# 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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## Introduction

The National Veterinary Institute, Technical University of Denmark (DTU Vet) is appointed as the European Union Reference Laboratory (EURL) for Fish Diseases, in accordance with the Commission Implementing Regulation (EU) No 135/2013 of 18 February 2013, the notification of grant decision for an action regarding the EURL for Fish Diseases – SI2.725290 and the corresponding grand decision (Ref. Ares(2016)854560 - 18/02/2016) as regards the Union financial aid for the year 2016 and 2017 to the EURL for Fish Diseases.

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern fish diseases listed as exotic diseases: epizootic haematopoietic necrosis (EHN) and fish diseases listed as non-exotic diseases: infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and koi herpes virus disease (KHVD). This report follows the format of the work programme adopted for the EURL for 2016 and 2017, describing activities and sub-activities and the status of on-going projects. The list of functions and duties of the EURL follows this introduction.

In 2017, the National Veterinary Institute moved from central Copenhagen, where it has been placed for more than 100 years, to a new building at DTU Campus in Kgs. Lyngby, 15 km north of the capital. Laboratories and tank facilities, specifically designed for research and surveillance of fish- and shellfish diseases, were designed and built. This new environment, placed door to door with the National Institute of Aquatic Resources (DTU Aqua), gives us new opportunities for collaborations and access to up to date laboratories, experimental facilities, and training and meeting facilities. Significant resources were allocated to this transfer and this is to some extend reflected in this report. We have chosen, however, to give it priority in order to ensure a strong basis for our future activities within the EURL.

The 21st Annual Meeting of the National Reference Laboratories (NRLs) for Fish Diseases was held in Kgs. Lyngby, Denmark, May 30th – May 31st, at the premises of the Veterinary Institute. A total of 63 participants from 35 countries attended over the two days period. There were five sessions with a total of 28 presentations, three of which were given by invited speakers, a working group session and a round table discussion.

Again this year, an inter-laboratory proficiency test was distributed to the NRLs mainly within the EU, however, there were also participants from countries outside EU. The proficiency test consisted of two tests, PT1 and PT2. The PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV. The PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV. Again in 2017, the identification of SAV was included in PT2 on a voluntary base. The proficiency test is covering all five listed exotic and non-exotic fish diseases. FortyfiveNRLs participated in the proficiency test. A report was submitted medio March 2017. The majority of the laboratories performed well.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. Diagnostic manuals for VHS, IHN, ISA, KHV and EHN are available at the EURL website.

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed and emerging diseases and their accreditation. One outcome of

these efforts was the publication and acceptance of a new validated conventional RT-PCR for detection of VHSV. Resources were also used to optimize and implement a real-time RT-PCR for detection of PRV-3; an emerging disease in European aquaculture.

During 2017, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL website (www.eurl-fish.eu) and finally to attend international meetings and conferences.

In 2017, Dr. Nikolaj Reducha Andersen took the responsibility as the Coordinator of the EURL – taking the tasks of organising workshop and training courses, updating our website, conducting in-vivo viral characterisations and strengthens our statistical capabilities. DVM Niccolò Vendramin, during his two year sabbatical leave from the EURL, kept the responsibility of planning, shipping and reporting the proficiency tests and the questionnaires on fish health status in Europe.

Kgs. Lyngby, 12 April 2018

Niels Jørgen Olesen
Professor, DVM
Head of EURL for Fish Diseases

**The functions and duties for the**
**European Union Reference Laboratory for Fish Diseases**
**According to Council Directive 2006/88/EC of 24 October 2006 - Annex VI**
**Period: 1 January 2017 – 31 December 2017**

## The functions and duties for the European Union Reference Laboratory for Fish Diseases (EURL)

The European Union reference laboratories shall:

(a) coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing the disease concerned, specifically by:

(i) typing, storing and, where appropriate, supplying strains of the pathogen of the relevant disease to facilitate the diagnostic service in the Community,

(ii) supplying standard sera and other reference reagents to the national reference laboratories in order to standardize the tests and reagents used in each Member State, where serological tests are required, L 328/48 EN Official Journal of the European Union 24.11.2006

(iii) organising periodic comparative tests (ring tests) of diagnostic procedures at Community level with the national reference laboratories designated by the Member States, in order to provide information on the methods of diagnosis used and the results of tests carried out in the Community;

(iv) retaining expertise on the relevant disease pathogen and other pertinent pathogens to enable rapid differential diagnosis;

(b) assist actively in the diagnosis of outbreaks of the relevant disease in Member States by receiving pathogen isolates for confirmatory diagnosis, characterisation and epizootic studies;
(c) facilitate the training or retraining of experts in laboratory diagnosis with a view to harmonising diagnostic techniques throughout the Community;
(d) collaborate, as regards methods of diagnosing animal diseases falling within their areas of competence, with the competent laboratories in third countries where those diseases are prevalent;
(e) collaborate with the relevant OIE reference laboratories with regard to exotic diseases listed in Part II of Annex IV under their responsibility;
(f) collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture.

**Work programme for 2017**

# Technical report

## *1. Coordination and training*

*1-1,1-2 Organise and prepare for the 21st Annual Workshop of the National Reference Laboratories for Fish Diseases (NRLs) and produce a report from the Annual Workshop*

**Organization of the 21st Annual Workshop**

The 21st Annual Workshop of the National Reference Laboratories for Fish Diseases was held 30th – 31st of May, at DTU Veterinary Institute, 2800 Kgs. Lyngby, Denmark. This annual workshop was the first to be held at our new premises in Kgs. Lyngby.

A total of 63 participants from 35 countries attended over the two days period. All presenters arrived to the workshop, thus, no last minute changes were made in the programme. There were five sessions with a total of 28 presentations, three of which were given by invited speakers; 1) Nadav Davidovich from Israel (Tilapia viruses), 2) Britt Bang Jensen from Norway (Cardio Myopathy Syndrome (CMS) in Atlantic salmon) and 3) Nicholas Stinton from United Kingdom (Use of tablet devices during aquaculture visits), a Working Group activity and a Round Table discussion. The scientific programme of the Annual Workshop was wide and covered many interesting topics.

The workshop was opened with “Welcome and announcements” by Head of the EURL for Fish Diseases, Niels Jørgen Olesen and EURL coordinator, Nikolaj Reducha Andersen. The scientific part was opened with the traditional Session 1 “Update on important fish diseases and their control”, in which participants had the opportunity to present new findings from their respective countries.

Initially, an overview of the disease situation and surveillance in Europe 2016 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire. A report compiling all information are available at the EURL website http://www.eurl-fish.eu/activities/survey_and_diagnosis. Secondly, the fish disease situation in Norway was presented; a detailed report in Norwegian is available at https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2017/fiskehelserapporten-2016. An English version will be available later. The two final presentations in Session 1 were an update on the disease situation in aquatic organisms in the Mediterranean and a presentation of aquaculture and disease threats in Australia with an update on Epizootic Haematopoietic Necrosis Virus (EHNV).

The second half of the morning was allocated to the Working Group activity, introduced for the first time during the Annual Workshop in 2014. Briefly, each participant was, before the workshop, asked to choose one of four groups, divided into fish species, and rate the different fish diseases for that certain fish species in his or hers country. All participants received

beforehand four tables listing the most renowned pathogens for the most important farmed fish species in Europe. Before the workshop, each participant had the opportunity to interact with different stakeholders and assess impact on production, economy, legislative consequences and risk of future significance for the different infectious diseases in 2016. During the Working Group activity, participants discussed and agreed on a common rating for all the diseases. Each Working Group lastly presented their results to the rest of the participants at the workshop. The second session of the Workshop was dedicated to emerging diseases. Firstly, a combined presentation with data from the Netherlands and Korea was given on Cyprinid Herpesvirus 2 (CyHV-2). This was followed by an update on Cardio Myopathy Syndrome (CMS) in Atlantic salmon from Norway. The following two presentations were both given by employees at DTU VET and addressed Piscine Orthoreovirus (PRV) in Europe and Red Mark Syndrome (RMS) in rainbow trout, respectively.

The third session on control and surveillance of relevant pathogens in the EU started with a presentation describing Carp Edema Virus Disease (CEVD) in Europe. This was followed by an update on the fish health situation in France. Then Niels Jørgen Olesen gave a thorough review on aquaculture in the new Animal Health Law, an update on listing of fish diseases in the EU legislation and an update on susceptible species from an OIE working group. The last part of session three was dedicated to data and handling by a presentation on how to collect and interpret disease data and secondly, how to use tablet devices in inspection and compliance visits of aquaculture facilities. In the evening of the first day, a banquet was held at Restaurant “Brdr. Price” in Tivoli.

The second and last day was opened with a session on results from ongoing research on listed and emerging fish diseases. Traditionally, this fourth session faced several different topics covering molecular characterization of pathogens, development of new diagnostic techniques, including serology, conventional PCR and Real Time PCR, cell cultures and characterization and description of new fish pathogens. The session started with three presentations addressing infectious salmon anemia (ISA) from Norway and the Faroe Islands. The virus, VHSV, was also addressed in this session with four presentations, mainly on molecular work and identification of virulence markers. An interesting case on recurrent unexplained mortalities in warm water fish species in Israel was also presented in this session. In another presentation, attention was given to barcoding of fish cell lines, and it was shown that the origins of these are not always correctly noted. The fourth session ended before lunch with the second activity, Round Table discussions. All participants had the opportunity to give a short pitch talk on a topic they believed needed discussion and would be of common interest. Two pitch talks were given; one on sample preservation for PCR and a second on the use of FTA cards in fish diagnostics. Both topics initiated interesting plenary discussions.

The Annual Workshop ended with the traditional fifth session on updates from the EURL. The results of the two proficiency tests sent out in 2016, PT1 and PT2, were presented. The programme and application system for the annual training courses, which will be provided by the EURL in October 2017, was described and participants were given the opportunity to suggest topics for future courses. The planned EURL activities in year 2017 were presented and proposals for the EURL work plan for 2018-2020 were discussed.

***The final report of the 21st Annual Workshop is available on the website***
**http://www.eurl-fish.eu/Activities/annual-meetings**

*1-3 Collect and report data on the fish diseases situation in EU, including all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2*

**Questionnaire on survey and diagnosis of fish diseases in Europe in 2016 (S&D)**

The questionnaire, which is collated annually, is the only comprehensive overview of the disease situation in aquaculture in Europe. The information has been made available on the EURL website (www.eurl-fish.eu), where all raw data can be obtained. In 2016 the S&D was changed significantly, where we asked each Member State to write a report and submit it to the EURL together with few questions. This year the same template was submitted but asking only for changes since the previous year in order to avoid duplicating earlier information. The questionnaire comprises 3 parts:

1. General data on aquaculture fish production: Number of fish farms, and the health categorization according to Council Directive 2006/88/EC and information on national surveillance programmes.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases (information on number of outbreaks and increase or decrease in number of infected farms and severity of outbreaks) but also including other diseases of interest.
3. Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.

**Production data from FEAP**

The data on the European aquaculture production was obtained from the "European aquaculture production report 2007-2015" Prepared by the FEAP secretariat October 2016. Again this year, we validated the data against the FIGIS database and concluded that there were no major discrepancies except for the common carp production estimated by FEAP to be only 1/3 of the production data we obtained from FIGIS. The report does not include information on the number of fish farms, and therefore these data were obtained directly in the questionnaire. The report only provides data from back to 2015 as data from 2016 was only available from autumn 2017. The total fish production in aquaculture in Europe increased again both in 2014 and in 2015 after a decrease in 2013 and is now at 2,359,705 t, the highest level ever. The increase, however, is almost only due to increases in non-EU Member States. Among the Member States the production has been almost horizontal in the past 10 years with a 30,000 t increase in 2015 to 674,493 t. The Atlantic salmon production, account for 1.57 mill ton against 1.55 mill ton in 2014, and is by far the largest contingency in Europe. The rainbow trout production is again below 400,000 t after steady increases in the previous years. The decrease is due to reduced production of table size rainbow trout, while production of large rainbow trout is increasing. After several years of increased production Turkey has experienced an almost 20% reduction from 2013 to 2015 but are still the largest contributor of table size rainbow trout with > 100,000 t production. The carp production is still mainly in the Eastern part of Continental Europe and is very stable with 57,610 t produced in all. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a production of 147,640 t and 158,479 t, respectively. Among other fish species of interest are eel (with 6,266 t in 2015 no significant increase since 2010), sturgeon (2,559 t) and caviar with 20% increase from 2014 to 127 t, turbot (decrease from 12,748 t to 7,823 t in 2013 and increase again to 11,270 in 2015), the cod production have collapsed from 22,729 t in 2009 to 78 t in 2015. The production of cleaner fish as lumpfish for lice control is increasing significantly, however, the total production has not been possible to retrieve.

**Health categorization of fish farms**

Many countries provided very clear and correct answers and almost all Member States did reply to the questionnaire, when compared to the previous year's, providing a rather complete overview of the status of fish health categorization in Europe. This year, in all 12,680 farms with susceptible species were included in the questionnaire as categorized while a total of 30,810 fish farms were reported. The number of categorized farms is, unfortunately, very variable from year to year which more reflects changes in the way of reporting than de-facto changes. There was a significant increase in the reported number of farms in categorized zones and compartments (From 8,505 in 2012 to 14,508 in 2015 for VHS and from 7,360 in 2012 to 12,130 in 2015 for KHV!) this was especially due to Germany who successfully included almost all their farms in categorized zones.

74% of the authorised trout farms in Europe are situated in category III zones

for VHS and 70% for IHN, with 23% and 27%, respectively, in Category 1. For both diseases the remaining 3% of the farms are situated in category II, IV or V. In all countries, except Norway, almost all salmonid farms are in Category I for ISA with 73% in Category I and 27% in category III. Only very few carp farms are approved KHV free in Category I (1%) and almost all are placed in Category III (96%) or in Category II 3%.
There are still several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in Category III or I when considering the risk of infection with VHSV from the marine environment?
Commission Decision 2015-1554 provide the guidelines for obtaining disease-free health statuses with regard to ISA and to contain infection with HPR deleted ISAV, saying that detection of ISAV HPR0 will not compromise the health status of a fish farm and is not notifiable to the EU (in contrast to OIE where detection of ISAV HPR0 is still notifiable). Some Member States do not include small registered APBs in the categorisation (e.g. hobby farms) but according to 2006/88/EC Annex III health categorisation comprise all APBs in the Member States, zones and compartments for each category. Only fish species listed as susceptible for the given listed disease shall be included in the categorization. Therefore, important aquaculture species as sea bass, sea bream, meagre, eel and pike-perch are not included in the European health surveillance for specific diseases.
The new Animal Health Law has now been adopted and includes all aquatic animals; in this connection the categorisation system will be simplified and be made more transparent.

**Outbreaks and severity of listed diseases in Europe**
Concerning the epidemiological data on the non-exotic diseases a moderate increase in the number of **VHS** infected farms and outbreaks were observed in Belgium (in brook trout farm!), Bavaria (nine new cases), a new outbreak of VHS in Romania and three outbreaks in Czech Republic. Decrease in severity observed in Mecklenburg-Western Pomeranian and in Saxony, and only one farm positive in Switzerland, no VHSV positive samples were found in Croatia and no other reports of changed severity of VHS was given.
For **IHN,** only few reports were given: increase in Saxony with three IHNV outbreaks without losses, one new IHN outbreak in the Netherlands and no new outbreaks in Croatia.
For **ISA,** Norway reported 12 new sites with ISAV HPRΔ and Faroe Islands reported one finding of HPRΔ in 2016 without clinical symptoms and with an outbreak in 2017. No other reports on ISA.
Concerning **KHV,** Germany reported an increases in number of infected farms in Saxony and Rhineland-Palatina. In Ireland and in Lithuania one outbreak in garden ponds reported, in the Netherlands two outbreaks in open water carps. In England and Whales 33 outbreaks were reported in 2016 (only 11 in 2015). In Croatia, KHV was reported from two farms and two ponds for sport fishing - Croatia have not encountered KHV before.

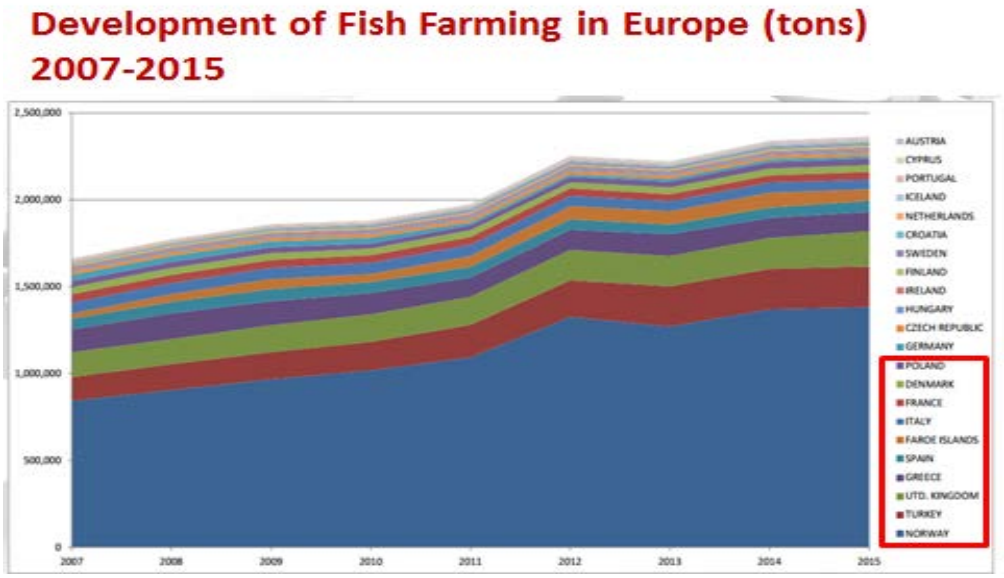
**Other fish disease problems in EU**

A whole range of other disease problems in 2016 were reported:

- In **rainbow trout**, the major concerns are flavobacteriosis (RTFS), red mark syndrome, puffy skin, enteric redmouth, and infectious pancreatic necrosis but also, lactococcosis, bacterial kidney disease, proliferative kidney disease, ichthyophthiriasis, saprolegniosis, columnaris and furunculosis (especially in brown trout).
- In **salmon** farming, it is sea lice, pancreatic disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome, amoebic gill disease, and moritella and in addition flavobacteriosis, furunculosis, and saprolegniosis (Baltic salmon). In Norway detection of PRV-*om* (=PRV-3) have been made in more than 100 samples.
- In **pike-perch** farming, one outbreak of perch rhabdovirus with 100% mortality in larvae.
- In **cleaner fish**, it is primarily vibriosis and *A. salmonicida* infection giving problems.
- In **carp**, it is primarily *Aeromonas hydrophila*, SVC (in Romania).
- In **seabass** and **seabream**, it is primarily VNN/VER, *Lernathropus kroyeri* infection, Microcotylosis and Rash syndrome.

In northern European countries the most common problems in the salmon production are thus sea lice, PD and AGD, in addition several countries reported finding of Winter Ulcer Disease in salmon caused by *Moritella viscosa.* In continental Europe it is primarily bacterial diseases like ERM and Aeromonas infections, AGD and RTFS – but also red mark syndrome is causing severe problems. Parasite infestations as Ich is still a very serious problem especially in view of the foreseen prohibition of use of formalin, while problems in the Mediterranean countries are the same as in continental except for Lactoccocosis which is more common in southern Europe and Nodavirus infection in mariculture which definitely plays an important role and as a bottleneck for especially the seabass production.

There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. Annex 5 provide the total number of laboratory examinations conducted in Europe in 2016 on VHSV, IHNV, ISAV, KHV, SVCV, CEV, IPNV, SAV and Nodavirus, respectively.

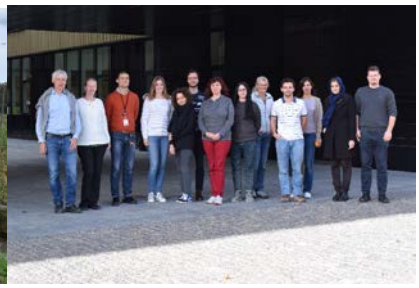



***A summary of the results for 2016 is presented on our website:***
**www.eurl-fish.eu/Activities/survey_and_diagnosis.aspx**

*1-4 Facilitate and provide training in laboratory diagnosis. The yearly training courses in methods used for diagnosis of fish diseases will be offered at the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.*

**Training**

Training courses:



The EURL Fish is yearly offering two training courses in diagnostic techniques for identification of listed fish diseases and introduction to histopathology on fish diseases.

The training courses took place in Kgs. Lyngby at the National Veterinary

Institute, Technical University of Denmark, Kemitorvet, building 202, 2800 Kgs. Lyngby, Denmark, from October the 9th to the 19th, 2017. Two courses were prepared: the first one, with nine trainees, was entitled "Methods for implementation of surveillance procedures for listed fish diseases" and took place from 9th to 13th October. The second course was entitled "Introduction to histopathology in fish diseases" and took place from the 16th to 19th October 2017 with 13 participants. A single person participated in both training courses.

The overall purpose of the training courses was to provide an opportunity for the NRLs to send employees for training in techniques relevant when working with fish diseases. Concerning the course on surveillance of listed fish diseases the staff of the EURL provided this training, together with teachers from the Danish Veterinary and Food Administration. For the course, that focused on histopathology, staff from the EURL and DTU VET, in cooperation with NVI-Oslo and Aquapri DK, constituted the tutor team. Knowledge-sharing and discussions between participants and teachers were important parts of both courses.

**Course 1: Methods for implementation of surveillance procedures for listed fish diseases**

The five day course in "Methods for implementation of surveillance procedures for listed fish diseases" was primarily based on practical work (hands on) in combination with theoretical presentations.

**Day 1** was dedicated to a fish farm inspection. Prof. Niels Jørgen Olesen and EURL coordinator Nikolaj Reducha Andersen participated from the EURL**.** As procedure does not allow a visit to a fish farm after working in our laboratory it was decided to meet with the participants at the entrance of their hotel and to drive to the Danish Veterinary and Food Administration offices in Vejen, where we were received by Henrik Korsholm. After the training course introduction by Niels Jørgen Olesen and presentations on Danish surveillance plans for fish diseases held by veterinarian Morten Fruergaard-Andreasen, the participants visited a rainbow trout farm, Vejen Store Vandmølle, located five km from Vejen. During the on-site visit, procedures for inspection and sample collection were demonstrated. Participants were taught on how fish necropsy techniques are performed in the field. All participants had the opportunity to perform necropsy on diseased fish collected at the farm. They collected relevant samples and brought them back to the laboratory in Copenhagen for further examination.

On **day 2** a detailed description of the course programme was presented by Niels Jørgen Olesen and discussed with the participants. After the introduction, a lecture on fish virology and the use of cell culture and qPCR for surveillance was given by Niels Jørgen Olesen. In the afternoon, the participants had to process the samples collected in the fish farm the day before. The processing of fish samples was demonstrated before the participants were asked to do it themselves.

**Day 3** was allocated to theoretical molecular techniques. The participants

attended presentations explaining the theoretical principles behind the different PCR techniques, methods of data analysis and a session addressing troubleshooting and pitfalls in real-time PCR, as well as routines to minimize the risk of (cross-) contamination. Practical exercises for the analysis and interpretation of qPCR results were carried on. During the afternoon session there was a lecture addressing the theory behind Sanger DNA sequencing, as a number of computer exercises where the students could learn how to read and assemble DNA sequences. The session included theoretical and practical (computer) exercises.

**Day 4** was dedicated to cell culture and started with the demonstration of the procedure implemented in the laboratory to prepare diagnostic trays with BF-2 and EPC cell cultures. Subsequently, all the participants prepared their own trays and inoculated the trays with the sample prepared at the second day. Titration procedure was also demonstrated and a talk on titre calculation and cell susceptibility test was given. The afternoon was allocated to provide the participants with knowledge in reading different fish cell lines inoculated with different viral pathogens, highlighting features of different cytopathogenic effects (CPE). As practical assignment participants were asked to read titration plates previously prepared, calculate the titre of the virus inoculated, and describe and draw different CPE's on infected cell cultures. The day ended with a series of presentations about the use of BLAST and the most important BLAST parameters, and about how to read, interpret and construct phylogenetic trees. The sessions included theoretical and practical (computer) exercises.

At **day 5** in the morning the participants were divided into two groups. One group were shown bacteriology techniques and visited the EURL facilities and the other group were reading their inoculated cell cultures - the groups thereafter switched. The afternoon was allocated to finalize the course, discussing both results obtained by the participants and different methods for performing surveillance of listed fish diseases in their countries of origin. Finally, a questionnaire for the course evaluation was given and the participants were asked to fill it anonymously.

The methods taught were primarily focused on the protocols given in the EU legislation and on the OIE guidelines from the Manual of Aquatic Animal Diseases, and included how to select proper controls, the typical pitfalls, troubleshooting, etc. Every activity had a team of tutors in order to provide an effective support to the trainees. For the practical cell culture activities Niccolò Vendramin, Didde Hedegaard Sørensen and Christina Flink Desler were assigned.

As get-together, a joint dinner the 10$^{th}$ of October was included.

**Course 2: Introduction to histopathology in fish diseases**
The four day course in histopathology and immunochemical techniques was divided into theoretical lectures on relevant topics, practical exercises both in necropsy room and microscopy laboratory.

**Day 1** started with an introduction to the course and practical information. Each participant had the opportunity to present themselves to the tutors and the other trainees. Practical demonstration on how to sample fish tissues and organs for proper histological examination followed, and each participant thereafter tried the technique on their own fish. Lectures given by Ole Bendik Dahle from NVI-Oslo on the normal histology and artefacts followed after lunch break.
**Day 2** was divided between practical observation of slides from confirmed cases at the microscope and theoretical lectures focusing on general pathology. Ole Bendik Dahle and Tine Moesgaard Iburg were in charge of the teaching.
The first part of **day 3** was dedicated to lectures on Immunohistochemistry (IHC) and in situ hybridisation (ISH), the different phases of sample preparation for staining techniques and troubleshooting and pitfalls during the process were discussed. This part of the programme was conducted by Tine Moesgaard Iburg, Torsten Snogdal Boutrup and Tim Kåre Jensen. Theoretical exercises in IHC were used as a platform for discussing.
**Day 4**, the last day of the course started directly at the microscopy room, diagnostic cases brought by participants were discussed and presented in an open forum, with supervision of tutors Ole Bendik Dahle and Tine Moesgaard Iburg. After lunch, there was a last round of group work before all the participants met up again for a last discussion about the subjects that had been covered earlier in the day. At the end of the course the participants were provided with a questionnaire to fill in as part of the evaluation. Furthermore, the participants were invited to give their opinions on the course. All in all, the course was very well-received, and the distribution of practical and theoretical lessons seemed well balanced.

Through lectures, exercises, discussions, and knowledge exchange between participants, the knowledge on histopathology, and troubleshooting related to this technique was increased. There was much fruitful discussion among participants and teachers during the course, both during the lectures, the breaks and the theoretical exercises.

As get-together, a dinner event on day 2 was held.

## Participant list

| Surname | Name | Country | Affiliation | Course 1 | Course 2 |
|---|---|---|---|---|---|
| Ekaterina | Mileva | Bulgaria | National Diagnostic & Research Veterinary Institute | x | x |
| Lubomir | Pojezdal | Czech Republic | Veterinary Research Institute, Brno | x | |
| Thorbjorg | Einarsdóttir | Iceland | Institute for Experimental Pathology, University of Iceland | x | |
| Nastaran | Shahbazian | Iran | Iran Veterinary organization | x | |
| Miriam | Abbadi | Italy | Italian health authority and research organization for animal health and food safety | x | |
| Monique | Oosterhof | Netherlands | Wageningen Bioveterinary Research (former CVI) of Wageningen UR | x | |
| Jorge | Freitas | Portugal | Madeira University, Madeira Chemical Center (CQM) | x | |
| Regula | Hirschi | Switzerland | National Fish Disease Laboratory, Centre for Fish and Wildlife Health (FIWI) | x | |
| Ava | Waine | United Kingdom | Centre for Environment, Fisheries & Aquaculture Science (CEFAS) | x | |
| Svetlina | Kirova | Bulgaria | National Diagnostic & Research Veterinary Institute | | x |
| Catherine | Graham | Canada | Animal Health Laboratory, Nova Scotia Department of Agriculture | | x |
| Kateřina | Matějíčková | Czech Republic | Veterinary Research Institute, Brno | | x |
| Sofie | Bjørnholt Binzer | Denmark | University of Copenhagen, Marine Biological Section | | x |
| Tuulia | Enbom | Finland | Finnish Food Safety Authority Evira | | x |
| Seifory | Parvaneh | Iran | Veterinary organization of Iran | | x |
| Nooshin | Zamannejad | Iran | Shahid Beheshti University Tehran Iran | | x |
| Liga | Ansonska | Latvia | Institute of Food Safety, Animal Health and Environment "BIOR" | | x |
| Darius | Nienius | Lithuania | National Food and Veterinary Risk Assessment Institute | | x |
| Paulo | Carvalho | Portugal | Pathology Department of the National Institute for Agrarian and Veterinary Research (INIAV) | | x |
| Silvia | Soares | Scotland | Marine Scotland Science | | x |
| Matthew | Green | United Kingdom | Centre for Environment, Fisheries and Aquaculture Science (Cefas) | | x |

***The final report is available on the EURL website***
***www.eurl-fish.eu/Activities/traning***

**Master and PhD students**

**Anna Luiza Farias Alencar**, was from July 1st 2016 enrolled as PhD student on a project entitled: Identification of virulence markers in two Novirhabdoviruses causing serious diseases in fish. With Professor Niels Jørgen Olesen as supervisor and Thomas Brun Rasmussen, Argelia Cuenca DTU Vet and Michel Bremont INRA, JOUY-EN-JOSAS, France as co-supervisors. Anna has a master in Veterinary medicine and natural science from University of São Paulo, USP. Her project will end on June 2019.

**Niccolò Vendramin** was from December 1st 2016 granted a 2 year sabbatical leave from his position as coordinator of the EURL for Fish Diseases for finalizing a PhD study entitled "Piscine orthoreovirus in salmonids: geographic distribution, molecular characterization, pathogenesis under experimental conditions" with Professor Niels Jørgen Olesen as main supervisor and Professor Espen Rimstad NMBU, Oslo, Norway as co-supervisor. Niccolò has a degree as DVM from University of Padua and have been employed as coordinator of the EURL for Fish Diseases since 2012.

**Sofie Hansen** was from December 1st 2017 granted a 3 year scholarship for a PhD project entitled: Vaccination of European seabass against a lethal viral disease and characterization of protective immunity, with Professor Niels Jørgen Olesen and Professor Niels Lorenzen as supervisors. Sofie has a degree as DVM from Copenhagen University.

**OIE collaboration**

N.J. Olesen is designated expert of the OIE reference laboratory for VHS, and in this function had consultancies and requests worldwide concerning VHS. The OIE chapter on VHS and related diseases in the diagnostic manual for aquatic animal diseases is revised and updated by the OIE reference laboratory for VHS.

In this connection an OIE Twinning project has been established between the National Fishery Products Quality Management Service (NFQS) of the Republic of Korea, as the candidate institute, being the National Reference Laboratory (NRL) for fish diseases in Korea and DTU Vet. The candidate laboratory wanted to improve the capabilities of performing its duties as the NRL for Viral Haemorrhagic Septicaemia (VHS). The twinning project ended December 2017 with NFQS applying for obtaining a status as OIE Reference Laboratory for VHS for the Asian region. In this colaboration Dr. Hyoung Jun Kim, PhD and his director National Fishery Products Quality Management Service, RoK, visited us two times in 2017.

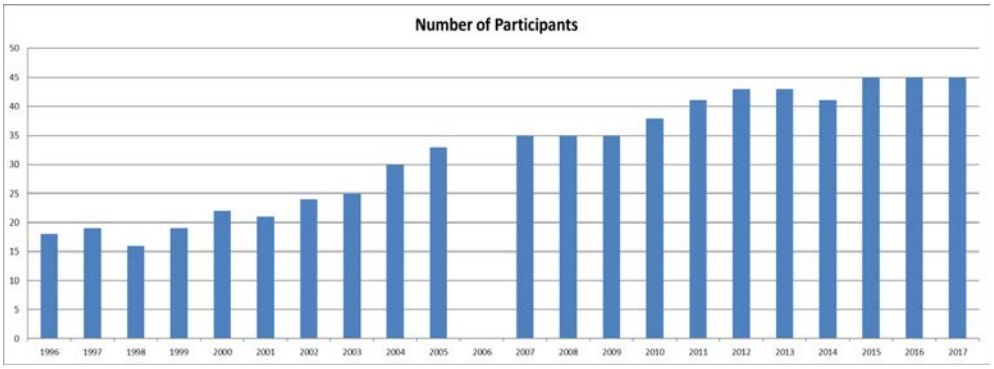
Niels Jørgen Olesen is member of the *ad hoc* group on susceptibility of fish species to infection with OIE listed diseases established by the OIE in 2016 and with regular meetings in Paris. Two 3-day meetings were held in 2017.

## *2. Proficiency tests*

*2-1 Prepare the Annual Inter-laboratory Proficiency Tests year 2017 for the NRLs. The tests include VHSV, IHNV, EHNV, ISAV, and KHV and in addition address other common viral pathogens in fish farming (IPNV, SVCV etc.).*

**The inter-laboratory Proficiency Tests 2017**

Since 1996, 21 inter-laboratory proficiency tests (PTs) have been organised by the EURL. The number of participants has increased from 18 to 45. The goal of these tests is to harmonise diagnostic methods between national reference laboratories and to ensure that the examination of a given sample leads to the same conclusions in any laboratory.




A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2). PT1 was designed to primarily assess the identification of the fish viruses causing notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and, in addition, other fish pathogenic viruses such as pike fry rhabdovirus (PFR), spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was designed for assessing the ability of participating laboratories to identify the fish viruses: infectious salmon anaemia virus (ISAV), salmonid alphavirus (SAV) and cyprinid herpesvirus 3 (CyHV-3) (otherwise known as *koi herpes virus* – KHV) by biomolecular methods (PCR based). A total of 45 laboratories participated in PT1 while 44 laboratories participated in PT2. Regarding PT1 and PT2, 42 and 38 laboratories, respectively, participated in identifying all viruses included. The tests were sent from the EURL mid of September 2017. Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043. The report submitted medio March 2017 covers both the results of PT1 and PT2.

**PT1** consisted of five coded ampoules (I-V). These ampoules contained VHSV (two isolates in two different ampoules), IHNV, European catfish ranavirus (ECV) and sterile cell culture supernatant from BF-2 cells. The proficiency test was designed to primarily assess the ability of participating laboratories to identify any of the fish viruses VHSV, IHNV and to be able to discriminate between the exotic listed EHNV from other ranaviruses

(Council Directive 2006/88/EC Annex IV part II and Commission Implementing Directive 2014/22/EU of 13 February 2014). Furthermore, the inter-laboratory proficiency test is also suitable for maintaining accreditation for identification of SVCV and IPNV; participants have to consider that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses or birnaviruses). In addition, the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Implementing Decision (EU) 2015/1554 and by using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.

If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2015. Laboratories were encouraged to identify VHSV and IHNV isolates by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al*. (2004) for VHSV and either method as mentioned in the IHN chapter of the 2013 version of the OIE manual on Aquatic Animal Diseases (Emmenegger et al. (2000)) or in Kurath et *al*. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

**PT2** consisted of four coded ampoules (VI-IX). One ampoule contained CyHV-3 (KHV), one contained SAV, one contained ISAV and one contained sterile cell culture supernatant from BF-2 cells,. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV and KHV (listed in Council Directive 2006/88/EC, Annex IV and Commission Implementing Directive 2014/22/EU) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test for SAV or not. The EURL team would then take care of calculating the score accordingly, overall 38 of 44 laboratories tested for SAV in 2017, which was an increase of one laboratory compared to 2016. Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. Regarding SAV analysis, participants can refer to the OIE manual Chapter 2.3.5b. — Infection with salmonid alphavirus. It was not mandatory to grow KHV and ISAV on cell cultures in this test, however, the viruses were not inactivated thus it might had been possible to replicate them in cell cultures.


Participating laboratories in PT1 and PT2 in 2017

*2-2 Collate and analyse information gained from the Inter-laboratory Proficiency Test*

## Outcome of the Inter-laboratory Proficiency Tests 2017

### Shipment and handling

Within one day, the tests were delivered to 18 participants; 18 more tests were delivered within the first week; five more within the first two weeks; one further within four weeks; due to delivery problems in the receiving countries three tests were seven – nine weeks in transit, this means that 40% of parcels were delivered by the shipping companies within one day after submission, 80% was delivered within one week and 91% was delivered within two weeks. The remaining four parcels took longer for delivery primarily due to border controls, the maximum time of shipment was 59 days.

### Concluding remarks on PT1

This year ECV was included in the proficiency test. Three out of the 45 countries do not test for Ranavirus. A total 38 participants provided the correct identification, two laboratories identified correctly the isolate as ECV by sequencing, however, they submitted the result as EHNV and two laboratories did no sequencing.

In the Report of the Inter-Laboratory Proficiency Test 2017, all the viral titres submitted by participants are compared to each other. In this way, the titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory is able to compare the sensitivity of its cell lines to the sensitivity of those used by the other participants. We recommend all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose, especially as it appears that the variations in titres between laboratories was very high – with more than eight log differences from the lowest to the most sensitive performances. Laboratories scoring low titres should definitely consider to exchange their cell lines with more sensitive strains or assess if the performance of their cells could be improved.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We will also take the opportunity to provide a comment to participants regarding submitted results if relevant. Furthermore, we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

Overall 39 out of 45 participants scored 100% success rate and five participants scored 90% due to sequencing of the content in ampoule IV (ECV) or contamination of ampoule contents. These points will be assessed directly with the single participants that has underperformed.

**Concluding remarks PT2**

The EURL have decided to include SAV in the panel of viruses included in PT2 since this was regarded as a proper initiative that strengthen the diagnostic capacities of the NRLs in detecting emerging pathogens. A total of 44 laboratories participated in PT2. Of those, 39 laboratories tested for SAV and 37 correctly identified the virus in Ampoule VI, one laboratory seems to have switched two of the ampoules and one laboratory answered 'negative'. Out of the 44 laboratories, 41 correctly identified the ISA virus in ampoule VII. One did not test for ISAV and two laboratories seems to have switched two of the ampoules. Of the 44 laboratories, 43 laboratories correctly identified the KHV in ampoule IX and one did not test for KHV. It has been a concern that two laboratories has identified the correct virus but not in the right ampoule, meaning that some mistakes in traceability of the ampoules during the working flow procedure has occurred. These points will be assessed directly with the single participants that has underperformed. It is appreciated, that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis, as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains, especially after the delisting of ISAV HPR0 (Commission Implementing Directive 2014/22/EU).

During the preparation of the PT report the EURL has acknowledged the big effort that many participants are putting in sequencing and genotyping the isolates of the PT panel. For this reason, the EURL has decided that from 2018 more focus and acknowledgement of the sequencing work conducted by the participants will be given. The EURL proposes to provide a separate scoring system for the genotyping results, which will be attached to the annexes which display the genotyping results provided by all participants. This topic will be explained and discussed at the next Annual Workshop.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they fare in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We take the opportunity to provide comments to participants regarding submitted results if relevant. Furthermore, we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

The results given in the report will be further presented and discussed at the 22$^{nd}$ Annual Workshop of the National Reference Laboratories for Fish Diseases to be held 30$^{th}$ - 31$^{st}$ of May 2018 in Kgs. Lyngby, Denmark.

***The final report is available on the EURL website***
***www.eurl-fish.eu/Activities/proficiency_tests***

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

## *3. Reagents and products*

**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. The number of laboratories receiving material and the specific material and number of units supplied by the EURL in 2017 are listed in Annex 1.

***Further details of the supplied materials are listed in Annex 1***

*3-2. Production of diagnostic reagents against selected pathogens when necessary*

**Production of antisera**

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 and other relevant putative emerging fish pathogens.*

**Virus library**

Isolates of the listed viruses ISAV, VHSV, IHNV and KHV were received and stored in our library during 2017. In addition, we received Ranavirus isolates, Flavivirus from lumpfish, tissue material from PRV-3 infected fish etc. All isolates were further sequenced and characterized genetically.

***Further details of the received materials are listed in Annex 2***

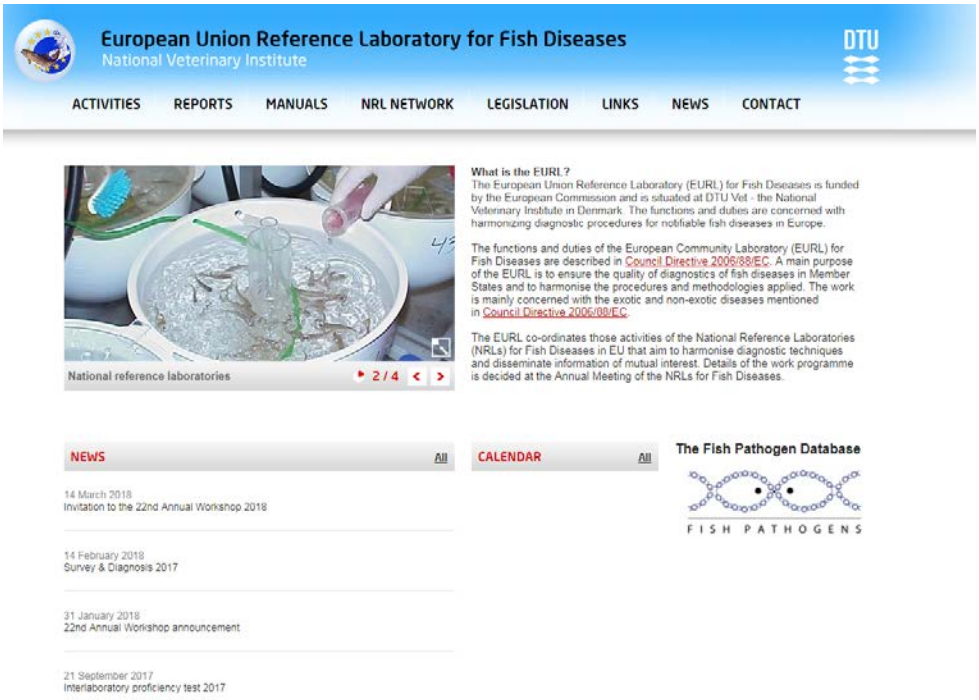
## *4. Scientific advice and activities*

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

**Update the webpage of the EURL**

All activities of the EURL for Fish Diseases are disseminated through our website www.eurl-fish.eu. We strive to have news and relevant updates available on the website as soon as possible and always within a few days. The website is mainly for use by European NRLs for fish diseases; however, we can register visits from countries all over the World after we have signed the website up for monthly Siteimprove.com reports.

Frontpage of www.eurl-fish.eu




Routinely, EURL reports such as the results of the proficiency test and Survey and Diagnosis, the report of the annual workshop and training courses and reports from expert meetings etc. are uploaded immediately after release. Contact details of the EURL have also been updated with new address after moving of the laboratory in 2017 and contact details of new employees. Furthermore, we have made a new sub site called “Scientific activities” that are updated with the newest information on current scientific achievements by the EURL. E.g. have a picture series, showing the development of the skin disease red mark syndrome been uploaded, to show visually, how the disease develop in rainbow trout.

The sub site “NRL Network” has been updated by contacting all EU Member State NRL, and asking them to review their contact information, ensuring that the EURL always has an updated list of all NRLs.

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

**Diagnostic manuals**

The procedures for the surveillance, sampling and diagnostics for each of the listed fish diseases are uploaded on the EURL Fish website for easy and comprehensive access to the details in the **Commission Decision 2015-1554**. As the decision only comprise the non-exotic diseases listed in the directive 2006/88/EC, a non-approved version of the sampling and diagnostic procedures for the exotic disease Enzootic Hematopoietic Necrosis (EHN) are included as are the methods for detection of EUS which was delisted previously but where the diagnostic procedures are still in force.

*4-3 FishRefLabNet: Maintain and further develop the interactive network with the NRLs, in order to promote a proactive data sharing and communication with and between reference laboratories in the Member States.*

**FishRefLabNet**

The email group has been updated and now contain more than 130 persons from 49 countries who are involved/interested in the work of the European Union reference laboratory for Fish Diseases. Official communication and updates of interest to the scientific community are still delivered periodically. To keep the email group up to date, we have asked the group to invite other people with interest in our work and given people the opportunity to unsubscribe from the group by replying to our newsletters. The email group has in 2017 been transferred to a CSV file, making us able to sort and filter contacts after e.g. country, NRL etc.

*4-4 Pathogen characterization: Identify and characterise selected isolates of listed viruses (pathogenicity testing in vivo and in-vitro, serological and genetic characterisation).*

**Studies conducted on pathogen characterization**

A total of 90 VHSV isolates from our repository representing all known genotypes were identified and propagated for full genome sequencing. The sequencing was conducted by Cefas, UK and ANSES, France. Hereby, the largest collection ever of VHSV full genome sequences (approx. 140) are now available for in depth studies of virulence determinants, molecular tracing and development of vaccines and specific diagnostic tools.

IHNV isolates from Korea were brought to the EURL in order to assess their virulence as they were provided from farms experiencing significant differences in IHN mortalities. In vivo virulence studies were conducted showing no significant differences under experimental conditions.

Finland reported outbreak of IHN in 2017. This is the first case in a Nordic country and isolates have been received for further characterisation in vivo (to be reported for 2018).
Samples of diseased lumpfish were received from Norway- as they were reported to suffer infection with Flavivirus-like viruses. No flaviviruses were detected and the studies are ongoing in 2018

**Identification and characterisation of selected virus isolates**

In 2017 a number of virus isolates, sera and other reagents were received for further characterisation at the EURL and for storing in our virus library as shown in the table beneath.

| Member States and countries outside EU | | |
|---|---|---|
| **Material received** | **Laboratories** | **Units** |
| Diagnostic material for virology | 5 | 97 samples |
| Diagnostic material for PCR | 6 | 39 samples |
| Diagnostic material for bacteriology | 1 | 60 samples |
| Diagnostic material for sequencing | 4 | 27 samples |
| Virus panels or PCR controls | 4 | 38 samples |
| Cell cultures | 2 | 7 sample |
| MAb/PAb | 1 | 6 samples |
| Screening of PRV | 6 | 363 samples |
| Other material | 1 | 8 samples |

***Further details are listed in Annex 2***

Below is listed samples, isolates and reagents received for identification, characterization and update of the virus library and diagnostic procedures applied for the relevant cases:

**CER Groupe Fish Diseases Laboratory, Belgium (François Lieffrig)**

- 50 tubes of blood from Atlantic salmon and sea trout to screen for the presence of PRV-1 and PRV-3 by Q-PCR. (DTU-VET 17-4258)

**National Research & Diagnostic Veterinary Institute, Bulgaria. (Ekaterina Mileva)**

- 10 tubes of purified RNA and 10 tubes of cell supernatant to be sequenced to assess the presence of IPN. (DTU-VET 17-16047)

**Laboratorio de Inmunologia Comparativa. Centro de Biotecnologia Acuicola (CBA), Chile (Kevin R. Maisey):**

- 5 tubes of supernatant of anti-salmon IFN-a1 (mouse IgM isotype)
- 1 tube of recombinant salmon IFN-a1 (DTU-VET 17-14242)

**Food and Veterinary Agency, Faroe Island (Kristín Baldvinsdóttir):**

- 8 tubes of Ranavirus isolates for the RANA panel. (DTU-VET 17-2000)

**Conservatoire national du saumon, France (Patrick Martin):**

- 30 tubes of Fish tissue in EMEM from Atlantic salmon and sea trout to screen for the presence of PRV-1 and PRV-3 by RT-qPCR. (DTU-VET 17-6259)

**University of Veterinary Medicine Hannover, Germany. (Mikolaj Adamek)**

- Kidney tissue obtained from rainbow trout preserved in RNAlater and cDNA obtained from kidney of rainbow trout for PRV-3 testing by RT-qPCR and sequencing. (DTU-VET 17-14683)

**Marine Institute, Ireland ( Neil Martin Ruane):**

- 122 samples for survey of PRV1 and PRV-3 in wild fish (DTU-VET 17-6222).

**Istituto Zooprofilattico Sperimentale delle Venezie IZSVe, Italy (Anna Toffan):**

- 37 tubes of homogenated organs from *Salmo trutta fario* (Brown Trout) for research of PRV-3. (DTU-VET 17-19266)
- 13 tubes of Italian VHS (selected and used for the NOVIMARK project) to be included in the VHSV panel. (DTU-VET 17-9375)

**National Veterinary Institute. Veterinærinstituttet Harstad, Norway (Stefanie):**

- 12 tubes of organs in medium to be testet for Flavivirus by PCR. All tested negative. No IHNV, IPNV and VHSV detected in cell culture.
- 20 tubes of organs in RNAlater (DTU-VET 17-477).

**National Veterinary Institute. Virology, Norway (Torfinn Moldal):**

- Heart, liver and kidney from Lumpfish in RNAlater and homogenates of the same organs + brain in transport media. For detection of flavivirus. (DTU-VET 17-6661).

**Norwegian University of Life Sciences, NMBU, Norway (Espen Rimstad):**

- PRV-3 purified from blood and PRV-3 infected blood from Rainbow Trout.
  To be used in the PRV-3 study. (DTU-VET 17-3602).

**Institute for Diagnosis and Animal Health, Romania (Mihaela Costea):**

- 4 samples with infected cell supernatant to confirm the presence of VHSV. VHSV was confirmed by ELISA. (DTU-VET 17-3602)

**Marine Scotland Science, Marine Laboratory, Scotland (Eann Munro):**

- Fish heart homogenate containing PRV-3 to be sequenced. (DTU-VET 17-16823)

**Institute of Veterinary Medicine of Serbia. (Vladimir, Ivan Radosavljevic):**

- 13 tubes of cell supernatant and 8 tubes of purified RNA/DNA for confirmation of virus. Tested for KHV, CEV, CyHV and SAV. Two samples were positive for SAV and two were positive for KHV, one was sequenced. (DTU-VET 17-8489).
- 4 vials containing supernatant of cell culture infected with Ranavirus from Serbia for viral growth on EPC for full genome sequences.

**University of Ljubljana, Veterinary Faculty, Slovenia (Diana Zele):**

- 30 pools of 5 Rainbow Trout kidneys to be checked for BKD to achieve BKD free status in fish farm. All found negative for BKD. (DTU-VET 17-2001)
- 30 pools of 5 Rainbow Trout kidneys to be checked for BKD to achieve BKD free status in fish farm. All found negative for BKD. (DTU-VET 17-10192)

**National Veterinary Institute, Sweden (Charlotte Axén):**

- 1 sample for confirmation of IPN by RT-PCR and sequencing. Found positive for IPNV. (DTU-VET 17-2629).
- 110 tubes with tissue (heart, skin or tissuelyzed organs) in RNAlater and 10 tubes with purified RNA to be screened for PRV-3 (DTU-VET 17-1611).
- 4 tubes of cell supernatant infected with virus from Sturgeons, for identification of virus. No CPE observed in WSSK, BF-2, GF, SSN or EPC cells. (DTU-VET 17-13337).

**University of Bern, Switzerland (Thomas Wahli):**

- 3 tube of cell supernatant and 3 tubes of purified RNA to be tested for VHSV and IHNV by RT-qPCR. (DTU-VET 17-1383)
- 8 slides (Originals IHC slides FIWI, Switzerland) for evaluation of PKD PT 2017. (DTU-VET 17-8515)

**Cefas Weymouth Laboratory Microbiology, UK-England, (Irene Cano Cejas):**

- Fish tissue + slides for AGD Proficiency Test (DTU-VET 17-9856)

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

**The pathogen database www.fishpathogens.eu**

During this year we have focused our efforts in data mining, completing records and included more comprehensive information, particularly regarding the geographical place of the different outbreaks, and to correct links to the correct gene bank sequences, as it is fundamental that our fishpathogens database is able to link results to the huge and widely used database in ncbi.

In addition, we are undergoing a change in the server hosting the fishpathogens database. Features regarding the use of whole genome sequences were implemented for VHSV. This in order to have a more appropriate platform for sharing this kind of sequences. Furthermore, we corrected some functions that will make it easier to make public the different isolates, and implemented an issue tracking Github. We updated the country list, as some countries were missing.

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

## Molecular epidemiology analysis

### VHSV in Iceland

Following the finding of VHSV in lumpfish from Icelandic waters in 2015, we have now proposed the creation of VHSV genotype IVc to include this isolate. Results from our molecular and pathogenicity studies were presented at the 21st EURL Annual Workshop from the EURL for Fish Diseases, as well as in the 10th International Symposium of Virus in Lower Vertebrates hold in Budapest on June 2017. In particular the last presentation generated a great deal of discussion with international colleges, particularly from USA and Canada, bringing forward the opportunity to cooperate in the generation of a better understanding of the evolution, dispersion and spread routes of VHSV in the North Atlantic Area. At the stage, the first manuscript describing the finding of the new VHSV strain in Iceland is in the final process of revision, hoping to submit in the close future. A second manuscript including a deep molecular characterization of the isolate, as well as a molecular tracing of VHSV in the North Atlantic is in preparation.

### Recent history of VHSV in Denmark

We performed an exhaustive analysis of the VHSV isolates found in Denmark from the 60's to their eradication in the country. This allowed us to detect migration routes from the virus, mostly by trade among farmers, but more interesting, we identified that most of the spreading of VHSV followed the placement of the slaughterhouses in the country. We also identified a number of events for export/import of VHSV to/from other countries during the 80's and 90's. Results of this research was presented in the 10th International Symposium of Virus in Lower Vertebrates hold in Budapest on June 2017.

### Evolutionary History of VHSV

To this stage, we have done the largest phylogenetic analysis of VHSV including all public available data with complete sequence for the G-protein (826 isolates after data curation). This is work in progress and we are supplementing our analysis with a large dataset of whole genome sequences. We are particularly interested to identify host/environmental changes and selection patters in VHSV. An additional point of interest is the identification of molecular markers for pathogenicity, both for diagnostic purposes, but also to understand adaptive trends in this virus.

### Piscine orthoreovirus 3 (PRV-3) in Europe

During 2017 the presence of PRV-3 was detected in Germany and Scotland

in rainbow trout, and there were suspicions that it may also be present in brown trout in Italy. Samples were send to our laboratory and the presence of PRV-3 was confirmed. At the same time, PRV-3 was found in rainbow trout reared in RAS in Denmark. A protocol for the sequencing the S1 protein were developed in our laboratory, and molecular analyses of these new PRV-3 isolates clearly indicate that the European PRV-3 is a different clade than the Norwegian PRV-3, been more closely related to PRV-3 strains found in Chile during the last years. At this stage we are not able to have a clear pattern of migration and spreading of the virus, as additional sampling is required. A manuscript done together with our collaborators in Norway has just been accepted (March 2018), describing the full genome of the Norwegian PRV-3 and the occurrence of PRV-3 in continental Europe.

*4-7 Assessment and standardization of real-time PCR tests for the diagnosis, identification and typing of emerging and listed non-exotic and exotic fish diseases*

**Real-time PCR**

1. We implemented and assessed the use of two different qPCR protocols for the detection of *Paramoeba perurans*, causing gill disease in salmon. We are expecting to be able to obtain positive material for validation of these procedures.
2. We tested and optimized some of our currently used diagnostic procedures based on RT-qPCR for piscine orthoreovirus 1 (in salmon) and 3 (in rainbow trout), as well as conventional RT-PCR and sequencing methods for both viruses.
3. We implemented a procedure diagnostic for *Piscirickettsia salmonis* based on nPCR targeting the 16S of these bacteria, followed for sequencing. Is in our plans to change for a faster and more specific protocol using qPCR.
4. We tested a protocol for discriminate the main VHSV genotypes without the need for sequencing. This protocol is based on the use of PNA probes and RT-qPCR, and was done in collaboration with colleges from Korea.
5. A new conventional RT-PCR protocol was developed for VHSV. This protocol has an increased sensitivity compared with the current RT-PCR used in diagnostic labs, and it is able to detect all four VHSV genotypes. The validation of this new protocol has been accepted for publication in Aquiculture.
6. Regarding the IHNV validation initiated in 2016, we have finished with the Analytical validation, and undergone two different proficiency tests (one with samples in FTA cards, and the second one with virus ampules). We are waiting for diagnostic samples to undergo the diagnostic validation of this protocol.
7. We have undergone a test for robustness for the protocol implemented to detect *Tetracapsuloides bryosalmonae*, the causative agents of proliferative kidney disease (PKD). This was done by a proficiency test including six European laboratories.
8. We have done a large project trying to determine which is the best diagnostic method to detect *renibacterium salmoninarum* (causing bacterial kidney disease in rainbow trout). Contradictory results are found using two PCR methods (nPCR vs qPCR), and this project is going to continue in 2018.

*4-8 Emerging diseases: In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases*

**Emerging diseases**

A novel flavivirus has been found in lumpfish in Norway. Diagnostic methods were not public available, as it was a private company to develop them. However, we have material that we believe is positive and, as new diagnostic methods have recently published (march 2018), we are already in the process to start implementation in our laboratory.

PRV-3 in Europe. Contrasting our 2016 screening where PRV-3 was not found in Denmark, in 2017 PRV-3 was detected for first time in several Danish farms, but also in Scotland and in Germany. PRV-3 is an orthoreovirus that causes heart inflammation in rainbow trout, but recently we have also found it in brown trout samples from Italy. Molecular characterization and phylogenetic analysis of these new PRV-3 isolates have been done (see 4.6), finding that these European isolates are different from the Norwegian PRV-3 strain, and more closely related to Chilean strains. We started a large screening for PRV-3 in Danish rainbow trout farms, as high mortality has been associated in some places, although it is not clear yet whether these mortalities are a direct effect of a PRV-3 infection.

Ranavirus in lumpfish. A ranavirus has been detected in lumpfish in the recent years. Phylogenetic analysis and pathogen characterization has been carried on, to see how this lumpfish ranavirus is placed respect to other ranaviruses. A manuscript describing the findings of Ranaviruses in the Atlantic is under preparation for publication in 2018.

*4-9 Producing virtual teaching material (e-learning)*

The aim of this task is to support the NRLs, by showing the correct laboratory techniques, to be used under the proficiency test send out by the EURL. The recording of videos were initiated during the training courses in 2017, where the first movie sequences were made to show sampling at a fish farm. The movie sequences for laboratory techniques are delayed due to our recent movement of laboratories in 2017. The movie sequences made during the training course has been made into a presentation video of the work we do as EURL, and has been shown during a theme day for the industry at DTU. The video will also be used at upcoming arrangements. The work with recording of video sequences and finalizing movies will continue in 2018.

*4-10 Molecular characterization of fish cell lines Perform molecular analysis to "bar-code" and certify cell lines routinely used for viral diagnostics*

As a continuation of what we have done last year, we have implemented a routine checkup of all our cell lines, this includes both the cell lines that are in our repository, but also all new cell lines received from other laboratories.

As reported in the previous year, some of our cell lines seemed to be contaminated. We asked for new batches in different laboratories which were also barcoded

1. ASK – Atlantic salmon kidney – from Norway – correctly barcoded and included in our repository
2. SBL – Sea bass larvae – from France – barcoded, resulting in species of origin being sea bream! This result is correct, and it seems a misidentification when the cell line was established. Included in our

repository.

3. E11 cells – received from IZSVe, Padua, Italy originated from striped snakehead. Barcoded correctly and included in our repository
4. GF cells – grunt fin cells – from blue striped grunt barcoded as common grunt. Included in our repository
5. ASE128 – unknown origin. Barcoded as at fathead minnow
6. KF-1 – koi fin, correct origin (koi carp)
7. SRTF 97 – unknown origin, barcoded as goldfish
8. PA4 – unknown origin, barcoded as fat head minnow.

## *5. Missions*

*5-1 Missions: Organizing missions to relevant laboratories. Missions 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*

**Missions to relevant laboratories**

In 2017 no classic missions were conducted. But during visits around the world laboratories were visited. The reason for missing missions was that no visits were needed due to underperformance at the proficiency tests 2016 and 2017. In addition with new staff and the movement more training was needed for conducting full missions.

**Visit in Korea 2017**

Prof. N.J. Olesen visited the National Fishery Products Quality Management Service, Busan, Republic of Korea in connection with a workshop organized by NFQS on VHS from May 7$^{th}$ to 12$^{th}$. Since 2011 NFQS participated successfully in the proficiency test organised by the EURL. The laboratory moved in 2016 from Seoul to Busan and was in 2017 fully up and running when visited. With very spacious facilities and small but dedicated staff the institute was fully able to cover the requirements for being a National reference laboratory - the facility, however, still lack access to experimental facilities and the division of tasks between NIFS and NFQS under the same ministry is still unclear with NIFS taking hold on surveillance and NFQS on import/exports.

**Visit in Peru**

Prof. N.J. Olesen visited the veterinary school, aquatic animal health in Lima while participating at the International Congress on Aquatic Health November 2017, Lima, Peru. The institute primarily studied and conducted diagnostics on bacterial diseases. It was recommended that more training is needed and scientist from the institute were invited for training courses at the EURL. Peru has a fast growing production of rainbow trout especially at altitudes above 3000 m. Most eggs are imported from Europe and training in biosecurity and diagnostics is needed in order to prevent serious disease outbreaks.

*5-2 International meetings. Attending missions, international*

**International meetings organized**

Contact with colleagues from other laboratories is a channel for exchange of information in the field of fish diseases, and an opportunity to keep abreast with new developments in the field. Of special interest are of course the

*meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.*

activities relating to VHS, IHN, KHV, ISA and EHN, but a number of emerging disease are coming up and need attention as well. Scientists at the EURL organized and/or participated in the following international meetings and conferences in 2017:

The 21st Annual Workshop of the National Reference Laboratories for Fish Diseases, Copenhagen, Denmark, May 30-31st 2017, with the following presentations:

- Overview of the disease situation in Europe, Niels Jørgen Olesen
- Update on the disease situation in aquatic organisms in the Mediterranean, Niccolò Vendramin
- Piscine Orthoreovirus (PRV) in Europe, Niccolò Vendramin
- Red Mark Syndrome (RMS) in rainbow trout, Jacob Günther Schmidt
- A: Aquaculture in the new Animal Health Law, B: Listing of fish diseases in EU legislation and C: Susceptible species – report from an OIE working group, Niels Jørgen Olesen/Knut Roenningen
- In the search for virulence markers of Viral Haemorrhagic Septicaemia Virus (VHSV), Anna Luiza Farias Alencar
- Barcoding of fish cell lines - the origin of cell lines is not always what we believe, Niels Jørgen Olesen
- New Viral Haemorrhagic Septicaemia (VHS) virus subtype in Europe, Argelia Cuenca Navarro

DAFINET workshop, Copenhagen, Denmark, October 25th 2017

The 18th International Conference on Diseases of Fish and Shellfish, Belfast, UK, September 2017, with the following presentations:

- Emergence of a novel VHSV genotype IV in lumpfish (Cyclopterus lumpus): Risk assessment for European aquaculture Cuenca A, Vendramin N, Sigurðardóttir H, Moesgaard Iburg T, Guðmundsdóttir S, Olesen N
- Development and validation of a novel one-step reverse transcription PCR method for detecting viral haemorrhagic septicaemia virus Kim H, Cuenca A, Olesen N
- Piscine orthoreovirus infection reduces susceptibility of Atlantic salmon (*Salmo salar* L.) to highly virulent infectious haematopoietic necrosis virus (IHNV), Vendramin N, Farias Alencar A, Iburg T, Dahle M, Olsen A, Olesen N
- Evidence for a Midichloria-like bacterium as causative agent of red mark syndrome in rainbow trout, Schmidt J, Madsen L, Iburg T, Boutrup T, Strube M, Henriksen N, Olesen N
- Rainbow trout red mark syndrome lesion development visualized, Schmidt J, Jørgensen L, Chen D, Buchmann K, Iburg T, Olesen N
- Red mark syndrome: Observations on disease development in a cohabitation model, Schmidt J, Iburg T, Madsen L, Henriksen N, Boutrup T, Olesen N
- Molecular tracing of viral haemorrhagic septicaemia outbreaks in

Denmark Cuenca A, Mikkelsen S, Skall H, Panzarin V, Schütze H, Fusaro A, Korsholm H, Olesen N

- Viral haemorrhagic septicaemia virus (VHSV) remains viable within water flea *Moina macrocopa* over several days but at low levels, Ito T, Olesen N
- Validation of histological tools for *Tetracapsuloides bryosalmonae* through interlaboratory proficiency test, Iburg T, Cuenca A, Vendramin N, Andersen N, Secombes C, Wahli T, Palenzuela O, Bron J, Madsen L, Olesen N
- Surveillance of BKD: Optimization of procedures for detection of Renibacterium salmoninarum, Iburg T, Cuenca A, Madsen L, Madsen S, Clausen T, Olesen N
- Epidemiology of infectious hematopoietic necrosis virus (IHNV) in the Netherlands since 2008, Haenen O, de Vos C, Boender G, Schuetze H, Cieslak M, Oldenburg S, Spierenburg M, Roozenburg-Hengst I, Voorbergen-Laarman M, van Gelderen B, Engelsma M, Olesen N
- Open EAFP workshop: "Neglected viral diseases affecting freshwater fish farming", Haenen O, Bigarré L, Ito T, Vendramin N
- Epizootiology of Koi herpes virus disease in Croatia, Zrncic S, Brnic D, Haenen O, Iburg T, Vendramin N, Sucec I, Oraic D
- Development of a DNA vaccine against SVCV and characterization of local and systemic immune responses after intramuscular and oral delivery, Embregts C, Boudinot P, Lunazzi A, Rigaudeau D, Lorenzen N, Veselý T, WiegertjesG, Forlenza M
- An IFN-inducible DNA vaccine against Viral Haemorrhagic Septicaemia in rainbow trout: evaluation of safety and immune protection profile, Sepúlveda D, Lorenzen E, Rasmussen J, Einer-Jensen K, Collet B, Secombes C, Lorenzen N
- Early dip vaccination against furunculosis (*Aeromonas salmonicida*) may improve productivity of sea-reared rainbow trout (Oncorhynchus mykiss), Frank Skall H, Henrik Henriksen N, Dalsgaard I, Bartkova S, Priess M, Lorenzen E, Madsen SB, Krossøy B, Lorenzen N

<u>International Congress on Aquatic Health 2017, Lima, Peru</u>, with the following presentation:

- Exchange of viral pathogens between farmed and wild fish, Niels Jørgen Olesen
- Advances in research on Viral Haemorrhagic Septicemia and Infectious Hematopoietic Necrosis, Niels Jørgen Olesen

<u>The 10th International Symposium on Viruses of Lower Vertebrates, Budapest, Hungary, June 2017</u>, with the following presentations:

- Novel piscine orthoreovirus from rainbow trout (*Oncorhynchus mykiss*) infects and causes heart pathology in rainbow trout, Niccolo Vendramin, Helena Hauge, Anne Berit Olsen, Torunn Taskdal, Øystein Wessel, Anna Luiza Farias Alencar, Maria K. Dahle, Niels

Jørgen Olesen

- First isolation of VHSV in lumpfish (*Cyclopterus lumpus*): genetic characterization and pathogenicity of a new subgroup of VHSV genotype IV, Argelia Cuenca, Niccoló Vendramin, Heiða Sigurðardóttir, Tine Moesgaard Iburg, Niels Jørgen Olesen, Sigríður Guðmundsdóttir
- Development of a novel one-step reverse transcription PCR method for detecting viral haemorrhagic septicaemia virus, Hyoung Jun Kim, Susie Sommer Mikkelsen, Niels Jorgen Olesen
- Viability of infectious viral hemorrhagic septicemia virus in water flea *Moina macrocopa*, Takafumi Ito and Niels Jørgen Olesen
- Isolation and characterisation of a new ranavirus isolated from lumpfish in the north Atlantic area, Hannah E. B. Stagg, Sigríður Guðmundsdóttir, Niccolò Vendramin, Neil Ruane, Heiða Sigurðardóttir, Debes H. Christiansen, Argelia Cuenca Navarro, Petra E. Petersen, Eann Munro, Niels Jørgen Olesen
- In the search for virulence markers of viral hemorrhagic septicemia virus (VHSV), Anna Luiza Farias Alencar, Argelia Cuenca Navarro, Thomas Bruun Rasmussen, Yannick Blanchard, Michel Bremont, Niels Jørgen Olesen

International workshop on VHS Research in Korea, Busan, RoK May 2017, with the following presentations:

- The Status of the OIE Laboratory Twinning Project for viral haemorrhagic septicaemia (VHS) with Korea and Denmark, Hyoung Jun Kim and Niels Jørgen Olesen
- Viral Haemorrhagic septicaemia virus (VHSV): on the search for determinants important for virulence in rainbow trout (Oncorhynchus mykiss), Niels Jørgen Olesen, Anna Luiza Farias Alencar, Argelia Cuenca Navarro, Hyoung Jun Kim and Takafumi Ito
- Severe mortality of farmed rainbow trout caused by infectious haematopoietic necrosis virus in Korea, Hyoung Jun Kim, Jung Jo Han, Torunn Taksdal, Ole Bendik, Niels Jørgen Olesen, Jeong Su Park and Se Ryun Kwon
- Assessment of a new conventional RT-PCR for VHSV detection in an inter-laboratory proficiency test, Hyoung Jun Kim and Niels Jørgen Olesen

*Peer reviewed publications Presentations and posters*

**Articles published in peer-reviewed journals**

*S. M. Bergmann, Q. Wang, W. Zeng, Y. Li, Y. Wang, M. Matras, M. Reichert,D. Fichtner, Th. Morin, L. Bigarre, N.J. Olesen, H.F. Skall, P.-Y. Lee, S.Monaghan, S. Reiche, W. Fuchs, M. Kotler, K. Way, G. Bräuer, K. Böttcher,A. Kappe and J. Kempter* (**2017**) Validation of an enzyme-linked immunosorbent assay (ELISA) to detect antibodies against koi herpesvirus (KHV) in sera obtained from carp or koi (*Cyprinus carpio* L.). Journal of Fish Diseases 12621.DOI: 10.1111/jfd.12621

*Hauge H, Vendramin N, Taksdal T, Olsen AB, Wessel Ø, Mikkelsen SS, Alencar ALF, Olesen NJ, Dahle M K* (**2017**) Infection experiments with novel Piscine orthoreovirus from rainbow trout (*Oncorhynchus mykiss*) in salmonids. PLoS ONE 12(7)

*Way K., O. Haenen, D. Stone, M. Adamek, S.M. Bergmann, L. Bigarré, N. Diserens, M. El-Matbouli, M.C. Gjessing, V. Jung-Schroers, E. Leguay, M. Matras, N.J. Olesen, V. Panzarin, V. Piačkov[3], A. Toffa[2], N. Vendramin, T. Veselý, T. Waltzek* (**2017**) Emergence of carp edema virus (CEV) and its significance to European common carp and koi *Cyprinus carpio* . Dis Aquat Org 126:155-166. https://doi.org/10.3354/dao03164

*Ito, T and N.J. Olesen* (**2017**) Viral haemorrhagic septicaemia virus (VHSV) remains viable for several days but at low levels in the water flea *Moina macrocopa* Dis. Aquat. Org. Vol. 127: 11–18, 2017

*Natalie K. Stilwell, Richard J. Whittington, Paul M. Hick, Joy A. Becker, Ellen Ariel, Steven van Beurden, Niccolò Vendramin, Niels J. Olesen, Thomas B. Waltzek1 (2017)* Validation of a TaqMan Real-Time Quantitative PCR for the Detection of Ranaviruses. Submitted to DAO Sept **2017**

*International scientific collaborative studies*

**Participation in international scientific collaborative studies**

**NOVIMARK: Identification of virulence markers in VHSV and development of tests for discriminating between virulence properties**
The group is partner in the ANIHWAS-ERA Net project NOVIMARK and work package leader of WP1.

The purpose of the project is to identify virulence markers in two loss-making Novirhabovirus as a key to improve diagnostic and strategic management in farmed rainbow trout. The participants are Institut National de la Recherche Agronomique (INRA); Agence Nationale de Sécurité Sanitaire (ANSES); Universidad de Santiago de Compostela (USC); Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) and Centre for Environment Fisheries and Aquculture Sciences (CEFAS). A PhD student, Anna Luiza Farias Alencar was employed in our team from July 1st 2016 and have conducted a number of cloning's and infection trials with selected VHSV isolates from our large repository.

**AQUAEXCEL$^{2020}$**
The DTU Fish diseases group is partner in AQUAEXCEL$^{2020}$. The objectives of AQUAEXCEL$^{2020}$ are to provide a wider and more efficient access to, and use of, the aquaculture research infrastructures existing in the EU. AQUAEXCEL2020 is a key vehicle in the improvement of aquaculture research practices. It will lead to a better management of animal experiments for research according to the 3 R's. One major feature of AQUAEXCEL2020 is its **Transnational access (TNA) program**, allowing external teams to

access the partners' infrastructures via submission of research proposals, and based on evaluation by an independent selection panel. Detailed information is available at http://www.aquaexcel2020.eu/
In 2017 two transnational access were conducted: one on protists as vectors for *Candidatus Midichloriaceae* in fish to fish transfection and one on the susceptibility of brown trout to PRV-3 and to prove Koch's postulate that PRV-3 is in fact the causative agent of HSMI-like disease in rainbow trout.

**ParaFishControl**
DTU is partner in the H2020 project ParaFishControl under the call "sustainable food security". The aim of the project is to 1) generate new knowledge on the most important parasites in fish farming including genome analysis, life cycles, and host parasite interactions especially in order to improve prophylaxis, 2) assess the impact of transfer of parasites between farmed and wild fish, 3) develop an array of new prophylactic measures, including vaccines and functional feed, 4) develop specific and sensitive diagnostic tools, 5) risk assessments, 6) identify zoonotic risks and 7) develop catalogue on GMP

The main objectives for DTU is:

1. Validation of diagnostic procedures for detection of parasites based on qPCR, ISH and IHC, by e.g. inter-laboratory proficiency tests.
2. Participate in development of "point of care" diagnosis.
3. Participate in publishing diagnostic protocols and standards.
4. Optimization of use of cleaner fish for controlling ectoparasites.
5. Treatment and management of infected fish farms.
6.

In 2017 inter-laboratory proficiency tests on PKD was conducted and 2 other proficiency tests were prepared.

# Annex 1 Reagents supplied by the EURL-Fish in 2017

| Country | Name | Institute | Date of receipt | Material | Amount | Remarks |
|---|---|---|---|---|---|---|
| **The Netherlands** | Olga L.M. Haenen | Bioveterinary Research of Wageningen UR | 18-01-2017 | Freeze dried fish virus | 5 stk | Ampoule I+II+III+IV+V (PT1) from 2016 |
| **Italy** | Chiara Bazzocchi | Università degli Studi di Milano | 23-01-2017 | Oprenset DNA | 8 stk. | 8 vials each containing 20µl of purified DNA in water. Extracted from rainbow trout skin. |
| **Iceland** | Sigridur Gudmundsdottir | Institute for Experimental Pathology University of Iceland | 25-01-2017 | Antiserum | 1 stk | Approx. 2 ml rabbit antiserum against Bohle-Irido-virus F53, 069/11-8-99 |
| **Belgium** | François Lieffrig | CERGroupe Fish Diseases Laboratory | 06-02-2017 | PFR - freeze-dried<br>EVEX virus | 1 ampoule<br>2 tubes | 1 ampoule with PFRV (Amp.I, PT16) to be forwarded to Anita Grinter at Bio-X Diagnostics S.A<br>2 cryotubes with EVEX, (Isolate received from J. Castric Brest), one harvested 08.08.1991 and one harvested 06.02.2017 |
| **Germany** | Heike Schütze | Friedrich-Loeffler-Institut (FLI) | 06-02-2017 | VHSV | 25 tubes | Medium panel of VHSV |
| **USA** | Thomas Waltzek | University of Florida | 20-02-2017 | Cell supernatant intected with Ranavirus | 13 tubes | 13 vials with cell supernatant infected with Ranavirus from Lumfish. Ranapanel |
| **Norway** | Anne Berit Olsen | Norwegian Veterinary Institute | 20-02-2017 | Glas slites | 7 slides | 7 histhological sections stained with Haematossilin and Eosin from experimental trial in Atlantic salmon with PRV and IHNV |
| **France** | Patrick Martin | Conservatoire national du saumon | 15-03-2017 | Eagels MEM | 50 tubes | 50 tubes with 4 ml in each. |
| **Ireland** | Neil Martin Ruane | Marine Institute Fish Health Unit | 04-04-2017 | GF cells | 1 flask | 1 small flask with GF cells |
| **Denmark**<br>**UK-England**<br>**Spain**<br>**Switzerland**<br>**UK-Scotland** | Lone Madsen<br>Richard Paley<br>O. Palazuela<br>Thomas Wahli<br>James Bron | NVI-DTU<br>CEFAS*<br>CSIC IATS *<br>University of Bern<br>University of Stirling* | 04-04-2017 | Kidney from 7 different Rainbow trout in RNAlatter.<br>Slides obtained from 7 | 7 tubes x 5 | PKD proficiency Test, Validation of diagnostic methods for Tetracapsuloides bryosalmonae-PKD.<br>Kidney in RNAlatter for Q-PCR and slides for histopathological examination. |

| Country | Name | Institute | Date of receipt | Material | Amount | Remarks |
|---|---|---|---|---|---|---|
| | C. Secombes | University of Aberdeen* | | different Rainbow Trouts | 3x 7 slides x 5 | *Also recived 40µl Plasmid PEX-A2-PKD, diluted $10^{-5}$ |
| **Norway** | Anne Berit Olsen | Norwegian Veterinary Institute | 06-04-2017 | Glas slites | 33 slites | 33 histhological sections stained with Haematossilin and Eosin from experimental trial in Atlantic salmon with PRV and IHNV |
| **Denmark**<br>**France**<br>**Germany**<br>**Italy**<br>**Croatia**<br>**China**<br>**Scotland** | Tine M. Iburg<br>Thierry Morin<br>Heike Schütze<br>Anna Toffan<br>Snjezana Zrncic<br>Shi Hong LIU<br>Eann Munro | NVI-DTU<br>ANSES<br>FLI<br>IZSVe<br>Croatian Vet. institute<br>AQSIQ<br>Marine Scotland | 28.04.2017 | FTA cards - IHNV | 7x 5 cards | Validation of a one-step real time RT-PCR for detection of IHNV.<br>5 FTA cards with 2 samples in duplicate on each |
| **Spain** | Oswaldo Palazuela | CSIC IATS | 02.05.2017 | Plasmid | 10 tubes | 10x 8µl Plasmid PEX-A2-PKD, diluted $10^{-5}$<br>Batch nr. 109/29.09.16-2 |
| **Ireland** | Orla Slattery | GMIT | 10.05.2017 | CHSE cells | 2 small flask | 2 small flask with CHSE cells |
| **Iceland** | Sigridur Gudmundsdottir | University of Iceland | 10.05.2017 | BF-2 cells<br>EPC cells | 2x 120ml<br>2x 120ml | 2 small flask with BF-2 cells<br>2 small flask with EPC cells |
| **UK - England** | Irene Cano | CEFAS | 23.05.2017 | Rainbow trout skin | 7 + 9 | 7 containers of Rainbow trout skin in 70% Ethanol for histology and 9 ampoules of Rainbow trout skin in 70% Ethanol for sequencing. |
| **South Korea** | Hyoung Jun KIM | NFQS | 07.06.2017 | Mab anti VHSV<br>Mab anti IHNV | 1x 3ml<br>1x 3ml | Monoclonal antibodies:<br>Mab anti VHSV IP5B11<br>Mab anti IHNV HYB 136-3 |
| **Czech Republic** | Lubomir Pojezdal | Veterinary Research Institute | 06.06.2017 | FTA cards - IHNV | 5 cards | Validation of a one-step real time RT-PCR for detection of IHNV.<br>5 FTA cards with 2 samples in duplicate on each |
| **Spain** | Carlos Zarza | Skretting España SA | 08.06.2017 | Culture swabs<br>Tubes with RNA later | | |

| Country | Name | Institute | Date of receipt | Material | Amount | Remarks |
|---|---|---|---|---|---|---|
| **USA** | Thomas Waltzek | University of Florida | 26-06-2017 | Cell supernatant intected with Ranavirus | 2x 8 tubes | 2x 4 vials with cell supernatant infected with Ranavirus from I. Nebulosus (from Serbia). Included in the Ranapanel |
| **UK - England** | Richard Keith Paley | Cefas Weymouth Laboratory | 26.06.2017 | VHSV infected cell supernatant | 33+1 tubes | 33 + 1 tubes with 1 ml cell supernatant infected with cloned VHS virus.For full genome sequences.VHS strains included in the investigations conducted in the Novimark project |
| **France** | Yannick Blanchard | ANSES | 26.06.2017 | VHSV infected cell supernatan | 50 tubes | 50 tubes with 1 ml cell supernatant infected with cloned VHS virus.<br>For full genome sequences.<br>VHS strains included in the investigations conducted in the Novimark project |
| **Iceland** | Sigridur Gudmundsdottir | Institute for Experimental Pathology University of Iceland | 03.07.2017 | BF-2 cells<br>EPC cells | 2x 120ml<br>2x 120ml | 2 small flasks with BF-2 cells<br>2 small flasks with EPC cells |
| **Spain** | Pilar Fernández | Somalo Ictiopathology Laboratorio Central de Veterinaria | 28.08.2017 | Antibodies | 2 tubes | Mab anti VHSV - 1P5B11 Mab anti IHNV HYB 136-3 |
| **Ireland** | Cathy Hickey / Neil Martin Ruane | Marine Institute | 29.08.2017 | EPC cells | 2x 120ml | 2 small flasks with EPC cells |
| **Iceland** | Torbjörg Einarsdóttir | Institute for Experimental Pathology University of Iceland | 13.10.2017 | BF-2 cells<br>EPC cells | 2x 120ml<br>2x 120ml | 2 small flasks with BF-2 cells<br>2 small flasks with EPC cells |
| **Bulgaria** | Ekaterina Mileva | National Research & Diagnostic Veterinary Institute | 13.10.2017 | BF-2 cells<br>EPC cells<br>FHM cells | 2x 120ml<br>2x 120ml<br>2x 120ml | 2 small flasks with BF-2 cells<br>2 small flasks with EPC cells<br>2 small flasks with FHM cells |
| **Switzerland** | Thomas Wahli | University of Bern | 13.10.2017 | PAb PERCH | 1x 2ml | Pab anti Perche-rhabdovirus<br>F28, 06.11.95,<br>Raised in Rabbit.<br>Collected 6/11-95 and heat-treated<br>Titer: 50% PNT against Perch Rhabdovirus |

| Country | Name | Institute | Date of receipt | Material | Amount | Remarks |
|---|---|---|---|---|---|---|
| | | | | | | > 5120 |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 30.10.2017 | Tissue in Formalin<br>Blood | 66 tubes<br>28 tubes | Fish tissue in Formalin 4% (Ester no.: 17-15043-5 to 17-15043-70).<br>Heparin glass containing blood from Rainbow trout and Brown trout |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology. | 30.10.2017 | Tissue in RNAlater | 196 tubes | 50-200 mg fish tissue in 500µl RNAlater (Ester no.: 17-15043-5 to 17-15043-70) |
| **Norway** | Ole-Bendik Dale | Norwegian Veterinary Institute Section for Immunology | 30.10.2017 | Antibody | 1 tube | Approx. 3 ml of Anti Bohle Irido virus (from Rabbit F53). |
| **Italy** | Anna Toffan | Istituto Zooprofilattico Sperimentale delle Venezie | 30.10.2017 | Plasmid | 1 tube | Approx. 100µl plasmid pEX-A2-IHNV APC. Diluted 100x |
| **Portugal** | Tiago Luís | Instituto Nacional de Investigação Agtária e Veterinária, IP | 06.11.2017 | BF-2 cells<br>RTG-2 cells | 2x 120ml<br>2x 120ml | 2 small flasks with BF-2 cells<br>2 small flasks with RTG-2 cells |
| **Poland** | Marek Matras | National Veterinary Research Institute, | 06.11.2017 | CCB cells | 2x 120ml | 2 small flasks with CCB cells |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 13.11.2017 | Tissue in Formalin<br>Blood | 56 tubes<br>28 tubes | Fish tissue in Formalin 4% (Ester no.: 17-15043-71 to 17-15043-126).<br>Heparin glass containing blood from Rainbow trout and Brown trout |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 13.11.2017 | Tissue in RNAlater | 168 tubes | 50-200 mg fish tissue in 500µl RNAlater (Ester no.: 17-15043-71 to 17-15043-126) |
| **Germany** | Uwe Fischer | Friedrich-Loeffler-Institut (FLI) | 13.11.2017 | Perch Rhabdovirus infected cell supernatant | 2 tubes | 2 tube (1,5 ml in each tube) with cell culture supernatant containing Perch rhabdovirus, the Reference strain of perch rhabdovirus recived from P. de Kinkelin (from France) in 1992 as “Virus de Perche”. |
| **Australia** | John Hoad | AAHL Fish Diseases Laboratory<br>CSIRO Australian | 13.11.2017 | Perch Rhabdovirus infected cell supernatant | 2 tubes | 2 tube (1,5 ml in each tube) with cell culture supernatant containing Perch rhabdovirus, the Reference strain of perch rhabdovirus recived from P. de Kinkelin (from France) in |

| Country | Name | Institute | Date of receipt | Material | Amount | Remarks |
|---|---|---|---|---|---|---|
| | | | | | | 1992 as “Virus de Perche”. |
| **Malaysia** | Marlinda Anim Bt Marham | Malaysia Ministry of Agriculture and Agro Based Industry | 24.11.2017 | PFRV<br>SVCV | 2 ampoules<br>2 ampoules | Freeze-dried fish viruses with 100 mg in each ampoul. 2 ampoules of each virus.<br>*Ampoule I, 2016*<br>Pike Fry Rhabdovirus<br>PFR<br>*Ampoule II, 2014*<br>Spring Viraemia of Carp virus (SVCV) |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 27.11.2017 | Tissue in Formalin<br>Blood | 56 tubes<br>28 tubes | Fish tissue in Formalin 4% (Ester no.: 17-15043-127 to 17-15043-182).<br>Heparin glass containing blood from Rainbow trout and Brown trout |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 27.11.2017 | Tissue in RNAlater | 168 tubes | 50-200 mg fish tissue in 500µl RNAlater (Ester no.: 17-15043-127 to 17-15043-182) |
| **Italy** | Simona Perulli | FATRO S.p.A. | 27.11.2017 | Bacterial culture | 3 tubes | Cryotube with 500 µl of E.coli (Top10) transformed with<br>• pVax-vhsG-1 (Derived from VHSV isolate DTU2015-18464-2 – Italy-67/10)<br>• pVax-vhsG-2 (Derived from VHSV isolate DTU2015-18464-2 – Italy-67/10)<br>• pVax-ihsG-2 (Derived from IHNV isolate DTU2015-18466-2 – Italy-86/5). |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 11.12.2017 | Tissue in Formalin<br>Blood | 56 tubes<br>28 tubes | Fish tissue in Formalin 4% (Ester no.: 17-15043-183 to 17-15043-238).<br>Heparin glass containing blood from Rainbow trout and Brown trout |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 11.12.2017 | Tissue in RNAlater<br>Purified RNA | 168 tubes<br>12 tubes | 168 tubes 50-200 mg fish tissue in 500µl RNAlater (Ester no.: 17-15043-183 to 17-15043-238) and 12 tubes with purified RNA from 17-15043 nr. 28, 29, 43, 54, 55, 57, 68, 99, 112, 126 - all heart and nr. 99 + 100 both spleen |
| **Norway** | Torfinn Moldal | Norwegian Veterinary Institute | 11.12.2017 | Cell supernatant intected with Aquareovirus | 3 tubes | Aqua Reovirus isolated from Atlantic Halibut in Iceland. |

| Country | Name | Institute | Date of receipt | Material | Amount | Remarks |
|---|---|---|---|---|---|---|
| | | Section for Virology | | | | 2 isolates juveniles farmed in 1999<br>1 isolate - isolated in Iceland in 2007 |
| **Norway** | Maria Dahle | Norwegian Veterinary Institute. Section for Immunology | 22.12.2017 | Plasma<br>Blood | 16 tubes<br>28 tubes | Plasma from Rainbow trout from suspected outbreak in a Danish fish farm (Ester no.: 17-20347, 1-10 post symptoms, 11-16 acute symptoms).<br>Heparin glass containing blood from Rainbow trout and Brown trout (Ester no.: 17-15043). |

# Annex 2 Reagents received in 2017

| Country | Name | Institute | Date of receipt | Material | Amount | Protocol No. | Remarks |
|---|---|---|---|---|---|---|---|
| **Norway** | Stefanie | Norwegian Veterinary Institute Veterinærinstituttet Harstad | 04.01.2017 | Organs | 32 vials | 17-477 | *Suspected flavivirus*: 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 Axén | National Veterinary Institute Section of fish | 18.01.2017 | Supernatant | 1 | 17-2629 | IPN strain for PCR and sequencing |
| **Sweden** | Charlotte Axén | 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 |
| **Faroe Island** | Kristín Baldvinsdóttir | Food and Veterinary Agency Department of Fish and Animal Diseases | 27.01.2017 | Cell supernatant | 8 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** | Lenaïg Louboutin | ANSES | 03.04.2017 | SBL cells | 1 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) |

| 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<br>PhD - Project (NIVEN) |
| **Ireland** | Neil Martin Ruane | Marine Institute<br>Fish Health Unit | 19.04.2017 | | 122 samples | 17-6222 | Survey of PRV in wild fish |
| **Norway** | Torfinn Moldal | Norwegian Veterinary Institute<br>Virology | 27.04.2017 | Fish tissue in RNAlater +<br>Homogenated tissue | 5 tubes<br>+<br>5 tubes | 17-6661 | Detection of Flavivirus |
| **Serbia** | Vladimir, Ivan Radosavljevic | Institute of Veterinary Medicine of Serbia<br>Department of Fish Diseases | 30.05.2017 | Cell supernatant<br>Purified RNA/DNA | 13 tubes<br>8 tubes | 17-8489 | Confirmation of Virus analysed for KHV, CEV, CyHV and SAV |
| **Switzerland** | Thomas Wahli | University of Bern<br>Centre for Fish and Wildlife Health | 31.05.2017 | Histology slites | 8 | 17-8515 | Originals slides for evaluation of PKD PT2017 |
| **Italy** | Anna Toffan | Istituto Zooprofilattico Sperimentale delle Venezie<br>Fish Virology Department | 14.06.2017 | Cell supernatant | 13 tubes | 17-9375 | To be included in the VHSV panel |
| **UK-England** | Irene Cano Cejas | Cefas Weymouth Laboratory | 27.06.2017 | Fish tissue + slides | | 17-9856 | AGD Proficiency Test |
| **Norway** | Hilde Sindre | Norwegian Veterinary Institute | 29.06.2017 | Infected cell supernatant | 5 tubes | 17-10023 | SAV for the Virus Bank |
| **Slovenia** | Diana Zele | University of Ljubljana, Veterinary Faculty | 30.06.2017 | Fish tissue (kidney) | 30 pools | 17-10192 | 30 pools of 5 Rainbow Trout kidneys to be checked for BKD to achieve BKD free status in fish farm |
| **Germany** | Mikolaj Adamek | Fish Disease Research Unit<br>University of Veterinary Medicine Hannover | 04.07.2017 | Fish tissue (gill)<br>gDNA | 1 tube<br>1 tube | 17-10228 | CEV positive controls |
| **The Netherlands** | Olga L.M. Haenen | Wageningen Bioveterinary Research of Wageningen UR, NRL for Fish Diseases | 11.07.2017 | Fish organs, suspension or in idexx | 6 tubes | 17-10754 | CEV positive controls |

| Country | Name | Institute | Date of receipt | Material | Amount | Protocol No. | Remarks |
|---|---|---|---|---|---|---|---|
| **The Netherlands** | Olga L.M. Haenen | 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 Axén | National Veterinary Institute Section of fish | 31.08.2017 | Supernatant | 4 tubes | 17-13337 | Identification of virus in Sturgeons |
| **Chile** | Kevin R. Maisey | Universidad de Santiago de Chile Santiago | 04.09.2017 | Anti-salmon IFN-a1 Recombinant salmon IFN-a1 | 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 Adamek | 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** | Lenaïg Louboutin | ANSES Plouzané | 22.09.2017 | SBL cells $PA_4$ cells | 1 flask 1 flask | | To have in stock |
| **Norway** | Espen Rimstad | NMBU Oslo | 28.09.2017 | Purified PRV-3 Infected blood | 1 vials 3 vial | 17-15043 | PRV PhD - Project (NIVEN) |
| **Bulgaria** | Ekaterina Mileva | NRL of Fish, Mollusc and Crustacean Disease National Research & Diagnostic Veterinary Institute | 10.10.2017 | Purified RNA Supernatant | 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) |