EURL-Fish training course: Methods for implementation of surveillance procedures for listed fish diseases. Copenhagen 5th to 9th October 2015

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 (5/10-9/10 2015)

Course content:

The 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 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 by tutors and each participant will have the possibility to try them.

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 practised. The CPE of different viruses will be shown and the participants 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 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.

Susie Sommer Mikkelsen (Molecular Biologist, 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)

Christina Flink Desler (sample preparation)

Troels Secher Rundqvist (real- time PCR)

Didde Hedegaard Sørensen (sample preparation)

Teena Vendel Klinge (real-time PCR)

European Union Reference Laboratory for Fish Diseases

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Draft programme (subject to changes)

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 Goodbyes** **Wrapping up of the course and questionnaire fill out (coffee at the tables).**