National Veterinary Institute

Technical University of Denmark

European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Copenhagen

Inter-Laboratory Proficiency Test 2015

for identification of VHSV, IHNV, EHNV SVCV and IPNV (PT1) and identification of CyHV-3 (KHV), ISAV and SAV (PT2)

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Content

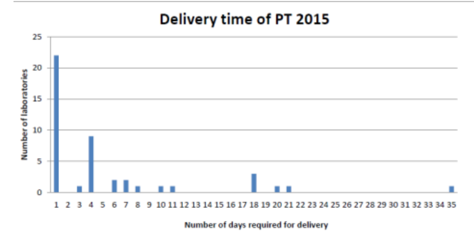
- Proficiency test 1, PT1
- Proficiency test 2, PT2
- Feedback from participants
- Proficiency test 2015

PT1 and PT2 was delivered to 46 laboratories

All NRL's for Fish Diseases in EU Member States

NRL's in:
Australia
Canada
Faroe Islands
Iceland
Japan
New Zealand
Norway
P.R. China (2)
Republic of Korea (2)
Switzerland
Turkey
USA (2)

Distribution of PT1 and PT2



Within one day, the tests were delivered to 22 participants; 14 more tests were delivered within the first week; 3 more within the first two weeks; 5 further within three weeks and the last test was delivered within 35 days (Figure 1).

PT1: Content of ampoules

Five ampoules containing virus/ lyophilised tissue culture supernatant

Code	Isolate
Ampoule I:	IHNV 32/87
Ampoule II:	VHSV strain 1P8
Ampoule III:	European Catfish virus (ECV), Isolate 562/92
Ampoule IV:	BLANK
Ampoule V:	VHSV strain, DK-5151 + IHNV 32/87

Testing PT1

- The proficiency test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043
- The titre and homogeneity of the samples was tested prior to sending out the test by *titration of 5 ampoules of each virus preparation in 4 cell lines.*
- The identity of the virus in the 5 ampoules was checked by ELISA, IFAT, PCR and serum neutralisation.
- The lyophilisation procedure caused a significant titre reduction for IHNV with 1-2 log reduction, while for VHSV, IPNV, SVCV and EHNV almost no reduction was observed.
- All titres of the lyophilised viruses were above detection level, except for IHNV on BF-2 cells. As participants, however, are expected to use at least two different cell lines, IHNV would have been detected on the other cell line.

Titres before and after lyophilization

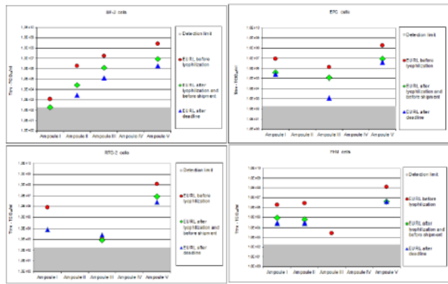


Figure 4. Virus titers in different cell lines:
Before lyophilisation, After lyophilisation-before shipment and After minimum 3 months after lyophilisation (storage 4°C in the dark) (1 ampoule).

PT1

- Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in Commission Decision 2015-1554

AMPOULE V 2015

During results receipt **24 of 44** participating laboratories reported the detection of an additional rhabdovirus in ampoule V, being a SVCV or a SVCV-like isolate.

- Direct RT-PCR on re-suspended content of 4 ampoule V replicates both using diagnostic and sequencing primer sets as described by Koutná et al. (2003) and Stone et al., (2003) all tested negative.
- Re-suspended content in ampoule V was inoculated on BF-2; EPC, RTG-2 and FHM cell lines.
- Harvested supernatant tested by SVCV-ELISA and both SVCV RT- PCR protocols tested negative. However an IFAT analysis performed using polyclonal antibody K42 raised against pike fry rhabdovirus (Jørgensen et al. 1989) provided a positive staining.
- Further examinations were then initiated as re-suspended content of ampoule V was inoculated on BF-2-; EPC-, RTG-2- and FHM cell lines, respectively with polyclonal neutralizing antisera against VHSV and IHNV and cells were incubated at 24°C, a temperature considered not permissive to the growth of VHSV and IHNV.

An isolate was finally obtained and tested with the two PCR protocols mentioned above, where only the more generic test performed with sequencing primers tested positive.
The amplicon was sequenced and the sequence analysis blasted against the ones retrieved from the other participants.
Sequence analysis finally confirmed that the additional isolate from Ampoule V obtained from cell culture at non permissive temperature for the growth of VHSV and IHNV, was 99% identical to the tench rhabdovirus S64 (Jørgensen et al. 1989).

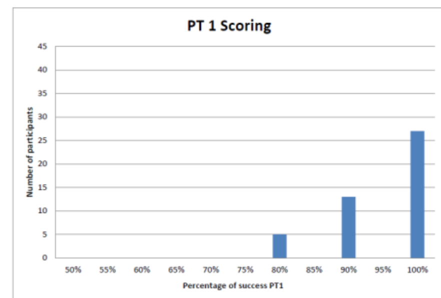
Laboratory score 46-24

Laboratory code number	Score
46	10-10
45	10-10
44	8-10
43	8-8
42	9-10
41	10-10
40	10-10
39	10-10
38	10-10
37	8-10
36	10-10
35	10-10
34	10-10
33	9-10
32	10-10
31	9-10
30	9-10
28	9-10
27	10-10
26	10-10
25	9-10
24	10-10

Laboratory score 23-1

Laboratory code number	Score
23	10-10
22	9-10
21	10-10
20	10-10
19	9-10
18	10-10
17	10-10
16	10-10
15	8-10
14	9-10
13	10-10
12	10-10
11	10-10
10	9-10
9	10-10
8	9-10
7	10-10
6	8-10
5	8-10
4	10-10
3	9-10
2	9-10
1	10-10

Laboratory scoring, PT1



Any comments/questions to PT1?

PT-2 Content of ampoules

Four ampoules containing pathogens / lyophilised tissue culture supernatant

Code	Isolate
Ampoule VI:	KHV-TP 30
Ampoule VII:	SAV 6
Ampoule VIII:	BF-2, cells Supernatant
Ampoule IX:	ISAV FO/01/01/HPR13

PT2 Virus identification participating laboratories

Laboratory score 46-24

Laboratory code number	Score
46	8-8
45	8-8
44	8-8
42	6-8
41	8-8
40	8-8
39	6-8
38	8-8
37	8-8
36	8-8
35	8-8
34	8-8
33	8-8
32	8-8
31	8-8
30	8-8
28	8-8
27	8-8
26	8-8
25	8-8

Laboratory score 23-1

Laboratory code number	Score
23	8-8
22	8-8
21	8-8
20	8-8
19	8-8
18	8-8
17	8-8
16	8-8
15	8-8
14	8-8
13	8-8
12	8-8
11	8-8
10	8-8
9	8-8
8	8-8
7	4-8
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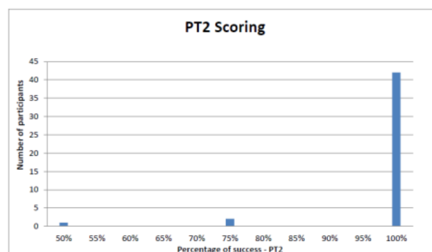
4	8-8
3	8-8
2	8-8
1	8-8

Laboratory scoring; PT2



Any comments/questions to PT2?

Feedback

Name of the National Reference Laboratory:			
Work area		Specific points to be adressed	Reply
Concerning the ampoules that you received:	1	Were they received safely and under proper conditions?	
	2	Were there enough time to perform the test?	
	3	Were instructions clear?	
	4	Were you able to use daily diagnostic procedures to analyse the content?	
	5	Any other comments?	
Concerning results and report?	6	Was it convenient for you to use the spreadsheet for submission of results?	
	7	Was the report straightforward to understand?	
	8	Was it easy to assess how you performed compared to other participants?	
	9	Were you satisfied with SAV beeing included in PT2?	
Future PT	10	Would you be in favour to include other pathogens in the PT in the future?	
If you have any other comments please fill in below:	11	Comments	

Feedback PT2015

Feedback from 23 countries out of 46

Concerning the ampoules that you received

1	Were they received safely and under proper conditions?
2	Were there enough time to perform the test?
3	Were instructions clear?
4	Were you able to use daily diagnostic procedures to analyse the content?

Feedback PT2014

5. Other comments
We were surprised to find 3 different viruses (especially the PFRV-like virus) from the ampoule V.
Please provide PT1 ampoules I to V in dublicate to PCR and cell cultivation

Feedback

6	Was it convenient for you to use the spreadsheet for submission of results?
	Yes, except for some technical faults, it was ok to use the spreadsheet. However, I think it would benefical to organize the sheets for PT1 and PT2 in the same way, ie. include information on the assays and Ct-values also for PT1.
7	Was the report straightforward to understand?
8	Was it easy to assess how you performed compared to other participants?
	yes, concerning the final results, but we would additionally be interested in which qRT-PCR tests other labs use for VHSV and IHNV, and their Ct values.
9	Were you satisfied with SAV beeing included in PT2?
	We haven't had the time to develop this PCR diagnostic tool.

FUTURE PT

Feedback

10	Would you be in favour to include other pathogens in the PT in the future?
	Yes - R. salmoninarum, G. salaris
	Yes but not for next year !
	Yes, if we are provided with protocols for testing of them
	I think nodavirus should be included.
	Nodavirus
	Yes, if they are relevant and methods are developed.
	From our experience, Tetracapsuloides bryosalmonae might be of interest,
	not yet, possibly CEV in PT2 in future
	yes - with the option to opt in or out depending on the pathogen

11	Comments
	We had problems with our delivery service because they asked first for shipement to be payed in order to deliver it to the laboratoy. So we lost some time in the payment procedure.
	Only question about scoring. Is it better not to do some tests compared with making as much possible with coincidental mistake, i.e. less without mistake is better than more with?
	I would like to include VER/VNN virus

Proficiency test 2016

- Aim: To send out the test in end of September 2016
- PT1: For identification of VHSV, IHNV and EHNV and in addition SVC, differentiating from other viruses as IPNV, Rana-viruses etc.
- PT2: Identification of ISAV, KHV and SAV (with option to opt in and out)

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