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 2013

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

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DANAK

Content

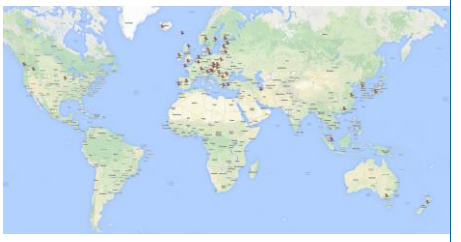
- Proficiency test 1, PT1
- Proficiency test 2, PT2
- Feedback from participants
- Proficiency test 2013 and 2014

The accreditation situation in 2013	
How many NRL's have regional laboratories in their country/region.	17/43
Have many NRL's organize inter-laboratory proficiency test annual for the regional laboratories their countries?	15 / 17

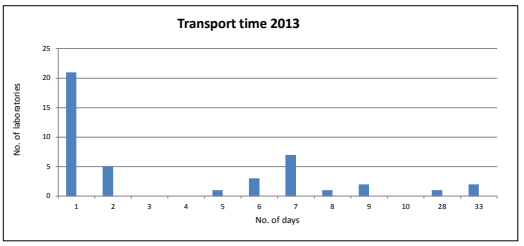
PT1 and PT2 was delivered to 43 laboratories

All NRL's for Fish Diseases in EU Member States

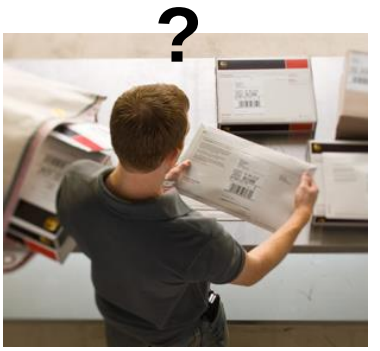
NRL's in:
Australia
Canada
Croatia
Faroe Islands
Iceland
Iran
Japan
New Zealand
Norway
P.R. China (2)
Republic of Korea *new*
Serbia
Switzerland
Singapore *new*
Turkey
USA (2)



Distribution of PT1 and PT2



Within two days, the tests were delivered to 26 participants, 18 participants in EU and 8 participants outside EU; due to a mistake in the delivery process by the shipping company 6 laboratories received their own parcels after 7 – 9 days. 2 laboratories (outside EU) received the test after 33 days; 1 participant collected the test directly from Denmark, finally one participant received an extra test because the first package was damaged during transport.



PT1: Content of ampoules

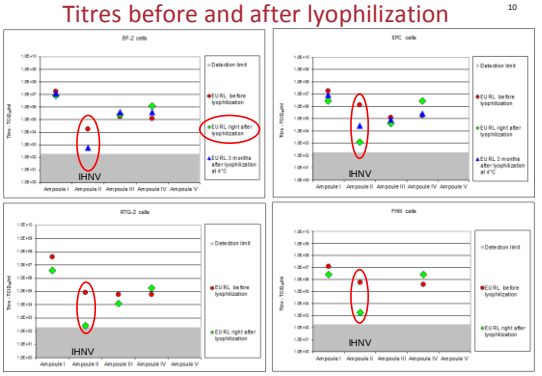
Five ampoules containing virus/ lyophilised tissue culture supernatant

Ampoule	Virus	Isolate	Species
I	IPNV + VHSV	Type strain Sp (Spjarup) DK-6137 (Hjarnø)	Rainbow trout Rainbow trout
II	IHNV	IHN virus 217/A	Rainbow trout
III	EHNV	Isolate 86/8774	Rainbow trout
IV	SVCV	SVCV strain 56/70 most likely identical to the S/30 isolate	Carp
V	No Virus	Sterile medium	

Testing PT1

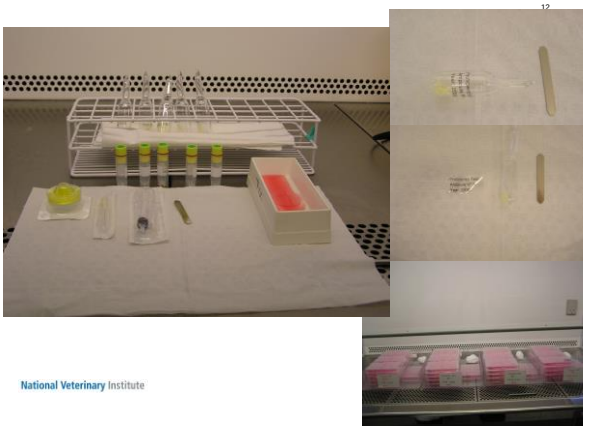
- The proficiency test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17043
- The titre and homogeneity of the samples was tested prior to sending out the test by *titration of 5 ampoules of each virus preparation in 4 cell lines.*
- The identity of the virus in the 5 ampoules 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



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

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Ampoule	Virus	Correct ID	Partly correct	Wrong ID	No ID
I	IPNV strain Sp + VHSV DK-6137 (Hjarnø)	40	3		
II	IHNV	43			
III	EHNV	39		2	2
IV	SVCV	42			1
V	EMEM	37		4	2

43 of 43 laboratories replied
34 participants out of 43 were able to identify all the pathogens they were supposed to

Virus identification participating laboratories

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Ampoule	Virus	Correct ID	Partly correct	Wrong ID	No ID
I	IPNV strain Sp + VHSV DK-6137 (Hjarnø)	40	3		
II	IHNV	43			
III	EHNV	39		2	2
IV	SVCV	42			1
V	EMEM	37		4	2

2 did not identify IPN
1 did not identify VHS

Sequences retrieved uncorrected

Virus isolated but not identified

Virus identification participating laboratories

15

Ampoule	Virus	Correct ID	Partly correct	Wrong ID	No ID
I	IPNV strain Sp + VHSV DK-6137 (Hjarnø)	40	3		
II	IHNV	43			
III	EHNV	39		2	2
IV	SVCV	42			1
V	EMEM	37		4	2

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Isolated and identified
Different viruses

Virus isolated but not identified

Laboratory scoring, PT1

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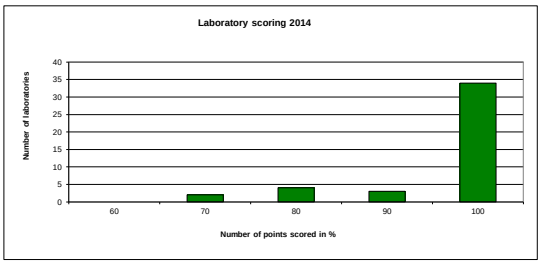

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Genotyping and sequencing; PT1

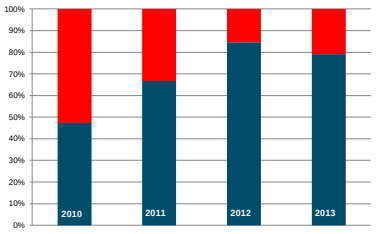
- The sequences were in general of high quality and usable for genotyping
- It is however important that all laboratories use their sequencing results to discriminate EHNV from the rest of the much related types of ranaviruses
- Furthermore, it is important that the remaining laboratories implement PCR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

Conclusions and remarks; PT1

- 34 of 43 got a success rate of 100%.
- 42/43 laboratories correctly isolated and identified VHSV
- 43/43 laboratories correctly isolated and identified IHNV
- 6/43 laboratories detected virus in Ampoule V that only contain MEM

- We encourage participants to evaluate the sensitivity of their cell lines
- In this and future proficiency tests a comment was/will be provided in the report to participants if this seemed relevant.
- We would like to encourage all NRLs to contact us on any question concerning the proficiency test as well as on any other questions in relation to diagnosis of listed fish diseases.

Success-rate of participating laboratories 2010 - 2013



Blue: % of labs with maximum score
Red: % of labs underperforming

Any comments/questions to PT1?

PT-2 Content of ampoules

Five ampoules containing pathogens / lyophilised tissue culture supernatant

Ampoule	Virus
VI	Ampoule VI: ISAV Glesvaer/2/90
VII	Ampoule VII: KHV-TP 30
VIII	Ampoule VIII: KHV 07/108b
IX	Sterile pyrogen free water
X	*Aphanomyces invadans* NJM9701

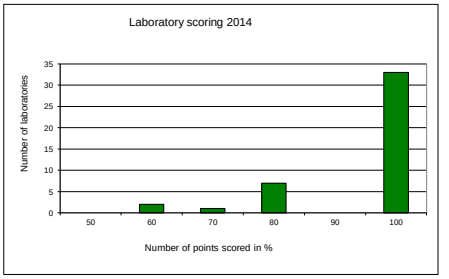
PT2 Testing the test

- 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

Ampoule	Virus	Correct ID	Wrong ID	Did not examine
VI	ISAV	40	1	2
VII	KHV (CyHV-3)	41	1	1
VIII	KHV (CyHV-3)	42		1
IX	Sterile pyrogen free water	40	3	
X	*Aphanomyces invadans*	32	4	7

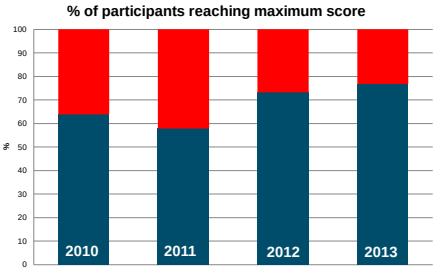
Laboratory scoring; PT2



Conclusions and remarks

- Very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these 4 years
- In autumn 2012 the European Commission decided to de-list EUS and it is officially no more considered as an exotic disease in the Union. It was agreed, however, to include it in PT2 for 2013, this will not be done for 2014

Success-rate of participating laboratories 2010 - 2013



Blue: % of labs with maximum score
Red: % of labs underperforming

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 PT2013

Feedback from 25 countries out of 43

Concerning the ampoules that you received

1. Were they received safely and under proper conditions?
Temperature out of range according to temperature logger.

2. Were there enough time to perform the test?
Timeframe was suitable for completion of tests, however other laboratory diagnostics were ceased during this time to ensure that the deadline was met. A longer timeframe would be less disruptive to ongoing laboratory activities.

3. Were instructions clear?
yes, instructions were clear for both PT-1 and PT-2.

4. Were you able to use daily diagnostic procedures to analyse the content ?
PT-1: Our lab does not routinely perform TCID 50 to determine virus concentration but rather uses plaque assays. Consequently, the TCID50 was done specifically for the inter-laboratory proficiency test.
yes (but further methods had to be included e.g. PCR methods which are not regularly used in our lab).

Feedback PT2013

5. Other comments
Occasionally the ampules do not break cleanly and could present a risk of cross-contamination. This is likely due to our lack of familiarity with handling such vials.
We are currently reanalyzing a new EUS ampoule.
It would be useful to receive more material for PT1 (2 ml) in order to perform duplicate tests for the qualification of the laboratory technicians for Quality requirements (UNI CEI EN ISO/IEC 17025)

Feedback

Concerning results and report

6. Was it convenient for you to use the spreadsheet for submission of results?
PT-2: Molecular - yes spreadsheet was fairly straightforward. Only minor suggestion would be to grey out fields that are not applicable i.e. RT-PCR and real-time RT-PCR for DNA pathogens. PT-1: use of spreadsheet was convenient.
Quite difficult to use the spreadsheet for submission of results
Yes, very convenient
It is rather complex and needs good checking, but it works. I cannot give alternatives which would work better, so, this is just an opinion. Thanks!

7. Was the report straightforward to understand?
Yes, complete and clear

8. Was it easy to understand how you performed compared to other participants?
Yes, but not so easy.

Feedback

If you have any other comments

9. Other comments
Additional analysis of all viral sequences would be useful
Thank you for including our laboratory as one of the participants of the inter-laboratory proficiency test.
In the EURL proficiency test we test for ISAV by PCR and sequencing only. In the OIE manual an ISAV-HPRdel detection by PCR and confirmation by sequencing without clinical signs is not adequate for a defintion of a suspect case of ISA (section 7.1). My question is why do we then test ISAV by PCR and sequencing only in the proficiency tests when we need some alternative methods to confirm a suspect ISAV case. To comply with the OIE manual I suggest that we at the next proficiency test do cell culture and PCR or other alternative methods as outlined in the manual.
*My only concern is that for this year the European Reference Laboratory for Crustacean diseases is planning to ran the proficiency test around October and the Annual Meeting during November . Since there are Labs with both duties it would be thoughtful to have some extra days.
Last year there was a brief discussion about a possible PT3 of non-listed diseases/national measures e.g. BKD, G. salaris etc. There was no conclusion to that and it would be good to discuss again at the next meeting or ask labs for a list of diseases they are interested in and perhaps different NRL could set up their own schemes together.
I really appreciated the sequence comparison. It would be nice to have it every year.

Feedback

If you have any other comments

9. Other comments
As you know, I received the point (19/20point) in your PT test. In some other PT test (such as VETQAS, KHV), the attented people were received a pass (correct) or fail (incorrect) score. What is meant the our score (19/20point)? The comparison of the EHN-viruses was very helpful.
no comments or suggestions. Thank you again!
In the report a paper by Johansson et al was indicated for the sequencing of IHNV while in the instructions Kurath et al is given as reference. We still have some problems with the sequencing of IHNV and get regularly incorrect results. For our sequencing we used two different methods one by Enzmann and one by Emmenegger but unfortunately in both cases we got other results than those provided in the report.
In the case of ISAV in ampoule VI would there also be other accession numbers indicating on the same isolate be o.k..

Proficiency test 2014

- 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

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