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

DTU

Inter-Laboratory Proficiency Test 2012

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

European Union Reference Laboratory for Fish Diseases

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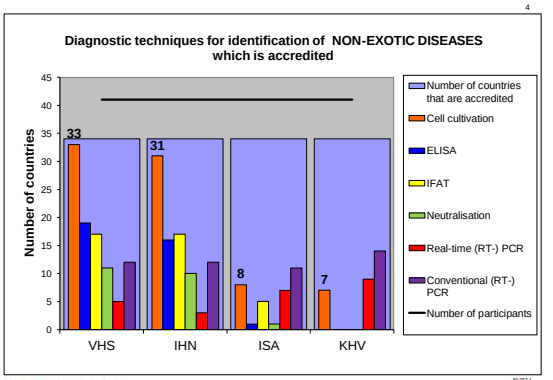
DANAK
PT Reg. no.: 555

Content

- Accreditation status in the NRL's
- Proficiency test 1, PT1
- Proficiency test 2, PT2
- Feedback from participants
- Proficiency test 2013 and 2014

Accreditation status in participating NRLs and regional laboratories

- Questionnaire send with Proficiency test:
- The Accreditation Situation in your Laboratory:
- Which diagnostic techniques for identification of EXOTIC DISEASES are accredited in your lab?
- Which diagnostic techniques for identification of NON-EXOTIC DISEASES are accredited in your lab?



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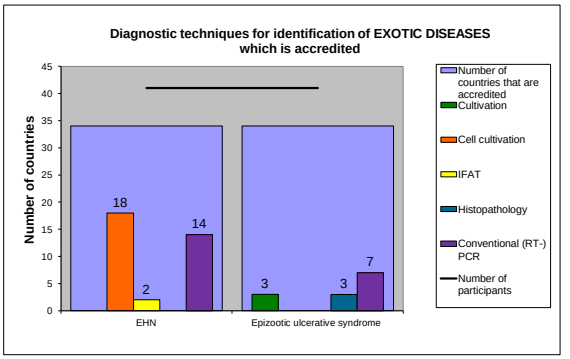

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How many NRL's have regional laboratories in their country/region.	16 / 41
Have many NRL's organize inter-laboratory proficiency test annual for the regional laboratories their countries?	15 / 16

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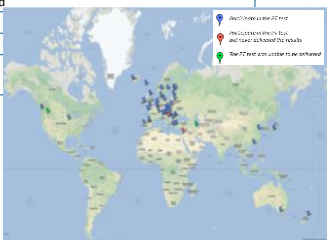
PT1 and PT2 was delivered to 41 laboratories		
All NRL's for Fish Diseases in EU Member States		
NRL's in:	Australia	Bosnia and Herzegovina
	Canada	Croatia
	Faroe Islands	Iceland
	Iran	Israel
	Japan	New Zealand
	Norway	P.R China
	Serbia	Switzerland
	Turkey	USA (1) USA (2)



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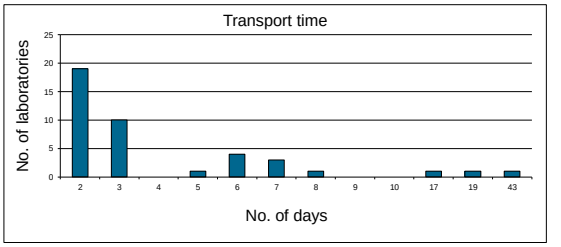
Distribution of PT1 and PT2



29 parcels were delivered by the shipping companies within 3 days after submission.

PT1: Content of ampoules

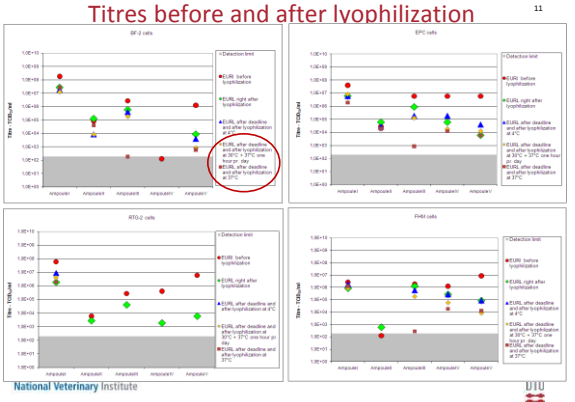
Five ampoules containing virus/ lyophilised tissue culture supernatant

Ampoule	Virus	Isolate	Species
I	IPNV	Type strain Sp (Spjarup)	Rainbow trout
II	EHNV	Isolate 86/8774	Rainbow trout
III	SVCV	SVCV strain 56/70 most likely identical to the S/30 isolate	Carp
IV	IHNV	IHN virus 217/A	Rainbow trout
V	VHSV	DK-6137 (Hjarnø)	Rainbow trout

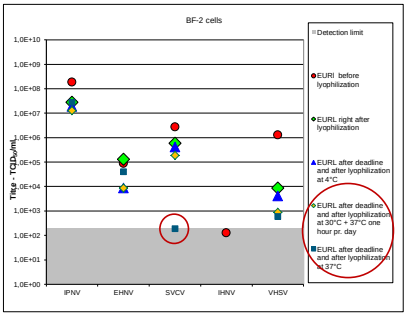
Testing the test, PT1

- 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 lyophilization procedures did cause a decrease in marine VHS virus titre and some for IHN and IPN.

Titres before and after lyophilization



Titres before and after lyophilization



The test, PT1

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- 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

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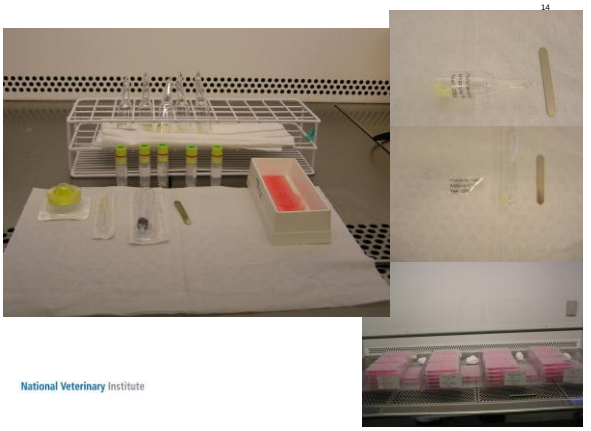

Virus identification participating laboratories

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Ampoule	Virus	Correct ID	Partly correct	Wrong ID	No ID
I	IPNV	36		2	1
II	EHNV	36	2		1
III	SVCV	38			1
IV	IHNV	39			
V	VHSV	39			

39 of 41 laboratories replied
36 participants out of 39 answered contend in all ampoules correctly

Virus identification participating laboratories

16

Ampoule	Virus	Correct ID	Partly correct	Wrong ID	No ID
I	IPNV	36	1	2	
II	EHNV	36	2		1
III	SVCV	38			1
IV	IHNV	39			
V	VHSV	39			

Dont analyse for the presence of IPNV

Identified ranavirus, but did not employ genomic analysis

Laboratory scoring, PT1

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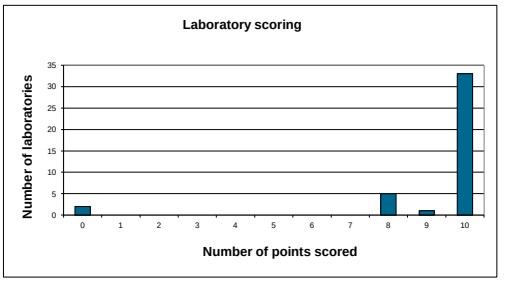

Cell lines used for titration

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Cell line	2012	2011	2010	2009	2008	2007
BF-2	34	34	32	29	29	29
EPC	37	35	34	34	33	33
RTG-2	15	13	14	12	12	10
FHM	12	15	16	10	9	9

- 6 laboratory used CHSE cells
- 7 laboratories used all four cell lines
- 6 laboratories used tree cell lines
- 26 laboratories used two cell lines

Virus titre; BF-2 cells

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Remarks:
What about outliers?

Virus titre; EPC cells

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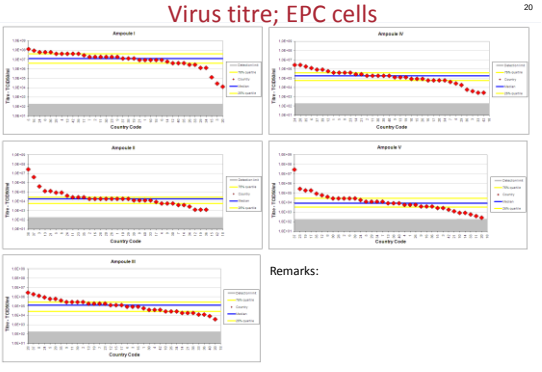
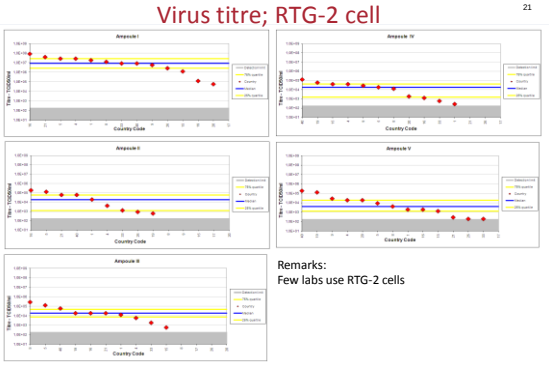


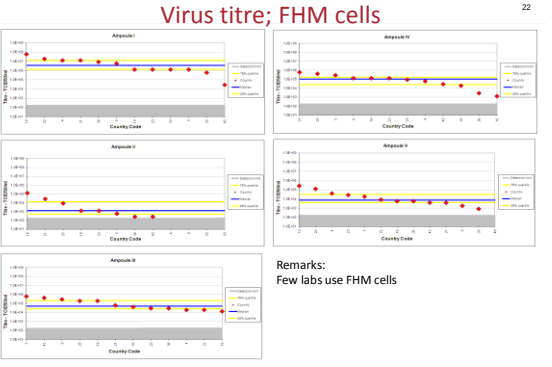
Remarks:

Virus titre; RTG-2 cell



Remarks:
Few labs use RTG-2 cells

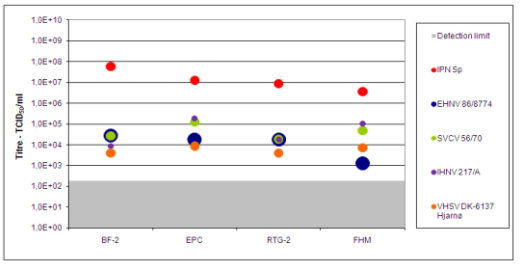
Virus titre; FHM cells



Remarks:
Few labs use FHM cells

Virus identification and titration

Median titre of viruses obtained by participants at different cell lines



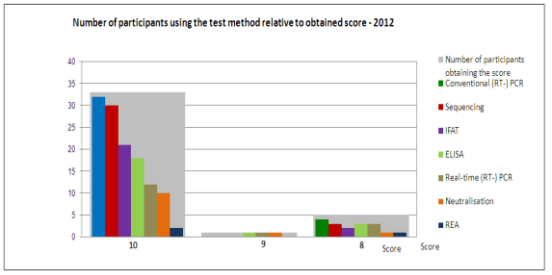
There there is a tendency that FHM cells are less efficient replication of IPNV and EHNV and that BF-2 and RTG-2 cells are less replication of IHNV. SVCV and VHSV grow on all cell lines.

Test method used by participants

Cell line	2012	2011	2010	2009	2008	2007
ELISA	22	22	25	24	23	22
IFAT	23	24	23	21	22	25
Neutralisation	12	11	8	11	11	9
PCR		**39**	35	34	29	20
Conventional (RT-) PCR	36					
Real-time RT-) PCR	16					
Sequencing	33	33	30	30	15	16

- 3 laboratory performed REA according to the OIE Aquatic Animals Manuals for identification of ranaviruses

Test method used by participants relative to obtained score



Genotyping and sequencing

Ampoule I = IPNV Sp

- 14 laboratories submitted sequences
- 9 laboratories genotyped the IPNV isolate as belonging to genogroup 5 (Sp)
- 1 laboratory genotyped the IPNV isolate as belonging to genogroup 3
- 4 laboratories did not provide a genogroup despite having sequenced the isolate
- All the laboratories used different references.

Genotyping and sequencing

Ampoule II - EHNV

- 33 laboratories performed sequencing to identify the virus in ampoule II with correct result
- 2 laboratories performed REA according to the OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.1 with correct result
- 1 laboratory performed restriction enzyme fragmentation
- 10 laboratories used primers described in Hyatt et *al*. (2000)
- 13 laboratories used primers described in the OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.1
- 3 laboratories used primers described in Holopainen et *al* (2009)
- 9 laboratories were using primers described in other references or they did not report the reference

Genotyping and sequencing

Ampoule III – SVCV

- 15 laboratories performed sequencing
- 7 laboratories identified the isolate as being genotype Id
- 8 laboratories did not give any genotype of the sequences
- 5 laboratories used primers described in the OIE Manual of Diagnostic Tests for Aquatic Animals. Chapter 2.3.8
- 5 laboratories used primers described in Stone et *al*. (2003)
- 5 laboratories were using primers described in other references or they did not report the reference

Genotyping and sequencing

29

Ampoule IV – IHNV

- 23 laboratories performed sequencing to identify the virus in ampoule IV with correct result
- 17 laboratories genotyped the IHNV isolate as belonging to genogroup M
- 1 laboratory genotyped the IHNV isolate as belonging to genogroup M or U
- 1 laboratory genotyped the IHNV isolate as belonging to genogroup U
- 1 laboratory genotyped the IHNV isolate as belonging to genogroup L
- 3 laboratories did not give any genotype of the sequences
- 7 laboratories used primers described in the OIE Manual of Diagnostic Tests for Aquatic Animals, chapter 2.3.4
- 4 laboratories used primers described in Emmenegger et *al*., 2000
- 11 laboratories were using primers described in other references or they did not report the reference

Genotyping and sequencing

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Ampoule V – VHSV genotype Ia

- 22 laboratories performed sequencing to identify the virus in ampoule V with correct result
- 19 laboratories identified the VHSV isolate as genotype Ia
- 2 laboratories identified the VHSV isolate but didn't write the genotype
- 1 laboratory identified the VHSV isolate as genotype I
- 17 laboratories did not genotype the VHSV
- 4 laboratories used the primers described in Einer-Jensen et *al*. (2004)
- 3 laboratories used the primers described in Snow et *al*. (2004)
- 3 laboratories used used primers described in the OIE Manual of Diagnostic Tests for Aquatic Animals, chapter 2.3.9
- 11 laboratories used primers described in other references or did not report the reference

Genotyping and sequencing; PT1

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Conclusion:

- The increasing number of laboratories that performed sequencing is very positive
- The sequences were 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

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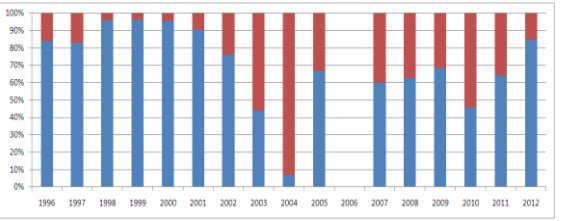
- 36 of 39 participants identified all viruses correctly.
- VHS and IHNV diagnosis is perfect with 39/39 correct answers!!
- All Laboratories should implement PCR diagnostic assays

Conclusions and remarks; PT1

- 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 1996 - 2012



Blue: Number of labs with score 10
Red: Number of labs with score <10

Any comments/questions to PT1?

PT-2 Content of ampoules

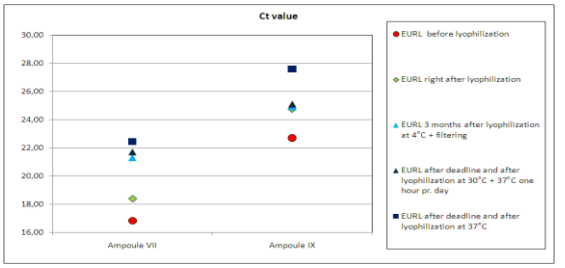
Five ampoules containing pathogens / lyophilised tissue culture supernatant

Ampoule	Virus	Isolate	Species
VI	*Aphanomyces invaans*	NJM901	
VII	KHV (CyHV-3)	KHV-TP 30	Carp
VIII	Sterile pyrogen free water		
IX	ISAV	ISAV Glesvaer 2/90	Atl. salmon

PT2 Testing the test

- Prior to sending out the test, the EURL tested 4 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

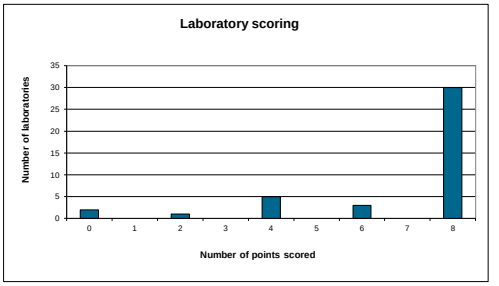
PT2 Ct value before and after lyophilization



PT2 Virus identification participating laboratories

Ampoule	Virus	Correct ID	Wrong ID	Did not examine
VI	*Aphanomyces invaans*	34		5
VII	KHV (CyHV-3)	36	2	1
VIII	Sterile pyrogen free water	32	1	
IX	ISAV	36	2	1

Laboratory scoring; PT2



Genotyping and sequencing

- 1 laboratory found the Ct value for *A. Invadans*
- 12 laboratories performed sequencing for *A. Invadans*

- 16 laboratories found the Ct value for KHV
- 16 laboratories performed sequencing for KHV

- 13 laboratories found the Ct value for ISAV
- 25 laboratories performed sequencing for ISAV

Test method used by participants

Methods used	2012	2011	2010
ISAV Real-time RT-PCR	16	15	12
ISAV RT-PCR	30	28	24
ISAV Real-time RT-PCR and ISAV T-PCR	9	8	4
KHV Real-time RT-PCR	17	13	14
KHV PCR	29	34	30
KHV Real-time PCR and KHV PCR	11	10	11
A. invandans PCR	30	31	-
A. invandans Real-time PCR	2		

Conclusions and remarks

- Considering that this was the first time that the EURL provided a proficiency test on *A. invadans* isolate identification, we find that most participants obtained satisfying results. Out of 34 laboratories testing for *A. invadans* all 34 identified the pathogen in ampoule VI.

- Out of 38 laboratories performing KHV identification, 36 laboratories identified KHV in ampoule VII.

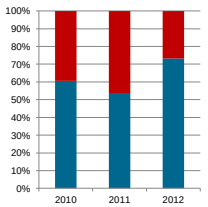
- Out of 38 laboratories performing ISAV identification 36 identified ISAV in ampoule IX.

Conclusions and remarks

- Very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these 3 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.

Success-rate of participating laboratories 2010 - 2012



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

Feedback from 20 countries out 39, concerning the ampoules that you received

Were there enough time to perform the test?
Because of the delay of customs clearance,It left us not much time to perform the test. Anyhow we provided the test results before the deadline.
No - not to carry out genotyping aswell

Were you able to use daily diagnostic procedures to analyse the content ?
Yes, only titration is normally done slightly different then we did now for the proficiency test (we normally get rid of the volume added virus before adding extra medium in the 96 well plate)
No (not normally diagnostics for ISA, EUS and EHN)
Some tests we had to bring on board for this proficiency, others were in our procedures

Other comments
I think the ampoules is a bit hard to handle. Sometimes it is difficult to breake them.

Feedback

Concerning results and report

There were no comments

Feedback

If you have any other comments

Other comments
Excellent organization
Thank you again for all the work!
Thank you very much for preparation,post of samples ,communication and solution of delay during the customs clearance.as for the delay,we talked to the Beijing AQSIQ headquaters and DTU VET,and we try to improve this situation this year.
Easy to read and understand the report. Good overview on results
This was our first time with this proficiency programme and we learnt a lot from it and can only improve for the next year! Would be good to have a bit more time to carry out all the tests and sequence analysis.

Proficiency test 2013

- Aim: To send out the test in September 2013
- 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 *A. invadans*

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