Report of the Inter-Laboratory Proficiency Test for National Reference Laboratories for Fish Diseases

2007

Organised by
the Community Reference Laboratory
for Fish Diseases,
Technical University of Denmark, National Veterinary Institute, Århus, Denmark

Introduction

A comparative test of diagnostic procedures was provided by the Community Reference Laboratory for Fish Diseases (CRL) to 35 Reference Laboratories (NRLs) in the end of September 2007.
The test contained five coded ampoules, with either viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV) or spring viraemia of carp virus (SVCV). The ringtest was designed to primarily assess the ability of participating laboratories to identify the non-exotic viruses: VHSV, IHNV, and SVCV but also to assess their ability to differentiate other fish viruses, as infectious pancreatic necrosis virus (IPNV), perch rhabdovirus etc. In addition the participants were asked to titrate the viruses in order to assess the cell susceptibility for virus infection in the respective laboratories.
Participants were asked to reply within 8 weeks of receiving the test.
Due to an ongoing discussion on sequencing as a tool for differentiation between various genotypes of the non-exotic viruses, all laboratories were asked to provide full-length G-gene sequences of the submitted rhabdoviruses in the test. This exercise will provide a good tool for assessing the quality of sequence data, by assessing the homogeneity of the sequences. The results of the sequencing and genotyping were set 2 weeks after the first deadline.
Each laboratory has been given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. An un-encoded version of the report will be sent to the Commission.

Participants

Five ampoules with lyophilised tissue culture supernatant were delivered to all NRLs in EU Member States including Denmark and likewise to the reference laboratories in Australia, Croatia, Faroe Islands, Iceland, Israel, Japan, Norway, Switzerland and Turkey according to a special agreement. The NRLs for UK in Aberdeen and in Weymouth received a test each. The Belgian NRL covers both Belgium and Luxembourg and likewise the Italian NRL for Cyprus and Malta. Bosnia & Herzegovina did not participate this year.

Content of ampoules

The viruses were propagated in each their preferred cell line, and when total cytopathic effect (CPE) was observed, the supernatants were collected and filter sterilised, before lyophilisation with equal volumes of 20% w/v lactalbumin hydrolysate solution. 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 concerning each virus.

Ampoule I: VHS virus DK-F1 Genotype I (Undiluted)	**VHSV DK-F1**. First Danish VHSV isolate (1962) from a rainbow trout. Cell culture passage number: 8 passages in RTG-2 + 4 passages in BF-2, total 12 passages. *Jensen MH* **(1965)** Research on the virus of Egtved disease. *Annals of the New York Academy of Sciences* **126**, 422-426. GenBank accession number of the vaccine (REVA) strain of F1 in passage number 256: AF345857 (G-gene) and AY356633 (N-gene).
Ampoule II: VHS virus DK-F1 Genotype I (Diluted 10^{-5})	**VHSV DK-F1**. Same isolate as in ampoule I. The virus was diluted 10^{-5} before lyophilisation. Virus concentration might have been close to detection level
Ampoule III: SVC virus 56/70 Genotype Id	**SVCV 56/70.** Isolate (1970) from carp. The isolate was received from Prof. Fijan in January 1979 in a tube named Rhabdo virus carpio 56/70 and given as the reference strain of SVC virus. Cell culture passage number: 2 passages in EPC cells at the National Veterinary Institute, total passage number unknown. The isolate is most likely identical to the S/30 isolate described in *Fijan N, Petrinec Z, Sulimanovic D, Zwillenberg LO* **(1971)** Isolation of the viral causative agent from the acute form of infectious dropsy of carp. *Veterinarski Archiv* **41,** 125-138. GenBank accession number: AJ538061 (S/30) and Z37505 (Fijan).
Ampoule IV: IHNV 32/87 First French isolate Genotype M	**IHNV 32/87.** First French isolate (April 1987) from rainbow trout. Cell culture passage number: 8 passages in EPC. *Hattenberger-Baudouy AM, Danton M, Merle G, Torchy C, de Kinkelin P* **(1989)** Serological evidence of infectious haematopoietic necrosis in rainbow trout from a French outbreak of disease. *Journal of Aquatic Animal Health* **1**, 126-134. *Baudin Laurencin F* **(1987)** IHN in France. *Bulletin of the European Association of Fish Pathologists* **7**, 104. GenBank accession number: AY524121 (G-gene).
Ampoule V: VHSV 4p101 Genotype III	**VHSV 4p101.** Marine isolate from whiting (*Merlangius merlangus*) caught in Skagerrak (1997). Cell culture passage number: 8 passages in BF-2. *Mortensen HF, Heuer OE, Lorenzen N, Otte L, Olesen NJ* **(1999)** Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. *Virus Research* **63**, 95-106. *Einer-Jensen K, Ahrens P, Forsberg R, Lorenzen N* **(2004)** Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal og General Virologi* **85**,1167-1179. *Snow M., Bain N, Black J, Taupin V, Cunningham CO, King JA,, Skall HF, Raynard RS* **(2004)** Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diseases of Aquatic Organisms* **61**, 11-21. GenBank accession number: AY546581 (G-gene) and AJ130918 (N-gene).

Testing the test

The inter-laboratory test 2007 was prepared and checked according to accredited protocols (DS/EN ISO/IEC 17025 and ILAC-G13: 2000). Prior to distributing the test, the CRL 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 (Table 2). The lyophilisation procedure caused none to 3 log decrease in virus titre in the cells, a quite significant titre reduction. On the other hand, when first lyophilised the virus was very stable at storing. A set of the test was titrated after 3 and 6 months storage in the dark at 4°C and room temperature. Virus was isolated from all 5 ampoules with no more than one log decrease

in their preferred cell line compared to just after lyophilisation (Table 2). Previous years the viruses were lyophilised in ampoules and closed with rubber lids. This procedure is relatively gentle to the virus with only little decrease in titre by freeze-drying, but the titres in these ampoules decreased significantly after short time storage. In order to ascertain a homogenous virus titre in the ampoules after shipment it was therefore decided this year to change to ampoules closed by melting. Virus in these ampoules has proven to be stable for decades.
The identities of the viruses in the 5 ampoules were also checked and confirmed by ELISA, IFAT, RT-PCR and serum neutralisation test.

Table 2. Titre of representative ampoules tested at the CRL for each virus in four cell lines before lyophilisation, immediately after lyophilisation (median titre of 5 replicates), and after 3 and 6 months storage in the dark at 4°C and at room temperature (1 replicate), respectively.

Ampoule No.	**Content**	**Cell line**	**Titre before lyophili-sation**	**Median titre right after lyophili-sation**	**Titre after lyophilisation (4ºC)**		**Titre after lyophilisation (Room temperature)**	
			$TCID_{50}$/ml	**$TCID_{50}$/ml**	**$TCID_{50}$/ml 3 months**	**$TCID_{50}$/ml 6 months**	**$TCID_{50}$/ml 3 months**	**$TCID_{50}$/ml 6 months**
Ampoule I	VHSV DK-F1 (undiluted)	BF-2	$4.0*10^9$	$1.9*10^6$	$5.9*10^6$	$1.9*10^7$	$1.3*10^6$	$5.9*10^6$
		EPC	$1.3*10^7$	$2.7*10^4$	$2.7*10^5$	$1.9*10^5$	$8.6*10^4$	$1.9*10^4$
		RTG-2	$4.0*10^8$	$2.7*10^6$	$1.3*10^7$	$2.7*10^7$	$4.0*10^6$	$8.6*10^6$
		FHM	$4.0*10^8$	$4.0*10^6$	$2.7*10^7$	$1.9*10^7$	$1.9*10^6$	$4.0*10^6$
Ampoule II	VHSV DK-F1 (diluted 10^{-5})	BF-2	$2.7*10^4$	$1.9*10^2$	$1.0*10^0$	$4.0*10^2$	$< 1.9*10^2$	$< 1.9*10^2$
		EPC	$4.0*10^2$	$< 1.9*10^2$	$< 1.9*10^2$	$< 1.9*10^2$	$< 1.9*10^2$	$< 1.9*10^2$
		RTG-2	$4.0*10^4$	$< 1.9*10^2$	$2.7*10^2$	$5.9*10^2$	$< 1.9*10^2$	$< 1.9*10^2$
		FHM	$8.6*10^4$	$< 1.9*10^2$	$1.9*10^2$	$1.9*10^2$	$< 1.9*10^2$	$< 1.9*10^2$
Ampoule III	SVCV 56/70	BF-2	$1.3*10^9$	$4.0*10^6$	$1.3*10^6$	$1.3*10^7$	$2.7*10^6$	$1.3*10^7$
		EPC	$1.3*10^8$	$2.7*10^6$	$1.9*10^6$	$8.6*10^6$	$4.0*10^6$	$4.0*10^6$
		RTG-2	$1.3*10^7$	$1.3*10^5$	$5.9*10^5$	$4.0*10^6$	$2.7*10^5$	$2.7*10^6$
		FHM	$1.9*10^8$	$2.7*10^6$	$8.6*10^6$	$1.3*10^7$	$2.7*10^6$	$8.6*10^6$
Ampoule IV	IHNV 32/87	BF-2	$1.3*10^5$	$1.9*10^5$	$1.3*10^5$	$1.3*10^5$	$1.9*10^4$	$5.9*10^3$
		EPC	$2.7*10^7$	$4.0*10^5$	$8.6*10^5$	$5.9*10^5$	$8.6*10^4$	$2.7*10^4$
		RTG-2	$8.6*10^5$	$4.0*10^4$	5.9*104	$1.9*10^5$	$8.6*10^3$	$1.9*10^4$
		FHM	$1.9*10^7$	$1.3*10^6$	$8.6*10^5$	$5.9*10^5$	$1.3*10^5$	$8.6*10^4$
Ampoule V	VHSV DK-4p101	BF-2	$5.9*10^8$	$8.6*10^7$	$1.9*10^6$	$8.6*10^6$	$8.6*10^6$	$5.9*10^6$
		EPC	$4.0*10^8$	$8.6*10^6$	$4.0*10^5$	$8.6*10^6$	$1.9*10^6$	$4.0*10^5$
		RTG-2	$5.9*10^5$	$1.3*10^4$	$2.7*10^3$	$2.7*10^7$	$2.7*10^3$	$2.7*10^3$
		FHM	$5.9*10^8$	$1.3*10^7$	$2.7*10^6$	$5.9*10^6$	$8.6*10^6$	$2.7*10^6$

Distributing the test

The test was sent out according to current international regulations for diagnostic specimens UN 3373, "Biological substance, Category B". We included thermo-loggers in 14 of the parcels (-40°C to +30ºC) and they revealed that at no point were any of the batches above a critical temperature while in storage. The thermo-loggers are returned immediately on receipt and a computer programme translates the data into a graph, showing the temperature inside the parcel for every 15 minutes during transport (See examples in annex 1). The loggers were set to switch on a light in case the temperature had exceeded 30°C during transportation. The loggers are giving more participants a reassurance that the temperature encountered during transport has not been detrimental to the viability of the virus in the test.
Except for one laboratory, where the temperature rose to 28°C, none of the laboratories exceeded 15°C.

Virus identification and titration

Participants were asked to identify the content of each ampoule according to the procedures described in the Commission Decision 2001/183/EC, i.e. by a neutralisation test, ELISA, and/or by immunofluorescence. Additional identification by PCR was an option as usual. 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 exact method of titration was described in the instructions enclosed with the test. All titres were calculated at the CRL based on the crude data received from each participant and are 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 2 ml cell culture medium)**.** The combined results received from participating laboratories are represented graphically for each cell line in figures 1 to 4 in the appendix of the individual reporst as the highest, the lowest and the median titre in the group as well as the 75% inter quartile range. The data for each laboratory have been plotted on top of the combined data and are presented for each cell line used in the respective laboratory. The specific titres are listed in the tables of raw data under the country's code number (Tables 4 to 8).
Furthermore, laboratories with the required facilities were encouraged to examine and identify the genotype of the isolated viruses.

Table 3. Inter-Laboratory Proficiency Test 2007 - Virus Identification

Laboratory code number	Score	Answer recived at CRL	Ampoule I VHSV	Ampoule II VHSV	Ampoule III SVCV	Ampoule IV IHNV	Ampoule V VHSV
1	9	05-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
2	8	05-11-07	VHSV/IHNV	VHSV	SVCV	IHNV	VHSV
3	10	07-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
5	8	14-11-07	VHSV	VHSV	SVCV	IHNV/VHSV	VHSV
6	9	16-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
8	9	15-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
9	10	14-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
10	9	14-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
11	10	16-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
12	10	16-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
13	10	11-10-07	VHSV	VHSV	SVCV	IHNV	VHSV
14	10	15-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
15	10	16-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
16	9	13-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
17	6	16-11-07	VHSV	VHSV	IPN Ab	IHNV	VHSV/IPNV Ab
18	10	16-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
19	9	23-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
20	10	25-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
21	10	14-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
22	10	15-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
23	9	26-10-07	VHSV	Virus not found	SVCV	IHNV	VHSV
24	10	14-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
25	10	12-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
26	5	16-11-07	VHSV	Virus not found	SVCV	IHNV/SVCV	VHSV/SVCV
27	10	15-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
28	10	15-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
29	10	14-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
30	9	08-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
31	10	16-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
32	10	08-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
33	10	30-10-07	VHSV	VHSV	SVCV	IHNV	VHSV
34	9	08-11-07	VHSV	Virus not found	SVCV	IHNV	VHSV
35	10	15-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
36	8	19-11-07	Virus not found	VHSV	SVCV	IHNV	VHSV
37	10	13-11-07	VHSV	VHSV	SVCV	IHNV	VHSV
Correct ID			33	25	34	33	33
No virus			1	10	0	0	0
Wrong ID			1	2	1	2	2
No ID			0	0	0	0	0
Not replied			0	0	0	0	0
Total			35	35	35	35	35

Findings

The individual code numbers are supplied to the respective laboratories with this report. An un-encoded version of the report will be sent to the Commission.

Participation

35 laboratories received the annual proficiency test, 14 of the laboratories also received a thermo-logger.

All participants, apart from two, answered within the deadline.

Shipment and handling

All parcels except 2 were delivered within 4 days of sending the test by courier.

Colleagues from several laboratories complained that the vacuum in the ampoules caused the virus to escape when cutting and breaking the glass ampoules in order to open them. We will assess how this can be avoided in future tests. Any ideas for solving the problems are most welcome.

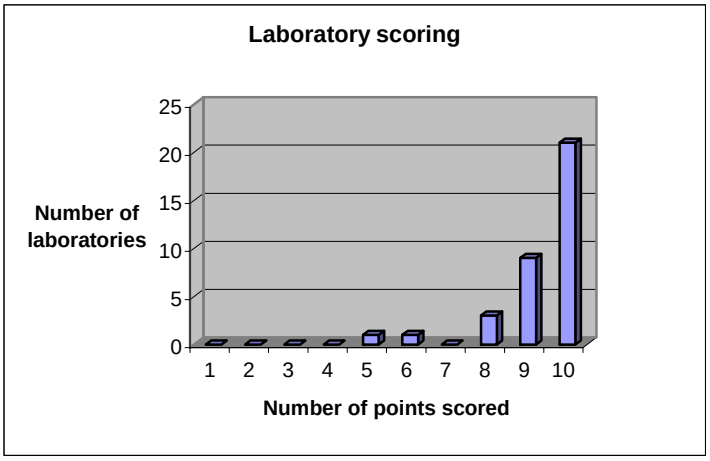
Identification of content

- 20 laboratories correctly identified all viruses in all ampoules.
- 11 laboratories did not isolate virus from ampoule II.
- 33 laboratories correctly identified the virus in ampoule I, IV and V
- 25 laboratories correctly identified the virus in ampoule II
- 34 laboratories correctly identified the virus in ampoule III
- 4 laboratories found double infection in one or two of the ampoules.
- 24 laboratories tested the ampoules with ELISA.
- 25 laboratories tested the ampoules with IFAT.
- 13 laboratories tested the ampoules with neutralisation tests.
- 26 laboratories tested the ampoules with PCR.
- 16 laboratories carried out sequencing and/or genotyping of the isolates.

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 2 points to each correct answer (Table 3), giving the possibility for obtaining a maximum of 10 points. For Ampoule 2 the result: “virus not found” was scored 1 point. Finding more than one virus type in the ampoules scored 0 even one of them was correct. Twenty-one laboratories obtained full points and nine laboratories did not isolate virus in ampoule 2 and thus received 9 points, saying that 30 of 35 laboratories identified all virus isolated correctly.

Figure 5. Points scored by the laboratories.



Genotypning

Participating laboratories were encouraged to examine and identify the genotype of the isolated viruses. Furthermore, participants were asked to provide the full-length G-gene sequences of the rhabdoviruses identified. As part of a study on sequencing as basis for molecular tracing of virus isolates, we wanted to assess the homology of the sequencing results between laboratories when using the same viruses in all laboratories. For molecular tracing of VHSV it is recommended to sequence the full length of the viral glycoprotein (G-gene), 1524 bp in all. The reliability of- and confidence in these sequence data are crucial for correct interpretation in epidemiology. Thus the assessment of the variability primarily due to technical differences must be recognised and taken into account. The results of this study will be presented at the next Annual Meeting and will be published later. In this report focus is on the results related to genotyping.

Sixteen of the 35 laboratories provided sequence data but only ten participants attempted to genotype the viruses in the 5 ampoules (Table 10). This may reflect a technical inability in the remaining laboratories, and if so, this is concerning in light of the ongoing discussion on genotyping as a basis for differentiation of notifiable viruses from others, and on including such typing in future legislation.

Ampoule I and ampoule II contained identical VHSV isolates: DK-F1 belonging to genotype I (Einar-Jensen et al. 2004). All ten laboratories genotyped the virus correctly as type I. In addition, two laboratories subgrouped the virus into 1d and 1b. The G-gene sequence of the DK-F1 isolate in ampoule I and II is not identical to the G-gene sequence of the published DK-F1 strain (GenBank acc. no. AF345857). This is most likely because the DK-F1 virus strain published in GenBank is the vaccine strain (REVA strain) of F1. This virus was passaged 256 times in cell cultures and cloned once. The F1 strain in ampoule I and II have been passaged only 12 times in cell culture with no cloning. The numerous passages might have lead to nucleotide substitutions in the vaccine strain compared to the more original isolate included in this test. The G-gene sequence of this DK-F1 (12 passages) will be submitted to GenBank in the near future. The REVA strain of DK-F1 is genotype I, but do not fit into any subgroup. Further conclusions and discussions with regard to subgrouping of the F1 isolates will be presented at the next Annual Meeting and will be published later when analysis of all sequence data have been performed.

Ampoule III contained the SVCV reference isolate 56/70 belonging to Genogroup I, subgroup Id (Stone et al. 2003). Twelve participants sequenced parts of the viral G-gene. Three participants correctly identified the virus as Id, two laboratories gave a virus reference without grouping the virus into Id. Only 6 laboratories thus attempted to type the virus despite the fact that this is to be done for correct identification of SVCV.

Ampoule IV contained the first French isolate of IHNV belonging to Genotype M (Enzmann et al. 2005). Fourteen participants obtained sequence data on the isolate. Five participants designated a name of the virus, provided a GenBank acc.no or submitted a phylogenetic tree, while only one laboratory directly indicated the virus as belonging to genotype M.

Ampoule V contained VHSV isolate 4p101 belonging to genotype III (Einer-Jensen et al. 2004). Fourteen participants submitted sequence data on this isolate. Of these, ten correctly designated the virus as a VHSV type III.

Especially, the SVCV and the IHNV isolates were designated according to alternative nomenclatures. For future studies it might be advisable to select one nomenclature that should be used in genotyping studies of SVCV and IHNV, e.g. as it is described by Stone et al. 2003 and Kurath et al. 2003, respectively).

In conclusion, the number of laboratories submitting an assessment of the genotypes included in the Proficiency test 2007 was not very impressive. Whether this low number was due to lack of technical skills or lack of resources allocated to the tests remain unknown. It is clear that the genotyping of VHSV is quite well established with only minor mistakes, while the genotyping of SVCV and IHNV were still not submitted the same way by all laboratories. Especially in the case of SVCV, where sequencing is demanded as a tool for definite identification of the notifiable disease SVC, the methods should be enforced. For IHNV, being a very homogenous virus, the typing is not crucial. For VHSV, the outcome of the discussions on splitting the VHSV genogroups in the legislation is very much dependent on the ability of all laboratories to be able to discriminate clearly between these.

Methods applied
Twenty-nine participants used BF-2 cell line, 33 used EPC, 10 used RTG-2, 9 used FHM and only one used CHSE cell line.
The viruses were primarily identified by ELISA or IFAT and 27 of 35 laboratories also included PCR, while only 12 laboratories included neutralisation.

The median titre of each ampoule in each cell line is illustrated in Figure 6. It appears that IHNV only replicate at very low level in BF-2. The marine VHSV genotype III, 4p101, had a slightly higher median titre in EPC and in FHM than in BF-2 cells, which was quite surprising. BF-2 is still the most sensitive cell line for the VHSV genotype I isolate F1.

Figure 6. Median titre of each ampoule in each cell line

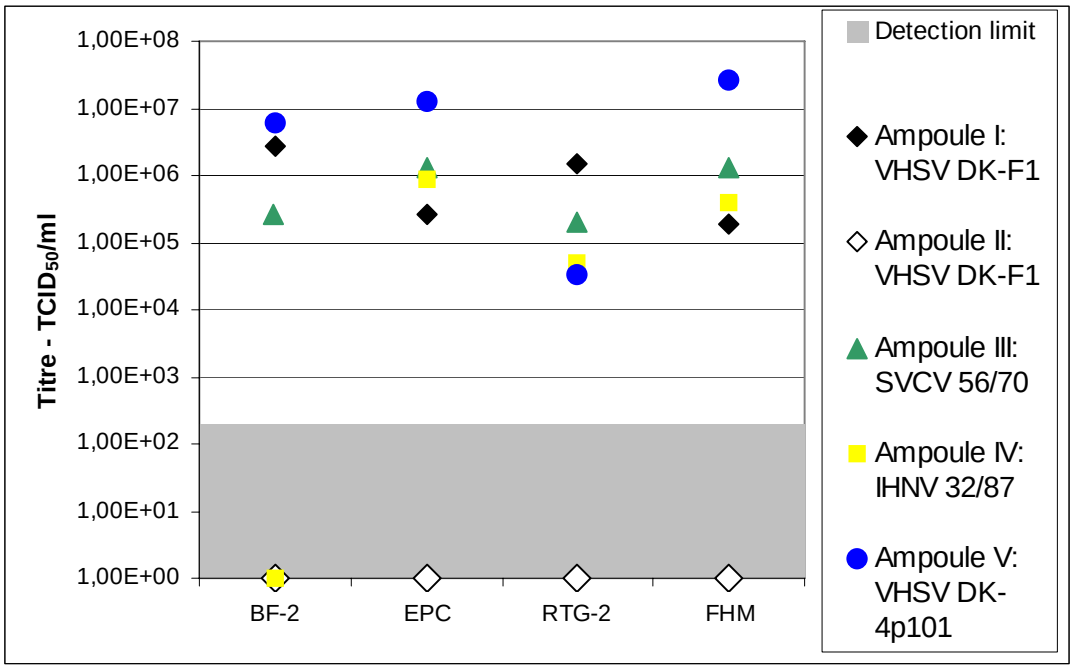


Table 4. Inter-Laboratory Proficiency Test 2007 – Identification and titration of ampoule I.

Ampoule I – VHSV						
Laboratory code number	Virus Identification	Titre in				
		BF-2	EPC	RTG-2	FHM	CHSE-214
1	VHSV	5.9E+06	2.7E+05	8.6E+02	2,7E+04	
2	VHSV/IHNV	8.6E+06	2.7E+05			
3	VHSV	2.7E+06	2.7E+04			
5	VHSV		1.9E+07			
6	VHSV	5.9E+04	4.0E+04			
8	VHSV		1.9E+05	8.6E+04	2,7E+05	
9	VHSV	1.3E+07	8.6E+04	8.6E+06	1,9E+07	
10	VHSV	4.0E+05	5.9E+04			
11	VHSV	8.6E+05	1.3E+05			
12	VHSV	2.7E+07	2.7E+05			
13	VHSV	2.7E+09	4.0E+04	5.9E+06		8,6E+05
14	VHSV	<1.9E+02	1.9E+03	8.6E+04	1,3E+05	
15	VHSV	4.0E+06	1.9E+05			
16	VHSV	2.7E+03	2.7E+02			
17	VHSV	1.9E+07	8.6E+05			
18	VHSV	2.7E+06	2.7E+05			
19	VHSV					
20	VHSV	4.0E+06	2.7E+04			
21	VHSV	8.6E+07	2.7E+05			
22	VHSV	1.9E+07	4.0E+05			
23	VHSV	1.3E+05	2.7E+05			
24	VHSV	5.9E+06	2.7E+05			
25	VHSV	5.9E+06	8.6E+04	8.6E+07	4,0E+04	
26	VHSV		8.6E+05	1.9E+05		
27	VHSV	2.7E+05	5.9E+03			
28	VHSV		<1.9E+02	8.6E+04	Tom	
29	VHSV	1.3E+06	1.3E+06	2.7E+06	2,7E+06	
30	VHSV	1.9E+06	2.7E+03			
31	VHSV	1.9E+07	2.7E+06	1.3E+07	1,9E+05	
32	VHSV	1.3E+06	1.3E+06			
33	VHSV	1.9E+07	5.9E+05			
34	VHSV		4.0E+05		8,6E+04	
35	VHSV	8.6E+05	2.7E+06			
36	Virus not found	<1.9E+02	<1.9E+02			
37	VHSV	1.9E+03			1,9E+06	
Number of laboratories		29	33	10	9	1
Median titre		2.7E+06	2.7E+05	1.4E+06	1.9E+05	8.6E+05
Maximum titre		2.7E+09	1.9E+07	8.6E+07	1.9E+07	8.6E+05
Minimum titre		<1.9E+02	<1.9E+02	8.6E+02	2.7E+04	8.6E+05
25% quartile titre		4.0E+05	4.0E+04	8.6E+04	8.6E+04	8.6E+05
75% quartile titre		1.3E+07	4.0E+05	7.9E+06	1.9E+06	8.6E+05

Table 5. Inter-Laboratory Proficiency Test 2007 – Identification and titration of ampoule II.

Ampoule II – VHSV						
Laboratory code number	Virus Identification	Titre in				
		BF-2	EPC	RTG-2	FHM	CHSE-214
1	Virus not found	<1.9E+02	<1.9E+02	<1.9E+02	<1.9E+02	
2	VHSV	5.9E+02	<1.9E+02			
3	VHSV	1.9E+02	<1.9E+02			
5	VHSV		1.3E+03			
6	Virus not found	<1.9E+02	<1.9E+02			
8	Virus not found		<1.9E+02	<1.9E+02	<1.9E+02	
9	VHSV	5.9E+02	1.9E+02	4.0E+02	5.9E+02	
10	Virus not found	<1.9E+02	<1.9E+02			
11	VHSV	<1.9E+02	<1.9E+02			
12	VHSV	5.9E+02	<1.9E+02			
13	VHSV	2.7E+03	<1.9E+02	<1.9E+02		<1.9E+02
14	VHSV	<1.9E+02	<1.9E+02	<1.9E+02	1.9E+02	
15	VHSV	1.9E+02	<1.9E+02			
16	Virus not found	<1.9E+02	<1.9E+02			
17	VHSV	1.3E+03	<1.9E+02			
18	VHSV	5.9E+02	<1.9E+02			
19	Virus not found					
20	VHSV	1.3E+03	1.3E+03			
21	VHSV	2.7E+03	<1.9E+02			
22	VHSV	8.6E+02	<1.9E+02			
23	Virus not found	<1.9E+02	<1.9E+02			
24	VHSV	<1.9E+02	<1.9E+02			
25	VHSV	<1.9E+02	<1.9E+02	1.9E+02	<1.9E+02	
26	Virus not found		<1.9E+02	<1.9E+02		
27	VHSV	<1.9E+02	<1.9E+02			
28	VHSV		<1.9E+02	<1.9E+02		
29	No VHSV/IPNV/SVCV/IHNV	<1.9E+02	<1.9E+02	<1.9E+02	<1.9E+02	
30	Virus not found	<1.9E+02	<1.9E+02			
31	VHSV	4.0E+02	1.9E+02	1.3E+03	1.9E+02	
32	VHSV	<1.9E+02	<1.9E+02			
33	VHSV	1.9E+03	<1.9E+02			
34	Virus not found		<1.9E+02		<1,9E+02	
35	VHSV	<1.9E+02	<1.9E+02			
36	VHSV	1.9E+02	<1.9E+02			
37	VHSV	<1.9E+02			<1,9E+02	
Number of laboratories		29	33	10	9	1
Median titre		<1,9E+02	<1,9E+02	<1.9E+02	<1.9E+02	<1.9E+02
Maximum titre		2,7E+03	1,3E+03	1.3E+03	5.9E+02	5.9E+02
Minimum titre		<1,9E+02	<1,9E+02	<1.9E+02	<1.9E+02	0
25% quartile titre		<1,9E+02	<1,9E+02	<1.9E+02	<1.9E+02	<1.9E+02
75% quartile titre		5,9E+02	<1,9E+02	1.4E+02	1.9E+02	1.5E+02

Table 6. Inter-Laboratory Proficiency Test 2007 – Identification and titration of ampoule III.

Ampoule III – SVCV						
Laboratory code number	Virus Identification	Titre in				
		BF-2	EPC	RTG-2	FHM	CHSE-214
1	SVCV	1.3E+06	1.3E+05	<1.9E+02	1,9E+04	
2	SVCV	1.9E+05	1.9E+06			
3	SVCV	2.7E+05	4.0E+06			
5	SVCV		2.7E+07			
6	SVCV	2.7E+04	1.9E+06			
8	SVCV		8.6E+05	2.7E+06	8.6E+05	
9	SVCV	4.0E+07	4.0E+06	2.7E+06	1.9E+07	
10	SVCV	1.3E+04	1.3E+06			
11	SVCV	1.3E+06	1.3E+06			
12	SVCV	5.9E+04	4.0E+05			
13	SVCV	1.9E+06	2.7E+07	2.7E+05		8.6E+05
14	SVCV	1.3E+04	5.9E+05	<1.9E+02	5.9E+05	
15	SVCV	1.9E+06	2.7E+06			
16	SVCV	1.3E+03	8.6E+02			
17	IPN Ab	2.7E+06	2.7E+06			
18	SVCV	1.9E+06	1.3E+06			
19	SVCV					
20	SVCV	8.6E+05	4.0E+05			
21	SVCV	8.6E+05	4.0E+04			
22	SVCV	1.9E+04	8.6E+04			
23	SVCV	2.7E+05	8.6E+05			
24	SVCV	2.7E+06	1.9E+05			
25	SVCV	4.0E+05	5.9E+06	4.0E+05	2.7E+06	
26	SVCV		1.3E+07	5.9E+04		
27	SVCV	5.9E+04	8.6E+03			
28	SVCV		5.9E+04	2.7E+03		
29	SVCV	1.3E+06	5.9E+06	2.7E+06	1.3E+06	
30	SVCV	<1.9E+02	1.9E+05			
31	SVCV	1.9E+05	1.3E+05	1.3E+05	2.7E+05	
32	SVCV	<1.9E+02	<1.9E+02			
33	SVCV	8.6E+05	8.6E+06			
34	SVCV		1.3E+06		1.3E+06	
35	SVCV	<1.9E+02	1.3E+06			
36	SVCV	4.0E+04	1.9E+05			
37	SVCV	<1.9E+02			1.9E+06	
Number of laboratories		29	33	10	9	1
Median titre		2,7E+05	1,3E+06	2.0E+05	1.3E+06	1.4E+06
Maximum titre		4,0E+07	2,7E+07	2.7E+06	1.9E+07	1.9E+06
Minimum titre		<1,9E+02	<1,9E+02	<1.9E+02	1.9E+04	8.6E+05
25% quartile titre		1,9E+04	1,9E+05	1.7E+04	5.9E+05	1.1E+06
75% quartile titre		1,3E+06	2,7E+06	2.1E+06	1.9E+06	1.6E+06

Table 7. Inter-Laboratory Proficiency Test 2007 – Identification and titration of ampoule IV.

Ampoule IV - IHNV						
Laboratory code number	Virus Identification	Titre in				
		BF-2	EPC	RTG-2	FHM	CHSE-214
1	IHNV	<1.9E+02	<1.9E+02	<1.9E+02	<1.9E+02	
2	IHNV	<1.9E+02	8.6E+05			
3	IHNV	4.0E+02	1.3E+06			
5	IHNV/VHSV		8.6E+06			
6	IHNV	<1.9E+02	2.7E+03			
8	IHNV		1.9E+06	5.9E+05	4.0E+05	
9	IHNV	1.3E+04	4.0E+05	1.3E+04	5.9E+05	
10	IHNV	<1.9E+02	2.7E+05			
11	IHNV	<1.9E+02	1.3E+05			
12	IHNV	<1.9E+02	5.9E+05			
13	IHNV	1.9E+04	1.9E+06	4.0E+05		5.9E+06
14	IHNV	<1.9E+02	8.6E+05	2.7E+03	1.9E+05	
15	IHNV	8.6E+03	1.9E+06			
16	IHNV	4.0E+03	1.3E+03			
17	IHNV	<1.9E+02	4.0E+06			
18	IHNV	2.7E+02	1.9E+05			
19	IHNV					
20	IHNV	1.3E+03	2.7E+05			
21	IHNV	1.9E+05	4.0E+06			
22	IHNV	<1.9E+02	<1.9E+02			
23	IHNV	1.3E+06	2.7E+05			
24	IHNV	<1.9E+02	1.3E+04			
25	IHNV	<1.9E+02	2.7E+06	2.7E+05	8.6E+04	
26	IHNV/SVCV		1.3E+05	8.6E+04		
27	IHNV	<1.9E+02	1.3E+05			
28	IHNV		8.6E+05	2.7E+03		
29	IHNV	1.3E+05	1.3E+06	1.3E+05	2.7E+05	
30	IHNV	<1.9E+02	1.3E+06			
31	IHNV	2.7E+03	1.9E+06	1.9E+03	1.3E+06	
32	IHNV	<1.9E+02	1.3E+04			
33	IHNV	8.6E+03	1.3E+07			
34	IHNV		5.9E+06		5.9E+06	
35	IHNV	4.0E+02	4.0E+06			
36	IHNV	<1.9E+02	2.7E+06			
37	IHNV	<1.9E+02			5.9E+06	
Number of laboratories		29	33	10	9	1
Median titre		<1,9E+02	8,6E+05	5.0E+04	4.0E+05	5.9E+06
Maximum titre		1,3E+06	1,3E+07	5.9E+05	5.9E+06	5.9E+06
Minimum titre		<1,9E+02	<1,9E+02	<1.9E+02	<1.9E+02	5.9E+06
25% quartile titre		<1,9E+02	1,3E+05	2.7E+03	1.9E+05	5.9E+06
75% quartile titre		4,0E+03	1,9E+06	2.4E+05	1.3E+06	5.9E+06

Table 8. Inter-Laboratory Proficiency Test 2007 – Identification and titration of ampoule V.

Ampoule V – VHSV						
Laboratory code number	Virus Identification	Titre in				
		BF-2	EPC	RTG-2	FHM	CHSE-214
1	VHSV	5.9E+06	1.9E+07	<1.9E+02	1.9E+07	
2	VHSV	2.7E+07	1.3E+07			
3	VHSV	1.9E+06	4.0E+06			
5	VHSV		1.3E+07			
6	VHSV	1.3E+06	2.7E+06			
8	VHSV		1.3E+07	5.9E+07	2.7E+07	
9	VHSV	8.6E+07	2.7E+07	4.0E+04	8.6E+06	
10	VHSV	2.7E+06	5.9E+06			
11	VHSV	5.9E+06	1.9E+07			
12	VHSV	4.0E+06	8.6E+06			
13	VHSV	4.0E+08	1.3E+06	2.7E+04		5.9E+03
14	VHSV	2.7E+04	1.3E+07	1.9E+04	5.9E+06	
15	VHSV	1.9E+07	1.3E+07			
16	VHSV	4.0E+04	4.0E+04			
17	VHSV/IPNV Ab	1.3E+07	8.6E+06			
18	VHSV	5.9E+06	8.6E+02			
19	VHSV					
20	VHSV	2.7E+06	1.9E+06			
21	VHSV	2.7E+07	1.9E+07			
22	VHSV	2.7E+05	5.9E+04			
23	VHSV	5.9E+06	4.0E+07			
24	VHSV	4.0E+06	8.6E+06			
25	VHSV	1.9E+08	4.0E+09	2.7E+04	1.3E+09	
26	VHSV/SVCV		1.9E+05	5.9E+04		
27	VHSV	5.9E+04	8.6E+06			
28	VHSV		1.3E+06	<1.9E+02		
29	VHSV	2.7E+07	1.3E+07	5.9E+06	2.7E+07	
30	VHSV	1.9E+07	1.9E+08			
31	VHSV	5.9E+05	2.7E+06	1.3E+06	4.0E+06	
32	VHSV	5.9E+05	1.3E+07			
33	VHSV	4.0E+07	4.0E+07			
34	VHSV		2.7E+07		4.0E+07	
35	VHSV	5.9E+06	4.0E+07			
36	VHSV	5.9E+05	1.9E+07			
37	VHSV	1.3E+05			4.0E+08	
Number of laboratories		29	33	10	9	1
Median titre		5,9E+06	1,3E+07	3.4E+04	2.7E+07	5.9E+03
Maximum titre		4,0E+08	4,0E+09	5.9E+07	1.3E+09	5.9E+03
Minimum titre		2,7E+04	8,6E+02	<1.9E+02	4.0E+06	5.9E+03
25% quartile titre		5,9E+05	2,7E+06	2.1E+04	8.6E+06	5.9E+03
75% quartile titre		1,9E+07	1,9E+07	9.9E+05	4.0E+07	5.9E+03

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

Laboratory code number	Score	Ampoule	ELISA	IFAT	Neutralisation	PCR	Genogroup	Other
1	9	Ampoule I				VHSV	Gene Sequenced	VHSV
		Ampoule II				Negative for VHSV,IHNV, IPNV,SVCV, EHNV		Not tested
		Ampoule III				SVCV	Gene Sequenced	Negativ
		Ampoule IV				IHNV	Gene Sequenced	Not tested
		Ampoule V				VHSV	Gene Sequenced	VHSV
2	8	Ampoule I	VHSV	VHSV/IHNV		VHSV/IHNV	part of G gene (758bp central G) of VHSV	
		Ampoule II	VHSV	VHSV		VHSV		
		Ampoule III	SVCV	SVCV		SVCV	part of G gene (606bp) of SVC- sample	
		Ampoule IV	IHNV	IHNV		IHNV	part of G gene (303bp mid-G) of INHV	
		Ampoule V	VHSV	VHSV		VHSV		
3	10	Ampoule I	VHSV	VHSV		VHSV		
		Ampoule II	VHSV	VHSV		VHSV		
		Ampoule III	SVCV	SVCV		-		
		Ampoule IV	IHNV	IHNV		IHNV		
		Ampoule V	VHSV	VHSV		VHSV		
5	8	Ampoule I	VHSV	VHSV	VHSV			VHSV
		Ampoule II	No dected	VHSV				VHSV
		Ampoule III	SVCV	SVCV	Not done			SVCV
		Ampoule IV	IHNV/VHSV	IHNV/VHSV	IHNV/VHSV			IHNV/VHSV
		Ampoule V	VHSV	VHSV	VHSV			VHSV
6	9	Ampoule I	X	X	X			
		Ampoule II	X	X	X			
		Ampoule III	X	X	X			
		Ampoule IV	X	X	X			
		Ampoule V	X	X	X			
8	9	Ampoule I	VHSV			VHSV		
		Ampoule II						
		Ampoule III	SVCV			SVCV		
		Ampoule IV	IHNV			IHNV		
		Ampoule V	VHSV			VHSV		
9	10	Ampoule I	VHSV		VHSV	VHSV	VHSV genogruppe I	VHSV Neutralization pattern I
		Ampoule II	VHSV		VHSV	VHSV	VHSV genogruppe I	VHSV Neutralization pattern I
		Ampoule III	SVCV		Not neutralised	SVCV	SVCV genogruppe Id	SVCV
		Ampoule IV	IHNV		IHNV	IHNV	IHNV genogruppe M	Not done
		Ampoule V	VHSV		VHSV	VHSV	VHSV genogruppe III	VHSV Neutralization pattern III

Laboratory code number	Score	Ampoule	ELISA	IFAT	Neutralisation	PCR	Genogroup	Other
10	9	Ampoule I	VHSV	VHSV	VHSV	VHSV		
		Ampoule II	Virus not found	Virus not found	Virus not found	Virus not found		
		Ampoule III	SVCV	SVCV	SVCV	SVCV		
		Ampoule IV	IHNV	IHNV	IHNV	IHNV		
		Ampoule V	VHSV	VHSV	VHSV	VHSV		
11	10	Ampoule I	VHSV	VHSV	VHSV	VHSV	X	
		Ampoule II	VHSV	VHSV	VHSV	VHSV	X	
		Ampoule III				SVCV	X	
		Ampoule IV	IHNV	IHNV	IHNV	IHNV	X	
		Ampoule V	VHSV	VHSV		VHSV	X	
12	10	Ampoule I	VHSV	not done		VHSV	VHSV, genotype I	
		Ampoule II	VHSV	not done		VHSV	VHSV, genotype I	
		Ampoule III	SVCV	not done		SVCV PCR not done not IHNV/VHSV		
		Ampoule IV	IHNV	IHNV		IHNV		
		Ampoule V	VHSV	not done		VHSV	VHSV, genotype III	
13	10	Ampoule I		VHSV	VHSV	VHSV		
		Ampoule II		VHSV	VHSV	VHSV		
		Ampoule III		SVCV	SVCV	SVCV		
		Ampoule IV		IHNV	IHNV	IHNV		
		Ampoule V		VHSV	VHSV	VHSV		
14	10	Ampoule I	VHSV	VHSV		VHSV	Full I-d	
		Ampoule II	VHSV	VHSV		VHSV	Full I-d	
		Ampoule III	Not VHSV/IHNV	SVCV		SVCV	not done	
		Ampoule IV	IHNV	IHNV		IHNV	Full German type	
		Ampoule V	VHSV	VHSV		VHSV	Full III	
15	10	Ampoule I	X	X				
		Ampoule II	X	X				
		Ampoule III	X	X				
		Ampoule IV	X	X				
		Ampoule V	X	X				
16	9	Ampoule I	VHSV		VHSV F1	VHSV	Genotype I-b	+
		Ampoule II	Virus not found			Positiv after deadline by agarose PCR		-
		Ampoule III	SVCV			SVCV	Fijan ref. Strain.	+
		Ampoule IV	IHNV			IHNV	X	+
		Ampoule V	VHSV		VHSV III	VHSV	Genotype III	+
17	6	Ampoule I		X				
		Ampoule II		X	X			
		Ampoule III		X	X			
		Ampoule IV		X				
		Ampoule V		X	X			

Laboratory code number	Score	Ampoule	ELISA	IFAT	Neutralisation	PCR	Genogroup	Other
18	10	Ampoule I	VHSV			VHSV	VHSV genotype I	
		Ampoule II	VHSV			VHSV	VHSV genotype I	
		Ampoule III	SVCV	SVCV		SVCV	SVCV	
		Ampoule IV		IHNV		IHNV	IHNV	
		Ampoule V	VHSV			VHSV	VHSV genotype III	
19	9	Ampoule I						
		Ampoule II						
		Ampoule III						
		Ampoule IV						
		Ampoule V						
20	10	Ampoule I		VHSV		VHSV		
		Ampoule II		VHSV		Negative for VHSV/IHNV		
		Ampoule III		SVCV		Negative for VHSV/IHNV		
		Ampoule IV		IHNV		IHNV		
		Ampoule V		VHSV		VHSV		
21	10	Ampoule I		VHSV		VHSV	VHS genogroup I	VHSV
		Ampoule II		VHSV		VHSV	VHS genogroup I	VHSV
		Ampoule III		SVCV or related virus		SVCV or related virus	SVC genogroup Id	SVCV
		Ampoule IV		IHNV		IHNV	IHNV	IHNV
		Ampoule V		VHSV		VHSV	VHS genogroup III	VHSV
22	10	Ampoule I		VHSV	VHSV?	VHSV		
		Ampoule II		VHSV	VHSV	VHSV		
		Ampoule III		SVCV	SVCV	SVCV		
		Ampoule VI				IHNV		
		Ampoule VI		VHSV	VHSV ?	VHSV		
23	9	Ampoule I	VHSV					
		Ampoule II	-					
		Ampoule III	SVCV					
		Ampoule VI	IHNV					
		Ampoule VI	VHSV					
24	10	Ampoule I		VHSV		VHSV	VHSV genogroup I	
		Ampoule II		-		VHSV	VHSV genogroup I	
		Ampoule III		-		SVCV	SVCV	
		Ampoule VI		IHNV		IHNV	IHNV	
		Ampoule VI		VHSV		VHSV	VHSV genogroup III	
25	10	Ampoule I	X			X	X	
		Ampoule II	X			X	X	
		Ampoule III	X				X	
		Ampoule VI	X			X	X	
		Ampoule VI	X			X	X	
26	5	Ampoule I			VHSV	VHSV		
		Ampoule II			Negative	Negative		

Laboratory code number	Score	Ampoule	ELISA	IFAT	Neutralisation	PCR	Genogroup	Other
		Ampoule III			SVCV	SVCV		
		Ampoule VI			IHNV/SVCV	IHNV/SVCV		
		Ampoule VI			VHSV/SVCV	VHSV/SVCV		
27	10	Ampoule I	VHSV	VHSV				
		Ampoule II	VHSV	VHSV				
		Ampoule III	SVCV	SVCV				
		Ampoule VI		IHNV				
		Ampoule VI	VHSV	VHSV				
28	10	Ampoule I	X	X		X	X	
		Ampoule II	Negative	Negative		X	X	
		Ampoule III	X	X		X	X	
		Ampoule VI		X		X	X	
		Ampoule VI	X	X		X	X	
29	10	Ampoule I	VHSV			VHSV	X	
		Ampoule II	no			VHSV	X	
		Ampoule III	SVCV			SVCV	X	
		Ampoule VI	IHNV			IHNV	X	
		Ampoule VI	VHSV			VHSV	X	
30	9	Ampoule I	VHSV	VHSV		VHSV		
		Ampoule II	Negativ	Negativ		Negativ		
		Ampoule III	SVCV	SVCV		Not IHNV/IPNV/VHSV		
		Ampoule VI	IHNV	IHNV		IHNV		
		Ampoule VI	VHSV	VHSV		VHSV		
31	10	Ampoule I	VHSV	VHSV	VHSV	VHSV		Serotype I
		Ampoule II	VHSV	VHSV	VHSV	VHSV		Serotype I
		Ampoule III		SVCV		SVCV		
		Ampoule VI	IHNV	IHNV	IHNV	IHNV		
		Ampoule VI	VHSV	VHSV	VHSV	VHSV		Serotype III
32	10	Ampoule I	X		X			
		Ampoule II	X		X			
		Ampoule III	X		X			
		Ampoule VI	X		X			
		Ampoule VI	X		X			
33	10	Ampoule I		VHSV		VHSV		
		Ampoule II		VHSV		VHSV		
		Ampoule III		SVCV				
		Ampoule VI		IHNV		IHNV		
		Ampoule VI		VHSV		VHSV		

Laboratory code number	Score	Ampoule	ELISA	IFAT	Neutralisation	PCR	Genogroup	Other
34	9	Ampoule I		VHSV		VHSV		
		Ampoule II		Virus not found		Virus not found		No CPE at EPC/FHM after 2 passage
		Ampoule III		SVCV		SVCV		
		Ampoule VI		IHNV		IHNV		
		Ampoule VI		VHSV		VHSV		
35	10	Ampoule I	VHSV	VHSV	VHSV			
		Ampoule II	VHSV	VHSV	VHSV			CPE after 2 passage on BF-2/EPC
		Ampoule III	SVCV	SVCV				
		Ampoule VI	IHNV	IHNV	IHNV			
		Ampoule VI	VHSV	VHSV	VHSV			
36	8	Ampoule I				Negative	Negative	
		Ampoule II	VHSV			Freshwater VHSV	VHSV Genotype I	
		Ampoule III	SVCV			SVCV	SVCV genotype Id	
		Ampoule VI		IHNV		IHNV	IHNV	
		Ampoule VI	VHSV			Marine VHSV	VHSV genotype III	
37	10	Ampoule I	VHSV	VHSV		VHSV	VHSV - genotype I	
		Ampoule II	VHSV	VHSV		VHSV	VHSV - genotype I	
		Ampoule III	SVCV	SVCV		SVCV	SVCV	
		Ampoule VI	IHNV	IHNV		IHNV	IHNV	
		Ampoule VI	VHSV	VHSV		VHSV	VHSV - genotype III	

Table 10. Overview of sequenced genes and genotyping results obtained by the laboratories. In the "DNA Seq." columns: "N": Partial N-gene was sequenced, "G"; Partial G-gene was sequenced and "G-Full": Full length G-gene was sequenced. In the column "Genotype": only genotyping results according to Einer-Jensen et al. 2004, Kurath et al. 2003, Snow et al. 2004 and Stone et al. 2003. In the column "Other Comments", comments from the laboratories regarding strain identity, accession number etc. are included.

Laboratory code number	**Ampoule I** VHSV DK-F1 (undiluted) Genotype I			**Ampoule II** VHSV DK-F1 (diluted 10^{-5}) Genotype I			**Ampoule III** SVCV 56/70			**Ampoule IV** IHNV 32/87 First French Isolate Genotype M			**Ampoule V** VHSV 4p101 Genotype III		
	DNA Seq.	Geno-type	Other Com-ments	DNA Seq.	Geno-type	Other Com-ments	DNA Seq.	Geno-type	Other Com-ments	DNA Seq.	Geno-type	Other Com-ments	DNA Seq.	Geno-type	Other Com-ments
1	N						G			N			N		
2	G						G			G					
9	G	VHSV genotype I	Probably isolate F1 (AF345857)	G	VHSV genotype I	Probably isolate F1 (AF345857)	G	SVCV genotype Id	Probably isolate S30 (AJ538061) or 880124 (AJ538078)	G	IHNV genotype M	100% identical with isolate Fsk/88	G	VHSV genotype III	Probably isolate 4p101 (AY546581)
11	G - Full.			G			G			G			G		
12	G-full M+N	VHSV genotype I	Closest relative: DK-F1 (acc no AF345857.1) is 99.5%	M+N	VHSV genotype I	Closest relative: DK-F1 (acc no AF345857.1) is 99.5%							M+N	VHSV, genotype III	
13	G-full	VHSV genotype I		G-full	VHSV genotype I					G		Phylogenetic Tree	G-full	VHSV, genotype III	
14	G-full	VHSV genotype I-d		G-full	VHSV genotype I-d					G-full		Full German type	G-full	VHSV, genotype III	Virus: DK-4p101 (AY546581)
16	N	VHSV Genotype I-b	Strain: Cod Ulcus '79, DK-				G		Fijan ref. Strain, ATCCC UR-1390	G		Strain: K '87	N	VHSV, genotype III	Strain: DK-4p101

Laboratory code number	**Ampoule I** VHSV DK-F1 (undiluted) Genotype I			**Ampoule II** VHSV DK-F1 (diluted 10^{-5}) Genotype I			**Ampoule III** SVCV 56/70			**Ampoule IV** IHNV 32/87 First French Isolate Genotype M			**Ampoule V** VHSV 4p101 Genotype III		
	DNA Seq.	Genotype	Other Comments	DNA Seq.	Genotype	Other Comments	DNA Seq.	Genotype	Other Comments	DNA Seq.	Genotype	Other Comments	DNA Seq.	Genotype	Other Comments
			M Rhabdo												
18	N	VHSV genotype I		N	VHSV genotype I		G			N			N	VHSV genotype III	
21	G	VHSV genotype I		G	VHSV genotype I		G	SVCV genotype Id		G			G	VHS genotype III	
24	G	VHSV genotype I	Isolate DK-F1, AF345847	G	VHSV genotype I	Isolate DK-F1, AF345847	G		Isolate ARH-98 Germany	G		X89213	G	VHSV genotype III	Isolate DK-4p101, AY546581
25	G - Full l.		Alignment to AJ233396	G - Full l.		Alignment to AJ233396									
28	N			N			G			G			N		
29	N		95% identity to Z93414.2	N		96% identity to Z93414.2	G		99% identity to AY540082.1	G		98% identity to X89213.1	N		96% identity to AJ130918
36				G - Full l.	VHSV Genotype I	99,61% ID to DK-F1	G	SVCV genotype Id	100% ID to S-30	N			G-Full	VHSV genotype III	99,93% ID to DK-4p101
37	G	VHSV genotype I		G	VHSV genotype I		G			G			G	VHSV genotype III	

Concluding remarks

The titre in Ampoule II was very low and in some case under detection level. Several participants only isolated the virus after subcultivation, and in future we encourage all participants to inoculate content of the ampoules onto cell plates equivalent to the plates used for diagnosis and surveillance in the respective laboratories and to subcultivate if no CPE is observed.

The CRL 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.

The results will be further presented and discussed at the Annual Meeting of National Reference Laboratories for Fish Diseases to be held 19-20 June 2008 in Århus, Denmark.

Nicole Nicolajsen, Søren Kahns and Niels Jørgen Olesen

European Community Reference Laboratory for Fish Diseases
Technical University of Denmark, National Veterinary Institute, March 2008

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