EURL for fish and crustacean diseases training course: Methods for implementation of surveillance procedures for listed fish diseases, 7th to 11th October 2019

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. 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 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;

- 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
- 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

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
Prof. Niels Jørgen Olesen (DVM, PhD) Diagnostic fish virology
Lone Madsen (DVM, PhD): Diagnostic fish bacteriology.
Argelia Cuenca Navarro (M.Sc., PhD): Molecular methods.
Niccoló Vendramin (DVM, PhD), Fish diagnostics
Jacob G. Schmidt (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)
Teena Vendel Klinge (Real Time PCR)

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 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, DFVF Vejen in Jutland.** **Start in front of Cabinn Hotel** **11:00 – 12:15** **Aquaculture surveillance and sampling procedures in Denmark, By DVM Morten Fruergaard, DFVF. Control of VHS in DK by NJ Olesen**	**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 - 12:10** **Team 1,2 and 3 - Cell observation** **Team 4, 5 and 6 - Fish bacteriology demonstration** ***Coffee break 10:30 - 10:50*** **Team 4, 5 and 6 - Cell observation** **Team 1,2 and 3 - 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 Hesselho Fish Farm **13:30 – 15:30** **Inspection and sampling** **15:30 – 19:00** **Transport by car to Hotel Cabinn, Frederiksberg**	**13:00 - 14:30** **Team 1,2 and 3: Sample preparation for cell culture, PCR and bacteriology on samples collected Monday** **Team 4, 5 and 6: Practical cell culture passaging and production of 24 well plates** ***Coffee break 14:30 - 14:45*** **14:45- 16.45 Change** **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**