EURL training courses

Copenhagen September 8th -17th 2014

Hosted by the European Union Reference Laboratory for Fish Diseases

Contents

General introduction

The training courses took place in Copenhagen at DTU National Veterinary Institute, Bülowsvej 27, 2700 Frederiksberg C Denmark, from September the 8th to the 17th, 2014. Two courses were prepared, the first one, with 10 trainees, was entitled "Methods for implementation of surveillance procedures for listed fish diseases" and took place from 8th to 12th September 2014. The second course was entitled "Real-time PCR for diagnostics and surveillance of Fish Diseases" and took place in Copenhagen 15th to 17th September 2014 with 13 participants. 3 persons participated in both training courses.

The overall purpose of the training course was to provide an opportunity for the NRLs to send employees for training in techniques relevant when working with fish diseases. Staff of the EURL provided this training, together with teachers from the Danish Veterinary and Food Administration and CVI, The Netherlands. Also, knowledge-sharing and discussions between participants and teachers were important parts of the courses.

Methods for implementation of surveillance procedures for listed fish diseases.

The 5-days 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**.**
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 our institute and to drive to the FVO offices in Vejle, where we were received by Dr. Korsholm. After the training course introduction by Niccolò Vendramin and presentations on Danish surveillance plan for fish diseases held by Dr. Korsholm, the participants visited a rainbow trout farm, Vingsted Dambrug, approx 25 km from Vejle.
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 program was presented by N. Vendramin and discussed with the participants. After the introduction, a lecture on fish virology was given by prof. Niels Jørgen Olesen and the morning was concluded with the introduction to the use of Real-Time PCR protocol for demonstrating freedom of listed fish diseases in Europe by Dr. Morten Bruun. The participants were then divided into two groups. As an assignment each participant had to process the samples collected in the fish farm and test it to rule out the presence of the listed disease VHS. Further on, participants were asked to investigate if other pathogens were present in the sample. The processing of fish samples collected the day before was demonstrated before the participants were asked to do it themselves.

Day 3 and 4 were replicated so that one group could follow the cell culture part one day and the Real time PCR the day after and vice versa for the other group. With this organization it was possible for all the trainees to participate in all the practical activities that were demonstrated.
Every activity had a team of tutors in order to provide an effective support to the trainees. For the cell culture activities Niccolò Vendramin, Betina Lynnerup and Lene Gertman were assigned, while for the Real-time PCR part, Morten Sichlau Bruun, Troels Secher Rundqvist and Anemone Ojala were the tutors.
The day dedicated to cell culture 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 the day before. 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 dedicated to Real-Time PCR started with a theoretical presentation of the principles of PCR. The practical laboratory exercises started with participants being demonstrated how to purify RNA from their samples, later on each participant had the chance to process his own sample. Preparation of Mastermix was demonstrated, and one of the participants of the group would prepare the Mastermix, and mix it with the samples. Finally samples were loaded in the real-time PCR machine. While the machine was processing the samples, participants had time to have a session on troubleshooting and pitfalls in Real-time PCR. At the end of the day, results of the analysis were collected and discussed.

Day 5 was allocated to finalize the course, discuss both results obtained by the participants and different methods for performing surveillance for listed fish diseases in their countries of origin. Finally a questionnaire for the course evaluation was given and participants asked to fill it anonymously.

The methods taught were primarily focused on the protocols given in EU legislation and on the OIE guidelines from the Manual of Aquatic Animal Diseases, and included how to select proper controls, the typical pitfalls, trouble shooting, etc. As get-together, a joint dinner the second evening was included.

Real-time PCR for diagnostics and surveillance of Fish Diseases.

The 3-days course in molecular techniques and bio-informatics was devoted to lectures on relevant topics, as well as theoretical exercises.

Day 1 was dedicated to the basic PCR principles, sample preparation and validation of new assays. Lectures on the history and basic principles of PCR, and how to set up a lab and an assay to prevent contamination was given by Marc Engelsma and lectures on sample preparation, transport and storage and validation of new assays was given by Susie Sommer Mikkelsen, all with a focus on fish diagnostics. Furthermore, there was a discussion about the application of the MIQE guidelines. The

day ended with theoretical exercises covering the areas taught earlier in the day. The participants were divided into three groups and given their own rooms to encourage discussion between them. Furthermore, both teachers moved between the groups to help answer questions and provide basis for more discussion.

Day 2 was primarily about the different PCR chemistries, primers and probes and controls. Lectures on the different PCR chemistries and primer and probe design together with a live demonstration on how to create new primers was given by Susie Sommer Mikkelsen, while Marc Engelsma gave a lecture on the appropriate controls and standards for real-time PCR assays for diagnostic purposes. After the last lecture the participants were provided with theoretical exercises and encouraged to try and design their own primers on their laptops. Furthermore, both teachers moved between the groups to help and to encourage discussion.

The first part of day 3 was dedicated to lectures on thresholds, cut-off values and analysis and interpretation of results, provided by Marc Engelsma using, exemplified using actual diagnostic cases. After the coffee-break, participants were invited to visit the facility for fish experiments at the Institute together with Torsten Snogdal Boutrup. 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, but participants generally would like more theoretical exercises to learn how to implement the knowledge gained from the lectures.

Through lectures, exercises, discussions, and knowledge exchange between participants, the knowledge on real-time PCR, 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, an optional dinner event on day 2 was held.

Participant List

Name	Country	Course 1	Course 2
Anna Maria Eriksson-Kallion	Finland		X
Bartolomeo Gorgoglione	Austria	X	
Charlotte Axén	Sweden	X	
Christel Männistu	Estonia	X	
Christine Dubreuil	France		X
Daina Čavare	Latvia	X	
Dante Mateo	Canada	X	X
Dragan Brnić	Croatia		X
Eva Borzym	Poland		X
Haakon Hansen	Norway		X
Jacqueline Savage	UK	X	
Julia Jurovcikova	Slovakia		X
Laura Hawley	Canada		X
Lénaïg Louboutin	France	X	X
Lone Madsen	Denmark		X
Nadav Davidovich	Israel	X	
Nastaran Shahbzian	Iran	X	X
Simona Pileviciene	Lithuania		X
Stamatina Arfara	Greece	X	
Valentina Panzarin	Italy		X

EURL course: Methods for implementation of surveillance procedures for listed fish diseases.

Description of the course "Methods for implementation of surveillance procedures for listed fish diseases", held at the European Union Reference Laboratory (EURL) for Fish diseases, Frederiksberg (8/9-12/9 2014)

Course content:

The 5-days course is primarily based on practical work (hands on) in combination with theoretical presentations.

This year the course will focus on the comparison between diagnostic techniques for listed fish diseases evaluating pros and cons of cell culture and Real-Time PCR methods.

The first day of the course all participants will take part in a fish farm visit. The trip will be supervised by the Danish Veterinary and Food Administration, Section for Aquaculture that will provide guidelines and protocols for inspection, necropsy and sample collection. Organ material will be collected on site for processing the following days.

During the farm visit sampling procedures will be demonstrated and conducted by each participant.

Starting from day 2, theoretical class room teaching will be given on methodologies, pitfalls and troubleshooting. After the introduction, the participants will be divided into groups.

Each group will start processing the samples collected in the farm. This process will include cell culture preparation, inoculation on monolayers, observation of cell culture and assessment of cytopathogenic effect.

All cell cultures used for isolation of the listed viruses will be demonstrated and procedures will be conducted by the participants themselves.

Participants will initially be introduced to basic cell culture work: 24-well plate preparation for diagnostic purpose as well as 96-well plates for titration and flasks maintenance will be demonstrated and subsequently prepared by participants. Inoculation of diagnostic samples on cell cultures will be practiced. The CPE of different viruses will be shown and the participant will practice reading of diagnostic trays. Titration procedures will be demonstrated and afterwards participants will have the opportunity to practice by themselves; finally course participants will calculate virus titers.

The application of novel validated and accredited Real-Time PCR protocols suitable for surveillance will be demonstrated and performed by participants.

The course is dialogue-based and sufficient time will be given for discussion under way and for evaluation of test results.

Quality assurance, cleaning and disinfection etc. will be an integral part of the practical demonstrations.

The taught methods will primarily focus on the protocols given within EU legislation and OIE guidelines from the Manual of Aquatic Animal Diseases, and include how to select proper controls, the typical pitfalls, trouble shooting, etc.

A social dinner will be organized the second evening. Further details are provided in the invitation letter.

General course objectives

The course aims to provide participants knowledge on the most used cell cultures available for diagnosis of important fish viruses. The course will focus on basic cell cultivation techniques, production of cells for different purposes (IFAT, diagnostic trays, titration etc.), cell susceptibility testing, inoculation of samples and sub-cultivation procedures, reading of cell cultures (including CPE) and virus titration.

To provide the participants with knowledge on the most used methods for diagnosis of important fish pathogens. The course will focus on Real-Time PCR protocols validated for surveillance of listed fish diseases.

To understand the underlying principles of the tests and to critically review them in order to assess pitfalls and to correctly interpret them

Learning objectives

The participants that will have fully followed all the course's objectives will be able to:

Maintain, cultivate, inoculate and read most used cell lines (BF-2, EPC, CCB and ASK) for fish disease diagnostic purposes.

Prepare cell cultures for different purposes, e.g. diagnosis, IFAT and virus titration.

Inoculate and sub-cultivate diagnostic samples.

Read diagnostic trays.

Titrate virus.

Apply Real-Time PCR for surveillance purposes.

Assess pitfalls and errors in test performances and designs.

The major focus will be on the viral fish diseases using VHS as model.

The course will provide a forum where pre-knowledge, experience and examples can be discussed between participants and teachers, and hereby raise the awareness of pitfalls when using the various techniques.

Intended learning outcomes

To increase the practical and theoretical knowledge of cell culture based and bio-molecular techniques used in fish virus diagnostics. The course also aims at providing a forum where (good and bad) experiences can be discussed among participants and teachers.

The core elements

Fish cell line cultivation

Real-Time PCR

Identification and discussion of pitfalls and perform trouble shooting.

Assessment

Each day will end with a short result and discussion follow-up in order to evaluate whether the participants picked up the treated core elements. Their results will be discussed. At the end of the course a questionnaire for course evaluation will be delivered to all participants.

The course material

Protocol for the hands-on exercises, power-point presentations as well as the original scientific papers describing the applied assays/techniques is included in the course binder. The supervisors

will collect the generated results and provide shared data overviews to be starting point of discussions.

The course participants

Since course attendants can come from very different experiences, during the general introduction (day 1), researchers and technicians will be asked to introduce themselves, their pre-experience in the laboratory and their expectations to the course in order to target the course content optimally, especially during the theoretical- and discussion workshops. Their starting point will therefore be mixed as some may have limited theoretical or practical experience, while others may be highly experience in some or all disciplines.

Course supervisors

Prof. Niels Jørgen Olesen experienced in fish virology and has been working with cell cultivation, ELISA and IFAT for >15 years, in cooperation with Henrik Korsholm (>15 years of experience in aquaculture surveillance plan) will take care about the farm visit.
Morten Sichlau Brunn (DVM Phd) will be supervisor for biomolecular techniques.
Niccoló Vendramin (DVM) will be supervisor for cell culture preparation and reading.
Technical help and assistance for running the laboratory courses will be given by:
Betina Lynnerup (Cell Culture)
Lene Gertman (Cell Culture)
Christina Flink Desler (sample preparation)
Troels Secher Rundqvist (Real Time PCR)
Anemone Ojala (Real time PCR)

Program: Methods for implementation of surveillance procedures for listed fish diseases

Day 1	Day 2	Day 3	Day 4	Day 5
Section 1 **FISH farm inspection**	**Section 2** **Laboratory introduction**	**Section 3** **Cell culture facility**	**Section 4** **qPCR analysis**	**Section 5** **Discussion and evaluation**
FISH FARM VISIT in collaboration with FVST (Danish Veterinary and Food Administration, Section for Aquaculture). The participants will be introduced to the contingency plan in Denmark, practical demonstration of farm inspection will be performed and samples for surveillance will be collected by participants	9:00-10:00 Participants will be introduced to the laboratory Coffee Break 10:00-10:30 10:30-12:00 Theoretical introduction to the use of cell culture and qPCR for surveillance programs for non-exotic listed disease in Europe.	8:30-10:00 Cell culture preparation for diagnostic purpose, titration and IFAT Coffee Break10:00-10:30 10:30-12:00 Titration procedure Viral titre calculation	8:30 - 12:00 Nucleic acid purification and master mix preparation	9:00- 9:30 Reading plates prepared by participants 9:30-10:30 Presentation of results obtained by participants 10:30-12:00 Scientific discussion and recommendations. Conclusion
	Lunch 12 -12:30	**Lunch12 -12:30**	**Lunch12 -12:30**	**Lunch12 -12:30**
	12:30 – 14:30 Samples processing collected following different diagnostics procedure (Cell culture and PCR) Coffee Break 14:30-15:00	12:30- 14:30 Cell observation Coffee Break 14:30-15:00 15:00-16:30 Cell observation 2	12:30-14:30 qPCR analysis Coffee Break 14:30-15:00 15:00-16:30 PCR results to be collected and discussed	12:30-15:00 Course evaluation Last minutes questions and Good Byes Wrapping up of the course and questionnaire fill out (coffee at the tables).

Evaluation: Methods for implementation of surveillance procedures for listed fish diseases

Table 1-3 and graphs 1-3 showing participant satisfaction level considering every section.

Fish Farm Inspection	Very Low	Low	Average	Good	Very Good
Teachers preparedness				1	10
Course relevance for you				1	9
Increase of your knowledge		1		2	7
Overall opinion of course				4	6

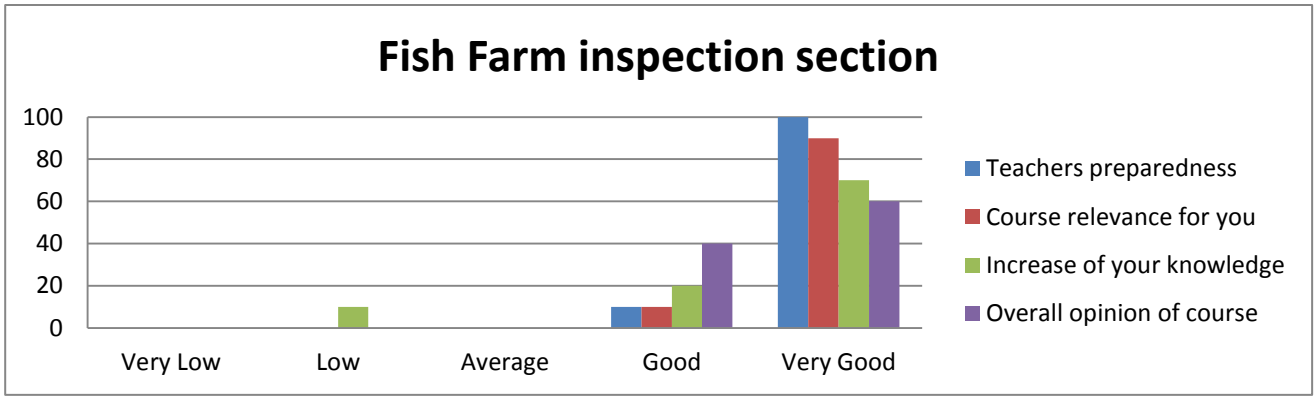
Table 1

Real Time PCR	Very Low	Low	Average	Good	Very Good
Teacher expertises			1	4	6
Teachers preparedness				5	6
Course relevance for you			1	7	2
Increase of your knowledge		1		7	3
Overall opinion of course				7	3

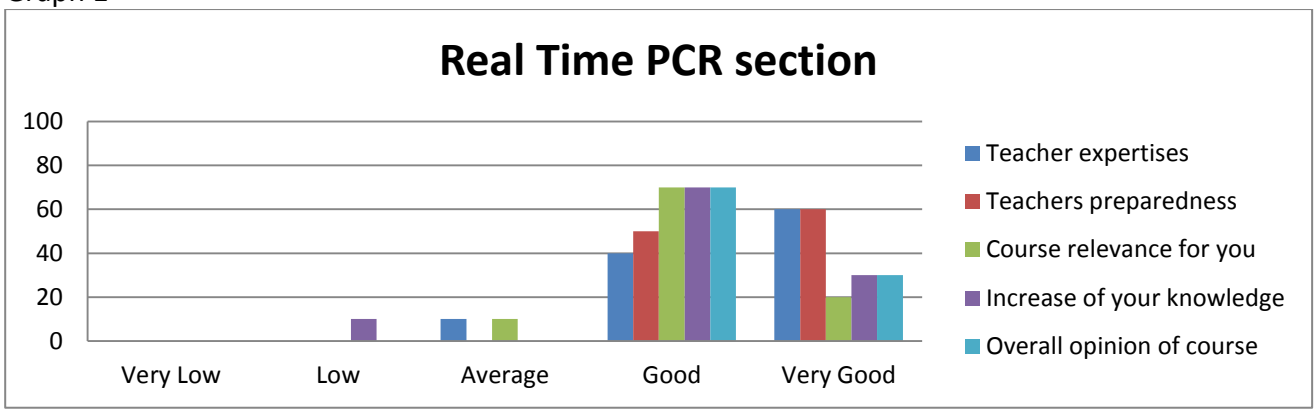
Table 2

CELL culture	Very Low	Low	Average	Good	Very Good
Teachers expertises				1	9
Teachers prepardness					9
Course relevance for you:				1	9
- Basic cell culture techiques				1	9
- Inoculation and subcultivation procedures				1	9
- Virus titration				2	8
- Reading of plates (CPE, toxic effect etc.)				1	9

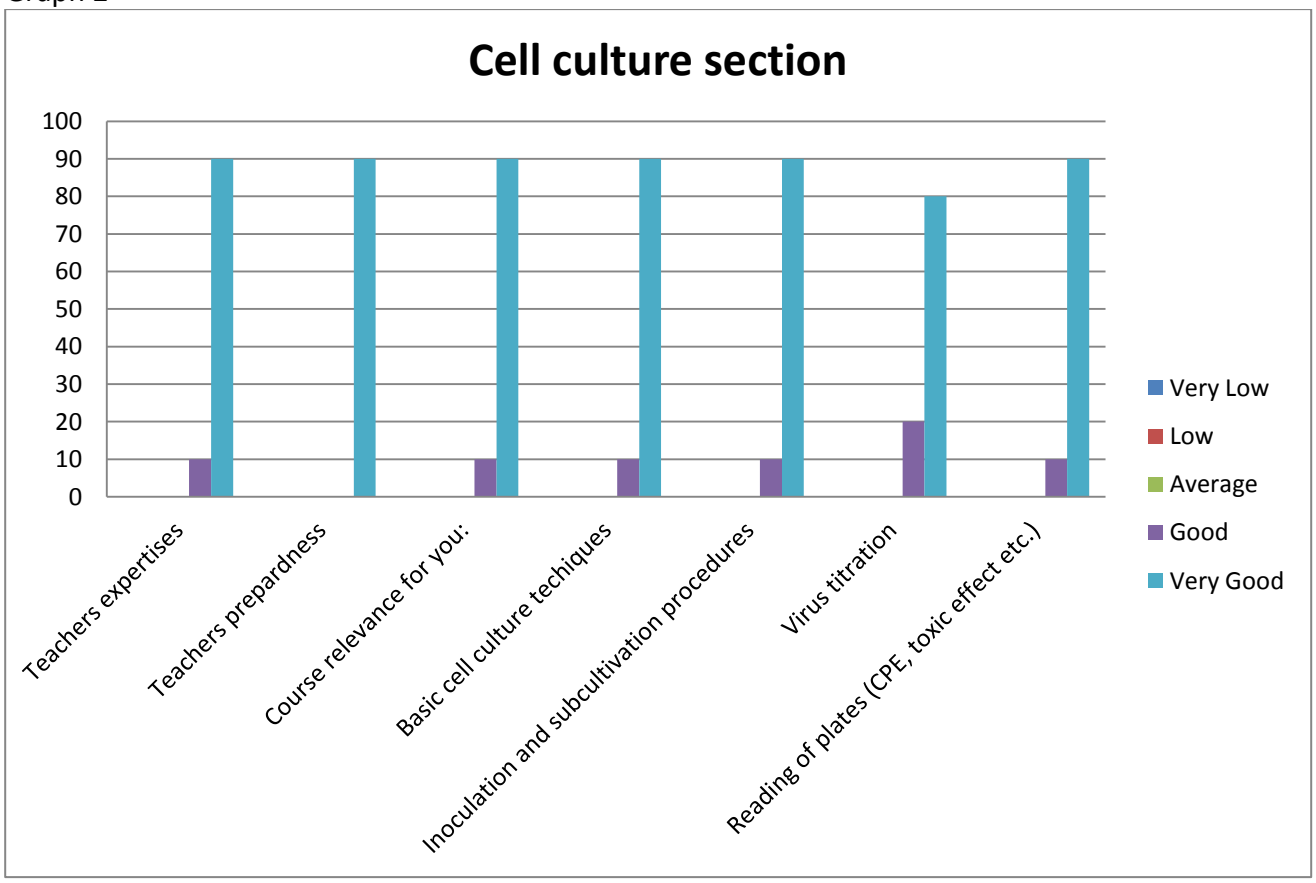
Table 3



Graph 1



Graph 2



Graph 3

EURL course: Real-time PCR for diagnostics and surveillance of Fish Diseases

Description of the course "Real-time PCR for diagnostics and surveillance of Fish Diseases", held at the European Union Reference Laboratory (EURL) for Fish diseases, Frederiksberg (15/9-17/9 2014)

Course content:
The 3-day course is primarily based on presentations in combination with theoretical exercises. There will be no practical lab work.
This year the course will focus on the real-time PCR technique used for diagnostics and surveillance of fish diseases.
The first day of the course the topics will focus on the sample preparation and validation of new real-time PCR assays for the lab. At the end of the day, the participants will be split into groups for theoretical exercises.
The second day will focus on the chemistry of real-time PCR as well as primer/probe design. The theoretical exercises will focus on what the participants has learned earlier in the day, including primer/probe design in silico.
The third day the focus will be on the analysis, interpretation and presentation of results, including familiarising the participants with the MIQE guidelines. The day will end with further theoretical exercises as well as general discussion and evaluation of the course.
A social dinner will be organized the second evening. Further details are provided in the invitation letter.

General course objectives
The course aims to provide participants knowledge on how to properly use real-time PCR in the lab for diagnostics and surveillance of fish diseases. This includes knowledge on how to validate new assays for the laboratory, different chemistries used and primer/probe design. To understand and interpret results as well as troubleshooting. To understand the underlying principles of the assays and to critically review them in order to assess pitfalls and to correctly interpret them. The course will be a mix of presentations by the teachers and theoretical exercises by the participants. The course will focus on assays for listed fish diseases.

Learning objectives
The participants that will have fully followed all the course's objectives should be able to:
Validate new real-time PCR techniques for the lab.
Design and evaluate new primer/probe sets.
Understand the basic chemistry behind PCR.
Understand how to analyse and interpret the results. Knowledge of the MIQE guidelines.
Troubleshooting.

The major focus will be on assays for listed fish diseases.
The course will provide a forum where pre-knowledge, experience and examples can be discussed between participants and teachers, and hereby raise the awareness of pitfalls when using the technique.

Intended learning outcomes

To increase the theoretical knowledge of real-time PCR for analysis of listed fish diseases in the lab. The course also aims at providing a forum where (good and bad) experiences with real-time PCR can be discussed among participants and teachers.

The core elements

Presentations of the real-time PCR technique.

Theoretical exercises by the participants.

Assessment

Each day there will be theoretical exercises by the participants that will be followed-up for discussion and evaluation. At the end of the course a questionnaire for course evaluation will be delivered to all participants.

The course material

Power-point presentations as well as the original scientific papers describing useful assays/techniques will be included in the course binder. For the theoretical exercises laptops will be necessary. Unfortunately these cannot be provided by the course responsible and the participants are required to bring their own from home. It might be necessary to download and install (free or trial) software for use in the theoretical exercises, and information of this will be provided before course start.

The course participants

Since course attendants can come from very different experiences, during the general introduction (day 1), researchers and technicians will be asked to introduce themselves, their pre-experience in the laboratory and their expectations to the course. Their starting point will therefore be mixed as some may have limited theoretical or practical experience, while others may be highly experience in some or all disciplines.

Course responsible and teacher*:*

Susie Sommer Mikkelsen (Molecular Biologist, PhD), National Veterinary institute, DTU, Denmark (susmi@vet.dtu.dk)

Teachers:

Marc Engelsma, Central Veterinary Institute of Wageningen, UR Lelystad, The Netherlands (marc.engelsma@wur.nl)

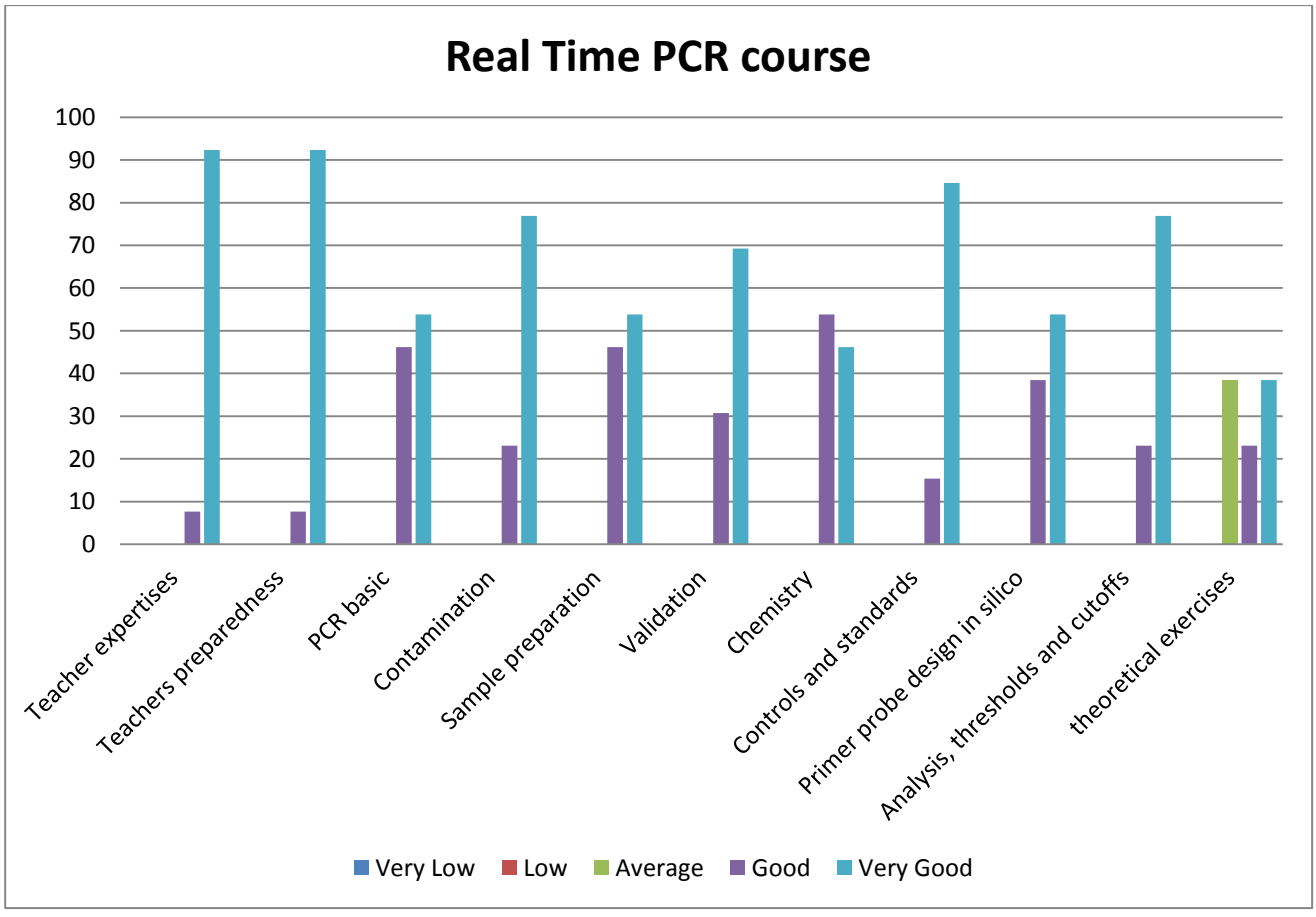
Program: Real-time PCR for diagnostics and surveillance of Fish Diseases

Day 1	Day 2	Day 3
Section 1	**Section 3**	**Section 5**
9:00-9:30 Registration and presentation of course 9:30-10:00 Presentation of participants 10:00-10:45 The basics… Coffee Break 10:45-11:15 11:15-12:00 …and beyond	9:00-10:00 Chemistry Coffee Break 10:00-10:30 10:30-12:00 PCR controls and standards	9:00-10:30 Thresholds, cut-offs and trouble Coffee Break 10:30-11:00 11:00-12:00 Fish facility visit
12:00 – 13:00 Lunch	**12:00 – 13:00 Lunch**	**12:00 – 13:00 Lunch**
Section 2	**Section 4**	**Section 6**
13:00-13:45 Sample preparation Extraction RNA/DNA 13:45-14:15 The PCR laboratory Coffee Break 14:15-14:45 14:45-15:45 Validation 15:45-17:00 Theoretical exercises RNA/DNA extraction Validation	13:00-14:00 Primer/probe design in silico Coffee Break 14:00 14:30 14:30-16:30 Theoretical exercises Primer design in silico	13:00-14:30 Theoretical exercises Analysis of results 14:30-15:00 General discussion and course evaluation 15:00 Cakes and Goodbyes

Table 4 and graph 4 showing participant satisfaction level during each section.

PCR	Very Low	Low	Average	Good	Very Good
Teacher expertises				1	12
Teachers preparedness				1	12
PCR basic				6	7
Contamination				3	10
Sample preparation				6	7
Validation				4	9
Chemistry				7	6
Controls and standards				2	11
Primer probe design in silico				5	7
Analysis, thresholds and cutoffs				3	10
theoretical exercises			5	3	5

Table 4



Graph 4

Closing remarks

The EURL training course 2014 was, based on the feedback from the participants, regarded as a success. The possibility to give financial support to participants made it possible to provide training to laboratories where the lack of funding usually makes it hard to find the resources to participate in such training courses. This way of funding the training courses therefore holds the possibility to increase the expertise in all laboratories within the EU.
Based on the experience of 2013, Training courses were provided in autumn in order to be able to clearly state which was the financial support for this activity and which applicant were entitled to obtain refunding.
Also for this year's course on Methods for implementation of surveillance procedures for listed fish diseases, it was decided to include an inspection to a fish farm, to demonstrate the whole process from sampling fish at a fish farm to final reading and interpretation of results, giving a holistic view and large span of topics that according to the evaluation schemes were well received.

DTU-Vet is acknowledged for offering training course facilities for free.
Dr. Henrik Korsholm, Veterinary and Food Administration, is deeply acknowledged for organizing the office and field visit and for teaching how to organize and implement surveillance and eradication programs and how to inspect and sample on fish farms.
Heinrich Werning, Vingsted Dambrug is acknowledged for his hospitality and for providing all information and facilities needed during the farm visit.
Dr. Marc Engelsma, Central Veterinary Institute of Wageningen, UR Lelystad, The Netherlands, is deeply acknowledged for his very enthusiastic and excellent lectures, which demanded a lot of preparation and work.
Finally all laboratory technicians and scientists in the fish diseases unit of DTU-VET are deeply acknowledged for delivering excellent teaching and training and help with practical issues.

Copenhagen, Tuesday, 21 October 2014
Niels Jørgen Olesen, Niccoló Vendramin, Morten, Sichlau Bruun, Susie Sommer Mikkelsen
EURL Fish Diseases