EURL for fish and crustacean diseases training course: Methods for implementation of surveillance procedures for listed fish diseases, 10th to 14th October 2022

Description of the course "Methods for implementation of surveillance procedures for listed fish diseases" held at the European Union Reference Laboratory (EURL) for fish and crustacean diseases

Course content

This 5 day course is primarily based on practical work (hands on) in combination with theoretical presentations. 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 organized in collaboration with veterinary services and aquaculture association. Protocols for inspection, necropsy and sample collection will be demonstrated. Sample collection will involve mandatory sampling for testing listed disease (virology and molecular assay) as well as testing for differential diagnosis (bacteriology). Starting from day 2, theoretical 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 accredited Real-Time PCR protocols for surveillance will be presented and discussed with the participants. Furthemore, focus will be put 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 concept of test specificity and sensitivity will be explained, and the students will learn how this affects validity of surveillance systems.

The course is dialogue-based and sufficient time will be given for discussions throughout the course and for the 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 WOAH 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 commonly used methods for diagnosis of important viral fish diseases. 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 commonly 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 and 6) epidemiological aspects to consider when designing surveillance for viral diseases in fish aquaculture.

Learning objectives

The participants that have followed all the course objectives will be able to;

- Explain the basic principles of the legislative framework for surveillance and control of listed fish disease in EU
- Describe basic principle of surveillance schemes or programs? for listed aquatic fish disease
- Sample and process material for diagnostic purpose
- 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
- understand basic principles of PCR and real time PCR techniques and their use in diagnostics and surveillance
- Genotype viral isolates by sequencing and blasting
- Troubleshoot test performances and designs.
- Demonstrate the implications of test sensitivity and specificity for surveillance programs

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

Sampling and processing fish tissue for diagnostic purpose
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 2), 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
Niccoló Vendramin (DVM, PhD), Fish virology and diagnostics
Argelia Cuenca Navarro (M.Sc., PhD): Molecular methods
Britt Bang Jensen (DVM, PhD) Epidemiology
Lone Madsen (DVM, PhD): Fish bacteriology and diagnostic
Jacob G. Schmidt (M.Sc., PhD) Course facilitator

Technical help and assistance for running the laboratory courses will be given by
Christina Flink Desler (sample preparation and cell culture)
List Christensen (cell culture)
Teena Vendel Klinge (Real Time PCR)

European Union Reference Laboratory for Fish and Crustacean 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 Veterinary sevices in Jutland**	**Section 2** **Laboratory introduction and sample preparation**	**Section 3** **PCR analysis and Cell culture methods**	**Section 4** **PCR, blast and phylogeny**	**Section 5** **Cell culture /bacteriology and evaluation**
8:00 – 11:00 **Transport by car to Danish Veterinary and Food Administration, in Jutland.** **11:00 – 12:15** **Introduction to surveillance programs** **Aquaculture surveillance and sampling procedures in Denmark**	**9:00 - 10:30** **Introduction and practicalities. Participants experience and expectations** ***Coffee break 10:30 - 10:50*** **10:50 - 12:15** **Theoretical introduction to sample preparation, cell cultivation, virus ID and qPCR for surveillance programs for the non-exotic listed fish disease in Europe**	**9:00 – 10:30 : PCR and real time PCR theory.** ***Coffee break 10:30 - 10:50*** **10:50 - 12:15** **Result analysis** **Practical exercises.**	**9:00 - 10:30** **PCR and Real Time PCR Troubleshooting.** **The diagnostic laboratory – PCR flow.** ***Coffee break 10:30 - 10:50*** **Sequencing theory and practical exercises**	**9:00 - 10:30** **Cell culture observation with different reference viral isolates** ***Coffee break 10:30 - 10:50*** **10:50-12:15** **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 – 13.30 Transport to Fish Farm **13:30 – 15:30** **Inspection and sampling** **15:30 – 19:00** **Return to Hotel**	**13:00 - 14:30** **Sample preparation for cell culture, PCR and bacteriology on samples collected Monday** ***Coffee break 14:30 - 14:45*** **14:45- 16.45 Practical cell culture passaging and production of 24 well plates** **19:00 -Social dinner**	**13:00- 16:30** **13:00-13:45 Reading cells and inoculation of samples** **13:45-14:30 Use of cell culture in fish virology** ***Coffee break 14:30 - 15:00*** ***15:00-16:00*** **Titration procedure, viral titre calculation. Barcoding cell lines**	**13:00 – 17:00** **Blast analysis and practical exercise** ***Coffee break 14:30 - 15:00*** **Introduction to phylogenetic analysis**	**13:00 – 14:45** **Assignment + presentation and assessment of data obtained by each group** **Discussion and recommendations** **Conclusion** **14:45-15:00** **Course evaluation, coffee and goodbyes**