



European Union Reference Laboratory for Fish Diseases

National Institute of Aquatic Resources, Technical University of Denmark



EURL-Fish work done in 2017



European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Copenhagen



Niels Jørgen Olesen

**Technical Report
2017**

from the
**European Union Reference Laboratory for
Fish Diseases**

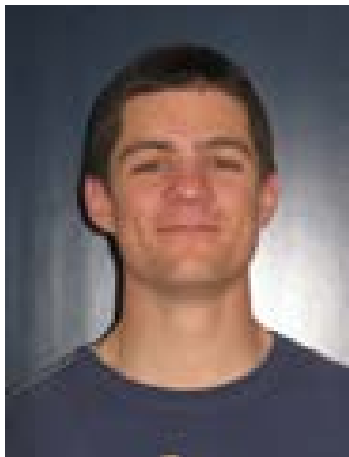


National Veterinary Institute
Technical University of Denmark
Kgs. Lyngby, Denmark
Content





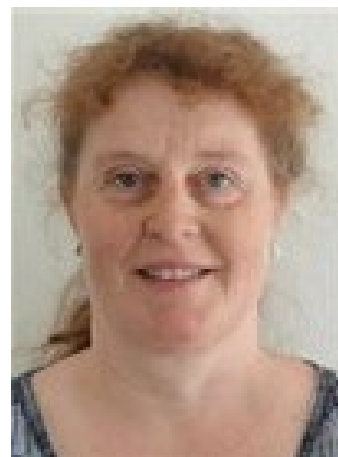
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Niccolò
Vendramin



Argelia Cuenca
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Tine Iburg



Lone Madsen



Jacob G., Schmidt



Nikolaj G. Andersen



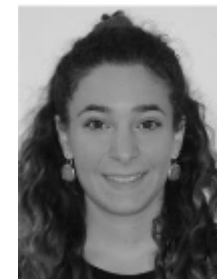
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EURL-Fish work program 2017

5 main objectives

- **1. Coordination and training**
- **2. Proficiency test**
- **3. Reagents and products**
- **4. Scientific advice and activities**
- **5. Missions**

20 subgoals

1-1,1-2 Organise and prepare for the 21st Annual Workshop for the NRLs and produce a report from the Annual Meeting



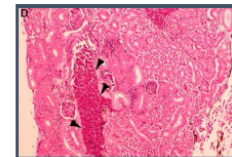
European Union Reference Laboratory for Fish Diseases
National Veterinary Institute, Technical University of Denmark

Report

21st Annual Workshop of the National Reference
Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

May 30th – 31st 2017



Tilapia disease in commercial hybrid
tilapia (Eyngor et al., 2014)



Red Mark Syndrome (RMS) in rainbow
trout

Organised by the European Union Reference Laboratory for Fish Diseases,
National Veterinary Institute, Technical University of Denmark, Kgs. Lyngby

1-4 Facilitate and provide training in laboratory diagnosis:

EURL training courses 2017

EURL course 1: “Methods for implementation of surveillance procedures for listed fish diseases” took place from 9th to 13th October 2017

EURL course 2: “Introduction to histopathology in fish diseases” and took place in Copenhagen 16th to 19th October 2017



2-1, 2-2 Prepare and report the Annual Inter-laboratory Proficiency Test.

PT1-2014 for identification of: **VHSV, IHNV, EHNV, SVCV and IPNV**

and PT2 identification of: **CyHV-3 (KHV), SAV and ISAV**



European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Copenhagen

EURL for Fish Diseases



Report of the Inter-Laboratory Proficiency Test 2017

for identification and titration of

VHSV, IHNV, EHNV, SVCV and IPNV (PT1)

and identification of

CyHV-3 (KHV), SAV and ISAV (PT2)

Organised by the

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

PT Rep. no.: 616



3-2 Production of antisera against selected isolates when necessary

In 2017 no new productions of antisera were needed and our stocks of supernatants from hybridoma cells producing monoclonal antibodies were sufficient for the year

3-3 Update and maintain a library of isolates of ISAV, VHSV and IHNV, KHV and EHN

- Several isolates of the listed viruses VHSV, IHNV and KHV were received and stored in our library during 2017.

Technical Report 2017 from the EURL for Fish Diseases

Annex 2 Reagents received in 2017

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.	Remarks
Norway	Stefanis	Norwegian Veterinary Institute Veterinærinstituttet Hovland	04.01.2017	Organs	32 vials	17-477	<i>Suspected flavivirus: Diagnostic</i>
Switzerland	Thomas Wahl	University of Bern Centre for Fish and Wildlife Health	13.01.2017	Supermaster Purified RNA	3 tubes	17-1383	VHS + IHV qPCR
Sweden	Charlotte Asan	National Veterinary Institute Section of fish	18.01.2017	Supermaster	1	17-2629	IPN strain for PCR and sequencing
Sweden	Charlotte Asan	National Veterinary Institute Section of fish	18.01.2017	Fish tissue Purified RNA	120 tubes 4 plates	17-4611	Screening for PRV
Norway	Anna B Mikalson	NMBU	19.01.2017	Tissue cDNA	2 vials 1 vial	17-1636	Calicivirus control
Faroe Islands	Kristin Baldvadsson	Food and Veterinary Agency Department of Fish and Animal Diseases	27.01.2017	Cell supernatant	6 tubes	17-2000	RANA viruses
Slovenia	Diana Zala	University of Ljubljana, Veterinary Faculty	27.01.2017	Pools of kidneys	30 pools	17-2001	BKD - ELISA
Italy	Anna Toffini	Istituto Zooprofilattico Sperimentale della Venezia	01.02.2017	E-11 cells	2 flasks	-	To have in stock
Romania	Mihails Costea	Institute for Diagnostic and Animal Health NRI for fish diseases	23.02.2017	Cell supernatant with Virus	4 vials	17-3602	Confirmation of VHSV
Belgium	François Liefbig	CERGroups	08.03.2017	Blood from Atlantic salmon and sea trout	2x 2 2x 2	17-4258	Screening for PRV1a and PRV1b (Virus-X)
France	Lanette Louboutin	ANSES	03.04.2017	SBL cells	1 small flask	-	To have in stock
Serbia	Vladimir Ivan Radovanovic	Institute of Veterinary Medicine of Serbia, Department of Fish Diseases	07.04.2017	Cell supernatant	4 tubes	17-1783	Viral growth on EPC (for full genome sequences in US)

Technical Report 2017 from the EURL for Fish Diseases

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.	Remarks
France	Patrick MARTIN	Conservatoire national des semences Lamm, Chateaugay	19.04.2017	Fish tissue in EMEM	30 tubes	17-6259	PRV P1D - Project (NIVEN)
Ireland	Niall Maria Renna	Marine Institute Fish Health Unit	19.04.2017		122 samples	17-6222	Survey of PRV in wild fish
Norway	Torfinn Moldal	Norwegian Veterinary Institute Virology	27.04.2017	Fish tissue in RNAlater + Homogenised tissue	2 tubes + 2 tubes	17-6661	Detection of Flavivirus
Serbia	Vladimir Ivan Radovanovic	Institute of Veterinary Medicine of Serbia, Department of Fish Diseases	30.05.2017	Cell supernatant Purified RNA/cDNA	13 tubes 8 tubes	17-0489	Confirmation of Virus analysed for IHNV, CEV, CyHV and SAV
Switzerland	Thomas Wahl	University of Bern Centre for Fish and Wildlife Health	31.05.2017	Histology slides	8	17-8313	Original slides for evaluation of PKD PT2017
Italy	Anna Toffini	Istituto Zooprofilattico Sperimentale della Venezia Fish Virology Department	14.06.2017	Cell supernatant	13 tubes	17-9373	To be included in the VHSV panel
UK-England	Tracey Cano Cajas	Celis Weymouth Laboratory	27.06.2017	Fish tissue + slides		17-9856	AGD Proficiency Test
Norway	Hilde Ståhre	Norwegian Veterinary Institute	29.06.2017	Infermed cell supernatant	3 tubes	17-10023	SAV for the Virus Bank
Slovenia	Diana Zala	University of Ljubljana, Veterinary Faculty	30.06.2017	Fish tissue (fillets)	30 pools	17-10192	30 pools of 5 Rainbow Trout fillets to be checked for BKD to achieve BKD free status in fish farm
Germany	Mikolaj Adamak	Fish Disease Research Unit University of Veterinary Medicine Hannover	04.07.2017	Fish tissue (gill) cDNA	1 tube	17-10228	CEV positive controls
The Netherlands	Olga L.M. Hoozen	Wageningen Bioveterinary Research of Wageningen UR, NRI for Fish Diseases	11.07.2017	Fish organs, suspension or in slices	6 tubes	17-10774	CEV positive controls

Technical Report 2017 from the EURL for Fish Diseases

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.	Remarks
The Netherlands	Olga L.M. Hoozen	Wageningen Bioveterinary Research of Wageningen UR, NRI for Fish Diseases	11.07.2017	Fish organs - suspension	1 tube	17-10755	IHNV positive control
Sweden	Charlotte Asan	National Veterinary Institute Section of fish	31.08.2017	Supermaster	4 tubes	17-13337	Identification of virus in Surgeons
Chile	Karin R. Muijar	Universidad de Santiago de Chile	04.09.2017	Anti-salmon IPN-1 Racoonium salmon IPN-1	1 tube 1 tube	17-14242	To have in stock
Italy	Anna Toffini	Istituto Zooprofilattico Sperimentale della Venezia	07.09.2017	E-11 cells	2 flasks	-	To have in stock
Germany	Mikolaj Adamak	Fish Disease Research Unit University of Veterinary Medicine Hannover	21.09.2017	Fish tissue cDNA	2 tubes 2 tubes	17-14683	PCR - PRV-3
France	Lanette Louboutin	ANSES Pleussac	22.09.2017	SBL cells PA cells	1 flask 1 flask	-	To have in stock
Norway	Espen Rimstad	NMBU Oslo	28.09.2017	Purified PRV-3 Infected blood	1 vial 3 vial	17-15043	PRV P1D - Project (NIVEN)
Bulgaria	Ekaterina Mileva	NRI of Fish, Mollusc and Crustacean Disease National Research & Diagnostic Veterinary Institute	10.10.2017	Purified RNA Supermaster	10 tubes 10 tubes	17-16047	Sequencing for IPN
Scotland	Emma Munro	Marine Scotland, Science Scottish Government, Marine Laboratory	12.10.2017	Fish heart homogenate	2 tubes	17-16623	Sequencing for PRV-3
Italy	Anna Toffini	Istituto Zooprofilattico Sperimentale della Venezia Fish Virology Department	21.11.2017	Homogenated organs	37 tubes	17-19266	Research of OutPRV (PRV-3)

4-1 Update the webpage for the EURL, www.eurl-fish.eu

The EURL website (www.eurl-fish.eu) is a notice board, where NRLs and other interested parties can access relevant information and previous reports concerning the activities coordinated by the EURL and relevant upcoming events in the Union.

European Union Reference Laboratory for Fish Diseases
National Veterinary Institute

DTU

ACTIVITIES REPORTS MANUALS NRL NETWORK LEGISLATION LINKS NEWS CONTACT

What is the EURL?
The European Union Reference Laboratory (EURL) for Fish Diseases is funded by the European Commission and is situated at DTU Vet - the National Veterinary Institute in Denmark. The functions and duties are concerned with harmonizing diagnostic procedures for notifiable fish diseases in Europe.

The functions and duties of the European Community Laboratory (EURL) for Fish Diseases are described in [Council Directive 2006/88/EC](#). A main purpose of the EURL is to ensure the quality of diagnostics of fish diseases in Member States and to harmonise the procedures and methodologies applied. The work is mainly concerned with the exotic and non-exotic diseases mentioned in [Council Directive 2006/88/EC](#).

The EURL co-ordinates those activities of the National Reference Laboratories (NRLs) for Fish Diseases in EU that aim to harmonise diagnostic techniques and disseminate information of mutual interest. Details of the work programme is decided at the Annual Meeting of the NRLs for Fish Diseases.

National reference laboratories 2 / 4

NEWS

- 14 March 2018
Invitation to the 22nd Annual Workshop 2018
- 14 February 2018
Survey & Diagnosis 2017
- 31 January 2018
22nd Annual Workshop announcement
- 21 September 2017
Interlaboratory proficiency test 2017

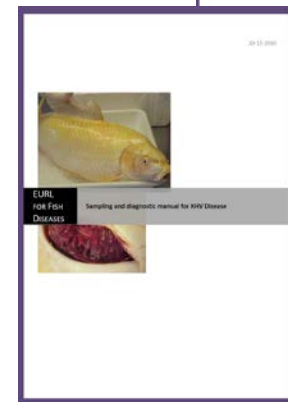
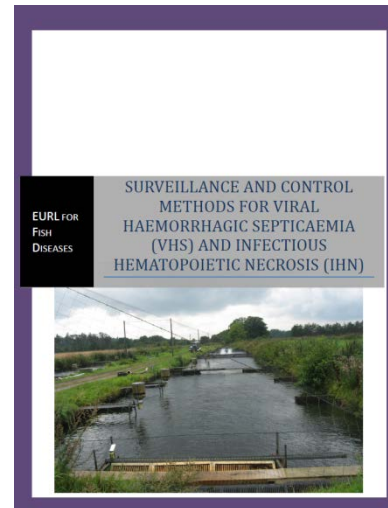
CALENDAR

The Fish Pathogen Database

FISH PATHOGENS

4-2 Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, and EHN on the EURL web page

- Commission Decision 2015-1554 finally adopted and implemented
- Easy access through our web page



4-3 Fishreflabnet: Maintain and further develop the interactive network with the NRLs, Fishreflabnet , in order to promote a more proactive data sharing and communication with and between reference laboratories in member states.

- This tool is used for newsletters, scientific updates and announcements from the EURL Fish like announcements and invitations for the Annual Workshop or publication of content in the ampoules from the proficiency test or on the final Inter-laboratory Proficiency test report. In addition the e-mail group is used for announcing other workshops, training courses and conferences and new publications of interest for the NRL Fish network

4-4 Identify and characterise selected isolates of listed viruses

- In 2017 a large number of virus isolates, sera and other reagents were received for further characterisation at the EURL
- and for storing in our virus library

4-5 Update and expand www.fishpathogens.eu with more pathogens

The database now consisted in 1194 VHSV records, of those 811 are public, and the rest are placed as restricted. Both betanoda and IHNV databases have numbers similar to last year, with 96 records for IHNV and 62 for betanoda. A new SAV database has been established. A number of new features are in the process to be added to all databases. A number of bugs were also corrected.

The screenshot shows the homepage of the Fish Pathogens Database. At the top, there is a navigation bar with links for Home, About, Contact, Terms and Conditions, F.A.Q., How to cite, Create an account, and Log in. Below the navigation bar is the logo for Fish Pathogens Database, which features a stylized DNA double helix and the text 'FISH PATHOGENS'. The main content area contains several paragraphs of text: 'Fishpathogens.eu offer a platform for sharing of available information on isolates of fish pathogens and their sequences to facilitate research on fish pathogens. The databases are free to use, but require subscription. One subscription covers all databases.' 'We encourage laboratories from all around the world to submit data of fish pathogens isolated in their laboratory, including as much isolate information as possible as well as genetic information if the isolate has been sequenced. It is not a requirement for upload to the database that the isolate has been sequenced.' 'For terms and conditions for using the database, please see Terms and Conditions. For information on how to use Fishpathogens.eu, please see the F.A.Q.' 'This website uses cookies. For more information about what a cookie is, go here. To see what cookies this website uses, go here. By use of this website, you agree to accept the use of these cookies.' Below the text are three buttons: 'VHSV >', 'IHNV >', and 'Betanodavirus >'. At the bottom of the main content area, there are four small images: a fish in a tank, a circular diagram, a close-up of a fish's head, and a fish in a natural environment.

4-6 Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens

- A standardization and validation of the **IHNV** real-time RT-PCR developed by Purcell et al. was initiated in 2016. The method is being translated and validated to a one-step procedure which is more convenient for use as a tool for surveillance of IHNV.

4-8 Emerging diseases:

Red Mark Syndrome (RMS) As presented earlier by Jacob G. Schmidt

Salmon gill poxvirus (SGPV)

Piscine orthoreovirus (PRV1 and PRV3)

Cardiomyopathy syndrome (CMS)

Flavivirus in lumpfish

4-10 Molecular characterization of fish cell lines: Perform molecular analysis to “barcode” and certify cell lines routinely used for viral diagnostics

Cell line	Name	Expected origin	De Facto origin
EPC*	<i>Epithelioma Papulosum Carpio</i>	Common carp	Fat Head Minnow
BF-2	Bluegill Fry	Bluegill	Green sunfish / bluegill
CHSE-214	Chinook Embryo	Salmon	Chinook Salmon
RTG-2	Rainbow trout gonad	Rainbow trout	Rainbow trout
FHM	Fat Head Minnow	Fat Head Minnow	Fat Head Minnow
CCO	Channel Catfish Ovary	Channel Catfish	Brown bullhead
EK-1	Eel Kidney	Pacific eel.	Japanese eel
ASK	Atlantic Kidney	Salmon	Atlantic Salmon
CCB	Common Carp Brain	Common Carp	Common Carp
SBL	Sea Bass Lymphoid	European seabass	Chinook Salmon
WSSK	White Sturgeon Skin-1	White sturgeon	white sturgeon

5-1 Missions: Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial. As collaboration with NRLs in 3rd countries from where EU is importing large amount of fish
Visits to the NRLs of Korea and Peru

5-2 Attending missions, international meetings and conferences in order to be updated on emerging and listed fish diseases.

Technical Report

- The Technical Report can be downloaded from our webpage WWW.eurl-fish.eu



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

Technical Report 2017

from the
**European Union Reference Laboratory for
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Content





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Workplan 2018 Suggestions for workplan 2019-21?

Niels Jørgen Olesen, Nikolaj Andersen, Niccolò Vendramin

22nd Annual Workshop

of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark, May 30 -31, 2018

2018 1-year workplan:

	Description	Objectives	Expected outputs
1. Coordination and training			
1-1	Annual workshop		
1-2	Annual workshop report		
1-3	Survey & Diagnosis		
1-4	Training courses in week 41 and 42		
1-5	Scientific working group 2018		

Suggestions for meetings?

Diagnostic manual for exotic diseases EHNV

Listing of fish diseases in the new legislation

2. Proficiency test

2-1

Proficiency tests

Suggestions for changes?

SAV continue

Betanodavirus?

More isolate variations

(KHV/ ISAV/ SAV)

**Demand for genotyping all
listed pathogens?**

2-2

PT reports

3. Reagents and products	
3-1	Supply of Reagents
3-2	Production of reagents
3-3	Pathogen library Important to keep up with library- virtual collection in sequence database (Fishpathogens.eu)

4. Scientific advice and activities

4-1	Webpage	Update
4-2	Diagnostic manuals	Update new RT-PCR for KHV?
4-3	FishRefLabNet	
4-4	Pathogen characterization	
4-5	www.fishpathogens.eu	
4-6	Molecular epidemiology	Approx 100 VHSV isolates full genome sequenced
4-7	Real-time PCR	IHNV
4-8	Emerging diseases	VHSV GIV?
4-9	Producing virtual teaching material (e-learning)	
4-10	Molecular characterization of fish cell lines	Barcoding, Use of gene expression in cells 3R?

5. Missions and international meetings

Missions

International meetings

Panels of viruses

- *VHSV reference set from EURL team*
- *ISAV?*
- *CyHV-3 ?*

- *Participating in Review on listing fish diseases in Annex to the new AHL*

Topics for training courses in 2019?

- Course will be held in Autumn 2019 in Lyngby (2x1 week).
- In 2019 EURL organize Aquaexcel2020 course in fish as experimental animals
- Topics: Cell cultivation, serology, molecular methods?
- Please indicate if any needs or special topics of interest!

2017-2018 Fish Pathogens Database

- In SAV still not finalized
- Future plans for including more pathogens:
- SVC
- Perhabdovirus
- ISA
- ?



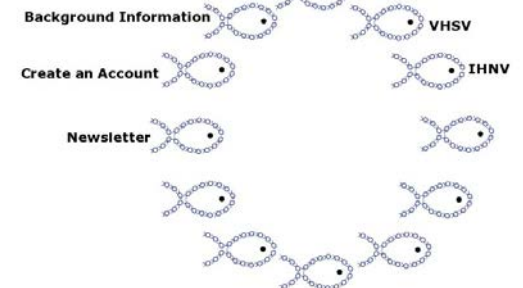
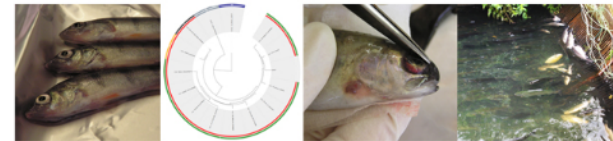
Fishpathogens.eu offer a platform for sharing of available information on isolates of fish pathogens and their sequences to facilitate research on fish pathogens. The databases are free to use, but require subscription. One subscription covers all databases.

We encourage laboratories from all around the world to submit data of fish pathogens isolated in their laboratory, including as much isolate information as possible as well as genetic information if the isolate has been sequenced. It is not a requirement for upload to the database that the isolate has been sequenced.

For terms and conditions for using the database, please see [Terms and Conditions](#). For information on how to use Fishpathogens.eu, please see the [F.A.Q.](#)

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[Betanodavirus »](#)
[IHNV »](#)
[SAV »](#)
[VHSV »](#)



23rd Annual Workshop 2019 DTU Campus in Lyngby?

- When?
- 27-29 May 2019 Back to back with NRLs for crustacean diseases?
- Alternative 12-14 June 2019

