National Veterinary Institute Technical University of Denmark DTU

European Union Reference Laboratory for Fish Diseases
National Veterinary Institute, Technical University of Denmark, Copenhagen

Inter-Laboratory Proficiency Test 2014

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

Niels Jørgen Olesen, Niccolò Vendramin and Anemone Ojala

DANAK
PT Reg. no.: 555

Content

- Proficiency test 1, PT1
- Proficiency test 2, PT2
- Feedback from participants
- Proficiency test 2014 and 2015

PT1 and PT2 was delivered to 41 laboratories

All NRL's for Fish Diseases in EU Member States

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

Figure 3. Worldwide distribution of the participants in the EURL proficiency test 2014.

Distribution of PT1 and PT2

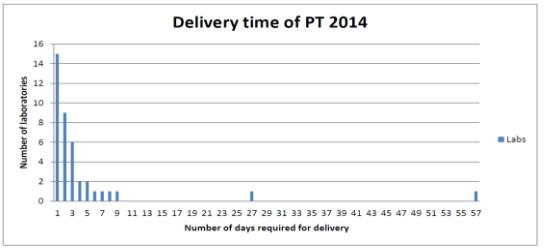


Figure 1. Transport time for the parcels to reach the participants.

Shipment and handling
Within three days, the tests were delivered to 30 participants; 8 more tests were delivered within 9 days; 1 further test was delivered within 27 days and the last within 57 days (Figure 1). All the parcels were sent without cooling elements. 6 countries outside EU had a logger in the parcel. One laboratory did not reported how long time the shipment last, however it is considered to be within 3 days from the shipment.

PT1: Content of ampoules

Five ampoules containing virus/ lyophilised tissue culture supernatant

Code	Isolate
Ampoule I:	IPNV strain Ab
Ampoule II:	SVCV 56/70
Ampoule III:	EHNV 86/8774
Ampoule IV:	IHNV 32/87
Ampoule V:	VHSV DK-6137 Hjarnø

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

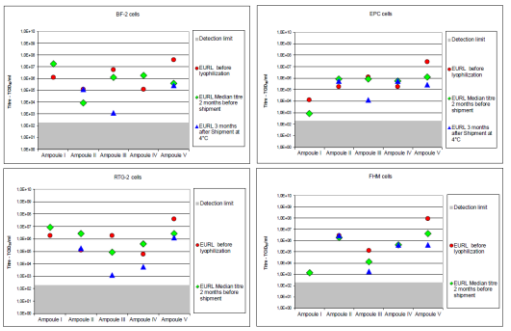
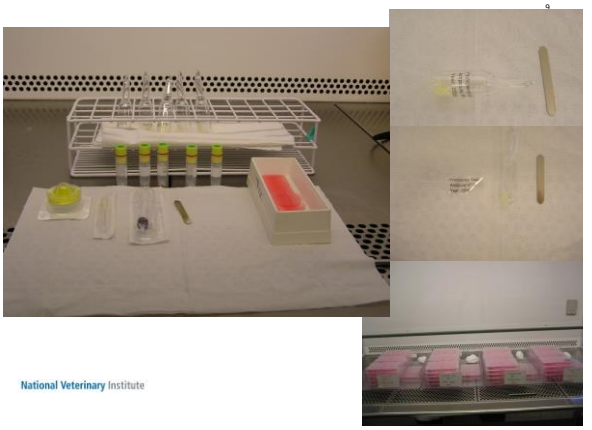


Figure 4. Virus titers in different cell lines before freeze drying, 2 months before- and 3 months after shipment.

PT1

- Participants were asked to examine the content of each ampoule virologically according to the procedures described in the Commission Decision 183/2001/EC:
  - Titration on preferred cell line followed by:
  - Neutralisation test
  - ELISA
  - IFAT
  - RT-PCR
  - PCR + sequence analyses
- But also to follow normal laboratory procedures



Virus identification participating laboratories

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

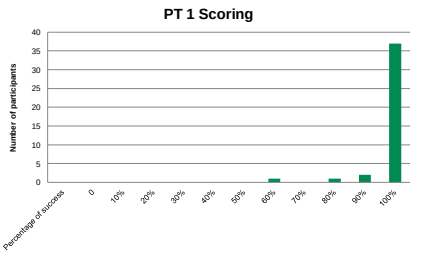
41 of 41 laboratories replied
35 participants out of 41 were able to identify all the pathogens they were supposed to

Virus identification participating laboratories

Laboratory code number	Score
23	10/10
24	10/10
25	10/10
26	10/10
27	10/10
28	10/10
29	10/10
30	9/10
31	10/10
32	10/10
33	10/10
34	10/10
35	10/10
36	10/10
37	8/10
38	10/10
39	10/10
40	10/10
41	10/10

41 of 41 laboratories replied
35 participants out of 41 were able to identify all the pathogens they were supposed to

Laboratory scoring, PT1



Genotyping and sequencing; PT1

13

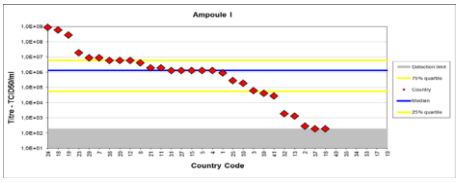
- The sequences were in general of high quality and usable for genotyping
- It is however important that all laboratories use their sequencing results to discriminate EHNV from the rest of the much related types of ranaviruses
- Furthermore, it is important that the remaining laboratories implement PCR and sequencing techniques in the laboratory as genotyping is the basis for differentiating several listed viruses from others

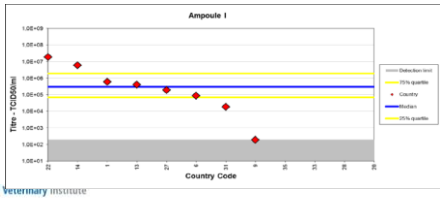
Conclusions and remarks; PT1
Stability of ampoule 1

14

Freeze drying process not fully effective.
Ampoules with clear signs were discarded
This might have affected the stability of the ampoule batch and the range of titre values retrieve from participants.

15





16

Any comments/questions to PT1?

PT-2 Content of ampoules

17

Four ampoules containing pathogens / lyophilised tissue culture supernatant

Code	Isolate
Ampoule VI:	KHV-TP 30 Diluted 1:3
Ampoule VII:	Blank
Ampoule VIII:	KHV-TP 30 Undiluted
Ampoule IX:	ISAV FO/01/01/HPR13

PT2 Testing the test

18

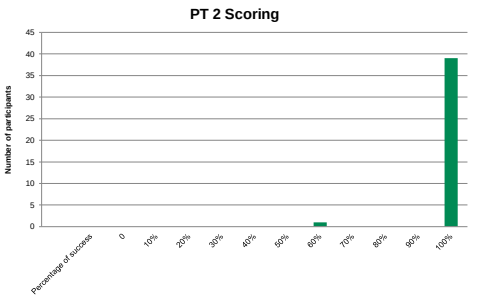
- Prior to sending out the test, the EURL tested 5 ampoules of each virus preparation by PCR (Bercovier et al. (2005)) and real-time PCR (Gilad et al. (2004)) for KHV and by RT-PCR (Mjaaland et al. (1997)) and real-time RT-PCR (Snow et al. (2006)) for ISAV, to ascertain identity, a satisfactory titre of the virus and homogeneity of the content in the ampoules
- Furthermore, conventional PCR/RT-PCR fragments were sequenced and so was the HPR region of the ISAV isolate

PT2 Virus identification participating laboratories

Laboratory code number	Score (Maximum 8/8)
1	8
2	8
3	8
4	
5	8
6	8
7	8
8	8
9	8
10	8
11	8
12	8
13	8
14	8
15	8
16	8
17	4/4
18	8
20	8
20	8
21	8
22	8
23	8
22	8
25	8
26	8
26	8
25	8
29	8
30	5
29	8
28	8
33	8
34	8
32	8
31	8
37	8
38	8
39	8
40	8
41	8

40 out of 41 laboratory delivered results
39 laboratories gained full score

Laboratory scoring; PT2



Conclusions and remarks

- Very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these 4 years

Any comments/questions to PT2?

Feedback

Work area		Inputs
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 understand how you performed compared to other participants?
If you have any other comments please fill in below	9	Comments

Feedback PT2014

Feedback from 14 countries out of 41

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 PT2013

5. Other comments
As noted in the reply on the proficiency test we detected very low amounts of virus in ampoule 1 - this gave rise to some concern regarding the possibility of this finding to be due to contamination or if it was the expected finding.
It may be more practical to send lyophilized virus in plastic vials rather than glass vials (difficult to open and possible source of cross-contamination)

Feedback

Concerning results and report

6. Was it convenient for you to use the spreadsheet for submission of results?
Ok, but a little tricky

7. Was the report straightforward to understand?

8. Was it easy to understand how you performed compared to other participants?

FUTURE PT

Feedback

9. Would you be in favour to include SAV in PT2 in 2015 ?
Due to lack of personnel i am a little bit hesitant
no
yes
Yes
Yes
no
yes, SAV has been listed in OIE manual, So it is necessary to assess the detected ability.
Maybe include SAV in an independent PT3?
Yes
YES, very much :o)
Yes
no
Yes
yes

Feedback

If you have any other comments

10. Other comments
I would very much like that PT2 was a bit more challenging meaning that the virus content in some of the samples was lower or that we were encouaged to do a 10 fold dilution of the samples before RNA extraction to test and compare the sensitivity of our PCR assays similar to the TCID50 experiment.
1) We found 2 isolates of KHV with two different genoytpes (ampoule VI : Japanese lineage / Ampoule VIII: USA/Israël lineage) while according to the EURL, only one genotype was put in these 2 ampoules. Did we make an error ? 2) We have developed a PCR which distinguishes EHNV from ECV by the size of the product, without the need for sequencing. It's now the third year that we performed it, in parallele to the traditional method (MCP amplification + sequencing), with excellent result. We have planned next year to use only this new method with the indication "PCR+ for EHNV". Is this possible? Will we be assessed in the same way? 3) We have observed a series of mismatches (at least 7) between the ISAV primers recommended by the OIE and some american strains published in Genbank . We would appreciate your comments on this fact at the next EURL meeting in 2015.
We are keen to have proficiency tests for diseases for which we have additional guarantees which apart from SVC also include BKD and *G. salaris* (for us molecular testing would be fine).
We find it difficult to open the ampoule using the knife in parcel. Could you offer other tool or method with detailed procedures?

Proficiency test 2015

- Aim: To send out the test in end of September 2014
- 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 (?)

Acknowledgements

- Anemone Ojala
- Troels Secher Rundqvist
- Susie Sommer Mikkelsen
- Betina Lynnerup

- ISAV: OIE reference laboratory, Oslo, Norway, Birgit Dannevig

- KHV: Institute of Medical Biotechnology, Central Taiwan University of Science and Technology, Dr. Peiyu Lee and Friedrich-Loeffler-Institut (FLI), Sven M. Bergmann