10^5$	$1,3*10^6$	$2,7*10^5$
	EPC	$8,6*10^5$	$2,7*10^3$	$8,6*10^3$	$5,9*10^3$
	RTG-2	$4,0*10^5$	$< 1,9*10^2$	$< 1,9*10^2$	$< 1,9*10^2$
	FHM	$1,9*10^8$	$1,9*10^6$	$1,3*10^7$	$5,9*10^6$
Ampoule II: EHNV Isolate 86/8774	BF-2	$5,9*10^4$	$5,9*10^5$	$2,7*10^5$	$2,7*10^5$
	EPC	$1,3*10^5$	$1,9*10^4$	$5,9*10^4$	$1,9*10^5$
	RTG-2	$8,6*10^4$	$4,0*10^3$	$4,0*10^4$	$1,9*10^4$
	FHM	$8,6*10^3$	$1,3*10^3$	$1,3*10^3$	$1,3*10^3$
Ampoule III: European Catfish virus (ECV) Isolate 562/92 Low titre	BF-2	$1,3*10^5$	$1,3*10^4$	$1,3*10^4$	$8,6*10^3$
	EPC	$5,9*10^4$	$1,3*10^4$	$8,6*10^3$	$8,6*10^3$
	RTG-2	$8,6*10^4$	$1,3*10^3$	$1,3*10^4$	$1,3*10^4$
	FHM	$1,3*10^3$	$1,3*10^3$	$< 1,9*10^2$	$< 1,9*10^2$
Ampoule IV: IHNV Isolate 32/87 + IPNV Strain Sp	BF-2	$4,0*10^6$	$2,7*10^5$	$1,9*10^6$	$4,0*10^6$
	EPC	$5,9*10^7$	$1,9*10^5$	$1,9*10^6$	$2,7*10^6$
	RTG-2	$2,7*10^5$	$8,6*10^4$	$2,7*10^5$	$8,6*10^5$
	FHM	$4,0*10^6$	$2,7*10^5$	$4,0*10^5$	$4,0*10^5$
Ampoule V: IPNV Strain Sp	BF-2	$1,3*10^8$	$5,9*10^6$	$2,7*10^7$	$4,0*10^5$
	EPC	$2,7*10^8$	$1,9*10^6$	$1,9*10^7$	$5,9*10^5$
	RTG-2	$5,9*10^7$	$8,6*10^5$	$1,3*10^7$	$4,0*10^5$
	FHM	$4,0*10^7$	$1,9*10^5$	$1,3*10^6$	$2,7*10^5$

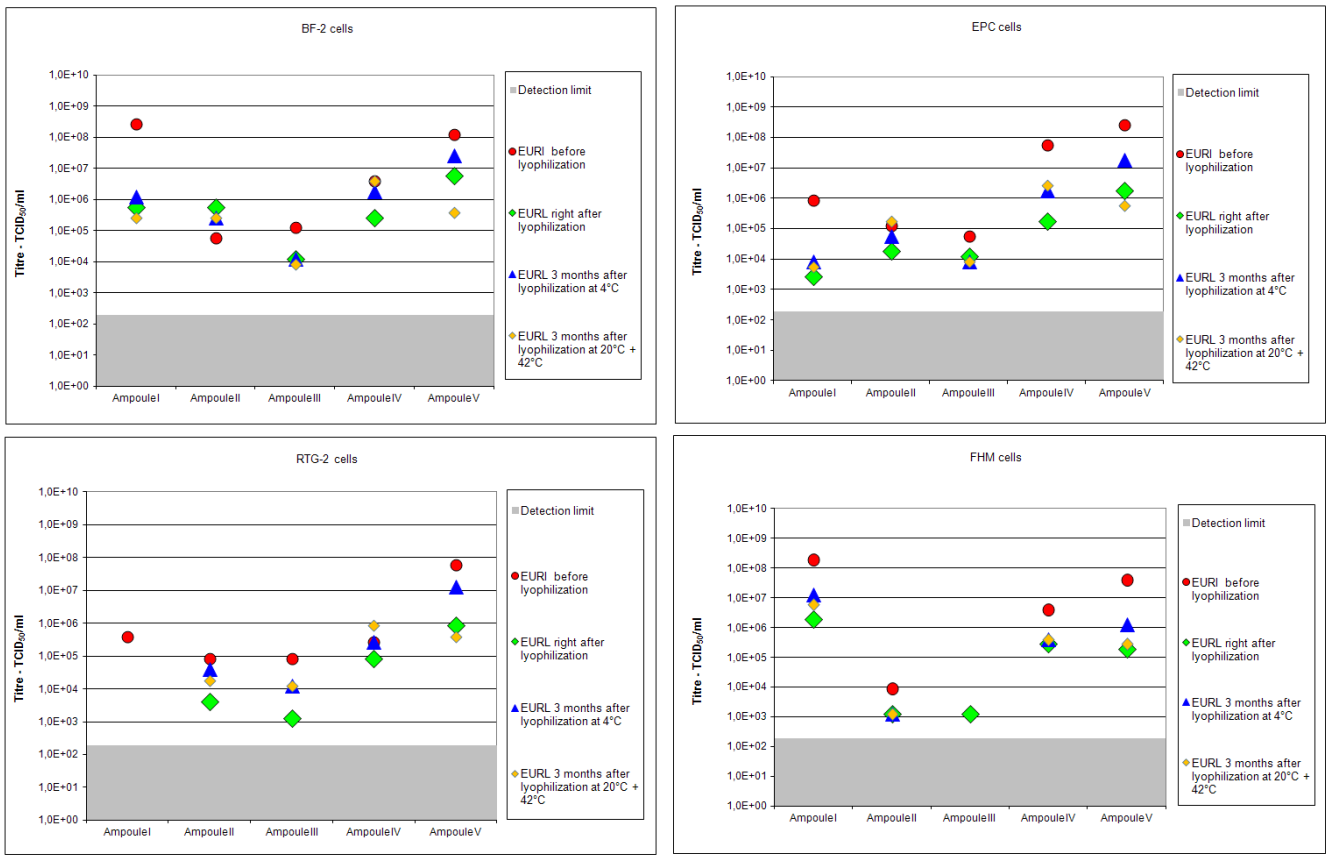

Figure 5. Virus titers in different cell lines before, right after, 3 months after lyophilisation and after staying at a temperature raised from 20-42°C over a period of 5 hours. Grey area is below detection level.

Virus identification and titration

Participants were asked to identify the content of each ampoule by the method used in their laboratory which should be according to the procedures described in the Commission Decision 2001/183/EC, i.e. by cell culture followed by ELISA, IFAT, neutralisation test and/or RT-PCR. Identification results of the content of the 5 ampoules for the participating laboratories are summarised in table 3.

Participants were also asked to titrate the contents of the ampoules. The method of titration was described in the instructions enclosed with the test. All titres were calculated at the EURL based on the crude data submitted by each participant and given as Tissue Culture Infective Dose 50% ($TCID_{50}$) per 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)**.** Titration results of the viruses of the 5 ampoules for the participating laboratories are summarised in tables 4 to 8. The titres obtained from each participating laboratory are represented graphically. In figures 6-9, all titres submitted by 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 three laboratories used these cells. Laboratories with the required facilities were encouraged to examine and 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, 2011 - Virus identification.

Laboratory code number	Score 10/10	Answer recived at EURL	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
			VHSV Isolate 1p8	EHNV Isolate 86/8774	Ranavirus, not EHNV European Catfish virus (ECV) 562/92 Low titre	IHNV and IPNV IHNV 32/87 + IPNV Strain Sp	IPNV IPNV Strain Sp
1	10	13-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
2[1]	8	29-11-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV	Not VHSV, IHNV and EHNV
3	10	12-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
4	7	16-12-11	VHSV	Ranavirus*	VHSV	IHNV and IPNV	IPNV
5	10	13-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
6	10	09-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
7[2]	6	16-12-11	VHSV	Not VHSV, IHNV, IPNV, SVCV	Not VHSV, IHNV, IPNV, SVCV	IHNV and IPNV	IPNV
8	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
9	10	23-11-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
10	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
11[3]	10	16-12-2011 21-12-2011	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
12	10	08-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
13	10	15-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
14	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
15	8	16-12-11	VHSV	Ranavirus*	Ranavirus*	IHNV and IPNV	IPNV
16	10	15-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
17	8	14-12-11	VHSV	EHNV	Ranavirus, not EHNV	IPNV	IPNV
18	0	n/a	No reply	No reply	No reply	No reply	No reply
19	10	16-12-11	VHSV	EHNV	Ranavirus, not EHVN	IHNV and IPNV	IPNV
20	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
21	9	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV	IPNV
22	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
23	6	14-12-11	VHSV	Ranavirus*	Ranavirus*	IPNV and Ranavirus*	IPNV
24	10	07-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
25	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
26	10	06-12-11	VHSV	EHNV	Ranavirus not EHNV	IHNV and IPNV	IPNV
27	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
28	0	n/a	No reply	No reply	No reply	No reply	No reply
29	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
30	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
31	8	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IPNV	IPNV
32	4	15-12-11	VHSV	Ranavirus*	Ranavirus* and SVCV	IHN and SVCV	not ranavirus, not IHNV, VHSV, SVCV
33	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
34	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
35	9	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV	IPNV
36	7	16-12-11	VHSV	Virus not identified	Ranavirus*	IHNV and IPNV	IPNV
37	10	13-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
38	8	16-12-11	VHSV	Ranavirus*	Ranavirus*	IHNV and IPNV	IPNV
39	10	07-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV
40	8	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV	not EHNV or other ranavirus, not IHNV, VHSV, SVCV
41	10	16-12-11	VHSV	EHNV	Ranavirus, not EHNV	IHNV and IPNV	IPNV

[1] Only tested by PCR

[2] Not tested for Ranavirus

[3] The laboratory submitted sequencing results after deadline but before ampoule contents were made public available. The result of this participant is therefore included in this report.

* Genomic analysis not performed

n/a: not applicable

Table 4. Inter-Laboratory Proficiency Test, PT1, 2011 – Results of titration of ampoule I.

Ampoule I - VHSV					
Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	VHSV	$2,7*10^4$	$1,3*10^3$	$5,9*10^5$	
2	VHSV	not performed	not performed	not performed	not performed
3	VHSV	$4,0*10^3$	$4,0*10^3$		
4	VHSV	$4,0*10^2$	$2,7*10^2$		
5	VHSV	$5,9*10^5$	$4,0*10^3$	$4,0*10^3$	$1,9*10^3$
6	VHSV	$5,9*10^5$	$1,9*10^3$	$< 1,9*10^2$	$4,0*10^6$
7	VHSV	$4,0*10^4$	$1,3*10^3$		
8	VHSV	$4,0*10^5$	$4,0*10^4$		
9	VHSV	$1,3*10^7$	$4,0*10^4$	$5,9*10^4$	
10	VHSV	$< 1,9*10^2$	$2,7*10^2$	$< 1,9*10^2$	$1,3*10^4$
11	VHSV	$4,0*10^5$	$5,9*10^3$		
12	VHSV	$1,3*10^5$	$1,3*10^5$		
13	VHSV	$1,3*10^5$	$< 1,9*10^2$		
14	VHSV	$1,9*10^5$	$5,9*10^3$		
15	VHSV	$5,9*10^5$	$2,7*10^2$		
16	VHSV		$2,7*10^4$	$1,3*10^3$	
17	VHSV	$1,3*10^6$	$4,0*10^5$	$8,6*10^4$	$1,3*10^6$
18	No reply				
19	VHSV	$1,3*10^4$	$< 1,9*10^2$		$< 1,9*10^2$
20	VHSV	$1,3*10^5$	$1,3*10^5$	$1,3*10^4$	$1,3*10^5$
21	VHSV		$1,9*10^4$	$5,9*10^2$	
22	VHSV	$8,6*10^5$	$2,7*10^4$	$2,7*10^5$	$1,3*10^4$
23	VHSV	$4,0*10^4$			$1,9*10^5$
24	VHSV		$1,9*10^3$		$1,9*10^3$
25	VHSV	$1,3*10^4$	$1,3*10^5$		
26	VHSV	$1,9*10^6$			$8,6*10^4$
27	VHSV	$1,3*10^5$	$5,9*10^3$	$< 1,9*10^2$	
28	No reply				
29	VHSV	$2,7*10^5$	$8,6*10^3$		
30	VHSV	$2,7*10^5$	$1,3*10^4$		
31	VHSV	$5,9*10^5$	$5,9*10^4$		
32	VHSV	$1,9*10^2$	$< 1,9*10^2$		
33	VHSV	$4,0*10^5$	$1,3*10^3$	$< 1,9*10^2$	$1,3*10^5$
34	VHSV	$1,9*10^4$	$1,9*10^2$		
35	VHSV				$< 1,9*10^2$
36	VHSV	$1,3*10^6$	$< 1,9*10^2$		
37	VHSV	$8,6*10^5$	$2,7*10^3$		
38	VHSV	$2,7*10^5$	$1,9*10^4$		
39	VHSV	$5,9*10^3$	$8,6*10^4$	$5,9*10^4$	$< 1,9*10^2$
40	VHSV	$2,7*10^5$	$2,7*10^2$		$4,0*10^3$
41	VHSV	$1,9*10^4$	$2,7*10^4$		$4,0*10^5$

Number of laboratories	34	35	13	15
Median titre	$2,3*10^5$	$5,9*10^3$	$4,0*10^3$	$1,3*10^4$
Maximum titre	$1,3*10^7$	$4,0*10^5$	$5,9*10^5$	$4,0*10^6$
Minimum titre	$<1,9*10^2$	$<1,9*10^2$	$<1,9*10^2$	$<1,9*10^2$
25% quartile titre	$2,1*10^4$	$7,7*10^2$	$<1,9*10^2$	$1,9*10^3$
75% quartile titre	$5,9*10^5$	$2,7*10^4$	$5,9*10^4$	$1,3*10^5$

Table 5. Inter-Laboratory Proficiency Test, PT1, 2011 – Results of titration of ampoule II.

Ampoule II - EHNV					
Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	EHNV	$1,9*10^5$	$5,9*10^4$	$2,7*10^5$	
2	EHNV	not performed	not performed	not performed	not performed
3	EHNV	$5,9*10^5$	$1,3*10^5$		
4	Ranavirus (not seq)	$1,3*10^3$	$4,0*10^4$		
5	EHNV	$1,3*10^3$	$5,9*10^4$	$4,0*10^3$	$4,0*10^3$
6	EHNV	$1,3*10^6$	$2,7*10^5$	$1,3*10^4$	$1,3*10^3$
7	Not VHSV, IHNV, IPNV, SVCV	$5,9*10^6$	$4,0*10^6$		
8	EHNV	$8,6*10^5$	$1,9*10^4$		
9	EHNV	$5,9*10^6$	$8,6*10^4$	$1,9*10^4$	
10	Ranavirus - EHNV	$4,0*10^4$	$1,9*10^4$	$< 1,9*10^2$	$< 1,9*10^2$
11	EHNV	$1,3*10^4$	$4,0*10^4$		
12	EHNV	$1,3*10^5$	$1,9*10^5$		
13	EHNV	$1,9*10^5$	$1,9*10^4$		
14	EHNV	$2,7*10^6$	$1,3*10^5$		
15	EHNV (not seq, therefore Ranavirus)	$5,9*10^5$	$1,9*10^5$		
16	EHNV		$1,3*10^3$	$4,0*10^3$	
17	EHNV	$1,9*10^6$	$4,0*10^5$	$2,7*10^5$	8,6*104
18	No reply				
19	EHNV	$4,0*10^4$	$< 1,9*10^2$		$< 1,9*10^2$
20	EHNV	$1,3*10^5$	$1,3*10^5$	$1,3*10^5$	$1,3*10^5$
21	EHNV		$4,0*10^6$	$1,9*10^4$	
22	EHNV	$2,7*10^6$	$1,3*10^6$	$4,0*10^6$	$1,9*10^6$
23	Ranavirus (not seq)	$4,0*10^4$			$1,3*10^3$
24	EHNV		$4,0*10^5$		$2,7*10^4$
25	EHNV	$1,3*10^3$	$2,7*10^5$	$4,0*10^4$	
26	EHNV	$1,3*10^5$			$4,0*10^5$
27	EHNV	$4,0*10^6$	$5,9*10^5$	$5,9*10^3$	
28	No reply				
29	EHNV	$4,0*10^4$	$1,9*10^5$		
30	EHNV	$2,7*10^5$	$1,3*10^5$		
31	EHNV	$4,0*10^5$	$2,7*10^4$		
32	EHNV (not seq, therefore Ranavirus)	$1,3*10^5$	$4,0*10^5$		
33	EHNV	$2,7*10^6$	$5,9*10^4$	$8,6*10^2$	$< 1,9*10^2$
34	EHNV	$5,9*10^4$	$4,0*10^3$		
35	EHNV				$1,3*10^4$
36	Virus not identified	$5,9*10^6$	$5,9*10^4$		
37	EHNV	$1,3*10^6$	$1,3*10^5$		
38	Ranavirus	$4,0*10^5$	$1,9*10^6$		
39	EHNV	$4,0*10^5$	$8,6*10^4$	$1,9*10^5$	$8,6*10^4$
40	EHNV	$5,9*10^6$	$4,0*10^4$		$2,7*10^4$
41	EHNV	$4,0*10^4$	$5,9*10^5$		$1,9*10^3$

Number of laboratories	34	35	14	15
Median titre	$3,4*10^5$	$1,3*10^5$	$1,9*10^4$	$1,3*10^4$
Maximum titre	$5,9*10^6$	$4,0*10^6$	$4,0*10^6$	$1,9*10^6$
Minimum titre	$1,3*10^3$	$<1,9*10^2$	$<1,9*10^2$	$<1,9*10^2$
25% quartile titre	$4,5*10^4$	$4,0*10^4$	$4,5*10^3$	$1,3*10^3$
75% quartile titre	$1,7*10^6$	$3,4*10^5$	$1,7*10^5$	$8,6*10^4$

Table 6. Inter-Laboratory Proficiency Test, PT1, 2011 – Results of titration of ampoule III.

Ampoule III - Ranavirus					
Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	ECV or ESV	$4,0*10^4$	$1,9*10^5$	$4,0*10^4$	
2	ECV or ESV	not performed	not performed	not performed	not performed
3	ECV or ESV	$4,0*10^4$	$4,0*10^4$		
4	VHSV	$4,0*10^2$	$2,7*10^2$		
5	ESV or ECV	$< 1,9*10^2$	$2,7*10^3$	$< 1,9*10^2$	$< 1,9*10^2$
6	ECV or ESV	$4,0*10^5$	$1,3*10^5$	$1,3*10^4$	$< 1,9*10^2$
7	Not VHSV, IHNV, IPNV, SVCV	$8,6*10^4$	$1,3*10^5$		
8	ECV or ESV	$8,6*10^4$	$1,3*10^4$		
9	Ranavirus - ECV	$4,0*10^5$	$1,9*10^5$	$8,6*10^2$	
10	Ranavirus - ESV	$1,9*10^3$	$1,3*10^4$	$< 1,9*10^2$	$< 1,9*10^2$
11	ECV or ESV	$8,6*10^3$	$4,0*10^3$		
12	ESV	$5,9*10^4$	$1,9*10^4$		
13	ECV or ESV	$2,7*10^4$	$4,0*10^3$		
14	ECV orESV	$1,9*10^5$	$2,7*10^5$		
15	EHNV (not seq, therefore Ranavirus)	$1,9*10^3$	$1,9*10^3$		
16	Ranavirus - ECV		$2,7*10^3$	$2,7*10^4$	
17	ECV or ESV	$1,3*10^5$	$4,0*10^4$	$1,3*10^5$	$1,3*10^3$
18	No reply				
19	Ranavirus	$1,9*10^3$	$< 1,9*10^2$		$< 1,9*10^2$
20	ECV or ESV	$1,3*10^5$	$1,3*10^5$	$1,3*10^5$	$1,3*10^5$
21	ECV		$2,7*10^3$	$5,9*10^2$	
22	Ranavirus not EHNV ESV or EC or DFV	$5,9*10^5$	$1,3*10^5$	$4,0*10^5$	$1,9*10^5$
23	Ranavirus (not seq)	$1,3*10^4$			$< 1,9*10^2$
24	ECV		$4,0*10^4$		$5,9*10^3$
25	ECV or ESV	$8,6*10^3$	$4,0*10^5$		
26	Ranavirus not EHNV	$< 1,9*10^2$			$1,3*10^4$
27	ESV	$1,9*10^6$	$1,9*10^5$	$< 1,9*10^2$	
28	No reply				
29	Iridovirus - ESV	$5,9*10^4$	$2,7*10^4$		
30	ECV or ESV	$2,7*10^3$	$1,9*10^4$		
31	ECV or ESV	$4,0*10^4$	$8,6*10^3$		
32	EHNV and SVC (not seq, therefore Ranavirus)	$< 1,9*10^2$	$4,0*10^5$		
33	ECV or ESV	$2,7*10^5$	$4,0*10^4$	$1,9*10^2$	$< 1,9*10^2$
34	Ranavirus - ESV	$2,7*10^3$	$1,3*10^3$		
35	ECV or ESV				$5,9*10^3$
36	Ranavirus	$4,0*10^5$	$8,6*10^4$		
37	ESV	$1,9*10^5$	$1,3*10^4$		
38	Ranavirus	$1,9*10^4$	$5,9*10^4$		
39	Ranavirus	$1,9*10^4$	$1,9*10^4$	$1,3*10^4$	$1,3*10^5$
40	ECV or ESV or Doctor fish virus	$4,0*10^5$	$8,6*10^3$		$1,3*10^4$
41	ECV or ESV	$5,9*10^4$	$1,3*10^5$		$4,0*10^2$

Number of laboratories	34	35	13	15
Median titre	$4,9*10^4$	$2,7*10^4$	$1,3*10^4$	$1,3*10^3$
Maximum titre	$1,9*10^6$	$4,0*10^5$	$4,0*10^5$	$1,9*10^5$
Minimum titre	$<1,9*10^2$	$<1,9*10^2$	$<1,9*10^2$	$<1,9*10^2$
25% quartile titre	$8,6*10^3$	$6,3*10^3$	$1,9*10^2$	$<1,9*10^2$
75% quartile titre	$1,9*10^5$	$1,3*10^5$	$4,0*10^4$	$1,3*10^4$

Table 7. Inter-Laboratory Proficiency Test, PT1, 2011 – Results of titration of ampoule IV.

Ampoule IV - IHNV and IPNV					
Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IHNV and IPNV	$1,9*10^5$	$2,7*10^5$	$4,0*10^5$	
2	IHNV	not performed	not performed	not performed	not performed
3	IHNV and IPNV	$1,9*10^5$	$1,9*10^5$		
4	IHNV and IPNV	$1,9*10^4$	$5,9*10^5$		
5	IHNV and IPNV	$1,9*10^6$	$1,9*10^6$	$1,9*10^6$	$5,9*10^5$
6	IHNV and IPNV	$4,0*10^6$	$8,6*10^5$	$1,3*10^6$	$5,9*10^5$
7	IHNV and IPNV	$8,6*10^6$	$8,6*10^6$		
8	IHNV and IPNV	$5,9*10^5$	$8,6*10^5$		
9	IHNV and IPNV	$1,3*10^6$	$5,9*10^6$	$1,3*10^6$	
10	IHNV and IPNV	$1,3*10^6$	$5,9*10^5$	$1,3*10^6$	$2,7*10^6$
11	IHNV and IPNV	$1,3*10^6$	$1,9*10^6$		
12	IHNV and IPNV	$2,7*10^4$	$1,9*10^4$		
13	IHNV and IPNV	$1,3*10^7$	$2,7*10^5$		
14	IHNV and IPNV	$4,0*10^6$	$1,9*10^6$		
15	IHNV and IPNV	$1,3*10^4$	$8,6*10^3$		
16	IHNV and IPNV		$8,6*10^4$	$4,0*10^5$	
17	IPNV	$1,9*10^8$	$1,9*10^6$	$2,7*10^6$	$5,9*10^6$
18	No reply				
19	IHNV and IPNV	$1,9*10^5$	$1,9*10^4$		$1,3*10^4$
20	IHNV and IPNV	$1,3*10^6$	$1,3*10^6$	$1,3*10^5$	$1,3*10^6$
21	IHNV		$1,9*10^6$	$1,3*10^5$	
22	IHNV and IPNV	$1,9*10^6$	$1,3*10^6$	$4,0*10^6$	$1,3*10^6$
23	IPNV and Ranavirus	$4,0*10^6$			$4,0*10^5$
24	IHNV and IPNV		$5,9*10^5$		$1,3*10^5$
25	IHNV and IPNV	$4,0*10^6$	$4,0*10^6$		
26	IHNV and IPNV	$4,0*10^7$			$5,9*10^6$
27	IHNV and IPNV	$5,9*10^6$	$1,9*10^6$	$2,7*10^5$	
28	No reply				
29	IHNV and IPNV	$4,0*10^6$	$1,3*10^4$		
30	IHNV and IPNV	$4,0*10^6$	$4,0*10^5$		
31	IPNV	$5,9*10^6$	$2,7*10^6$		
32	IHN and SVC	$4,0*10^2$	$2,7*10^6$		
33	IHNV and IPNV	$2,7*10^6$	$2,7*10^5$	$2,7*10^5$	$4,0*10^5$
34	IHNV and IPNV	$8,6*10^4$	$2,7*10^4$		
35	IHNV				$4,0*10^4$
36	IHNV and IPNV	$4,0*10^6$	$5,9*10^7$		
37	IHNV and IPNV	$1,9*10^6$	$5,9*10^6$		
38	IHNV and IPNV	$8,6*10^5$	$8,6*10^5$		
39	IHNV and IPNV	$1,3*10^3$	$1,3*10^6$	$8,6*10^4$	$8,6*10^3$
40	IHNV	$2,7*10^6$	$1,9*10^4$		$1,9*10^5$
41	IHNV and IPNV	$8,6*10^6$	$5,9*10^6$		$8,6*10^4$

Number of laboratories	34	35	13	15
Median titre	$1,9*10^6$	$8,6*10^5$	$4,0*10^5$	$4,0*10^5$
Maximum titre	$1,9*10^8$	$5,9*10^7$	$4,0*10^6$	$5,9*10^6$
Minimum titre	$4,0*10^2$	$8,6*10^3$	$8,6*10^4$	$8,6*10^3$
25% quartile titre	$2,9*10^5$	$2,7*10^5$	$2,7*10^5$	$1,1*10^5$
75% quartile titre	$4,0*10^6$	$1,9*10^6$	$1,3*10^6$	$1,3*10^6$

Table 8. Inter-Laboratory Proficiency Test, PT1, 2011 – Results of titration of ampoule V.

Ampoule V - IPNV					
Laboratory code number	Virus Identification	Titre in			
		BF-2	EPC	RTG-2	FHM
1	IPNV	$8,6*10^7$	$1,9*10^7$	$8,6*10^7$	
2	Not VHSV, IHNV and EHNV	not performed	not performed	not performed	not performed
3	IPNV	$8,6*10^6$	$8,6*10^6$		
4	IPNV	$1,3*10^5$	$5,9*10^6$		
5	IPNV	$4,0*10^7$	$2,7*10^7$	$1,3*10^8$	$1,3E+0^7$
6	IPNV	$8,6*10^6$	$1,3*10^6$	$8,6*10^5$	$8,6*10^5$
7	IPNV	$5,9*10^7$	$5,9*10^7$		
8	IPNV	$1,3*10^8$	$1,3*10^7$		
9	IPNV	$2,7*10^6$	$1,3*10^7$	$5,9*10^5$	
10	IPNV	$8,6*10^4$	$1,9*10^5$	$2,7*10^5$	$4,0*10^5$
11	IPNV	$1,3*10^8$	$1,9*10^7$		
12	IPNV	$8,6*10^6$	$5,9*10^6$		
13	IPNV	$2,7*10^7$	$2,7*10^5$		
14	IPNV	$8,6*10^5$	$2,7*10^5$		
15	IPNV	$1,9*10^7$	$5,9*10^6$		
16	IPNV		$5,9*10^5$	$5,9*10^6$	
17	IPNV	$2,7*10^9$	$4,0*10^9$	$5,9*10^8$	$1,9*10^9$
18	No reply				
19	IPNV	$5,9*10^7$	$1,3*10^6$		$8,6*10^6$
20	IPNV	$2,7*10^5$	$1,3*10^6$	$1,3*10^5$	$1,3*10^6$
21	IPNV		$2,7*10^8$	$1,9*10^6$	
22	IPNV	$1,3*10^8$	$5,9*10^7$	$2,7*10^7$	$2,7*10^7$
23	IPNV	$4,0*10^6$			$2,7*10^6$
24	IPNV		$2,7*10^6$		$5,9*10^4$
25	IPNV	$5,9*10^7$	$8,6*10^7$		
26	IPNV	$1,9*10^6$			$1,9*10^4$
27	IPNV	$5,9*10^7$	$4,0*10^7$	$2,7*10^6$	
28	No reply				
29	IPNV	$8,6*10^5$	$5,9*10^3$		
30	IPNV	$1,3*10^3$	$1,3*10^3$		
31	IPNV	$2,7*10^8$	$4,0*10^7$		
32	Virus not identified	$4,0*10^2$	$1,3*10^7$		
33	IPNV	$4,0*10^8$	$1,3*10^7$	$1,9*10^6$	$1,9*10^7$
34	IPNV	$5,9*10^5$	$1,3*10^6$		
35	IPNV				$2,7*10^3$
36	IPNV	$2,7*10^9$	$5,9*10^9$		
37	IPNV	$1,9*10^6$	$1,9*10^6$		
38	IPNV	$2,7*10^7$	$4,0*10^7$		
39	IPNV	$1,9*10^6$	$8,6*10^6$	$1,9*10^6$	$< 1,9*10^2$
40	not EHNV or other ranavirus, not IHNV, VHSV or SVCV	$2,7*10^7$	$1,3*10^4$		$8,6*10^5$
41	IPNV	$1,3*10^7$	$1,3*10^6$		$1,3*10^4$

Number of laboratoriums	34	35	13	15
Median titre	$1,6*10^7$	$8,6*10^6$	$1,9*10^6$	$8,6*10^5$
Maximum titre	$2,7*10^9$	$5,9*10^9$	$5,9*10^8$	$1,9*10^9$
Minimum titre	$4,0*10^2$	$1,3*10^3$	$1,3*10^5$	$<1,9*10^2$
25% quartile titre	$1,9*10^6$	$1,3*10^6$	$8,6*10^5$	$3,9*10^4$
75% quartile titre	$5,9*10^7$	$3,4*10^7$	$2,7*10^7$	$1,1*10^7$

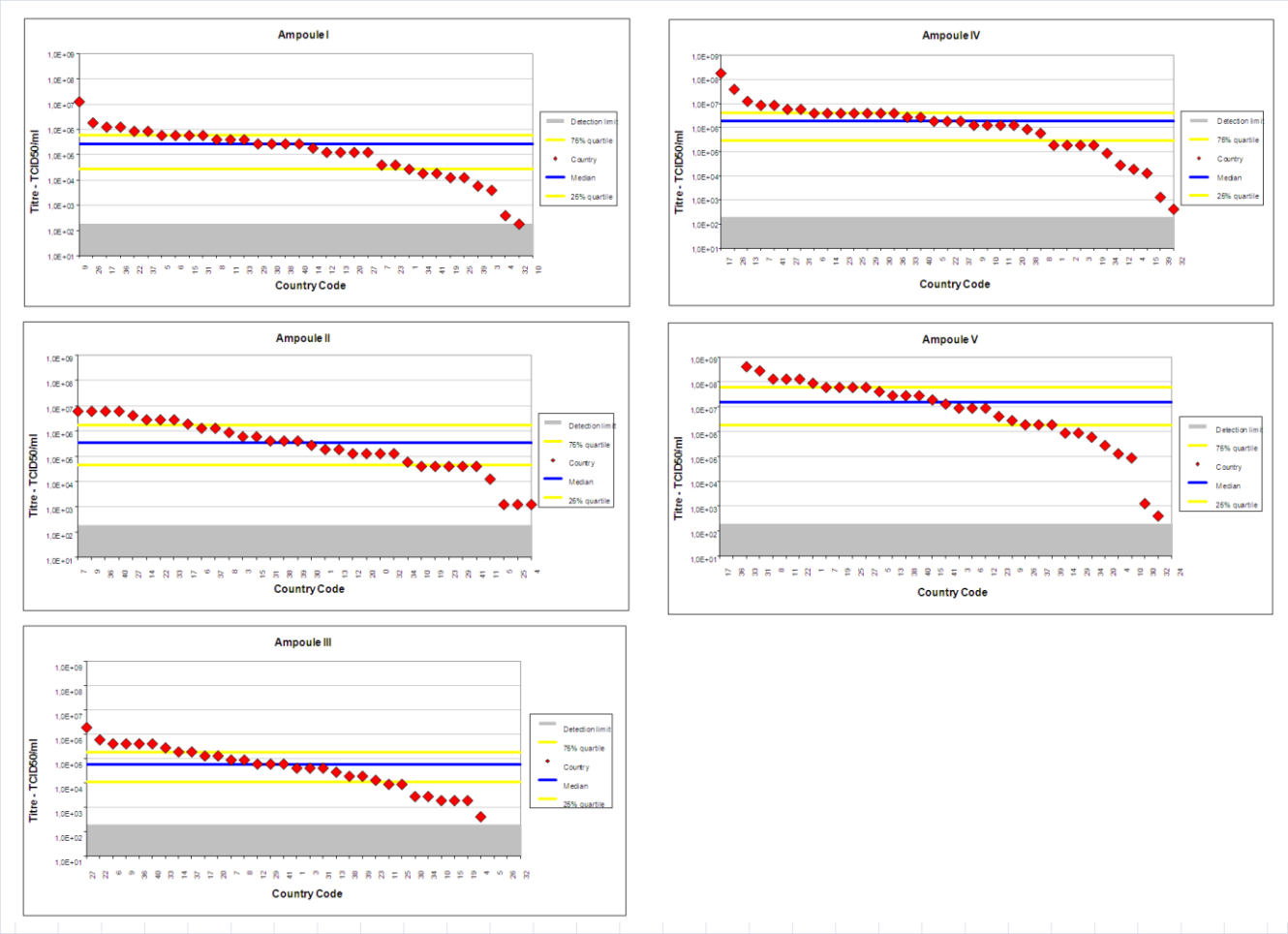

Figure 6. Virus titres obtained in BF-2 cells. The titre (red diamond) of each participating laboratory (country code) using BF-2 cells illustrated for ampoule I, II, III, IV and V. The detection level (gray shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line) are plotted on all graphs. For participants failing to obtain any titre, no red diamond is shown.

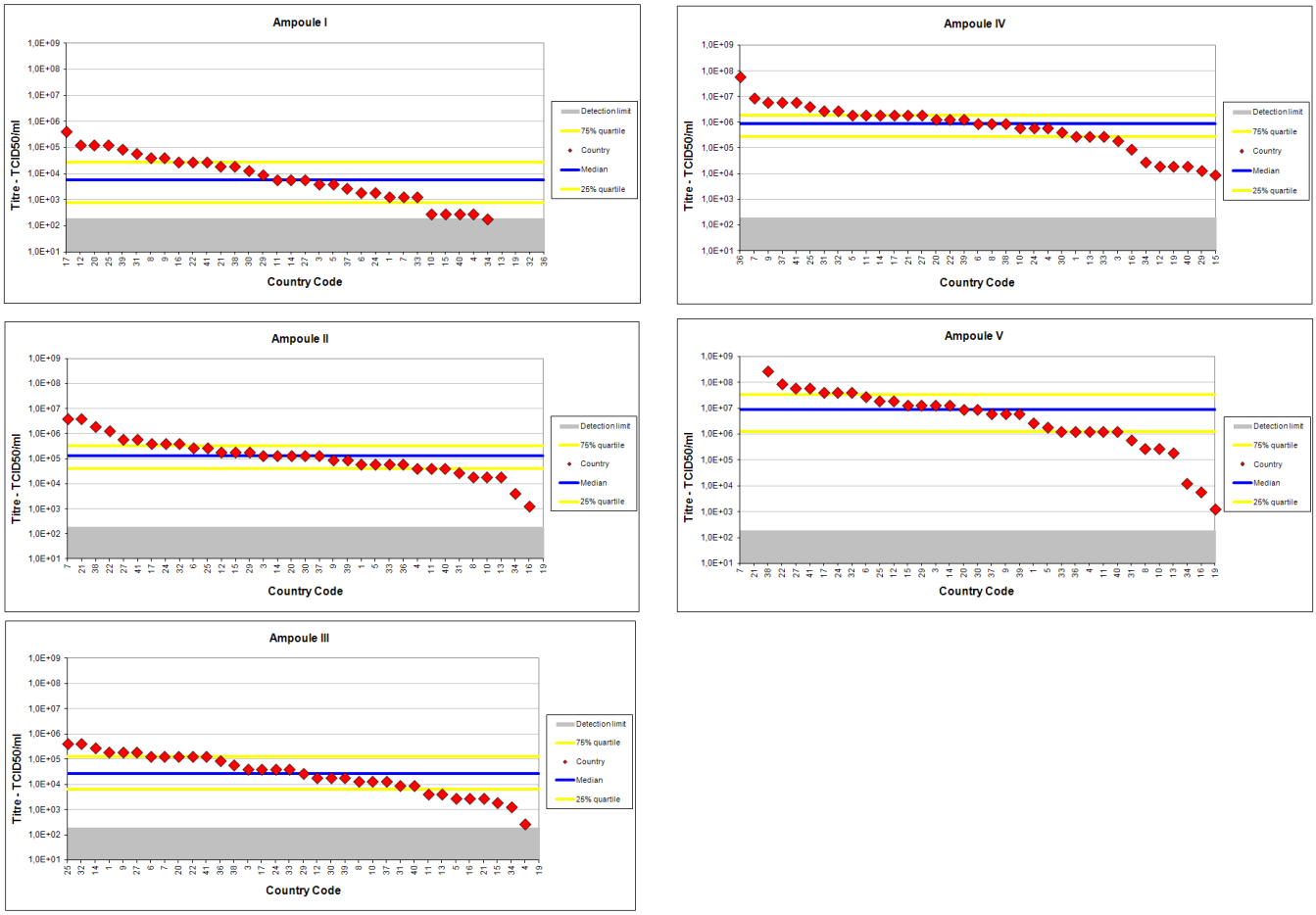

Figure 7. Virus titres obtained in EPC cells. For further details see Figure 6

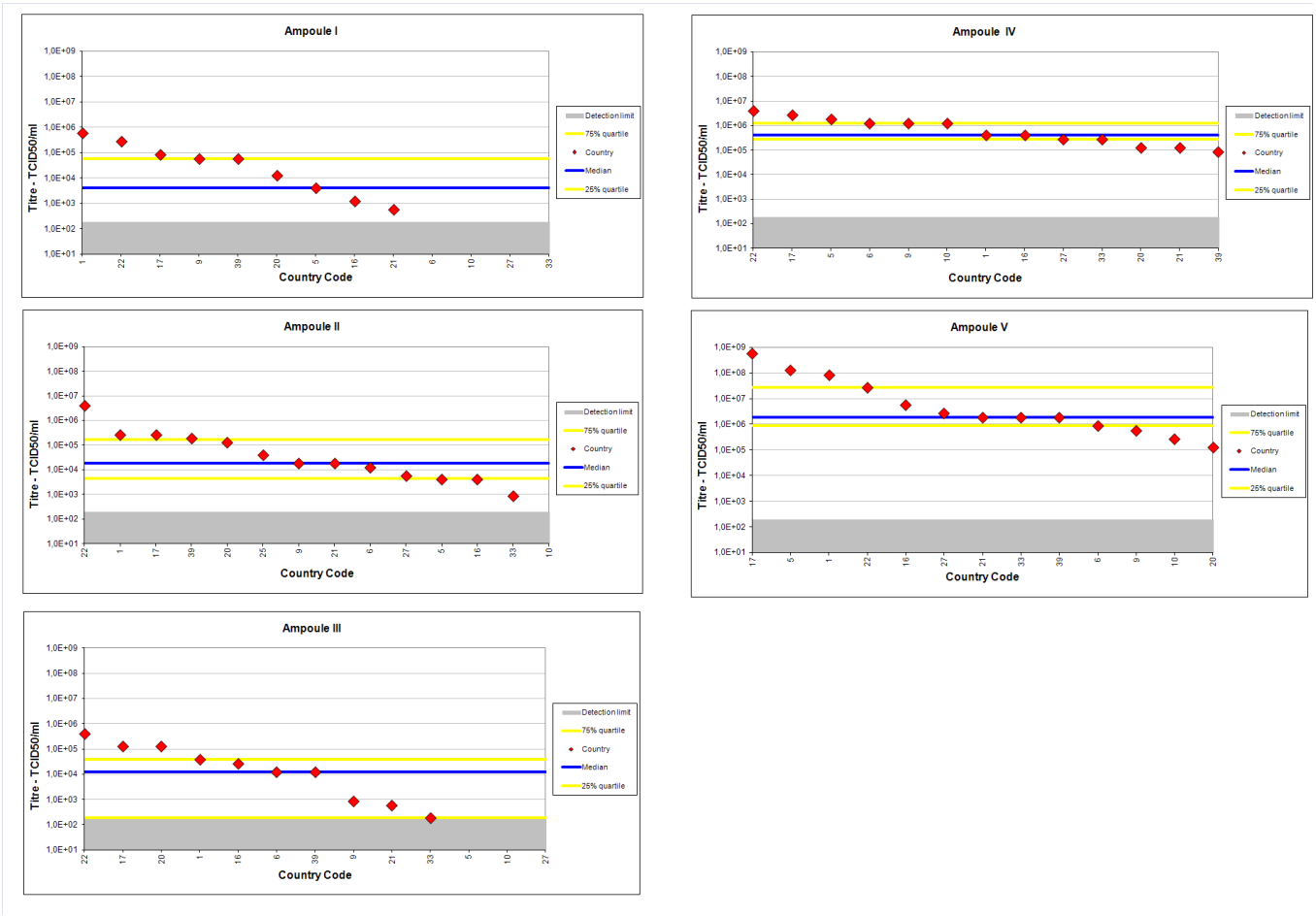

Figure 8. Virus titre obtained in RTG-2 cells. For further details see Figure 6

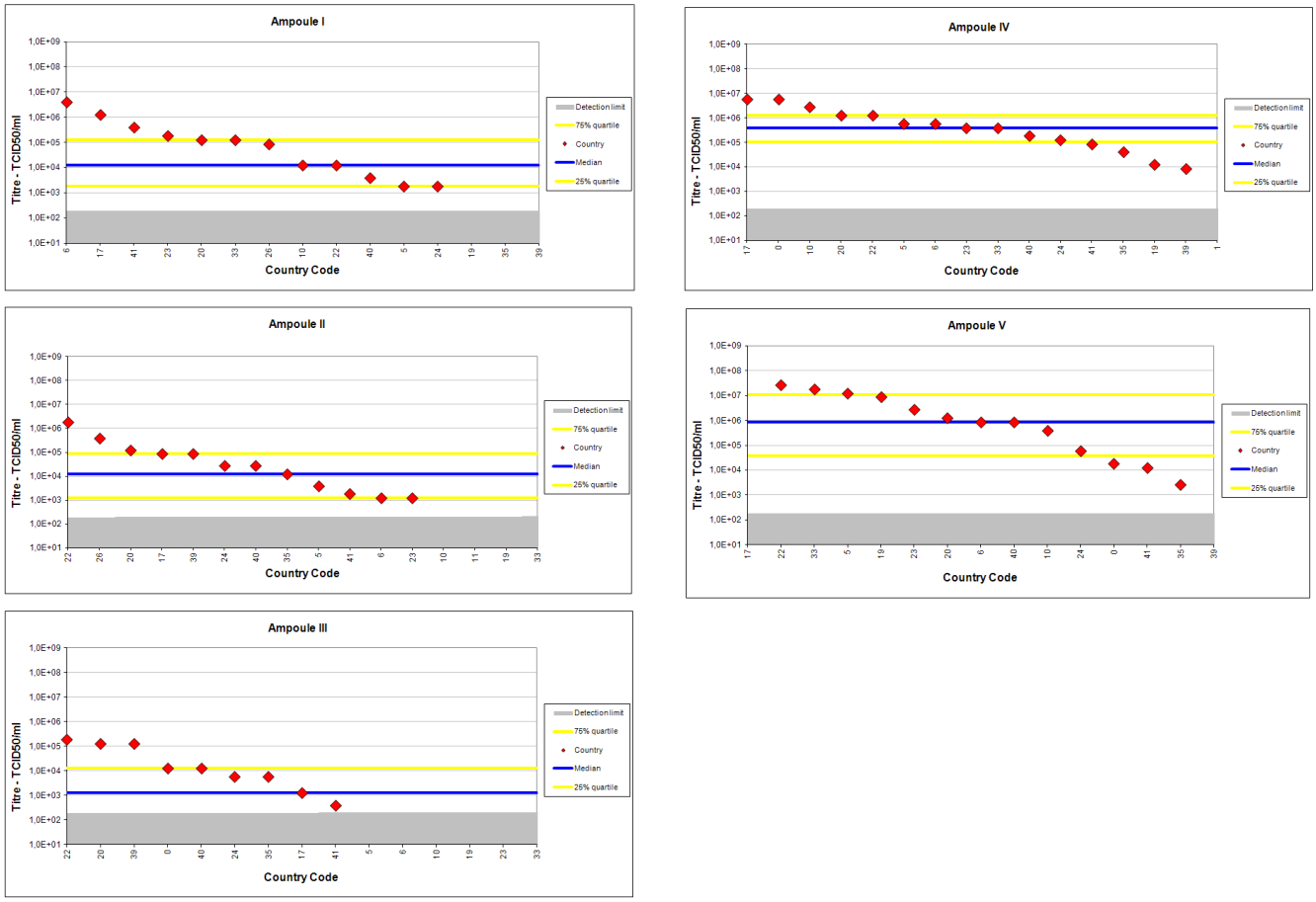

Figure 9. Virus titres obtained in FHM cells. For further details see Figure 6

Identification of content

- 26 laboratories correctly identified all viruses in all ampoules
- 2 laboratories did not reply to this test

Ampoule I – VHSV

- 39 laboratories correctly identified VHSV

Ampoule II – EHNV

- 32 laboratories correctly identified EHNV
- 5 laboratories identified ranavirus, but did not employ genomic analysis
- 2 laboratories found virus but did not identify it

Ampoule III - Ranavirus, ECV (or ESV), not EHNV

- 32 laboratories correctly identified Ranavirus, not EHNV
- 4 laboratories identified ranavirus, but did not employ genomic analysis
- 1 laboratory identified VHSV
- 1 laboratory identified ranavirus (no sequencing) and SVCV
- 1 laboratory found virus but did not identify it

Ampoule IV – IHNV and IPNV

- 31 laboratories correctly identified IHNV and IPNV
- 4 laboratories identified only IHNV
- 2 laboratories identified only IPNV
- 1 laboratory identified IPNV and ranavirus
- 1 laboratory identified IHNV and SVCV

Ampoule V – IPNV

- 36 laboratories correctly identified IPNV
- 2 laboratories identified the virus as not ranavirus, IHNV, VHSV or SVCV
- 1 laboratory examined by PCR only and did not employ IPN RT-PCR

Scores

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

Ampoule I: VHSV identification was given the score 2. VHSV not identified was given the score 0.

Ampoule II: EHNV identification backed up by genomic analysis was given the score 2. EHNV identification not backed up by genomic analysis or ranavirus/iridovirus as the only answer for this ampoule was given the score 1. In case of no genomic analysis the result is stated as ranavirus* in table 3.

Ampoule III: Identification of "ranavirus/iridovirus, not EHNV" or Catfish/sheatfish iridovirus was given the score 2 (stated as ranavirus, not EHNV in table 3). Ranavirus/iridovirus as the only answer for this ampoule was given the score of 1 (if no genomic analysis was performed).

Ampoule IV: Identification of IHNV and IPNV was given the score 2, identification of IHNV only was given the score 1 and identification of IPNV only was given the score 0.

Ampoule V: Identification of IPNV was given the score 2, and identification of virus as "not VHSV, IHNV or EHNV" was given the score 1.

Incorrectly finding of "no virus" or additional types of viruses than those included in the ampoules scored 0 even though included virus was amongst the identified viruses.

Of the laboratories that submitted results, 26 out of 39 correctly identified all viruses in all ampoules and obtained the maximum score 10. Of the laboratories that did identify ranavirus in ampoule II and ampoule III, 5 were not able to identify the ranavirus as EHNV in ampoule 2 and 5 were not able to identify the ranavirus as ranavirus, not EHNV in ampoule III. Additional viruses than present in the ampoules were observed by 2 laboratories. A diagram of the scoring obtained by the laboratories is shown in figure 10.

Serotyping and genotyping of VHSV and IHNV and submission of sequencing results are not a mandatory part of the test and is not included in the score of participants.

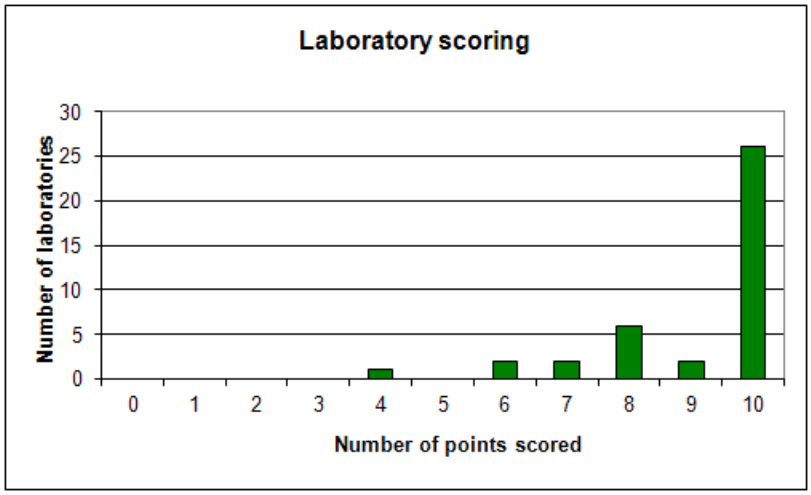

Figure 10. Scores obtained by participants.

Cells applied for solving the test

The following cell lines were used by the participants:

- 34 laboratories used BF-2 cells
- 35 laboratories used EPC cells
- 13 laboratories used RTG-2 cells
- 15 laboratories used FHM cells
- 3 laboratory used CHSE-214 cells
- 8 laboratories used four cell lines
- 6 laboratories used tree cell lines
    - 3 laboratories used BF-2 cells in combination with EPC cells and RTG-2 cells
    - 3 laboratories used BF-2 cells in combination with EPC cells and FHM cells
- 23 laboratories used two cell lines:
    - 18 laboratories used BF-2 cells in combination with EPC cells
    - 2 laboratories used RTG-2 cells in combination with EPC cells
    - 2 laboratories used BF-2 cells in combination with FHM cells
    - 1 laboratory used EPC cells in combination with FHM cells
- 1 laboratory use only FHM cells
- 1 laboratory did not use any cell line, they only performed PCR

The combination of EPC and FHM cells is not valid according to Commission Decision 2001/183/EC. The laboratories using these combinations are encouraged to include the use of BF-2 cells.

The median titre calculated from the submitted results of each ampoule and in each cell line is illustrated in Figure 11. It appears that all ampoules replicates on all cell lines, however there is a tendency that BF-2 cells are better for replication of VHSV genotype Ib, and BF-2 and EPC cells are better for ranaviruses and IPNV than the other cell lines. No conclusions regarding IHNV can be made based on this comparison as the ampoule with this virus also included IPNV.

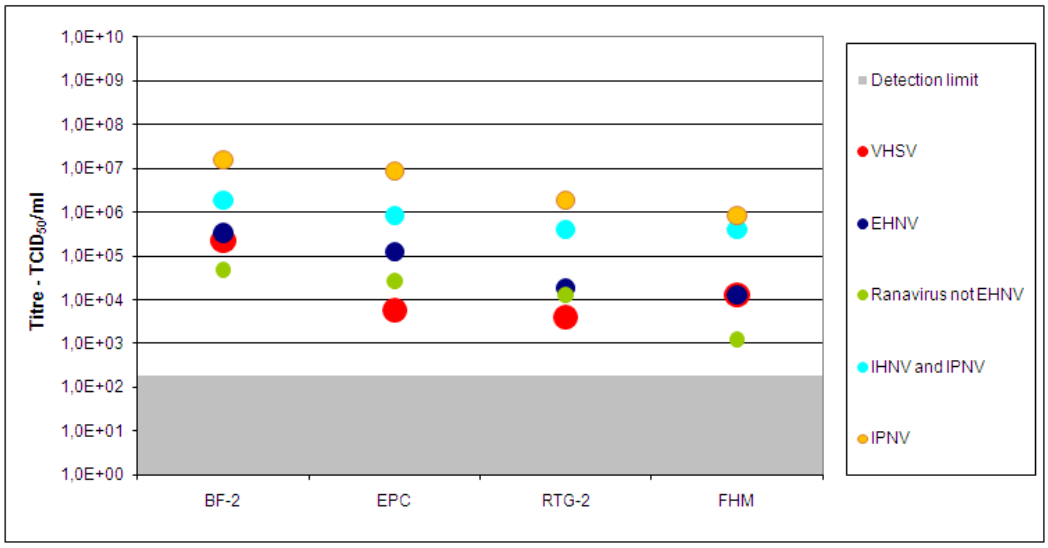

Figure 11. Median virus titres obtained by participants at different cell lines.

Methods used for identification of viruses

(Table 9)

- 22 laboratories used ELISA for identification of viruses.
- 25 laboratories used IFAT for identification of viruses.
- 12 laboratories used neutralisation tests for identification of viruses.
- 39 laboratories used PCR for identification of viruses.
- 33 laboratories performed sequencing for identification of viruses.
- 2 laboratory performed REA according to the OIE Aquatic Animals Manuals for identification of ranaviruses.

Table 9. Results obtained by different test methods in participating laboratories.

Laboratory code number	Score (max. 10)	ELISA	IFAT	Neutralisation	PCR	Sequence ampoule no.
1	10	X	X	X	X	I, II, III,IV and V
2*	8				X	II and III
3	10	X	X		X	II and III
4	7	X	X	X	X	
5	10	X			X	I, II, III and IV
6	10	X	X	X	X	I, II, III and IV
7**	6	X	X		X	
8	10	X			X	I, II, III and IV
9	10		X		X	I, II, III and IV
10	10	X	X	X	X	I, II, III and IV
11***	10	X			X	II and III
12	10	X		X	X	II and III
13	10	X	X		X	I, II, III,IV and V
14	10		X		X	I, II, III and IV
15	8	X	X		X	
16	10	X	X		X	II, III and IV
17	8	X	X		X	I, II, III,IV and V
18	n/a					
19	10	X	X	X	X	REA
20	10	X	X		X	I, II, III,IV and V
21	9				X	I, II, III,IV and V
22	10		X		X	I, II, III and IV
23	6	X		X	X	
24	10		X		X	II and III
25	10	X	X		X	I, II, III,IV and V
26	10	X	X		X	I, II, III,IV and V
27	10				X	I, II, III,IV and V
28	n/a					
29	10	X	X		X	I, II, III,IV and V
30	10				X	I, II, III,IV and V
31	8			X	X	II and III
32	4				X	
33	10				X	I, II, III,IV and V
34	10		X	X	X	I, II, III and IV
35	9				X	II and III
36	7	X			X	III
37	10		X		X	I, II, III and IV
38	8	X	X		X	II and III, REA
39	10		X	X	X	I, II, III,IV and V
40	8		X	X	X	I, II and III
41	10		X	X	X	I, II, III,IV and V

A graph was constructed to illustrate the association between the methods used by participants for virus identification and the obtained score (Figure 12). For participants scoring lower than 10, the deficiency in virus identification cannot directly be assigned to improper use of a single identification method. Rather mistakes might be related to performance of the overall procedure. It is clear, however, that if sequencing or REA is not used, a correct answer cannot be made for ampoule II and III. This accounts for 6 of the laboratories where 4 laboratories have reported ranavirus, due to lack of sequencing and 1 laboratory reported ranavirus due to lack of translation of the sequences and one laboratory likewise reported ranavirus due to low quality sequence and no conclusion was possible based on REA results. Finally, 3 out of the 13 participants scoring lower than 10 identified false positive viruses in ampoules indicating that cross contamination could have occurred at some point in the diagnostic process. Furthermore 8 out of the 13 participants scoring lower than 10 only found one out of two viruses in ampoule IV.

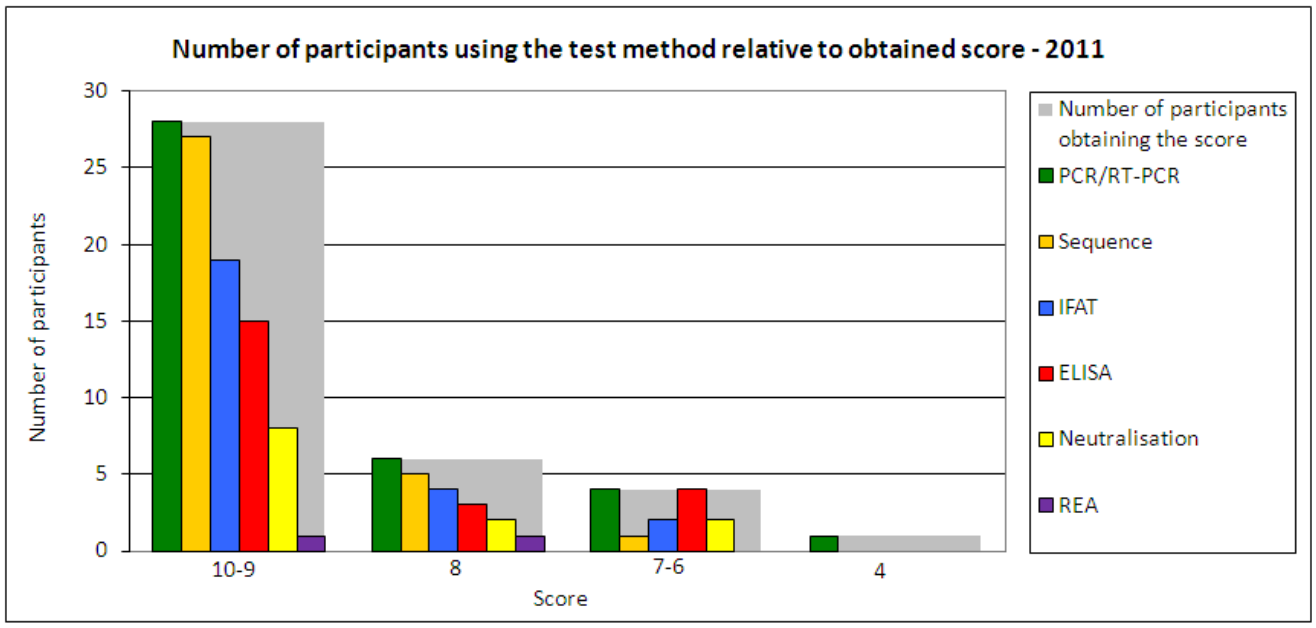

Figure 12. Methods used by participants for identification of viruses in relation to the obtained score.

Genotypning 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) for IHNV but this was not an obligatory task.

Ampoule I – VHSV genotype Ib

- 23 laboratories performed sequencing to identify the virus in ampoule I
- 6 laboratories used the primers described in Einer-Jensen et al. (2004)
- 6 laboratories used the primers described in Snow et al. (2004)
- 11 laboratories used primers described in other references or did not report the reference
- 20 laboratories identified the VHSV isolate as genotype Ib
- 1 laboratory identified the VHSV isolate as genotype Ie
- 2 laboratories did not genotype the VHSV

Ampoule II – EHNV

- 31 laboratories performed sequencing to identify the virus in ampoule II with correct result
- 1 laboratory performed REA according to the OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.1 with correct result
- 1 laboratory performed both REA and sequencing. From this laboratory it was not possible to identify which type of ranavirus the isolate belong to
- 10 laboratories used primers described in Hyatt et al. (2000)
- 11 laboratories used primers described in the OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.1
- 2 laboratories used primers described in Holopainen et al (2009)
- 10 laboratories were using primers described in other references or they did not report the reference

Within the OIE diagnostic manual for EHN, two sequence based methods are recommended to use in order to discriminate EHNV from the other non-listed ranaviruses. A total of 32 laboratories used sequencing analyses for identification of EHNV whereas one used REA. Of the laboratories that sequenced the isolate in ampoule II all but one identified the virus correctly as EHNV.

Ampoule III - Ranavirus, ECV (or ESV), not EHNV

- 31 laboratories submitted sequences that were either identical to or most similar to sheetfish or catfish iridovirus
- 1 laboratory performed REA according to the OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.1 with correct result
- 1 laboratory performed both REA and sequencing. From this laboratory it was not possible to identify which type of ranavirus the isolate belong to
- 1 laboratory sequenced the isolate but were not able to tell which type of ranavirus the isolate belong to
- 10 laboratories used primers described in Hyatt et al. (2000)
- 12 laboratories used primers described in OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.1
- 4 laboratories used primers described in Holopainen et al. (2009)
- 9 laboratories were using primers described in other references or they did not report the reference

Ampoule IV – IHNV and IPNV

- 23 laboratories submitted sequences
- IHNV
  - 20 laboratories genotyped the IHNV isolate as belonging to genogroup M
  - 1 laboratory did not provide a genogroup despite having sequenced the isolate
- IPNV
  - 4 laboratories genotyped the IPNV isolate as belonging to genogroup 5 (Sp)
  - 1 laboratories genotyped the IPNV isolate as belonging to genogroup 3
  - 2 laboratories did not provide a genogroup despite having sequenced the isolate
- For IHNV 4 laboratories used primers described in Emmenegger et al. (2000)
- For IHNV 5 laboratories used primers described in the OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.4
- For IPNV 2 laboratories used the primers described in Taksdal et al. (2001)
- For both IHNV and IPNV, 16 laboratories were using primers described in other references or they did not reported the reference

Ampoule V – IPNV

- 13 laboratories submitted sequences
- 4 laboratories genotyped the IPNV isolate as belonging to genogroup 5 (Sp)
- 1 laboratory genotyped the IPNV isolate as belonging to genogroup 3
- 8 laboratories did not provide a genogroup despite having sequenced the isolate
- 2 laboratories used the primers described in Taksdal et al. (2001)
- 11 laboratories were using other primers or they did not report the primers used

Of the 39 laboratories that delivered results to this PT 32 laboratories used either sequence analysis or REA to identify it the ranavirus was EHNV or not. It is important that the remaining laboratories implement PCR and sequencing techniques in the laboratory as genotyping is the basis for differentiating several listed viruses from others.

Table 10. Genotyping results on viruses in ampoule I-V submitted by participating laboratories**.**

Laboratory code number	Score (max. 10)	Ampoule I VHSV Isolate 1p8	Ampoule II EHNV Isolate 86/8774	Ampoule III Ranavirus not EHNV European Catfish virus (ECV) 562/92 Low titre	Ampoule IV IHNV and IPNV IHNV 32/87 and IPNV strain Sp	Ampoule V IPNV IPNV strain Sp
1	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M	IPNV
2*	8		EHNV (Seq)	ESV-ECV (Seq)		
3	10		EHNV (Seq)	ESV-ECV (Seq)		
4	7					
5	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M	
6	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M	
7**	6					
8	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M	
9	10	VHSV Ib	EHNV (Seq)	Ranavirus-ECV (Seq)	IHNV Genogroup M	
10	10	VHSV Ib	EHNV (Seq)	Ranavirus-ECV (Seq)	IHNV Genogroup M	
11***	10		EHNV (Seq)	ESV-ECV (Seq)		
12	10		EHNV (Seq)	ESV (Seq)		
13	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M IPNV Genogroup 5 (Sp)	IPNV Genogroup 5 (Sp)
14	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M	
15	8					
16	10		EHNV (Seq)	Ranavirus-ECV (Seq)	IHNV Genogroup M	
17	8	VHSV	EHNV (Seq)	ESV-ECV (Seq)	IPNV	IPNV
18	n/a					
19	9		EHNV (REA)	Ranavirus (REA)		
20	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M IPNV Genogroup 5 (Sp)	IPNV Genogroup 5 (Sp)
21	9	VHSV	EHNV (Seq)	ESV-ECV (Seq)	IHNV	IPNV
22	10	VHSV Ib	EHNV (Seq)	ESV-ECV-DFV (Seq)	IHNV Genogroup M	
23	6					
24	10		EHNV (Seq)	ECV (Seq)		
25	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M IPNV A2	IPNV
26	10	VHSV Ib	EHNV (Seq)	Ranavirus not EHNV (Seq)	IHNV Genogroup M IPNV Genogroup 5 (Sp)	IPNV Genogroup 5 (Sp)
27	10	VHSV Ib	EHNV (Seq)	ESV (Seq)	IHNV Genogroup M	IPNV
28	n/a					
29	10	VHSV Ib	EHNV (Seq)	Ranavirus ESV	IHNV Genogroup M	IPNV
30	10	VHSV Ie	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M IPNV Genogroup 5 (Sp)	IPNV Genogroup 5 (Sp)
31	8		EHNV (Seq)	ESV-ECV (Seq)		
32	3					
33	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M IPNV Genogroup III	IPNV Genogroup III
34	10	VHSV Ib	EHNV (Seq)	Ranavirus ESV (Seq)	IHNV Genogroup M	
35	9		EHNV (Seq)	ESV-ECV (Seq)		
36	7			Ranavirus (Seq)		
37	10	VHSV Ib	EHNV (Seq)	ESV (Seq)	IHNV Genogroup M	
38	8		Ranavirus (Seq, REA)	Ranavirus (Seq, REA)		
39	10	VHSV Ib	EHNV (Seq)	Ranavirus (Seq)	IHNV Genogroup M	IPNV
40	8	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)		
41	10	VHSV Ib	EHNV (Seq)	ESV-ECV (Seq)	IHNV Genogroup M	IPNV

Concluding remarks PT1

The inter-laboratory proficiency test 2011 was conducted without major constrains. Most parcels were delivered by the shipping companies within 3 days after submission. It was, however, unfortunate that one parcel was 44 days on the way before delivered to the laboratory primarily due to border controls.

In 2009 EHNV was included in the proficiency test for the first time and 32 participants were able to correctly identify the virus. This year EHNV was included as well as ECV, both belong to the ranavirus family. Of the laboratories performing PCR based methods, 31 laboratories performed sequencing only for ampoule II and 32 for ampoule III. Of these laboratories all correctly identified the content in ampoule II as EHNV and 31 correctly identified the content in ampoule III as ECV/ESV. One laboratory performed both sequencing and REA for both ampoule II and III without being able to identify which type of ranavirus the isolates belong to. One laboratory performed REA only for both ampoule II and III and was able to identify the isolate as either EHNV or ranavirus, not EHNV.

In this report (figures 6-9), all titres submitted by participants for each cell line and ampoule, respectively 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 should be able to compare the sensitivity of their cell lines to the sensitivity of those used by the other participating laboratories. We recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose.

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

The results presented in this report will be further presented and discussed at the 16th Annual Meeting of National Reference Laboratories for Fish Diseases to be held 30-31 May 2012 in Aarhus, Denmark.

Proficiency test 2, PT2

Five ampoules with lyophilised cell culture supernatant or *A. invadans spores* were delivered to the same laboratories as 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 20% w/v lactalbumin hydrolysate solution and lyophilized in glass ampoules. Produced *A. invadans* spores were treated the same way as supernatants. Before the ampoules were sealed by melting, the pathogen concentration was analysed by real-time PCR for KHV (protocol described by Gilad et al. (2004)), real-time RT-PCR for ISAV (protocol described by Snow et al. (2006)) and conventional PCR for *A. invadans* (protocol describe by Kurata et al. (2000)).

The details of the virus isolates used in the proficiency test are outlined in table 11.

Table 11. Content of each ampoule with reference to culture conditions and major publications of the included pathogens.

PT2	
Code	**Specifications**
Ampoule VI: ISAV Low titre	**ISAV Glesvaer/2/90** Received from Dr. B. Dannevig, ISA OIE Reference Laboratory, Oslo, Norway References: Mjaaland S, Rimstad E, Falk K & Dannevig BH (1997). Genomic characterization of the virus causing infectious salmon anemia in Atlantic salmon (*Salmo salar* L.): an orthomyxo-like virus in a teleost. *Journal of Virology* **71**, 7681-7686. 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.
Ampoule VII: ISAV High titre	Same as Ampoule VI
Ampoule VIII: KHV High titre	**KHV-TP 30** Koi Herpesvirus (CyHV-3): KHV-TP 30 (syn: KHV-T (for Taiwan)). KHV-TP 30 was isolated from koi in Taiwan and cloned for producing large plaques by Dr. Peiyu Lee, Institute of Medical Biotechnology, Central Taiwan University of Science and Technology, Dakeng, BeiTung District, TaiChung City 406, Taiwan in-2005. The isolate was provided by Dr. Sven M. Bergmann, Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Südufer 10, 17393 Greifswald-Insel Riems, Germany
Ampoule IX: *Aphanomyces invadans*	***Aphanomyces piscicida/invadans* spores NJM9701** Received from Dr. Kishio Hatai, Lab Fish Diseases NVLU Tokyo, Japan Reference: Kurata O., Kanai H. & Hatai K. (2000) Hemagglutinating and hemolytic capacities of Aphanomyces piscicida. *Fish Pathology - Gyobyo Kenkyu* **35**, 29-33.
Ampoule X: KHV Low titre	Same as Ampoule VIII

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 PCR (Bercovier et al. (2005)) and real-time PCR (Gilad et al. (2004)) for KHV and by RT-PCR (Mjaaland et al. (1997)) and real-time RT-PCR (Snow et al. (2006)) for ISAV, to ascertain identity, a satisfactory titre of the virus and homogeneity of the content in the ampoules (Table 12). Furthermore, conventional PCR/RT-PCR fragments were sequenced and so was the HPR region of the ISAV isolate.

KHV and ISAV were prepared in different concentrations that were well above detection level.

The lyophilisation procedure caused a significant virus reduction as detected by real-time PCR or real-time RT-PCR by approximately 4-5 Ct values (Table 13). Furthermore, after lyophilisation the content of the ampoules were tested for stability over time. Each virus preparation was stored three months in the dark and then kept for a period of 5 hours at temperatures rising from 20°C to 42°C, furthermore the viruses were tested with and without filtration through a 45 µm filter. These conditions did not decrease Ct values of neither KHV nor ISAV. *A. invadans* went through the same treatment, but only conventional PCR was done, and there was no change in the size of the band.

For each ampoule the presence of pathogens other than the expected was not detected.

Table 12. Ct-value of representative ampoules of no. VI to VIII and X tested at the EURL; tested before lyophilisation, immediately after lyophilisation, and after 3 months of storage in the dark at 4°C and rising from 20°C to 42°C for 5 hours (1 replicate), respectively. For ampoule IX the presence of a specific band after conventional PCR is stated.

Ampoule No.	**Ampoule**	**Ct value/presence of band before lyophilisation undiluted**	**Ct value presence of band right after lyophilisation**	**Ct value presence of band 3 months after lyophilisation (4°C, dark conditions)**	**Ct value presence of band 3 months after lyophilisation (4°C, dark conditions)**	**Ct value presence of band 3 months after lyophilisation (up to 42°C, dark conditions)**	**Ct value presence of band 3 months after lyophilisation (up to-42°C, dark conditions)**
				+ Filtration	- Filtration	+ Filtration	- Filtration
Ampoule VI: ISAV Glesvaer/2/90 Low titre	a	28,00	33,61	32,56	32,63	32,56	32,43
	b	27,90	33,42				
	c	27,91	33,89				
	d		33,00				
	e		34,03				
	Average	27,94	33,59				
Ampoule VII: ISAV Glesvaer/2/90 High titre	a	24,02	29,79	28,62	28,70	28,82	28,56
	b	24,14	29,56				
	c	24,27	30,08				
	d		29,98				
	e		29,89				
	Average	24,14	29,86				
Ampoule VIII: KHV KHV-TP 30 High titre	a	22,72	25,00	25,70	24,83	24,99	24,67
	b	22,84	25,15				
	c	21,62	24,92				
	d		25,14				
	e		25,28				
	Average	21,73	25,10				
Ampoule IX: *Aphanomyces invadans* NJM 9701	a	+	+	+	+	+	+
	b	+	+				
	c	+	+				
	d		+				
	e		+				
Ampoule X: KHV KHV-TP 30 Low titre	a	25,68	29,36	29,37	29,34	29,12	29,03
	b	25,74	29,60				
	c	26,03	29,73				
	d		29,25				
	e		29,27				
	Average	25,82	29,44				

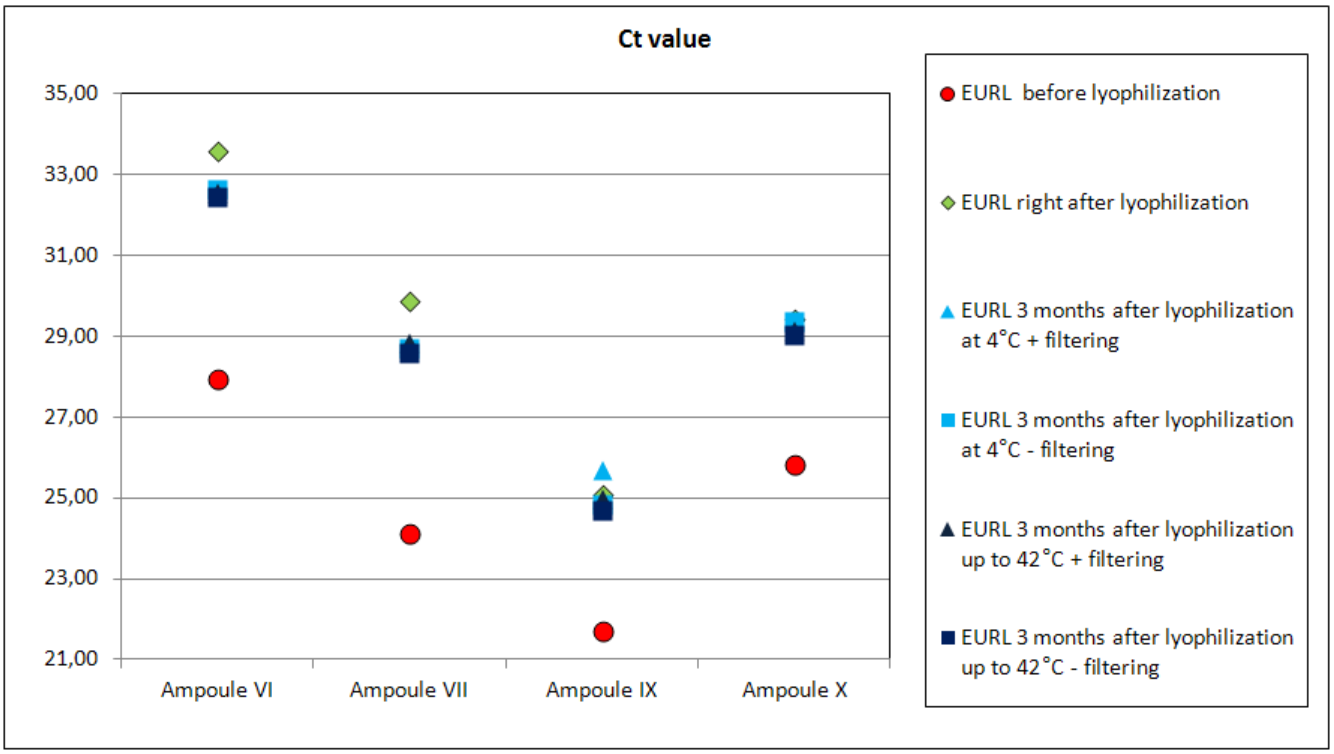

Figure 13. Ct values before, right after and 3 months after lyophilisation at different cell lines. "EURL before lyophilisation" correspond to the Ct value of the undiluted virus.

Virus identification

In PT2, Participants were asked to identify any of the fish viruses ISAV and KHV (both listed in Council Directive 2006/88/EC) if present in the ampoules, 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. In order to obtain uniform answers, participants were requested to download a spreadsheet available from the EURL web page, insert results in this and return by email. The results from participating laboratories are shown in table 13.

All laboratories were encouraged to sequence the HPR region of ISAV isolates. However, this was not a mandatory task.

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

Table 13. Inter-Laboratory Proficiency Test, PT2, 2011 - Virus identification.

Laboratory code number	Score (max. 10)	Answer recieved at EURL	Ampoule VI ISAV (low titer)	Ampoule VII ISAV (high titer)	Ampoule VIII KHV (high titer)	Ampoule IX *A.invadans*	Ampoule X KHV (low titer)
1	10	13-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
2	4	29-11-2011	ISAV (Seq)	ISAV (Seq)	KHV; ISAV (weak positive) (Seq)	*A. invadans* ISAV (weak positive) (Seq)	KHV, ISAV and *A. invadans* (weak positive) (Seq)
3	8	12-12-2011	ISAV, *A. invadans* (Seq)	ISAV (Seq)	KHV	*A. invadans*	KHV
4	4	16-12-2011	*A. invadans*		KHV		KHV
5	8	13-12-2011	ISAV	ISAV	KHV		KHV
6	10	09-12-2011	ISAV (Seq)	ISAV (Seq)	KHV	*A. invadans*	KHV
7	8	16-12-2011	ISAV	ISAV	KHV	not KHV, ISAV	KHV
8	8	16-12-2011	ISAV	ISAV	KHV	*A. invadans* negative	KHV
9	10	23-11-2011	ISAV (Seq)	ISAV (Seq)	KHV	*A. invadans*	KHV
10	8	16-12-2011	not ISAV, KHV, SVCV, EUS	ISAV (Seq)	KHV	*A. invadans*	KHV
11	10	16-12-2011	ISAV	ISAV	KHV	*A. invadans*	KHV
12	10	08-12-2011	ISAV	ISAV	KHV	*A. invadans*	KHV
13	10	15-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
14	10	16-12-2011	ISAV	ISAV	KHV	*A. invadans*	KHV
15	8	16-12-2011	ISAV	ISAV	KHV	Not ISAV and KHV	KHV
16	8	15-12-2011	ISAV	ISAV	KHV		KHV
17	10	14-12-2011	ISAV	ISAV	KHV	*A. invadans*	KHV
18	0	n/a	No reply	No reply	No reply	No reply	No reply
19	6	16-12-2011	Not IHNV, VHSV, KHV, Ranavirus, CyHV, SVCV and ISAV	ISAV	KHV	Not IHNV, VHSV, KHV, Ranavirus, CyHV, SVCV and ISAV	KHV
20	10	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
21	10	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
22	10	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
23	6	14-12-2011			KHV	*A. invadans*	KHV
24	10	07-12-2011	ISAV	ISAV	KHV (Seq)	*A. invadans*	KHV (Seq)
25	10	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
26	10	06-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
27	8	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	Not ISAV and KHV	KHV (Seq)
28	0	Did not participate	No reply	No reply	No reply	No reply	No reply
29	10	16-12-2011	ISAV (Seq)	ISAV	KHV (Seq)	*A. invadans*	KHV (Seq)
30	10	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans* (Seq)	KHV (Seq)
31	4	16-12-2011	ISAV	ISAV			
32	6	15-12-2011	*A. invadans*? *ISAV*?	*A. invadans*? ISAV?	KHV	*A. invadans*	KHV
33	10	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV	*A. invadans*	KHV
34	6	16-12-2011	ISAV	ISAV	KHV	KHV	no virus detected
35	8	16-12-2011	none	ISAV	KHV	*A. invadans*	KHV
36	10	16-12-2011	ISAV	ISAV	KHV	*A. invadans*	KHV
37	10	13-12-2011	ISAV	ISAV	KHV	*A. invadans*	KHV
38	n/a	16-12-2011	No reply	No reply	No reply	No reply	No reply
39	10	07-12-2011	ISAV (Seq)	ISAV (Seq)	KHV	*A. invadans* (Seq)	KHV
40	10	16-12-2011	ISAV	ISAV	KHV	*A. invadans* (Seq)	KHV
41	10	16-12-2011	ISAV (Seq)	ISAV (Seq)	KHV (Seq)	*A. invadans*	KHV (Seq)

[1] ISAV identification is performed by another NRL

[2] Analysed for the presence of ISAV only

[3] The concluding results were not written, the EURL has therefore written the concluding results in the sheet, the *A. invadans* in ampoule VI and VII may be due to a typing mistake in the received sheet

[4] The laboratory submitted sequence results after deadline, but before ampoule content were made public available. The result of this participant is therefore included in this report.

[5] Did not participate in PT2

n/a: *not applicable*

Identification of content

- 38 laboratories submitted results
- 22 laboratories correctly identified all five ampoules
- 29 laboratories tested for all three listed pathogens
- 36 laboratories tested for ISAV
- 37 laboratories tested for KHV
- 31 laboratories tested for *A. invadans*
- 1 laboratory tested for ISAV only
- 6 laboratories did not test for *A. invadans* but did for ISAV and KHV
- 2 laboratories did not test for ISAV but did for *A. invadans* and KHV
- 1 laboratory tested for ISAV by cell culture and IFAT, not by RT-PCR methods
- 3 laboratories did not submit any results

Ampoule VI – ISAV Low titre

- 31 laboratories correctly identified ISAV
- 1 laboratory identified ISAV and *A. invadans*
- 2 laboratories identified *A. invadans*
- 4 laboratories tested for and did not identify ISAV, one of these laboratories tested by cell culture only
- 2 laboratories did not examine for ISAV

Ampoule VII – ISAV High titre

- 35 laboratories correctly identified ISAV
- 1 laboratory identified *A. invadans*
- 1 laboratory tested for and did not identify ISAV
- 2 laboratories did not examine for ISAV

Ampoule VIII – KHV High titre

- 36 laboratories correctly identified KHV
- 1 laboratory identified KHV and ISAV (weak positive)
- 1 laboratory did not examine for KHV

Ampoule IX – Aphanomyces invadans

- 27 laboratories correctly identified *A. invadans*
- 1 laboratory identified *A. invadans* and ISAV (weak positive)
- 1 laboratory identified KHV
- 3 laboratories tested for but did not identify *A. invadans*
- 7 laboratories did not examine for *A. invadans*

Ampoule X – KHV Low titre

- 35 laboratories correctly identified KHV
- 1 laboratory identified KHV, *A. invadans* and ISAV (weak positive)
- 1 laboratory tested for and did not identify KHV
- 1 laboratory did not examine for KHV

Scores

We have assigned a score of 2 for each correct answer (Table 13), giving the possibility for obtaining a maximum score of 10. Incorrectly finding of pathogens not present in the ampoules gives the score 0.

Of the 38 laboratories submitting results 22 laboratories correctly identified all ampoules and obtained maximum score. One laboratory examined for ISAV only, this laboratory obtained the score 4 out of 4 possible, and one laboratory did not examined for ISAV, this laboratory obtain the score 6 out of 6 possible. Six laboratories did not examine for *A. invadans*, of these laboratories the maximum score of 8 was obtained by 4 of them. Tree laboratories did not submit any results and obtained the score 0. Laboratories not scoring for them the maximum possible score either lacked identification or identified pathogen(s) not present. Genotyping of ISAV HPR region and submission of sequencing results was not a mandatory part of the test and is not included in the score of participants.

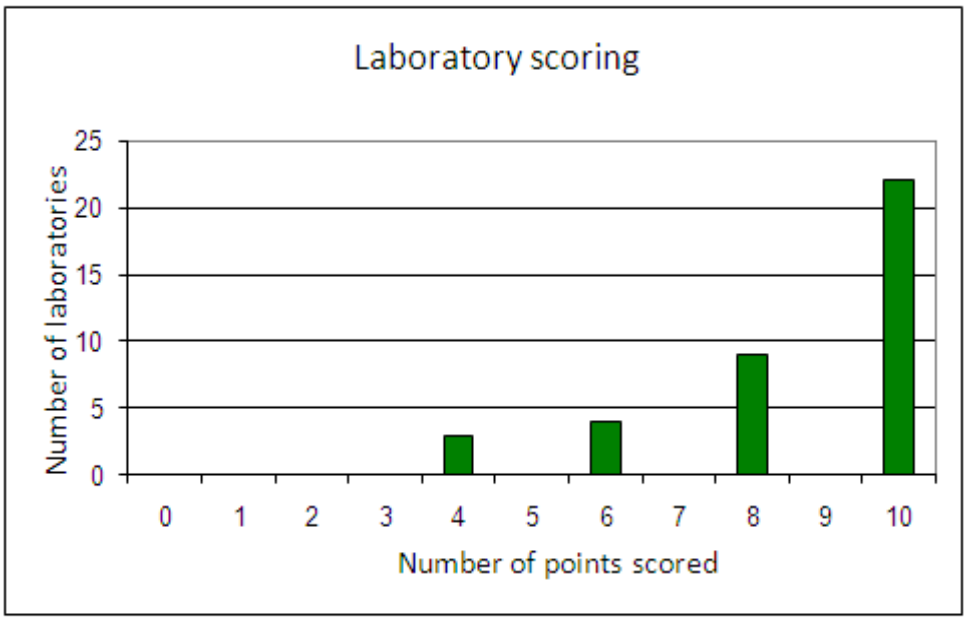

Figure 14. *Obtained score by participants.*

Methods applied

The following methods were used by the participants:

- 15 laboratories used ISAV real-time RT-PCR
- 28 laboratories used ISAV RT-PCR
- 8 laboratories used both ISAV real-time RT-PCR and ISAV RT-PCR
- 13 laboratories used KHV real-time RT-PCR
- 34 laboratories used KHV PCR
- 10 laboratories used both KHV real-time PCR and KHV PCR
- 31 laboratories used *A. invandans* PCR

Laboratory code number	Score (max. 10)	Answer recieved at EURL	ISAV real-time RT-PCR	ISAV RT-PCR	KHV real-time RT-PCR	KHV PCR	*A. invadans* PCR	Sequence ampoule no.
1	10	13-12-2011		X	X	X	X	VI,VII,VIII,IX and X
2	4	29-11-2011		X		X	X	VI,VII,VIII,IX and X
3	8	12-12-2011	X	X		X	X	VI and VII
4	4	16-12-2011		X		X	X	
5	8	13-12-2011		X		X	X	
6	10	09-12-2011	X	X	X	X	X	VI and VII
7	8	16-12-2011	X			X		
8	8	16-12-2011		X		X	X	
9	10	23-11-2011	X	X	X	X	X	VI and VII
10	8	16-12-2011		X		X	X	VII
11	10	16-12-2011		X		X	X	
12	10	08-12-2011	X		X	X	X	
13	10	15-12-2011	X	X	X	X	X	VI,VII,VIII,IX and X
14	10	16-12-2011	X			X	X	
15	8	16-12-2011		X		X		
16	8	15-12-2011	X		X			
17	10	14-12-2011		X		X	X	
18	0	n/a	No reply	No reply	No reply	No reply	No reply	
19	6	16-12-2011				X		
20	10	16-12-2011		X		X	X	VI,VII,VIII,IX and X
21	10	16-12-2011		X		X	X	VI,VII,VIII,IX and X
22	10	16-12-2011		X	X	X	X	VI,VII,VIII,IX and X
23	6	14-12-2011			X		X	VIII and X
24	10	07-12-2011	X		X	X	X	VIII and X
25	10	16-12-2011		X		X	X	VI,VII,VIII,IX and X
26	10	06-12-2011	X	X	X	X	X	VI,VII,VIII,IX and X
27	8	16-12-2011	X	X	X	X		VI,VII,VIII,IX and X
28	0	Did not participate	No reply	No reply	No reply	No reply	No reply	
29	10	16-12-2011		X		X	X	VI, VIII and X
30	10	16-12-2011	X			X	X	VI,VII,VIII,IX and X
31	4	16-12-2011	X					
32	6	15-12-2011						
33	10	16-12-2011		X		X	X	VI and VII
34	6	16-12-2011	X	X		X		
35	8	16-12-2011		X		X	X	
36	10	16-12-2011		X		X	X	
37	10	13-12-2011		X	X	X	X	
38	n/a	16-12-2011	No reply	No reply	No reply	No reply	No reply	
39	10	07-12-2011		X		X	X	VI, VII and IX
40	10	16-12-2011	X	X	X		X	IX
41	10	16-12-2011		X		X	X	VI,VII,VIII and X

A graph was constructed to illustrate the association between the methods used by participants for pathogen identification and the obtained score (Figure 15). The conventional PCR and RT-PCR was the most frequently used method compared to the equivalent real-time assays. For ISAV and KHV identification, approximate half the number of laboratories used real-time assays compared to the number of laboratories using conventional assays. This approximate ratio seems more or less conserved for laboratories scoring max point as well as for laboratories scoring lower points. Therefore, for participants scoring lower than 10, the deficiency in virus identification cannot directly be assigned to improper use of a single identification method. Rather mistakes might be related to performance of the overall procedure.

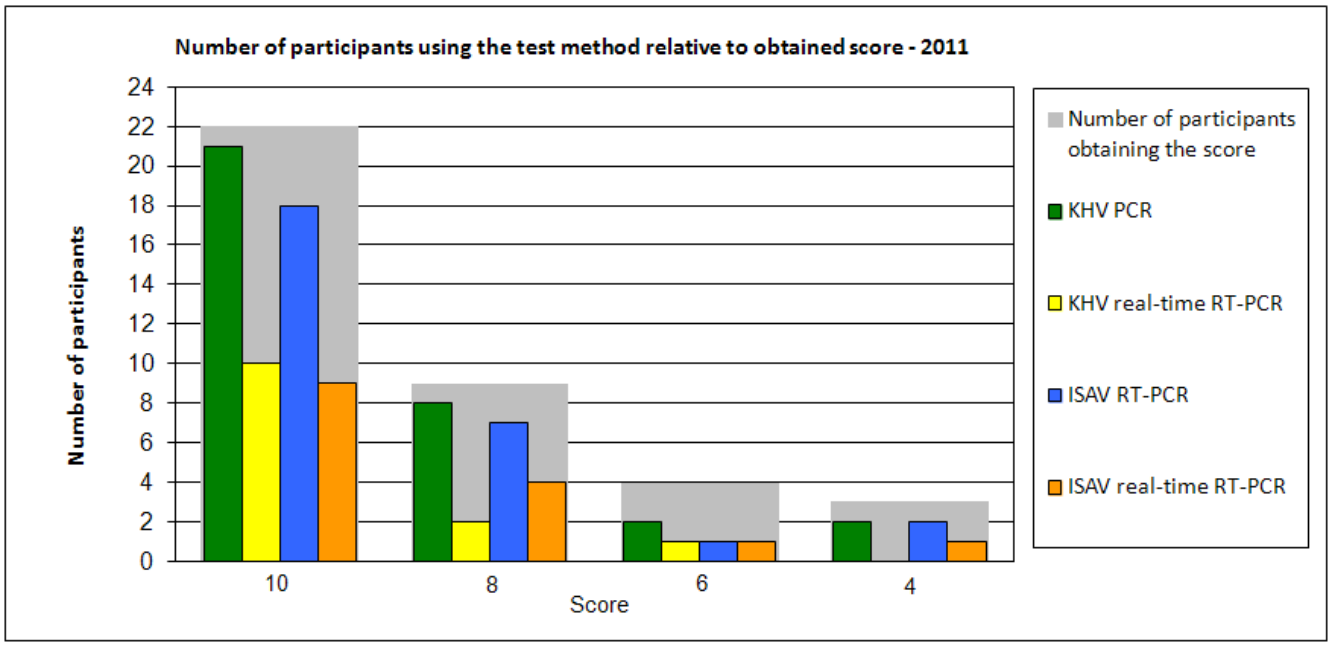

Figure 15. Methods used by participants for virus identification in PT2

Genotypning and sequencing

Participants were encouraged to sequence the HPR region of possible ISAV isolates though it was not a mandatory task.

- 18 laboratories performed sequencing of the ISAV isolate
- 14 laboratories performed sequencing of the KHV isolate
- 12 laboratories performed sequencing of the *A. invadans* isolate

It is positive that many laboratories performed sequencing of isolates as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains containing deletion in the HPR region and HPR0 strains. It was not described according to what notification the genotype of viruses should be performed reflecting the various way of reporting isolate genotypes. In future tests we will clarify which notification the genotyping should follow.

Concluding remarks PT2

The inter-laboratory proficiency test 2011 was conducted without major constrains. Most parcels were delivered by the shipping companies within 3 days after submission. It was, however, unfortunate that one parcel was on the way for 44 days before delivered to the laboratory primarily due to border controls.

Considering that this was the second time that the EURL provided a proficiency test on ISAV and KHV identification, and the first time that the EURL provided a proficiency test on *A. invadans*, we consider that most participants obtained satisfying results. Out of 36 laboratories performing ISAV identification 32 identified ISAV in ampoule VI containing low titre ISAV, and 35 identified ISAV in ampoule VII containing high titre ISAV. All 37 laboratories testing for KHV identified KHV in ampoule VIII containing high titre KHV, and 36 of them identified KHV in ampoule X containing low titre KHV. Out of 31 laboratories testing for *A. invadans* 28 identified the pathogen in ampoule IX.

Lowering the titre of the virus caused only one laboratory to miss identification of KHV in the low titered ampoule X. As this laboratory stated that KHV was identified in ampoule IX whereas no virus was identified in ampoule X, it may be due to either a typing mistake or a mix-up of ampoule IX and X as this laboratory did not test for *A. invadans* and not that the low titered virus was under detection level in this particular laboratory.. If this is the reason then all laboratories testing for KHV were able to identify both the high titer and the low titer KHV. For ISAV, one laboratory missed identification in the high titer ampoule and for the low titer ampoule three further laboratories did not succeed in the identification.

A couple of laboratories identified pathogens not present in the ampoules. E.g. one laboratory identified ISAV in all ampoules but only with a weak positive reaction in the ampoules where ISAV was not present.

A critical point in PCR based diagnostic tools is avoiding false positive and false negative results. To decrease the risk of having false negative results, it is always recommended that laboratories use the most sensitive tool available, validate the sensitivity of their diagnostic tools and use proper controls. To decrease the risk of false positive results laboratories have to be very aware of the risk of cross contaminations.

Many laboratories performed sequencing of ISAV and KHV isolates. However, it was not described which notification should be used for genotyping of viruses. This might reflect the various way of reporting isolate genotypes. In future tests we will clarify which notification the genotyping should follow.

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 presented in this report will be further presented and discussed at the 16th Annual Meeting of National Reference Laboratories for Fish Diseases to be held 30-31 May 2012 in Aarhus, Denmark.

Nicole Nicolajsen and Niels Jørgen Olesen

European Union Reference laboratory for Fish diseases
National Veterinary Institute, Technical University of Denmark, 12 March 2012

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