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

2009

Organised by
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for Fish Diseases,
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Introduction

A comparative test of diagnostic procedures was provided by the Community Reference Laboratory (CRL) for Fish Diseases to 36 National Reference Laboratories (NRLs) in the start of September 2009. The test contained five coded ampoules. Four contained viral haemorrhagic septicaemia virus (VHSV) genotype Ie and IVa, infectious haematopoietic necrosis virus (IHNV) genogroup L and epizootic haematopoietic necrosis virus (EHNV), respectively. Furthermore, one ampoule did not contain any virus, only medium. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish viruses VHSV, IHNV and ENHV (all listed in Council Directive 2006/88/EC). It was decided at the 13th Annual Meeting of the NRLs for Fish Diseases in Copenhagen 26-28 May 2009, that testing for EHNV for the first time should be included in this test.
In addition the participants were asked to titrate the viruses to assess the cell susceptibility for virus infection in the respective laboratories. 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 monolayered cell cultures followed by e.g. neutralisation test, immunofluorescence, ELISA or PCR. If ranaviruses should be present in any of the ampoules, it was mandatory to perform a sequence analysis of the isolate in order to determine if the isolate is EHNV. We recommend following the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2009, of which a new version has just been released.

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 that were used for genotyping of isolates.

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 provided comments to participants if relevant. An un-encoded version of the report is sent to the Commission.

In this proficiency test it was possible to download an excel sheet for filling in results. Participants could submit a filled scheme electronically or on paper. Furthermore, participants were asked to fill an extended questionnaire in order to obtain more information on the methodology used by the laboratories. Participants were asked to reply latest 13 November 2009

Participants

Five ampoules with lyophilised tissue culture supernatant were delivered to all NRLs in EU Member States, including Denmark, and likewise to the National reference laboratories in Australia, Bosnia and Herzegovina, Canada, Croatia, Faroe Islands, Iceland, Israel, Japan, Norway, P.R China, Serbia and Switzerland. The Belgian NRL covers both Belgium and Luxembourg and likewise the Italian NRL covers Italy, Cyprus, Malta and Greece.

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 virus.

Ampoule I: EHNV	Reference strain of EHNV Isolate 86/8774 from rainbow trout Received from Dr. R.J. Whittington, EHNV OIE reference laboratory, Chair Farm Animal Health, Faculty of Veterinary Science, University of Sydney, 425 Werombi Road, Private Bag 3, Camden NSW 2570, Australia Cell culture passage number 6 References: Langdon JS, Humphrey JD & Williams LM (1989). Outbreaks of an EHNV-like iridovirus in cultured rainbow trout, *Salmo gairdneri* Richardson, in Australia. *Journal of Fish Diseases* **11**, 93-96. Marsh IB, Whittington RJ, O'Rourke B, Hyatt AD & Chisholm O (2002). Rapid differentiation of Australian, European and American ranaviruses based on variation in major capsid protein gene sequence. *Molecular and Cellular Probes* **16**, 137-151.
Ampoule II: IHNV	IHNV Genotype L Isolate COL-80 from chinook salmon Received from Dr. Jim Winton, U.S. Geological Survey, Western Fisheries Research Center, 6505 NE 65th St., Seattle, WA 98115, USA Cell culture passage number unknown References: Nichol ST, Rowe JE & Winton JR (1995). Molecular epizootiology and evolution of the glycoprotein and non-virion protein genes of infectious hematopoietic necrosis virus, a fish rhabdovirus. *Virus Research* **38**, 159-173. Kurath G, Garver KA, Troyer RM, Emmenegger EJ, Einer-Jensen K & Anderson ED (2003). Phylogeography of infectious haematopoietic necrosis virus in North America. *Journal of General Virology* **84**, 803-814.
Ampoule III: VHSV	VHSV genotype Ie Turkish isolate TR-WS13G (=TR-SW13G) from turbot (*Psetta maxima*) Received from Dr. Toyohiko Nishizawa and Dr. Mamoru Yoshimizu. Graduate School of Fisheries Sciences, Hokkaido University, Hakodate 041-8611 Japan Cell culture passage number 5 References: Nishizawa T, Savas H, Isidan H, Üstündag C, Iwamoto H & Yoshimizu M (2006). Genotyping and pathogenicity of viral hemorrhagic septicemia virus from free-living turbot (*Psetta maxima*) in a Turkish coastal area of the Black Sea. *Applied and Environmental Microbiology* **72**, 2373-2378.
Ampoule IV: VHSV	VHSV genotype IVa Received as RBV (Ray Brunson virus from the Makah hatchery) isolate from coho salmon Received from Dr. Jim Winton, U.S. Geological Survey, Western Fisheries Research Center, 6505 NE 65th St., Seattle, WA 98115, USA Cell culture passage number is unknown References: Brunson R, True K & Yancey J (1989). VHS virus isolated at Makah National Fish Hatchery. *American Fisheries Society Fish Health Section Newsletter* **1**7, 3-4. Winton JR, Batts WN & Nishizawa T (1989). Characterization of the first North American isolates of viral hemorrhagic septicemia virus. *American Fisheries Society Fish Health Section Newsletter* **17**, 2-3. Winton JR, Batts WN, Deering RE, Brunson R, Hopper K, Nishizawa T & Stehr C (1991). Characteristics of the first North American isolates of viral hemorrhagic septicemia virus. *Proceedings of the Second International Symposium on Viruses of Lower Vertebrates*, 43-50.
Ampoule V: No virus	Pure cell culture medium

Testing of the test

The inter-laboratory test 2009 was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17025 and ILAC-G13:08/2007 standards. Prior to distribution 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 and homogeneity of content of ampoules (Table 2).

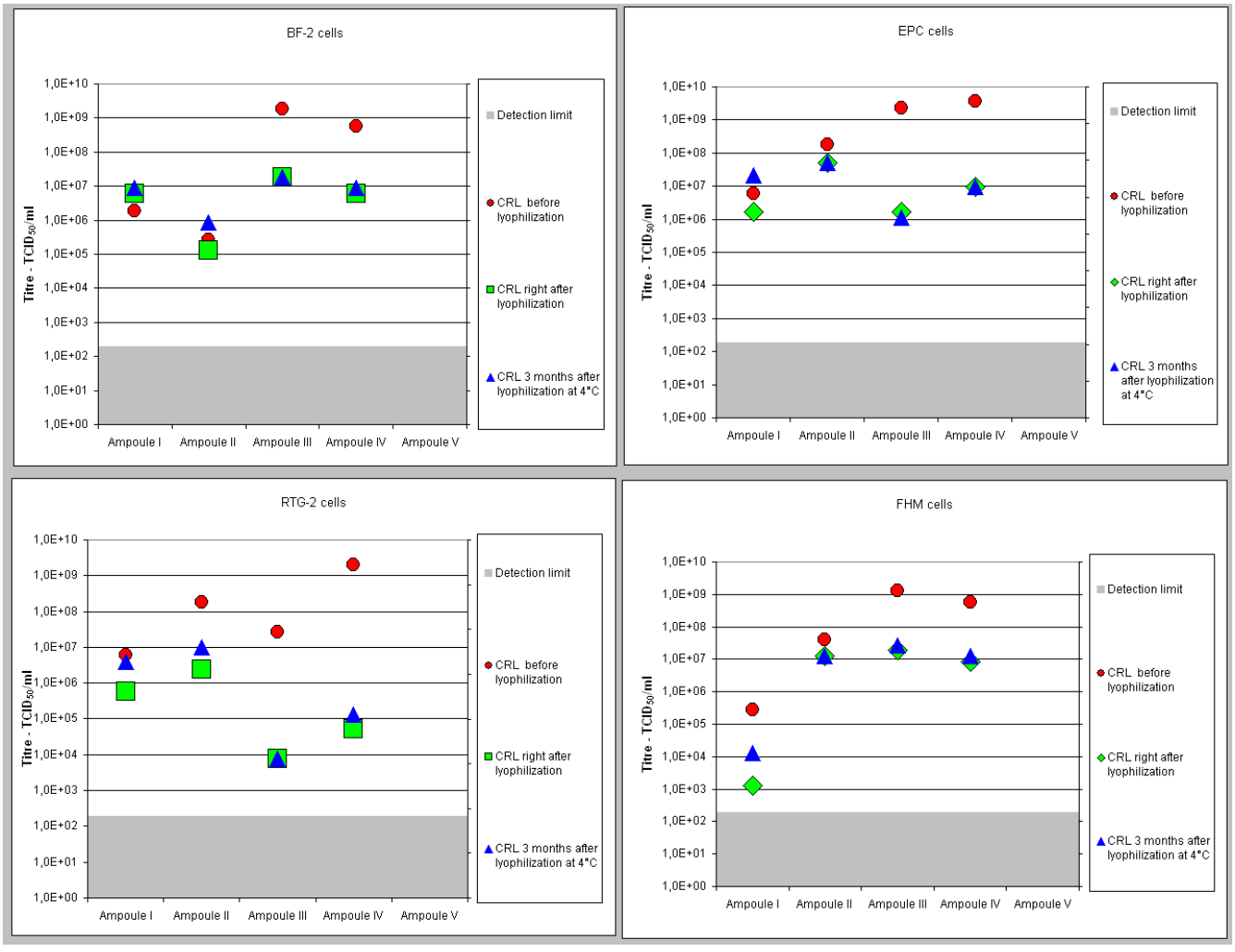
The lyophilisation procedure caused a significant titre reduction, especially for the viral haemorrhagic septicaemia virus (VHSV) where a 2-4 log reduction was observed (figure 1). For the infectious haematopoietic necrosis virus (IHNV) and epizootic haematopoietic necrosis virus (EHNV) a titre reduction between 0-2 log occurred. However, all titres of the lyophilised viruses were above detection level. Furthermore, when lyophilised the viruses were very stable at storing, tested by titration of one ampoule of each virus preparation after 3 months storage in the dark at 4°C. We have previously shown that lyophilised virus kept in these 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; the report is available at http://www.crl-fish.eu/upload/sites/crl-fish/reports/proficiency/report_2007.pdf).
The identities of the viruses in all 5 ampoules were checked and confirmed by ELISA, IFAT, RT-PCR and serum neutralisation tests for VHSV, IHNV, IPNV and SVCV and by PCR and sequencing for EHNV, and IFAT for ranavirus. Presence of viruses other than the expected in each ampoule were not observed.

Table 2. Titre of representative ampoules of no. I to V tested at the CRL 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 (1 replicate), respectively.

Ampoule No.	Content	Cell line	Titre before lyophilisation	Median titre right after lyophilisation	Titre 3 months after lyophilisation (4°C, dark conditions)
			$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule I	Reference strain of EHNV	BF-2	$1,9*10^6$	$5,9*10^6$	$8,6*10^6$
		EPC	$1,3*10^6$	$4,0*10^5$	$4,0*10^6$
		RTG-2	$2,7*10^5$	$4,0*10^4$	$1,9*10^5$
		FHM	$2,7*10^5$	$1,3*10^3$	$1,3*10^4$
Ampoule II	IHNV Genotype L	BF-2	$2,7*10^5$	$1,3*10^5$	$8,6*10^5$
		EPC	$2,7*10^7$	$8,6*10^6$	$8,6*10^6$
		RTG-2	$4,0*10^6$	$1,3*10^5$	$4,0*10^5$
		FHM	$4,0*10^7$	$1,3*10^7$	$1,3*10^7$
Ampoule III	VHSV genotype Ie	BF-2	$1,9*10^9$	$1,9*10^7$	$1,9*10^7$
		EPC	$2,7*10^8$	$4,0*10^5$	$2,7*10^5$
		RTG-2	$8,6*10^5$	$1,3*10^3$	$1,3*10^3$
		FHM	$1,3*10^9$	$1,9*10^7$	$2,7*10^7$
Ampoule IV	VHSV genotype IVa	BF-2	$5,9*10^8$	$5,9*10^6$	$8,6*10^6$
		EPC	$4,0*10^8$	$1,9*10^6$	$1,9*10^6$
		RTG-2	$2,7*10^7$	$5,9*10^3$	$1,3*10^4$
		FHM	$5,9*10^8$	$8,6*10^6$	$1,3*10^7$

Ampoule No.	Content	Cell line	Titre before lyophilisation	Median titre right after lyophilisation	Titre 3 months after lyophilisation (4ºC, dark conditions)
			$TCID_{50}$/ml	$TCID_{50}$/ml	$TCID_{50}$/ml
Ampoule V	Medium	BF-2		$< 1{,}9*10^2$	$< 1{,}9*10^2$
		EPC		$< 1{,}9*10^2$	$< 1{,}9*10^2$
		RTG-2		$< 1{,}9*10^2$	$< 1{,}9*10^2$
		FHM		$< 1{,}9*10^2$	$< 1{,}9*10^2$

Figure 1. Titration before, right after and 3 months after lyophilisation at different cell lines. For ampoule V no CPE was observed when titrated.



Distribution of the test

The test was sent out according to current international regulations for 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 when the test had been shipped.

Thermo-loggers were included in 15 of the parcels (-40°C to +30ºC). 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 15 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 assure more participants that the temperature encountered during transport has not been detrimental to the viability of the virus in the test.

In parcels that had to go through longer transport time, cooling elements were included.

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 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 method of titration was described in the instructions enclosed with the test. All titres were calculated at the CRL 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. On figures 2-5, 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 one laboratory 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 analysis of the isolate in order to determine if the isolate is EHNV.

Table 3. Inter-Laboratory Proficiency Test 2009 - Virus identification.

Laboratory code number	Score	Answer recived at CRL	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
			EHNV	**IHNV**	**VHSV**	**VHSV**	**No Virus**
2	10	12-11-09	EHNV	IHNV	VHSV	VHSV	No virus
3	10	09-11-09	EHNV	IHNV	VHSV	VHSV	No virus
4	10	10-11-09	EHNV	IHNV	VHSV	VHSV	No virus
5	6	02-11-09	Virus not found	Virus not found	VHSV	VHSV	No virus
6	9	13-11-09	Ranavirus	IHNV	VHSV	VHSV	No virus
7	0	no reply	no reply	no reply	no reply	no reply	no reply
8	6	13-11-09	EHNV and VHSV	IHNV	VHSV	VHSV	VHSV
9	10	12-11-09	EHNV	IHNV	VHSV	VHSV	No virus
10	10	05-10-09	EHNV	IHNV	VHSV	VHSV	No virus
11	8	13-11-09	Virus not identified	IHNV	VHSV	VHSV	No virus
12	10	13-11-09	EHNV	IHNV	VHSV	VHSV	No virus
13	10	06-11-09	EHNV	IHNV	VHSV	VHSV	No virus
14	10	06-10-09	EHNV	IHNV	VHSV	VHSV	No virus
15	10	16-10-2009 21-10-2009	EHNV	IHNV	VHSV	VHSV	No virus
16	10	09-11-09	EHNV	IHNV	VHSV	VHSV	No virus
17	10	13-11-09	EHNV	IHNV	VHSV	VHSV	No virus
18	10	13-11-09	EHNV	IHNV	VHSV	VHSV	No virus
19	8	10-09-09	EHNV	IHNV	VHSV	VHSV	VHSV
20	10	12-11-09	EHNV	IHNV	VHSV	VHSV	No virus
21	10	13-11-09	EHNV	IHNV	VHSV	VHSV	No virus
22	10	12-11-09	EHNV	IHNV	VHSV	VHSV	No virus
23	8	13-11-09	EHNV	IHNV	VHSV	VHSV	VHSV
24	8	13-11-09	EHNV	IHNV	VHSV	VHSV	IPNV
25	10	09-011-09	EHNV	IHNV	VHSV	VHSV	No virus
26	10	12-11-09	EHNV	IHNV	VHSV	VHSV	No virus
28	9	13-11-09	Ranavirus	IHNV	VHSV	VHSV	No virus
29	10	13-11-09	EHNV	IHNV	VHSV	VHSV	No virus
30	10	10-11-09	EHNV	IHNV	VHSV	VHSV	No virus
31	8	20-11-2009*	EHNV	IHNV	VHSV	VHSV	VHSV
32	10	10-11-09	EHNV	IHNV	VHSV	VHSV	No virus
33	9	05-11-2009 12-11-2009	Ranavirus	IHNV	VHSV	VHSV	No virus
34	10	10-11-09	EHNV	IHNV	VHSV	VHSV	No virus
35	10	09-11-09	EHNV	IHNV	VHSV	VHSV	No virus
37	10	12-11-09	EHNV	IHNV	VHSV	VHSV	No virus
38	10	05-11-09	EHNV	IHNV	VHSV	VHSV	No virus
39	8	04-11-09	EHNV / IPNV	IHNV	VHSV	VHSV	No virus

	Ampoule I	Ampoule II	Ampoule III	Ampoule IV	Ampoule V
	EHNV	IHNV	VHSV	VHSV	No Virus
Correct ID	28	34	35	35	30
Correct virus group	3				
No virus	1	1	0	0	0
Wrong ID	2	0	0	0	5
No ID	1	0	0	0	0
Not replied	1	1	1	1	1
Total	36	36	36	36	36

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

Table 4. Inter-Laboratory Proficiency Test 2009 – Identification and titration of ampoule I.

Ampoule I – EHNV					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
2	EHNV	2,7E+08	4,0E+04		4,0E+05
3	EHNV	2,7E+06	2,7E+06	1,3E+06	
4	EHNV	5,9E+07	2,7E+06		
5	Virus not found	<1,9E+02	< 1,9E+02		
6	Ranavirus		2,7E+07		
7	no reply				
8	EHNV and VHSV	5,9E+06	8,6E+05		
9	EHNV	1,3E+04	1,3E+04	1,3E+03	1,3E+03
10	EHNV	1,3E+07	8,6E+05	1,3E+05	4,0E+03
11	Virus not identified	2,7E+05	4,0E+04		
12	EHNV	2,7E+05	8,6E+05	2,7E+04	
13	EHNV	5,9E+06	2,7E+05		
14	EHNV	4,0E+07	1,3E+04	4,0E+03	
15	EHNV	4,0E+04	5,9E+05	1,3E+05	<1,9E+02
16	EHNV	4,0E+05	1,3E+06		
17	EHNV	5,9E+06	2,7E+06		
18	EHNV	4,0E+05	8,6E+04		
19	EHNV	2,7E+07	1,3E+06		
20	EHNV	4,0E+07	1,3E+06		
21	EHNV	8,6E+05	1,3E+05		
22	EHNV		1,3E+06	1,3E+04	
23	EHNV	5,9E+05	8,6E+04		
24	EHNV		1,9E+05	1,9E+03	
25	EHNV	2,7E+06	2,7E+06	5,9E+03	1,9E+03
26	EHNV		2,7E+04	4,0E+04	
28	Ranavirus	4,0E+05	8,6E+04		
29	EHNV	2,7E+03	8,6E+04		
30	EHNV	4,0E+05	1,3E+06		2,2E+05
31	EHNV	1,9E+06	1,3E+05		
32	EHNV	4,0E+07	5,9E+06	1,9E+07	8,6E+05
33	Ranavirus	1,3E+05			1,3E+03
34	EHNV	4,0E+08	1,9E+07		
35	EHNV		1,3E+05		8,6E+04
37	EHNV	8,6E+06	5,9E+05		
38	EHNV	2,7E+07	4,0E+06		2,7E+05
39	EHNV / IPNV		1,3E+06	5,9E+06	
Number of laboratories		29	34	12	10
Median titre		2,7E+06	8,6E+05	3,4E+04	4,5E+04
Maximum titre		4,0E+08	2,7E+07	1,9E+07	8,6E+05
Minimum titre		<1,9E+02	1,3E+04	1,3E+03	<1,9E+02
25% quartile titre		4,0E+05	8,6E+04	5,4E+03	1,4E+03
75% quartile titre		2,7E+07	1,3E+06	4,1E+05	2,6E+05

Table 5. Inter-Laboratory Proficiency Test 2009 – Identification and titration of ampoule II.

Ampoule II - IHNV					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
2	IHNV	1,9E+03	8,6E+05		1,9E+05
3	IHNV	1,3E+03	1,9E+07	8,6E+05	
4	IHNV	4,0E+04	2,7E+07		
5	Virus not found	<1,9E+02	<1,9E+02		
6	IHNV		1,9E+07		
7	no reply				
8	IHNV	1,9E+03	8,6E+05		
9	IHNV	1,3E+03	1,3E+06	1,3E+04	1,3E+04
10	IHNV	4,0E+05	4,0E+06	1,9E+06	4,0E+07
11	IHNV	1,3E+03	2,7E+05		
12	IHNV	1,3E+03	4,0E+06	1,9E+06	
13	IHNV	1,9E+05	8,6E+06		
14	IHNV	8,6E+03	1,9E+07	8,6E+04	
15	IHNV	<1,9E+02	5,9E+06	1,3E+03	1,9E+05
16	IHNV	1,9E+02	4,0E+06		
17	IHNV	4,0E+06	2,7E+07		
18	IHNV	1,9E+05	8,6E+05		
19	IHNV	8,6E+06	5,9E+07		
20	IHNV	2,7E+06	4,0E+07		
21	IHNV	1,3E+04	1,9E+06		
22	IHNV		1,3E+07	5,9E+06	
23	IHNV	5,9E+04	2,7E+05		
24	IHNV		5,9E+04	<1,9E+02	
25	IHNV	4,0E+05	1,9E+07	8,6E+04	1,9E+06
26	IHNV		4,0E+03	1,9E+04	
28	IHNV	5,9E+03	1,3E+04		
29	IHNV	4,0E+02	4,0E+05		
30	IHNV	7,1E+06	1,3E+07		2,2E+06
31	IHNV	<1,9E+02	1,9E+03		
32	IHNV	1,9E+05	1,9E+07	2,7E+06	1,9E+07
33	IHNV	1,9E+03			1,3E+06
34	IHNV	1,9E+07	2,7E+06		
35	IHNV		8,6E+06		2,7E+07
37	IHNV	2,7E+05	1,9E+07		
38	IHNV	1,3E+07	5,9E+07		1,9E+08
39	IHNV		<1,9E+02	<1,9E+02	
Number of laboratories		29	34	12	10
Median titre		1,3E+04	4,0E+06	8,6E+04	2,1E+06
Maximum titre		1,9E+07	5,9E+07	5,9E+06	1,9E+08
Minimum titre		<1,9E+02	<1,9E+02	<1,9E+02	1,3E+04
25% quartile titre		1,3E+03	5,2E+05	9,8E+03	4,6E+05
75% quartile titre		4,0E+05	1,9E+07	1,9E+06	2,5E+07

Table 6. Inter-Laboratory Proficiency Test 2009 – Identification and titration of ampoule III.

Ampoule III - VHSV					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
2	VHSV	1,9E+05	1,9E+05		5,9E+04
3	VHSV	1,3E+07	8,6E+05	5,9E+06	
4	VHSV	1,9E+05	1,9E+05		
5	VHSV	2,7E+05	5,9E+04		
6	VHSV		1,3E+05		
7	no reply				
8	VHSV	1,3E+04	4,0E+05		
9	VHSV	2,7E+05	1,9E+06	8,6E+06	1,3E+07
10	VHSV	8,6E+06	8,6E+05	1,3E+07	1,3E+07
11	VHSV	4,0E+06	1,9E+06		
12	VHSV	4,0E+06	5,9E+05	4,0E+02	
13	VHSV	1,3E+07	1,9E+06		
14	VHSV	2,7E+09	2,7E+06	2,7E+05	
15	VHSV	2,7E+05	8,6E+04	1,3E+03	5,9E+04
16	VHSV	1,9E+04	4,0E+03		
17	VHSV	8,6E+07	5,9E+06		
18	VHSV	2,7E+06	8,6E+04		
19	VHSV	4,0E+07	4,0E+06		
20	VHSV	8,6E+06	2,7E+06		
21	VHSV	1,9E+07	1,3E+06		
22	VHSV		2,7E+06	<1,9E+02	
23	VHSV	1,3E+06	8,6E+05		
24	VHSV		8,6E+04	<1,9E+02	
25	VHSV	1,3E+07	8,6E+05	5,9E+02	1,3E+07
26	VHSV		2,7E+06	2,7E+04	
28	VHSV	2,7E+05	1,3E+05		
29	VHSV	1,3E+05	1,9E+02		
30	VHSV	1,3E+05	2,2E+05		1,3E+04
31	VHSV	5,9E+06	1,9E+06		
32	VHSV	8,6E+06	1,3E+06	1,3E+07	5,9E+05
33	VHSV	1,9E+06			8,6E+06
34	VHSV	5,9E+08	1,3E+06		
35	VHSV		1,9E+05		2,7E+05
37	VHSV	8,6E+06	2,7E+06		
38	VHSV	5,9E+07	1,3E+07		1,3E+05
39	VHSV		<1,9E+02	<1,9E+02	
Number of laboratories		29	34	12	10
Median titre		4,0E+06	8,6E+05	1,4E+04	4,3E+05
Maximum titre		2,7E+09	1,3E+07	1,3E+07	1,3E+07
Minimum titre		1,3E+04	<1,9E+02	<1,9E+02	1,3E+04
25% quartile titre		2,7E+05	1,4E+05	3,0E+02	7,6E+04
75% quartile titre		1,3E+07	1,9E+06	6,6E+06	1,2E+07

Table 7. Inter-Laboratory Proficiency Test 2009 – Identification and titration of ampoule IV.

Ampoule IV - VHSV					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
2	VHSV	2,7E+05	1,9E+05		1,9E+05
3	VHSV	8,6E+06	1,3E+06	5,9E+05	
4	VHSV	1,9E+05	4,0E+06		
5	VHSV	1,3E+05	5,9E+03		
6	VHSV		8,6E+06		
7	no reply				
8	VHSV	1,3E+07	2,7E+07		
9	VHSV	2,7E+06	4,0E+06	2,7E+05	1,9E+07
10	VHSV	5,9E+07	5,9E+06	8,6E+06	5,9E+07
11	VHSV	2,7E+05	4,0E+05		
12	VHSV	1,3E+06	4,0E+05	8,6E+03	
13	VHSV	5,9E+06	8,6E+06		
14	VHSV	4,0E+08	4,0E+06	2,7E+04	
15	VHSV	2,7E+04	4,0E+05	1,3E+03	5,9E+04
16	VHSV	5,9E+05	4,0E+05		
17	VHSV	5,9E+06	8,6E+06		
18	VHSV	2,7E+06	8,6E+05		
19	VHSV	2,7E+07	8,6E+05		
20	VHSV	4,0E+06	8,6E+06		
21	VHSV	8,6E+05	1,9E+05		
22	VHSV		1,9E+07	5,9E+03	
23	VHSV	4,0E+06	5,9E+05		
24	VHSV		2,7E+04	<1,9E+02	
25	VHSV	5,9E+05	1,3E+06	1,9E+03	2,7E+06
26	VHSV		1,9E+04	2,7E+04	
28	VHSV	1,3E+05	1,3E+04		
29	VHSV	2,7E+07	8,6E+04		
30	VHSV	1,3E+03	1,3E+03		1,3E+03
31	VHSV	1,3E+05	5,9E+05		
32	VHSV	4,0E+06	1,3E+07	2,7E+06	2,7E+06
33	VHSV	1,9E+05			1,9E+06
34	VHSV	5,9E+06	1,3E+06		
35	VHSV		1,9E+07		8,6E+06
37	VHSV	5,9E+06	1,3E+07		
38	VHSV	8,6E+06	1,9E+07		1,9E+07
39	VHSV		<1,9E+02	<1,9E+02	
Number of laboratories		29	34	12	10
Median titre		2,7E+06	1,1E+06	1,8E+04	2,7E+06
Maximum titre		4,0E+08	2,7E+07	8,6E+06	5,9E+07
Minimum titre		1,3E+03	<1,9E+02	<1,9E+02	1,3E+03
25% quartile titre		2,7E+05	2,4E+05	1,7E+03	6,0E+05
75% quartile titre		5,9E+06	8,6E+06	3,5E+05	1,6E+07

Table 8. Inter-Laboratory Proficiency Test 2009 – Identification and titration of ampoule V.

Ampoule V					
Laboratory code number	**Virus Identification**	**Titre in**			
		BF-2	**EPC**	**RTG-2**	**FHM**
2	No virus	<1,9E+02	<1,9E+02		<1,9E+02
3	No virus	<1,9E+02	<1,9E+02	<1,9E+02	
4	No virus	<1,9E+02	<1,9E+02		
5	No virus	<1,9E+02	<1,9E+02		
6	No virus		<1,9E+02		
7	no reply				
8	VHSV	2,7E+03	1,3E+03		
9	No virus	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
10	No virus	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
11	No virus	<1,9E+02	<1,9E+02		
12	No virus	<1,9E+02	<1,9E+02	<1,9E+02	
13	No virus	<1,9E+02	<1,9E+02		
14	No virus	<1,9E+02	<1,9E+02	<1,9E+02	
15	No virus	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
16	No virus	<1,9E+02	<1,9E+02		
17	No virus	<1,9E+02	<1,9E+02		
18	No virus	<1,9E+02	<1,9E+02		
19	VHSV	<1,9E+02	<1,9E+02		
20	No virus	<1,9E+02	<1,9E+02		
21	No virus	<1,9E+02	<1,9E+02		
22	No virus		<1,9E+02	<1,9E+02	
23	VHSV	<1,9E+02	<1,9E+02		
24	IPNV		<1,9E+02	<1,9E+02	
25	No virus	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
26	No virus		<1,9E+02	<1,9E+02	
28	No virus	<1,9E+02	<1,9E+02		
29	No virus	<1,9E+02	<1,9E+02		
30	No virus	<1,9E+02	<1,9E+02		<1,9E+02
31	VHSV	<1,9E+02	<1,9E+02		
32	No virus	<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
33	No virus	<1,9E+02			<1,9E+02
34	No virus	1,3E+03	<1,9E+02		
35	No virus		<1,9E+02		<1,9E+02
37	No virus	<1,9E+02	<1,9E+02		
38	No virus	<1,9E+02	<1,9E+02		<1,9E+02
39	No virus		<1,9E+02	<1,9E+02	
Number of laboratories		29	34	12	10
Median titre		<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
Maximum titre		2,7E+03	1,3E+03	<1,9E+02	<1,9E+02
Minimum titre		<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
25% quartile titre		<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02
75% quartile titre		<1,9E+02	<1,9E+02	<1,9E+02	<1,9E+02

Figure 2. Titre obtained in BF-2 cells

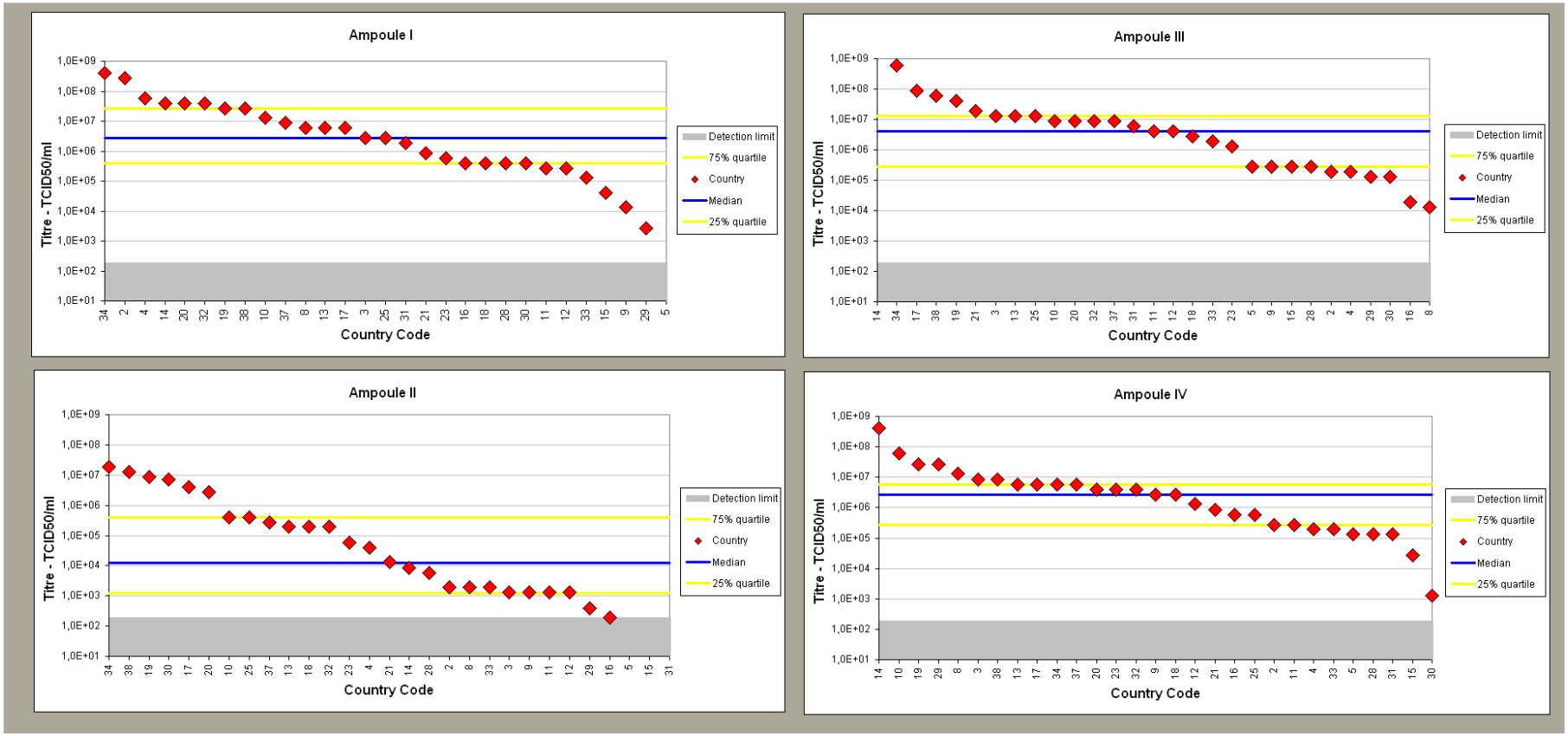


Figure 2. The titre (red diamond) of each participating laboratory (country code) using BF-2 cells illustrated for ampoule I, II, III and IV. On all graphs are plotted the detection level (gray shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line). For participants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

Figure 3. Titre obtained in EPC cells

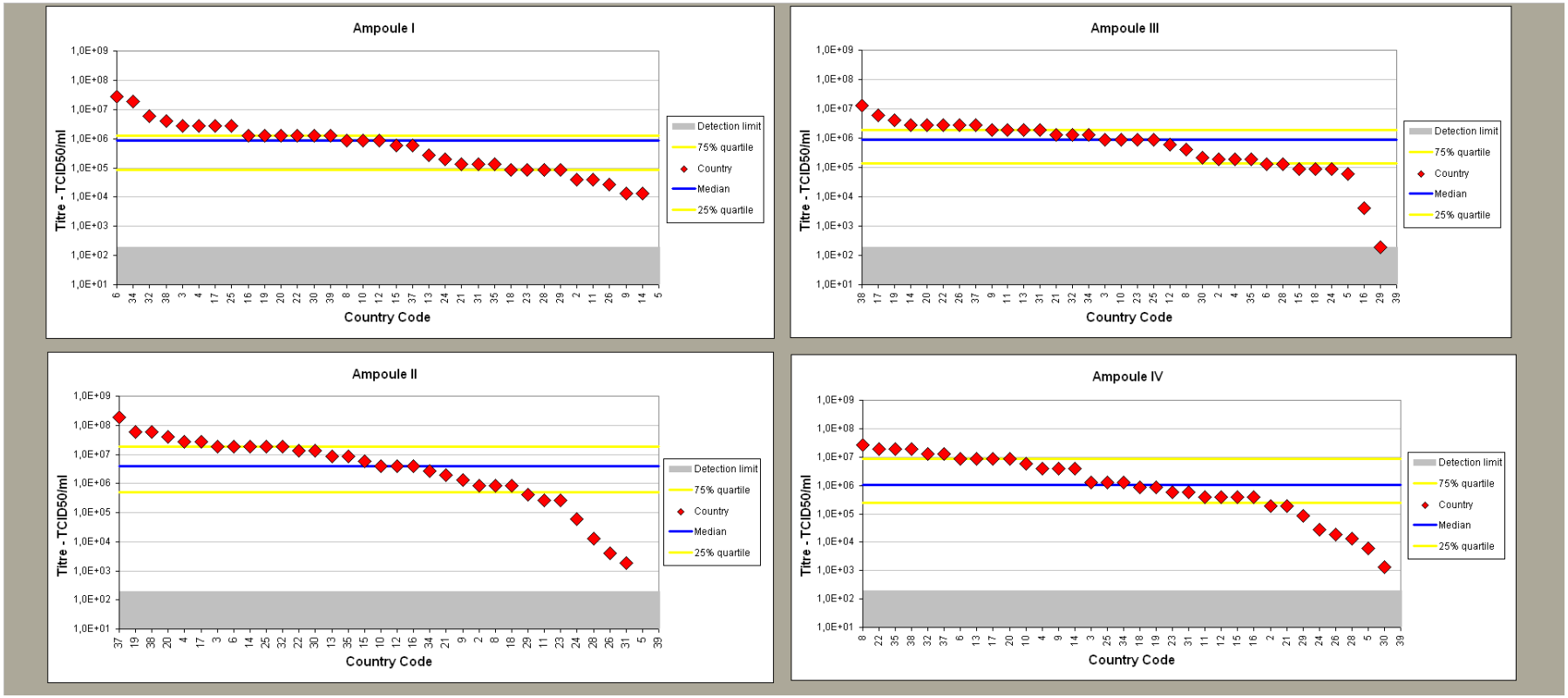


Figure 3. The titre (red diamond) of each participating laboratory (country code) using EPC cells illustrated for ampoule I, II, III and IV On all graphs are plotted the detection level (gray shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line). For participants failing to obtain any titre, no red diamonds is shown.

Figure 4. Titre obtained in RTG-2 cells

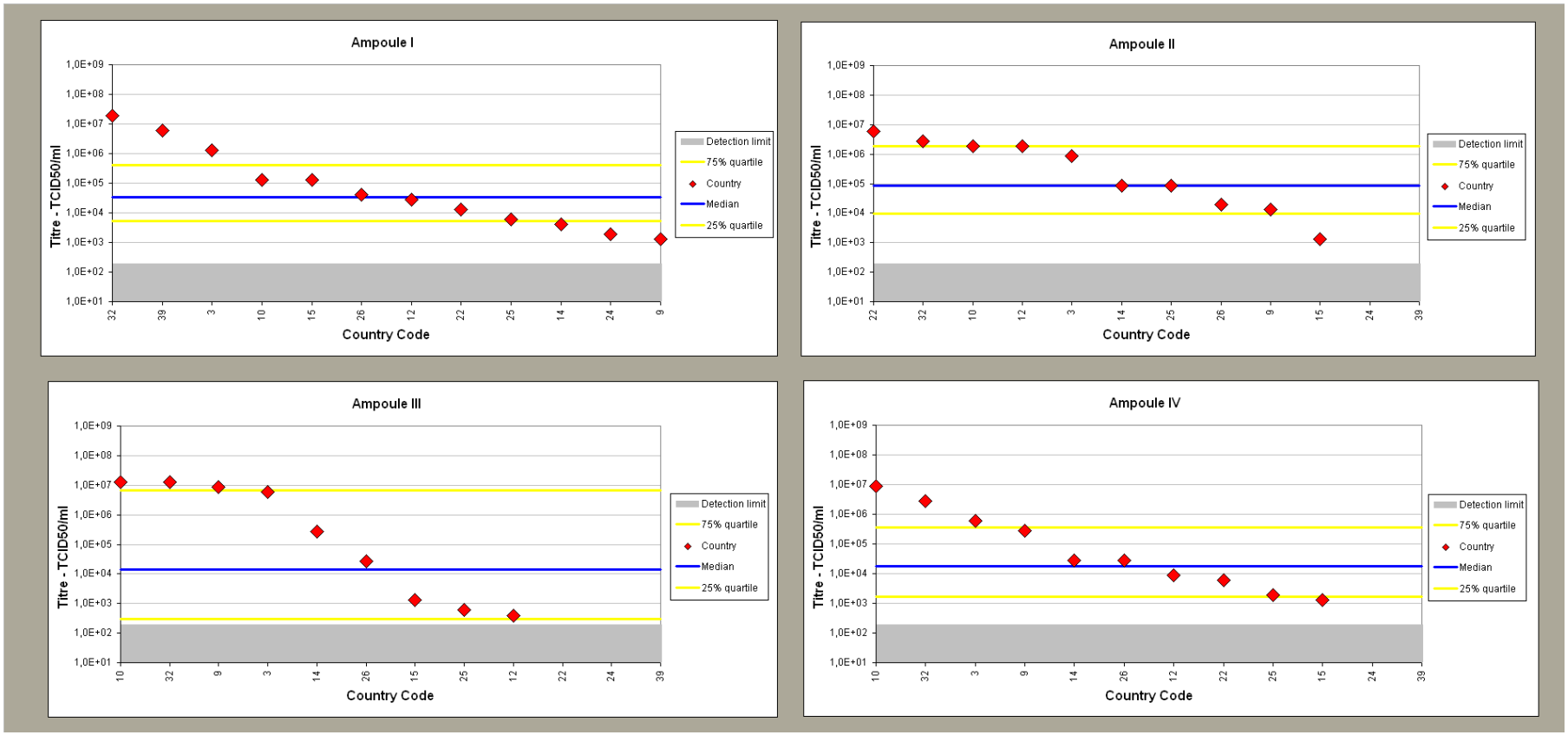


Figure 4. The titre (red diamond) of each participating laboratory (country code) using RTG-2 cells illustrated for ampoule I, II, III and IV. On all graphs are plotted the detection level (gray shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line). For participants failing to obtain any titre, no red diamonds is shown.

Figure 5. Titre obtained in FHM cells

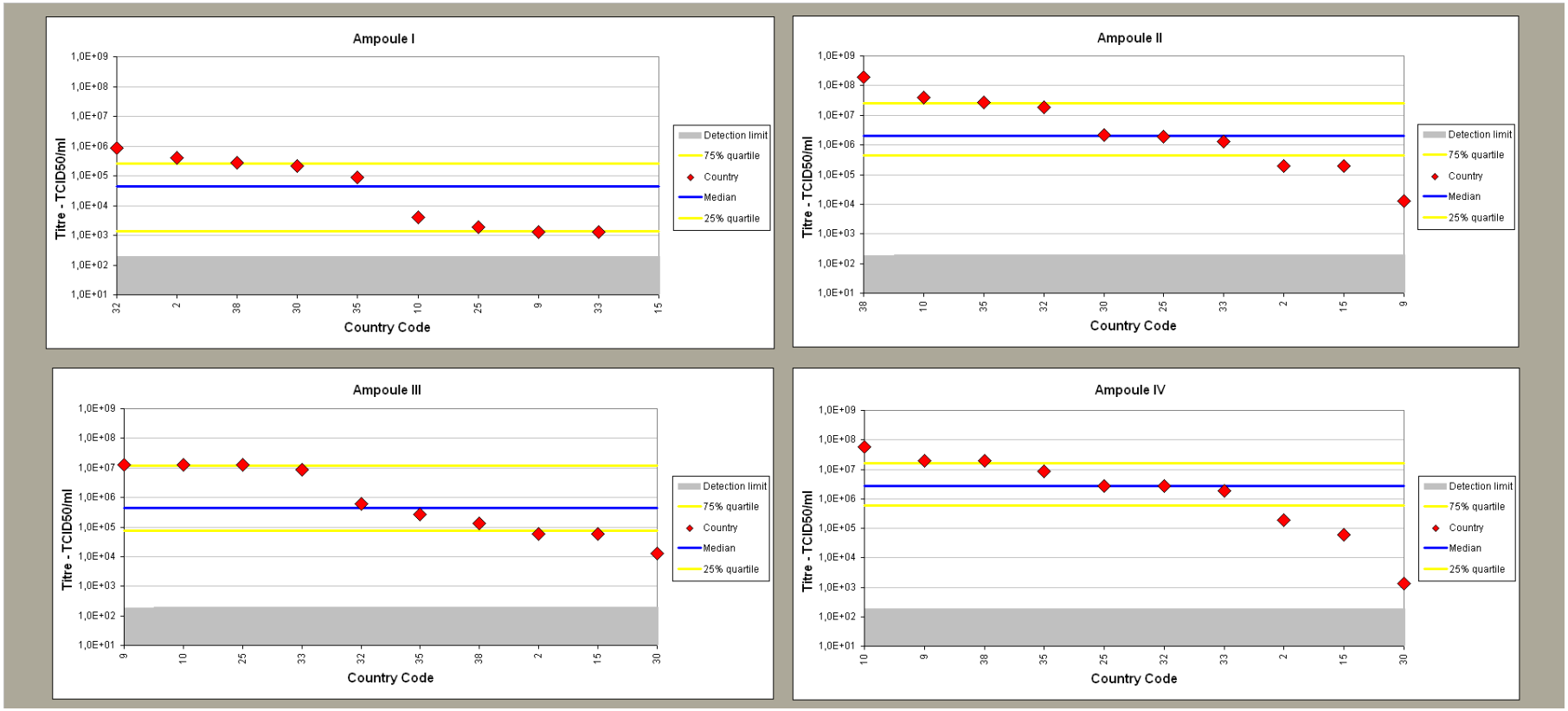


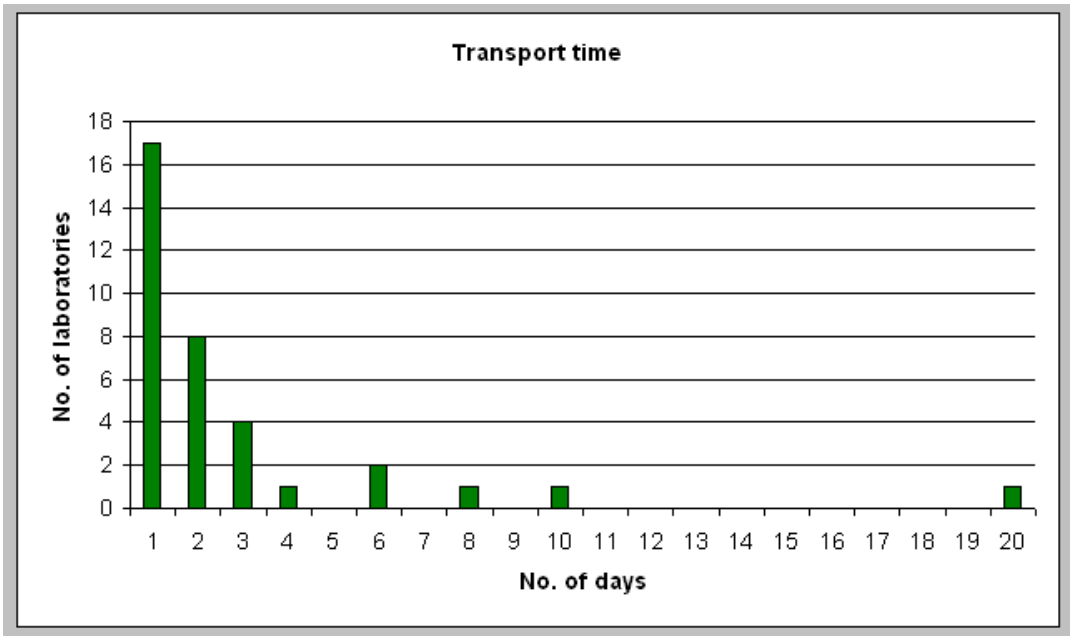
Figure 5. The titre (red diamond) of each participating laboratory (country code) using FHM cells illustrated for ampoule I, II, III, and IV On all graphs are plotted the detection level (gray shadow), median (blue line), 75% quartile (upper yellow line) and 25% quartile (lower yellow line). For participants failing to obtain any titre, no red diamonds is shown.

Findings

Participation
36 laboratories received the annual proficiency test, 34 participants submitted results within the deadline. One participant submitted results 7 days after deadline but before the content of the ampoules were made public available. One participant did not submit results.

Shipment and handling
All proficiency tests were delivered by courier and when possible, participants were provided with a tracking number when the test had been shipped. A thermo-logger was included in 15 of the parcels. Within three days, 29 proficiency tests were delivered to participants; 3 tests were delivered within 7 days and 4 tests within three weeks. The average temperatures for the transports without cooling elements were (for 9 countries) 17.8°C and the temperature only exceeded 25.5°C for one transports for two hours upon arrival. The remaining transports (6 countries) were send with cooling elements because of longer travel time. These transports had an average temperature of 13.9°C. The temperature of four of the transports did not exceed 24°C whereas it reached 29.5°C for very short periods of time for the last two. The laboratory receiving ampoules having reached highest temperature for longest exposure time was however, able to obtain virus titres significantly above background. Therefore, the temperature rise during transportation is not considered to have lowered the virus titres considerably.

Figure 6. Transport time for the parcel to reach the participants.



Identification of content

- 24 laboratories correctly identified all viruses in all ampoules.

Ampoule I – EHNV

- 28 laboratories correctly identified EHNV.
- 3 laboratories identified ranavirus.
- 2 laboratories found more isolates than were present.
- 1 laboratory did not find any virus.
- 1 laboratory found virus but did not identify it.

Ampoule II - IHNV

- 34 laboratories correctly identified IHNV.
- 1 laboratory did not find any virus.

Ampoule III - VHSV

- 35 laboratories correctly identified VHSV.

Ampoule IV – VHSV

- 35 laboratories correctly identified VHSV.

Ampoule V – No virus

- 30 laboratories correctly identified that there was no virus.
- 5 laboratories identified a virus.

One laboratory did not submit any results

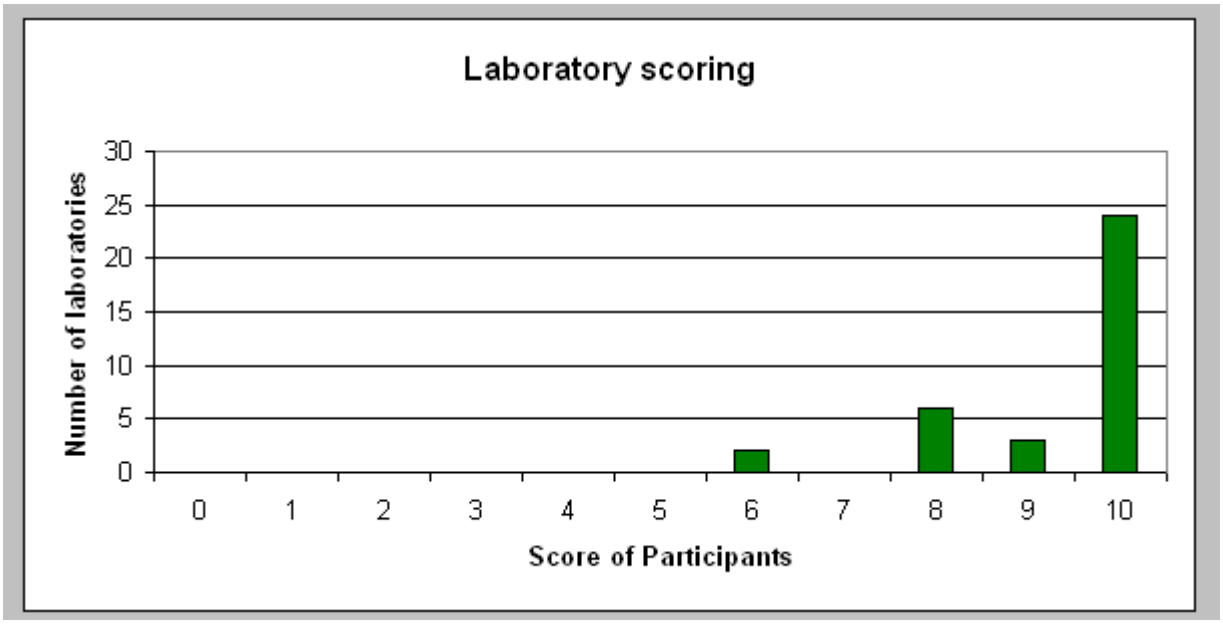
Scores

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 (Table 3), giving the possibility for obtaining a maximum score of 10. Identification of "ranavirus" as the virus in Ampoule 1 was given the score of 1. If no sequence analysis was performed, the participant would not be able to identify the ranavirus as EHNV and would be given the score of 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.

24 laboratories out of 36 correctly identified all viruses in all ampoules and obtained maximum score. Three laboratories scored 9 because the virus in ampoule I was identified as a ranavirus. 6 laboratories identified a virus in one or more ampoules that were not present. Two laboratories did not identify virus in one or more ampoules where a virus was present. Finally, one laboratory did not submit their results. A diagram of the scoring obtained by the laboratories is shown in figure 7. Serotyping, genotyping of VHSV and IHNV and submission of sequencing results is not a mandatory part of the test and is not included in the score of participants.

Figure 7. Obtained score by participants.



Methods applied

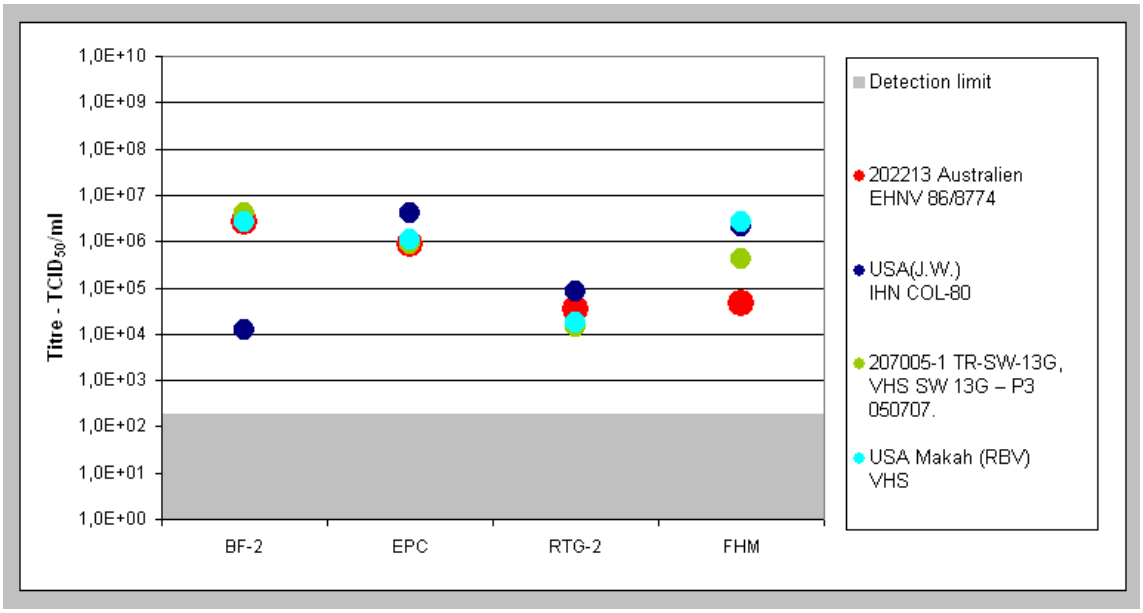
The following cell lines were used by the participants:

- 29 laboratories used BF-2 cells
- 34 laboratories used EPC cells
- 12 laboratories used RTG-2 cells
- 10 laboratories used FHM cells
- 5 laboratories used four cell lines
- 5 laboratories used tree cell lines
  - 3 laboratories used BF-2, EPC and FHM
  - 3 laboratories used BF-2, EPC and RTG-2
- 23 laboratories used two cell lines:
  - 17 laboratories used BF-2 cells in combination with EPC cells
  - 4 laboratories used RTG-2 cells in combination with EPC cells
  - 1 laboratory used BF-2 cells in combination with FHM cells
  - 1 laboratory used EPC cells in combination with FHM cells
- 1 laboratory used only EPC cells

The combination of EPC and FHM cells is not valid according to Commission Decision 2001/183/EC, neither is the use of EPC cells alone. 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 8. It appears that VHSV (Ampoule III and IV) replicates well on BF-2, FHM and EPC cells but less efficient on RTG-2 cells. IHNV (ampoule II) replicates well on EPC and FHM cells, and less efficiently on BF-2 and RTG-2 cells. Finally, EHNV (ampoule I) replicates well on EPC and BF-2 cells whereas lower titres were observed on RTG-2 cells and FHM cells.

Figure 8 Median titre of viruses obtained by participants at different cell lines. For ampoule V the median was below detection level and is not shown.



Methods used for identification of viruses (Table 9)

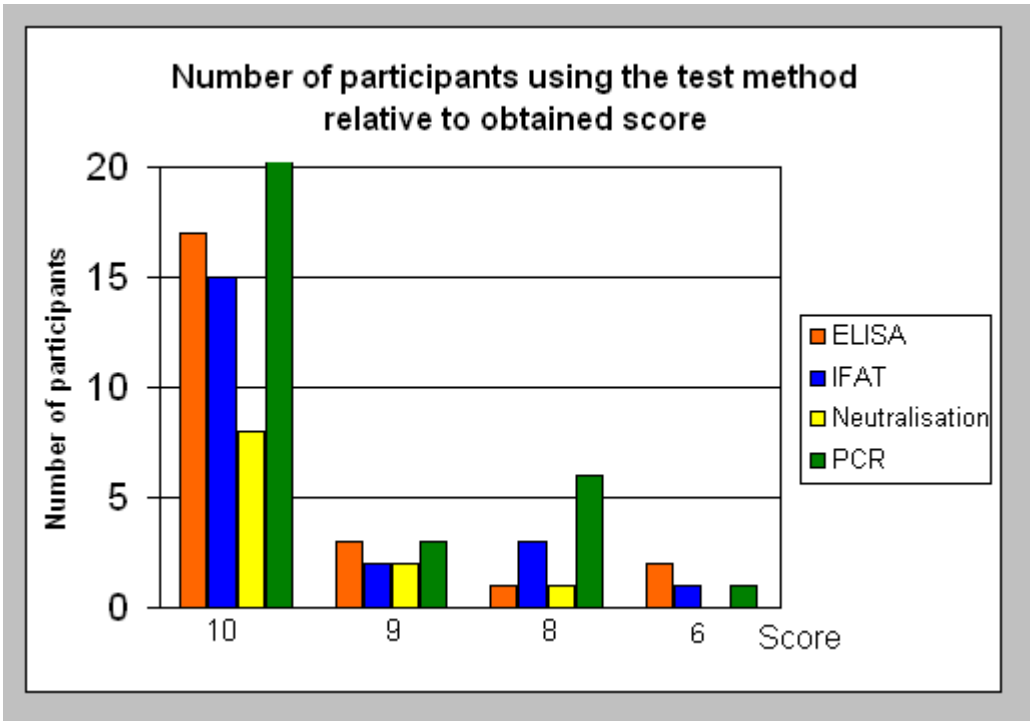
- 24 laboratories used ELISA for identification of viruses.
- 21 laboratories used IFAT for identification of viruses.
- 11 laboratories used neutralisation tests for identification of viruses.
- 34 laboratories used PCR for identification of viruses.
- 30 laboratories performed sequencing for identification of viruses.

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

Laboratory code number	Score Top score 10	ELISA	IFAT	Neutralisation	PCR	Sequence	Sequence ampoule no.
2	10				X	X	I, II, III, IV
3	10	X	X		X	X	I, II, III, IV
4	10	X			X	X	I
5	6	X					
6	9	X	X	X	X		
7							
8	6	X	X		X	X	I, II, III, IV, V
9	10	X			X	X	I
10	10	X	X	X	X	X	I, II, III, IV
11	8	X	X		X		
12	10	X	X		X	X	I, II, III, IV
13	10	X			X	X	I, III, IV
14	10		X	X	X	X	I, II, III, IV
15	10	X	X	X	X	X	I, II, III, IV
16	10	X		X	X	X	I, II, III, IV
17	10			X	X	X	I, III, IV
18	10	X	X		X	X	I, II, III, IV
19	8		X		X	X	I, II
20	10				X	X	I, II, III, IV
21	10	X	X	X	X	X	I, II, III, IV
22	10	X			X	X	I, II
23	8		X	X	X	X	I, III, IV
24	8				X	X	I, II
25	10	X	X		X	X	I, III, IV
26	10			X	X	X	I, II, III, IV
28	9	X	X		X		
29	10	X	X		X	X	I
30	10	X	X		X	X	I, II, III, IV
31	8	X			X	X	I, II, III, IV
32	10	X	X	X	X	X	I, II, III, IV
33	9	X		X	X		
34	10		X		X	X	I, II
35	10		X		X	X	I
37	10	X	X		X	X	I, II, III, IV
38	10	X	X		X	X	I, II, III, IV
39	8				X	X	I
Number of laboratories		24	21	11	34	30	

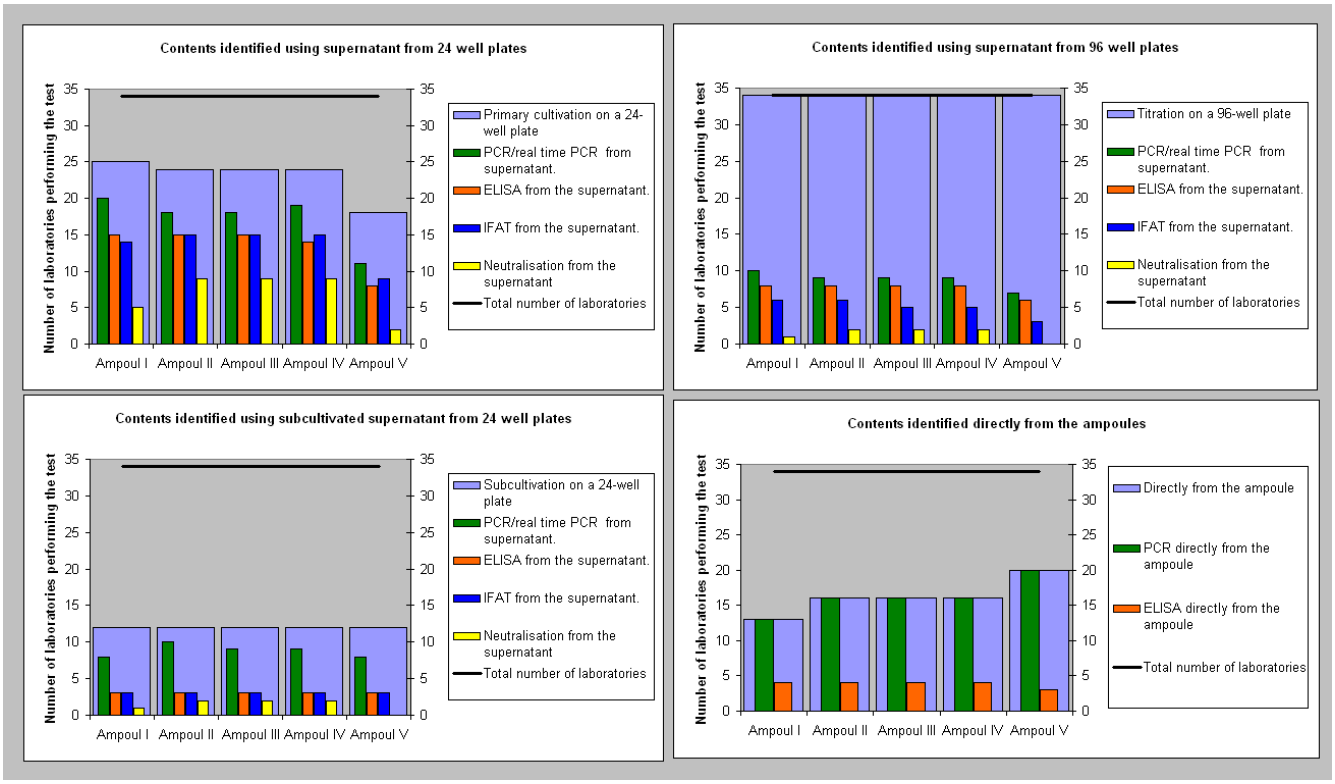
A graph was constructed to illustrate the association between the methods used by participants for virus identification and the obtained score (Figure 9). The PCR is the most frequently used method by participants and only one participant did not use this method. Participants scoring 9 all used PCR for identification of the virus in ampoule 1 as ranavirus but did not perform sequencing analyses to further identify the virus as EHNV. For participants scoring lower than 9, the deficiency in virus identification can not directly be assigned to improper use of an identification method. Rather mistakes might be related to performance of the overall procedure. 6 out of the 8 participants scoring lower than 9 identify false positive viruses in the ampoule indicating that cross contamination could have occurred at some point in the diagnostic process. The last two participants are unable to identify viruses in one or more ampoules. In one of these laboratories this might occur because cells are not sensitive towards detection of these viruses as no titres are reported. In another laboratory there was detectable titre but missing identification which may be due to the identification method (EHNV PCR) has not been implemented.

Figure 9. Methods used by participants for identification



At this year's proficiency test, participants were asked to fill out a questionnaire regarding the diagnostic methods used in the laboratory. The results are summarized in figure 10 A-D. All participating laboratories used 96 well plates for titration. Primary cultivation on 24 well plates was done by 18-25 participants and 12 participants subcultivated on 24 well plates. PCR was the most used method for identification of virus. On supernatant from subcultivated 24 well plates or directly on ampoule content, PCR was used more than double as frequently as any other method. However, when virus was identified from supernatant from primary cultures of either 24 or 96 well plates ELISA and IFAT was used at an only slightly lower frequency than the PCR. The reason for the different pattern in methods used for virus identification on primary and subcultivated cells is unclear but might reflect different usage of identification methods in the lower number of laboratories using subcultivation. The high proportion of laboratories using of PCR for identification of virus directly in the ampoule most likely reflect that PCR is a tool that can be very easily used for this purpose.

Figure 10. Diagnostic methods used for identification of viruses. A) Method used for identification using supernatant from primary cultivation from 24 well plates. B) Same as A but from subcultivated plate. C) Method used for identification using supernatant from primary cultivation from 96 well plates. D) Method used for identification directly on content in the ampoules.



Genotypning and sequencing

In previous proficiency tests provided by the CRL, we have encouraged participants to genotype the identified viruses though it has not been a mandatory task. As ranaviruses was for the first time included in the test, it is mandatory to do sequence analyses 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 but this was not an obligatory task.

Ampoule I - EHNV

- 30 laboratories sequenced to identify EHNV.
- 3 laboratories performed only PCR and no sequence analyses.
- 1 laboratory did not identify the virus
- No laboratory reported having performed RFLP analyses.

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. 30 laboratories used sequencing for identification of EHNV whereas none used RFLP. Of these, 29 used sequencing of the partial MCP gene as recommended by the OIE diagnostic manual for EHN whereas two participants sequenced the polymerase gene as described by Holopainen et al. 2009. Three laboratories identified the virus by PCR but not did not perform sequencing analyses.

Ampoule II-IV

25 out of 35 laboratories sequenced parts of the genome of either IHNV or VHSV isolates. This is five laboratories less than did sequencing of the EHNV. Both full length and partial N- and G-genes were used for virus/genotype identification (see table 10).

Ampoule II - IHNV Genotype L

- 21 laboratories performed sequencing
- 11 laboratories genotyped the IHNV isolate as belonging to genogroup L
- 6 laboratories used alternative genotyping notification or showed blast results
- 4 laboratories did not give any genotype of the sequences

11 laboratories genotyped the IHNV isolate as belonging to genogroup L as described in Kurath et al. 2003 (Table 10). This is a more laboratories compared to genotyping IHNV in proficiency test 2008. One laboratory genotyped the isolate according to another publication. Furthermore, five laboratories showed indirect isolate relatedness to genogroup L either by presenting a phylogenetic tree or by showing blast results.

Ampoule III - VHSV genotype Ie

- 21 laboratories performed sequencing
- 14 laboratories identified the VHSV isolate as genotype I
- 6 laboratories subtyped the isolates as a genotype Ie
- 2 laboratories subtyped the isolates as a genotype Ib
- 1 laboratory identified the VHSV isolate as genotype III
- 4 laboratories showed blast results
- 2 laboratories did not give any genotype of the sequences

14 laboratories correctly identified the isolate as belonging to genotype I. 6 laboratories correctly subtyped the isolate as belonging to the Ie subgroup (according to Einer-Jensen et al 2004) whereas two identified it as a genotype Ib. Ib genotype might come up because laboratories uses different genes and if a certain gene is not sequenced for genotype Ie isolates, a blast result will show a related

subgenotype. One laboratory identified the isolate as belonging to genotype III. This could be because the genotype notification is according to Nishizawa et al. 2002? Four laboratories indicated genotype of isolate by showing blast result.

Ampoule IV - VHSV genotype IVa

- 20 laboratories performed sequencing
- 12 laboratories identified the VHSV isolate as genotype IV
- 10 laboratories subtyped the isolates as a genotype IVa
- 1 laboratory identified the VHSV isolate as genotype I
- 1 laboratory identified the VHSV isolate as genotype Ie
- 1 laboratory identified the VHSV isolate as a mixture of genotype IVa and III
- 3 laboratories showed blast results
- 2 laboratories did not give any genotype of the sequences

12 laboratories correctly identified the isolate as belonging to genotype IV and 10 laboratories correctly subtyped the isolate as belonging to the IVa subgroup. One laboratory identified the isolate as belonging to genotype I which might be because the genotype notification in Nishizawa et al. 2002 is used. One laboratory identified the isolate as genotype Ie with identical sequence to the sequence of the isolate in ampoule III and therefore double sequencing of VHSV in ampoule III might have occurred. Four laboratories indicated the genotype of the isolate by showing blast result.
Interestingly, one laboratory report that a mixture of two VHSV isolates is present in ampoule IV. Direct sequencing of a PCR fragment identified the VHSV as a genotype IVa. Cloning of three independent clones identified one genotype IVa isolate and two genotype III isolates. The sequence of the genotype III isolate is very closely related to the marine 4p168 isolate (Einer-Jensen et al. 2004, Mortensen et al. 1999). The reason for this finding is not clear though it seems reasonable that a contamination have taken place. No other laboratories report of genotype III present in ampoule IV, indicating that a contamination might have occurred in the laboratory. However as the reported genotype III was from cloned sequences, it is also a possibility that primers favouring genotype III amplification could have amplified traces of genotype III RNA present as contaminating viruses in the sealed ampoule and that these sequences have been cloned into the sequencing vector.

In general, it is positive that more laboratories performed sequencing than at last year's proficiency test and that sequences were of high quality and usable for genotyping. This high number of laboratories performing sequencing might reflects that EHNV has been included in the test. It is important that the remaining laboratories implement the technique in the laboratory as genotyping is the basis for differentiating notifiable viruses from others. Genotyping of VHSV and IHNV were performed according to different notifications although references were provided on what notification should be used. In future proficiency tests it will again be specified for all listed disease according to which references, the genotyping should be performed.

Table 10. Genotyping, results on viruses in ampoule II-IV submitted by participating laboratories**.**

Laboratory code number	Score	Ampoule II - IHNV		Ampoule III - VHSV		Ampoule IV - VHSV	
		Genotype	Gene sequenced	Genotype	Gene sequenced	Genotype	Gene sequenced
2	10	Genogroup L Subgroup 2	Partial G	Genotype Ie	Partial G	Genotype Ie	Full length G
3	10	(98 % similar to Col-80…)	Partial N	(100% identical to TR-SW13G…)	Partial G	(99 % identical to ME03….)	Partial G
8	6		Partial N		Partial N		Partial N
10	10	Genogroup L	Partial G	Genotype Ie	Full G	Genotype IVa	Partial G
12	10	Genogroup L	Partial G	Genotype Ie	Partial G	Genotype IVa	Partial G
13	10			Genotype I	Partial G	Genotype IVa	Partial G
14	10	Phylogenetic tree	Partial N	Genotype I	Partial G = direct sequencing Full length G = 3 clone	2 X Genotype III 2 X Genotype Iva	Partial G = direct sequencing = genotype IV
15	10	Genotype L	Full length G	Genotype I	Full length G	Genotype IVa	Full length G
16	10	(100 % identical to AY442509)		(96% identical to Z93414)			
17	10			Genotype III	Partial N	Genotype I	Partial N
18	10	Genogroup L	Partial N Partial G	Genotype I	Partial N	Genotype IV	Partial N
19	8	Genogroup L	Partial G Partial NV				
20	10	Genogroup L	Partial G	Genotype Ie	Partial G	Genotype Iva	Partial G
21	10		Partial N		Partial N		Partial N
22	10	Genogroup L	Partial G				
23	8			Genotype Ib	Partial G	Genotype IVa	Partial G
24	8		Partial N				
25	10			Similar to AY546619	Full length G	Similar to AB490792	Full G
26	10	Genogroup L	Partial G	Genotype Ib	Partial N	Genotype IVa	Partial N
30	10		Partial N	Genotype Ie	Partial N	Genotype IVa	Partial N
31	8	(100 % homology with L40874)	Partial G	(98% hology to Z93412)	Partial G	(100% homology to DQ401192)	Partial G
32	10	Genogroup U+L	Partial G	Genotype I	Full length G	Genotype IV	Full length G
34	10	(100 % homology with AY442509)	Full length G				
37	10	Genogroup L	Partial N	Genotype I	Partial G	Genotype IVa	Partial G
38	10	Genogroup L	Partial N	Genotype Ie	Partial G	Genotype IVa	Partial G

Concluding remarks

The inter-laboratory proficiency test 2009 was conducted without major constrains. Most parcels were delivered by the shipping companies within 3 days after submission; it was, however, unfortunate that one of the parcels made op to 3 weeks before delivering to the laboratories (primarily due to border controls).

In 2009 EHNV was included in the proficiency test and 28 participants were able to correctly identify the virus. This is considered to be a relatively large number of participants as it is the first time EHNV is part of the test and because identification of the virus include sequence analyses which has not been mandatory to use in previous tests. Nevertheless, EHN is a listed disease and all laboratories are obliged to implement diagnostic tools for identifying EHNV as soon as possible.

The IHNV within this test replicates well on EPC and FHM cells, and less efficiently on BF-2 and RTG-2 cells (figure 8) making all the valid combinations of cell in Commission Decision 2001/183/EC suitable.
EHNV replicates well on EPC and BF-2 cells whereas lower titres were observed on RTG-2 cells and FHM cells (figure 8). Therefore the combination of RTG-2 and FHM cells seems less suitable.
It appears that the two VHSV isolates in this test replicates equally well on BF-2, FHM and EPC cells but less efficient on RTG-2 cells. This is valid for the two VHSV isolates included in this proficiency test but other VHSV isolates prefer BF-2 cells compared to EPC cells and therefore laboratories are still encouraged to use a combination of cells as described in Commission Decision 2001/183/EC. The bad performance in several laboratories of their RTG-2 cell lines for growth of VHSV (Figure 4) is worrying as is it described in Commission Decision 2001/183/EC that RTG-2 cells can be used instead of BF-2 cells. Based on these observations, we recommend that laboratories use BF-2 cells and not RTG-2 cells for replication/survey of/for VHSV.
In conclusion we recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose.

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. This year however, we 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 will be further presented and discussed at the 14th Annual Meeting of National Reference Laboratories for Fish Diseases to be held 26-28 May 2009 in Århus, Denmark.

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National Veterinary Institute, Technical University of Denmark, 12 February 2010

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