



EURL-Fish training course: Methods for implementation of surveillance procedures for listed fish diseases, 8th to 12th October 2018

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

Course content

This 5 day 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 by evaluating pros and cons of cell culture and Real-Time PCR methods.

At 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 in the lab the following days. During the farm visit sampling procedures will be demonstrated and afterwards 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 at 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.

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

The application of novel validated and accredited Real-Time PCR protocols suitable for surveillance will be presented and discussed with the participants. This year there will be more focus on genetic characterization of listed fish viruses with theoretical lectures and practical exercises on sequencing, Blast tool and phylogenetic analysis. For this reason each participant is required to bring his/her own laptop.

The course is dialogue based and sufficient time will be given for discussions throughout the course and for evaluation of test results. Quality assurance, cleaning, disinfection, etc. will be an integral part of



the practical demonstrations. The taught methods will primarily focus on the protocols given within the EU legislation and OIE guidelines from the Manual of Aquatic Animal Diseases, and include how to select proper controls, the typical pitfalls and troubleshooting, etc. A social dinner will be organized on the second evening. Further details are provided in the invitation letter.

General course objectives

The course aims to provide the participants with knowledge on the most used methods for diagnosis of important fish viruses. The course will focus on; 1) 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, 2) providing the participants with knowledge on the most used methods for diagnosis of important fish pathogens, 3) Real-Time PCR protocols validated for surveillance of listed fish diseases, 4) genotyping the most important viral pathogens by sequencing and blasting and 5) information on the underlying principles of the tests and how to critically review them in order to assess pitfalls.

Learning objectives

The participants that have followed all the course 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
- genotype important viral isolates by sequencing and blasting
- 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

PCR / Real-Time PCR

Sequence analysis and use of BLAST tool

Identification and discussion of pitfalls and how to perform troubleshooting



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, PowerPoint 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 with very different backgrounds, 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 experienced in some or all disciplines.

Course supervisors

Prof. Niels Jørgen Olesen (DVM, PhD) >15 years of experience in fish virology and cell cultivation, ELISA and IFAT.

Lone Madsen (DVM, PhD): Diagnostic fish bacteriology.

Argelia Cuenca Navarro (M.Sc., PhD): Molecular methods.

Nikolaj Reducha Andersen (M.Sc., PhD): Course facilitator

Technical help and assistance for running the laboratory courses will be given by

Betina Lynnerup (cell culture)

Christina Flink Desler (sample preparation)

Troels Secher Rundqvist (Real Time PCR)

Teena Vendel Klinge (Real Time PCR)



European Union Reference Laboratory for Fish Diseases

National Institute of Aquatic Resources, Technical University of Denmark

(Draft programme, subject to changes)

Day 1	Day 2	Day 3	Day 4	Day 5
Section 1 Visit to fish farm and DVFA in Jutland	Section 2 Laboratory introduction	Section 3 qPCR analysis	Section 4 Cell culture and phylogeny	Section 5 Cell culture /bacteriology and evaluation
8:00 – 11:00 Transport by car to Danish Veterinary and Food Administration in Jutland	9:00 - 10:30 Participants will be introduced to the laboratory	9:00 - 12:15 PCR and Real Time-PCR theory	9:00 - 10:00 Cell culture preparation for diagnostic purpose, titration and IFAT	9:00 - 12:10 (Split in to two teams)
11:00 – 12:15 Surveillance and control of fish diseases in Denmark	<u>Coffee break</u> 10:30 - 10:50	Results analysis	Team 1 - Cell observation	Team 2 - Fish bacteriology demonstration
	10:50 - 12:15 Theoretical introduction to the use of cell culture and qPCR for surveillance programs for non-exotic listed fish disease in Europe	<u>Coffee break</u> 10:30 - 10:50	<u>Coffee break</u> 10:30 - 10:50	Team 2 - Cell observation
		Practical exercises	10:50 - 12:15 Titration procedure, viral titre calculation	<u>Coffee break</u> 10:30 - 10:50
		PCR and Real Time PCR Troubleshooting		Team 2 - Cell observation
				Team 1 - Fish bacteriology demonstration
Lunch: 12:15 – 13:00	Lunch: 12:15 - 13:00	Lunch: 12:15 - 13:00	Lunch: 12:15 - 13:00	Lunch: 12:15 - 13:00
13:00 – 15:00 Visit to Vejen Mølle fish farm for sampling	13:00 - 15:00 Sample preparation for different diagnostics procedures (Cell culture and PCR)	13:00- 15:00 The diagnostic laboratory – PCR Flow	13:00- 13:30 Use of cell culture in fish virology	13:00 - 15:00 Scientific discussion and recommendations
15:00 – 18:00 Transport by car to hotel Cabinn	<u>Coffee break</u> 14:30 - 15:00	<u>Coffee break</u> 14:30 - 15:00	13:30 – 17:00 Blast analysis and practical exercise	Conclusion
	<u>Coffee break</u> 14:30 - 15:00	15:00 - 16:30 Sequencing theory and practical exercises	<u>Coffee break</u> 14:30 - 15:00	Course evaluation
	19:00 Social dinner		Introduction to phylogenetic analysis	