

#### **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 DTU

DTU

물



Niels Jørgen Olesen



Niccoló Vendramin



Argelia Cuenca Navarro



**Tine Iburg** 



Lone Madsen







Nikolaj G. Andersen

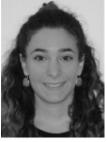


Anna L. F. Alencar



Sofie Barsøe





Valentina Laura Juliane Sørensen Donati



Didde Sørensen



**Betina Lynerup** Thorbjørn Tonndorff



**Christina Flink Desler** 



**Troels Secher** Rundqvist



Teena Klinge



Niels Lorenzen Professor



Dagoberto Andres Sepulveda Araneda Post Doc.



Inger Dalsgaard Ass. professor



Mie Johanne Hansen Post Doc.



Kári Karbech Mouritsen

#### 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 21<sup>st</sup> 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 21<sup>st</sup> Annual Workshop of the National Reference Laboratories for Fish Diseases

> Kgs. Lyngby, Denmark May 30<sup>th</sup> – 31<sup>st</sup> 2017



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

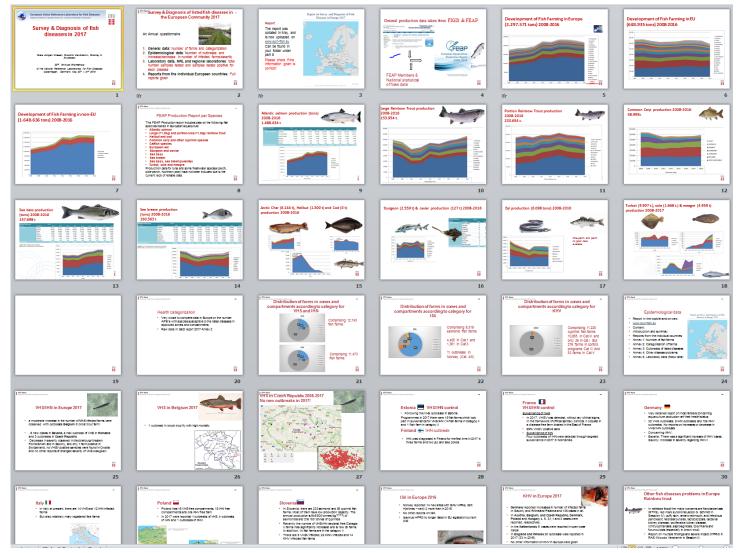


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Red Mark Syndrome (RMS) in rainbow trout

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

#### **1-3 Collect and report data on the fish diseases situation in EU**



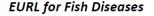
1-4 Facilitate and provide training in laboratory diagnosis:
EURL training courses 2017
EURL course 1: "Methods for implementation of surveillance procedures for listed fish diseases" took place from 9th to 13th October 2017
EURL course 2: "Introduction to histopathology in fish diseases" and took place in Copenhagen 16<sup>th</sup> to 19<sup>th</sup> October 2017

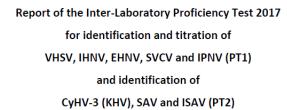


### 2-1, 2-2 Prepare and report the Annual Inter-Iaboratory Proficiency Test. PT1-2014 for identification of: VHSV, IHNV, EHNV, SVCV and IPNV and PT2 identification of: CyHV-3 (KHV), SAV and ISAV



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





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







# **3-1 Supply reference reagents to the NRLs in Member States**

#### Materials supplied by the EURL

On request, the EURL supplies material to other laboratories in Member States and Third Countries to aid in the diagnosis and characterisation of fish diseases.

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# **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 EHNV

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

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.	Remarks
Norway	Stefinie	Norwegian Veterinary Institute Veterinarinstituttet Harstad	04.01.2017	Organs	32 vials	17-477	Suspected flavivinus: Diagnostic
Switzerland	Thomas Wahli	University of Bern Centre for Fish and Wildlife Health	13.01.2017	Supernatant Purified RNA	3 tubes 3 tubes	17-1383	VHS + IHN qPCR.
Sweden	Charlotte Axen	National Veterinary Institute Section of fish	18.01.2017	Supermatant	1	17-2629	IPN strain for PCR and sequencing
Sweden	Charlotte Axen	National Veterinary Institute Section of fish	18.01.2017	Fish tissue Purified RNA	120 tubes 4 plates	17-1611	Screening for PRV
Norway	Aase B Mikalsen	NMBU	19.01.2017	Tissue cDNA	2 vials 1 vial	17-1636	Calicivirus control
Farce Island	Kristin Baldvinsdöttir	Food and Veterinary Agency Department of Fish and Animal Diseases	27.01.2017	Cell supernatant	S tubes	17-2000	RANA viruses
Slovenia	Diana Zele	University of Ljubljana, Veterinary Faculty	27.01.2017	Pools of kidneys	30 pools	17-2001	BKD - ELISA
Italy	Anna Toffan	Istituto Zooprofilattico Sperimentale delle Venezie	01.02.2017	E-11 cells	2 flasks	-	To have in stock
Romania	Mihaela Costea	Institute for Diagnosis and Animal Health NRL for fish diseases	23.02.2017	Cell supernatant with virus	4 vials	17-3602	Confirmation of VHSV
Belgium	François Lieffrig	CERGroupe	08.03.2017	Blood from Atlantic salmon and sea trout	2x 2 23x 2	17-4258	Screening for PRVss and PRVom (Virus-Y)
France	Lenzig Louboutin	ANSES	03.04.2017	SBL cells	l small flask		To have in stock
Serbien	Vladimir, Ivan Radosavljevic	Institute of Veterinary Medicine of Serbia. Department of Fish Diseases	07.04.2017	Cell supernatant	4 tubes	17-5793	Viral growth on EPC (for full genome sequences in US)

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Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.	Remarks
France	Patrick MARTIN	Conservatoire national du saumon Larma. Chanteuges	19.04.2017	Fish tissue in EMEM	30 tubes	17-6259	PRV PhD - Project (NIVEN)
Ireland	Neil Martin Ruane	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 + Homogenated tissue	5 tubes + 5 tubes	17-6661	Detection of Flavinizus
Serbia	Vladimir, Ivan Radosavljevic	Institute of Veterinary Medicine of Sarbia Department of Fish Diseases	30.05.2017	Cell supernatant Purified RNA/DNA	13 tubes 8 tubes	17-8489	Confirmation of Virus analysed for KHV, CEV, CyHV and SAV
Switzerland	Thomas Wahli	University of Bern Centre for Fish and Wildlife Health	31.05.2017	Histology slites	8	17-8515	Originals slides for evaluation of PKD PT2017
Italy	Anna Toffan	Istinuto Zooprofilattico Sperimentale delle Venezie Fish Virology Department	14.06.2017	Cell supernatant	13 tubes	17-9375	To be included in the VHSV panel
UK-England	Irene Cano Cejas	Ceffis Weymouth Laboratory	27.06.2017	Fish tissue + slides		17-9856	AGD Proficiency Test
Norway	Hilde Sindre	Norszegian Veterinary Institute	29.06.2017	Infected cell supernatant	5 tubes	17-10023	SAV for the Virus Bank
Slovenia	Dinna Zele	University of Ljubljana, Veterinary Faculty	30.06.2017	Fish tissue (kidney)	30 pools	17-10192	30 pools of 5 Rainbow Trout kidne 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) gDNA	1 tube 1 tube	17-10228	CEV positive controls
The Netherlands	Olga L.M. Hasnen	Waganingan Biovaterinary Research of Waganingan UR, NRL	11.07.2017	Fish organs, suspension or in	6 tubes	17-10754	CEV positive controls

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Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.	Remarks
The Netherlands	Olga L.M. Haanan	Wageningen Bioveterinary Research of Wageningen UR, NRL for Fish Diseases	11.07.2017	Fish organs - suspension	1 tube	17-10755	IHNV positive control
Sweden	Charlotte Axan	National Veterinary Institute Section of fish	31.08.2017	Supercontext	4 tubes	17-13337	Identification of virus in Storgeons
Chile	Kevin R. Maisey	Universidad de Santingo de Chile Santingo	04.09.2017	Anti-salmon IFN-al Recombinent salmon IFN-al	5 tubes 1 tube	17-14242	To have in stock
Italy	Anna Toffan	Istituto Zooprofilattico Sperimentale delle Venezie	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	Lenzig Louboutin	ANSES Plouzané	22.09.2017	SBL cells PAs cells	l flask l flask		To have in stock
Norway	Espen Rimstad	NMBU Oslo	28.09.2017	Purified PRV-3 Infected blood	l vials 3 vial	17-15043	PRV PhD - Project (NIVEN)
Bulgaria	Ekaterina Mileva	NRL of Fish, Mollins: and Crustneam Disease National Research & Diagnostic Veterinary Institute	10.10.2017	Purified RNA Supernaturt	10 tubes 10 tubes	17-16047	Sequencing for IPN
Scotland	Eann Munro	Marine Scotland. Science Scottish Government. Marine Laboratory	12.10.2017	Fish heart homogenate	2 tubes	17-16823	Sequencing for PRV-3.
Italy	Anna Toffan	Istituto Zooprofilattico Sperimentale delle Venezie Fish Virology Department	21.11.2017	Homogenated organs	37 tubes	17-19266	Research of OmPRV (PRV-3)

## 4-1 Update the webpage for the EURL, <u>www.eurl-</u> <u>fish.eu</u>

The EURL website (<u>www.eurl-fish.eu</u>) 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.





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 dubies 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 <u>Council Directive 2007680EC</u>. 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 <u>Council Directive 2006/08/EC</u>.

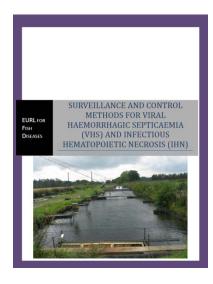
The EURL co-ordinates those activities of the National Reference Laboratories (NRLs) for Fish Diseases in EU that aim to harmonise diagnostic techniques and disseminate information of mutual inferest. Details of the work programme is decided at the Annual Meeting of the NRLs for Fish Diseases.

٧S	All	CALENDAR	All	The Fish Pathogen Database
rch 2018 on to the 22nd Annual Workshop 2018				FISH PATHOGENS
pruary 2018 y & Diagnosis 2017				FISH FATHOGEN:
nuary 2018 Annual Workshop announcement				
Annual Workshop announcement				

Interlaboratory proficiency test 2017

# 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.

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 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 Interlaboratory 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 <u>www.fishpathogens.eu</u> 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.





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 th FAQ

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

The development of Fishpathogens eu was funded by the FP6-2004-Food-3-A project EPIZONE. Further maintenance and development is funded by the European Commission financial aid for running the European Reference Laboratory for Fish Diseases.

#### 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*	Epithelioma Papullosum Carpio	Common carp	Fat Head Minnow
BF-2	Bluegill Fry	Bluegill	Green sunfish / bluegill
CHSE- 214	Chinook Salmon Embryo	Chinook Salmon	Chinook Salmon
RTG-2	Rainbow trout gonad	Rainbow trout	Rainbow trout
FHM	Fat Head Minnow	Fat Head Minnow	Fat Head Minnow
ссо	Channel Catfish Ovary	Channel Catfish	Brown bullhead
EK-1	Eel Kidney	Pacific eel.	Japanese eel
ASK	Atlantic Salmon Kidney	Atlantic Salmon	Rainbow trout
ссв	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
 <u>WWW.eurl-fish.eu</u>



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 Fish Diseases



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



#### **European Union Reference Laboratory for Fish Diseases**

National Institute of Aquatic Resources, Technical University of Denmark

## Workplan 2018 Suggestions for workplan 2019-21?

Niels Jørgen Olesen, Nikolaj Andersen, Niccolò Vendramin

22<sup>nd</sup> 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. Coordinati	ion and training
1-1	Annual w	orkshop	
1-2	Annual w	orkshop report	
1-3	Survey&	Diagnosis	
1-4	Training	courses in week 41	1 and 42
1-5	Scientific	working group 20	018
	<b>Suggestion</b>	s for meetings?	
		manual for exotic d	iseases EHNV
		sh diseases in the r	

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)			

4-1	Webpage	Update
4-2	Diagnostic manual	s T-PCR for KHV?
4-3	FishRefLabNet	
4-4	Pathogen characte	rization
4-5	www.fishpathogens	s.eu
4-6	Molecular epidemi genome sequenced	ology Approx 100 VHSV isolates full
4-7	Real-time PCR	IHNV
4-8	<b>Emerging diseases</b>	VHSV GIV?
4-9	Producing virtual t	eaching material (e-learning)
4-10		erization of fish cell lines of gene expression in cells 3

#### **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
- ?

#### Fish Pathogens Database

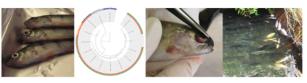
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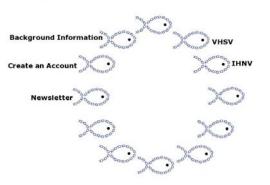
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Betanodavirus » I IHNV » SAV » VHSV »





### 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

