EURL training course 2012

Molecular techniques for identification of listed fish diseases (week 4)

General Virology (week 5)

Aarhus,
24/1-3/2 2012

Hosted by the European Union Reference Laboratory for Fish Diseases

Contents

General introduction

The training course took place at DTU Veterinary, Hangøvej 2, DK-8200 Aarhus N, 24/1-3/2 2012. The course was divided in two parts where one or both parts could be followed. Part 1 "Molecular techniques for identification of listed fish diseases" took place 24/1-27/1 and 12 persons participated. Part two "General Virology" took place 30/1-3/2 and 9 persons participated. 4 persons participated in both parts of the training course.

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, but also knowledge sharing between participants was prioritised so that everyone could learn from good and bad experiences of all participants. This year financial support from the EURL was given to several of the course participants so that also staff from laboratories with low budgets was given the chance to receive training.

The 4-day course in molecular techniques was equally devoted to hands-on laboratory work and theoretical workshops.
The experimental work was based on two realistic case stories where molecular based diagnostic methods were used for identification and characterisation of different EC- and OIE listed fish viruses. Participants performed manual purification of viral RNA and detected the viral agent by RT-PCR. In addition, robot based DNA purification was demonstrated, and participants subsequently performed real time RT-PCR. Gel electrophoresis, purification of PCR products, quantification of the concentration, and sample preparation for sequencing was also included in the hands-on laboratory work.
The techniques used during the practical experiments as well as the participants pre-experience were the starting points of the theoretical workshops. Through lectures, exercises, discussions, and knowledge exchange between participants, the knowledge on molecular techniques and troubleshooting related to these was increased. The facilitated discussions and exercises included focus on the EC/OIE recommended protocols, how to select proper controls, the typical pitfalls, and trouble shooting, retrieving genetic information from relevant databases, and performing phylogenetic analysis of selected sequence data.
As get together, a joint dinner the first evening was included, while an optional dinner event on day 3 was held.

The 5-day course in general virology was primarily based on practical work (hands on) in combination with theoretical presentations.
During the introduction to the course the participants were divided into small groups of 2. As an assignment each group received 2 blinded ampoules containing lyophilized putative fish pathogenic viruses to be identified during the course. All cell culture based and immunochemical methods used for isolation and identification of these viruses was demonstrated and conducted by the participants themselves.
Each group were initially introduced to basic cell culture work, and then produced their own flasks, 24 well trays, and 96-well plates for titration and immunoflourescence. The participants were then introduced to cell freezing- and thawing procedures followed by mycoplasma testing. Inoculation of diagnostic samples on cell cultures was practised. The CPE of different viruses was shown and the participant practised reading of diagnostic trays. Titration procedures was demonstrated for the participants and practised by themselves and titre calculation was practised. Medium production, cell sensitivity tests and test of calf serum batch before general use in cell medium was discussed.

Concerning ELISA and immunofluorescence each group designed and performed the practical testing in order to be able to identify the distributed virus isolates, following theoretical class room teaching on methodologies, pitfalls and error findings.
The course was dialogue based and sufficient time was given for discussion under way and for evaluation of test results.
In addition each group received a collection of slides for studying characteristic IFAT results of ISAV, KHV and other relevant viruses.
Concerning IHC the participants were taught basic methodologies and was given the opportunity to take part in practical performance for staining (like PAP ringing, microwave treatment etc.). A slide collection was distributed to each group for studying pathology and staining patterns in fish tissues subjected to differences in handling during sampling and staining.
Quality assurance, contamination, cleaning and disinfection etc. was an integral part of the practical demonstrations.
The methods teached 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.

Participants

Name	Country	Mol. Tech.	Gen. Vir.
Vera Deme	Bulgaria	x	x
Petya Orozova	Bulgaria	x	
Tiago Miguel Baeta Luís	Portugal	x	x
Athanasios Prapas	Greece	x	
Eleni Papalexiou	Greece	x	x
Thierry Morin	France	x	x
Siiri Poldma	Estonia	x	
Ülle Pau	Estonia		x
Laura Sneitz	Finland		x
Michelle Geary	Ireland	x	
Magdalena Stachnik	Poland		x
Eva Blomkvist	Sweden		x
Rita Granta	Latvia	x	
Kirsten Liland Bottolfsen	Norway	x	
Xu Ye	China		x
Hanne K Nilsen	Norway	x	
Thomas Wahli	Switzerland	x	

Course description - Molecular techniques for identification of listed fish diseases

4-day course at the EURL laboratories at DTU-Vet, Section for Fish Diseases, Hangøvej 2, DK-8200 Aarhus N, Denmark

24/1-27/1 2012

Overall objective:
To increase the knowledge of molecular techniques used in fish diagnostics. Furthermore, the course aimed at providing a forum where knowledge and experience could be discussed between participants and teachers.

Learning aims:
The aim was that participants of the course should be able to more critical evaluate their work when performing molecular techniques for diagnosis of fish diseases. This was achieved both by hands on laboratory work and theoretical sessions.

Course content:
The two first days of the course was mainly based on hands on laboratory work. Participants performed DNA/RNA purification (manually as well as using magnapure robot), conventional RT-PCR, gel electrophoresis, PCR product purification, sending for sequencing, and real-time PCR.
The last two days of the course was a theoretical mini-workshop. Through teaching, exercises, discussions, and knowledge exchange participants increased their theoretical knowledge on molecular techniques and troubleshooting related to this.

Course responsible: Søren Peter Jonstrup.
Teachers:
Maj-Britt Christophersen, Technician, mbch@vet.dtu.dk
Marianne Lajer, Technician, mlaj@vet.dtu.dk
Katja Einer-Jensen, PhD, Molecular Biologist, kaei@vet.dtu.dk
Søren Peter Jonstrup, PhD, Biologist, spjo@vet.dtu.dk

Program
(Time table is only guiding)
(Morning and afternoon coffee breaks will appear when possible)

24/1	25/1	26/1	27/1
8.30-9.00 Registration 9.00-10.30 General introduction to the course, safety in the lab, etc. 10.30-12.00 DNA/RNA purification.	9.00 – 12.00 Agarose gel electrophoresis Real-time PCR	8.30-9.00 Registration 9.00 – 12.00 Theoretical workshop. Focus on purification, PCR, Real-time PCR, proper controls and behaviour, trouble shooting, recommended protocols etc.	9.00 – 12.00 Theoretical workshop (continued). Focus on database, sequencing, alignment and simple phylogeny
12.00 - 12.30 Lunch	12.00 - 12.30 Lunch	12.00 - 12.30 Lunch	12.00 - 12.30 Lunch
12.30 – 15.30 DNA/RNA purification (continued). Conventional PCR setup 15.30-16.00 Follow up on work done	12.30 – 15.30 Purification of PCR product and sending for sequencing. Real-time PCR (continued) 15.30-16.00 Follow up on work done	12.30 – 16.00 Theoretical workshop (continued). Focus on purification, PCR, Real-time PCR, proper controls and behaviour, trouble shooting, recommended protocols etc.	12.30 – 16.00 Theoretical workshop (continued). Focus on database, sequencing, alignment and simple phylogeny

Evaluation of Molecular techniques for identification of listed fish diseases

Overall evaluation scheme for the Molecular Techniques course

	Very low	Low	Average	Good	Very good
Teachers expertises	0.0%	0.0%	0.0%	0.0%	100.0%
Teachers preparedness	0.0%	0.0%	0.0%	16.7%	83.3%
Course relevance for you*	0.0%	0.0%	0.0%	18.2%	81.8%
Increase of your conventional PCR knowledge	0.0%	0.0%	8.3%	25.0%	66.7%
Increase of your real-time PCR knowledge	0.0%	0.0%	16.7%	25.0%	58.3%
Increase of your sequencing and phylogeny knowledge	0.0%	0.0%	16.7%	16.7%	66.7%
Overall opinion of course	0.0%	0.0%	0.0%	0.0%	100.0%

* only 11/12 answers

What did you find good about the course	
P20	• Discussions and presentations
P21	• Selection of topics • Division between practical and theory • Overall atmosphere, engagement of the teachers
P22	• Very interactive • A practical and a theoretical part • Very friendly, a lot of exchange and discussion • Very clear
P23	• A lot of useful knowledge
P24	• The possibility to both improve practical skills and our theoretical knowledge at the same time
P26	• The practical work at the lab
P27	• Socially very nice! • To meet people with the same king of work. • Getting insight in/learn to move further on academically.
P28	• Very good interaction amongst the teachers and participants. • Thank you very much for the very relevant topics. • I improved my knowledge about fish viruses diagnostics and also overall knowledge about laboratory work.
P29	• Open-minded and good mood ambience
P30	• Good explanations. Very clear delivery of all topics. Great experience and lab time organised well to show + experience as much as possible.
P31	• Very good teachers! And very hospitable employees are working in your institute
P20	• Better arrangement of the time
Suggestions for improvements	
P21	• Probably for some topics additional handouts
P22	• Perhaps make one course only focused on sequencing and phylogeny
P23	• Everything was perfect
P27	• More time for sequencing and phylogeny knowledge
P28	• It would be nice to have a course more concentrated on phylogeny.

P30	• A little more time spent on sequencing + analysis would have been great.
P31	• Keep your course! Everything was perfect :o)
Further Comments	
P21	• Follow up course on specific topics (e.g. phylogeny work)
P22	• I will come back. Thank you very much!
P23	• The training might be a day longer, so to include some more subjects to learn and discuss

Course description - General Virology

5-day course at DTU Vet, Section for Fish Diseases, Hangøvej 2, DK-8200 Aarhus N, Denmark (40 hours)

Jan-Feb 2012

Overall objective:

I. To provide participants knowledge on the most used cell cultures available for diagnosis of important fish pathogens. The course focused on basic cell cultivation techniques, production of cells for different purposes (IFAT, diagnostic trays, titration etc.), freezing and thawing of cells, mycoplasma testing, cell susceptibility testing, inoculation of samples and subcultivation procedures, reading of cell cultures (including CPE) and virus titration
II. To provide participants knowledge on the most used immunochemical methods used for diagnosis of important fish pathogens. The course focused on ELISA, immunofluorescens and immunohistochemistry.

Learning aims:

The participants that fully have followed all objectives of the course will be able to:
Take care of the most used cell cultures (BF-2, EPC, CCB and ASK) in a cell culture bank.
Freeze and thaw cells.
Produce cells for different purposes, e.g. diagnosis, IFAT and virus titration.
Inoculate and subcultivate diagnostic samples.
Read diagnostic trays.
Titrate virus.
Design, perform and assess results of IFAT.
Design, perform and assess results of ELISA.
Design, perform and assess results of IHC for detection of in situ presence of pathogens.
Be able to assess pitfalls and errors in test performances and designs.
Focus will be on the listed diseases.

Course content:

Participants were divided into smaller groups.
Each group were introduced to basic cell culture work. The course was based on practical work (hands on). The participant produced their own 24 well trays and flasks and passage cells. The participant also froze down cells and thawed them again. Mycoplasma testing by Hoechst DNA dyeing was introduced and the participants were given positive and negative slides for identification. Inoculation of diagnostic samples on cell cultures was also practised. The CPE of different viruses was shown and the participant practised reading of diagnostic trays. Titration procedures was demonstrated for the participants and practised by themselves and titer calculation was practised. Medium production, cell sensitivity tests and test of calf serum batch before general use in cell medium was discussed.
Each group received blinded ampoules containing putative fish pathogenic viruses, to be identified by ELISA and/or IFAT and for inoculation on cell cultures.

Each group performed the practical testing following theoretical class room teaching on methodologies, pitfalls and error findings.
The course was dialogue based and sufficient time was given for discussion under way and for evaluation of test results.
In addition each group received a collection of slides for studying characteristic IFAT results of ISAV, KHV and other relevant viruses.
Concerning IHC the participants were taught basic methodologies and were given the opportunity to take part in practical performance for staining (like PAP ringing, microwave treatment etc.). A slide collection was distributed to each group for studying pathology and staining patterns in fish tissues subjected to differences in handling during sampling and staining.
Quality assurance, contamination, cleaning and disinfection etc. was an integral part of the practical demonstrations.

Course responsible: Niels Jørgen Olesen.

Teachers:
Helle Frank Skall, PhD, DVM (hfsk@vet.dtu.dk). Topic: cell cultivation
Niels Jørgen Olesen, professor, PhD, DVM (njol@vet.dtu.dk). Topic: Cell cultivation and related procedures, ELISA
Torsten Snogdal Boutrup, PhD, DVM (tosb@vet.dtu.dk). Topic: IHC
Niels Lorenzen, PhD, (nilo@vet.dtu.dk). Topic: IFAT, Monoclonal antibody production
Ellen Lorenzen, PhD, (ello@vet.dtu.dk). Topic: IHC
Jette Mølgaard, technician (jetm@vet.dtu.dk). Topic: cell cultivation
Marianne Lajer, technician (mlaj@vet.dtu.dk). Topic: cell cultivation, IFAT
Mette Eliassen, technician (meel@vet.dtu.dk**).** Topic: ELISA, titration, cell culture inoculation
Nicole Nicolajsen, technical engineer (nnic@vet.dtu.dk**).** Topic: IHC

Program

Day 1 Monday	Day 2 Tuesday	Day 3 Wednesday	Day 4 Thursday	Day 5 Friday
8.30-9.00 Registration. **9.00-9.30** Welcome, introduction to the course, safety in the lab, quality assurance, grouping etc. (coffee at the tables). **9.30-12.00** **Basic cell culture techniques, production of 24 well plates and cells for IFAT.** **Freezing of cells** The procedure for freezing of cells will be demonstrated. Each person will prepare their own cells for freezing. **Mycoplasma testing, setup** The procedure will be demonstrated and each person will set up their own test.	**8.30 – 12.00** **Thawing of cells** The procedure will be demonstrated and each person will revive their own frozen cells. **Inspection of produced 24 well plates and flasks from day 1** The plates will be inspected in the microscope and the health of the cells will be discussed. **Inoculation of samples and subcultivation procedures** The procedure will be demonstrated and each person will inoculate their own plates using samples from ELISA testing.	**8.30-9.30** **Inspection of thawed cell** The plates will be inspected in the microscope and the health of the cells will be discussed. **Inspection of inoculated cells** The plates will be inspected in the microscope and the health of the cells will be discussed. **9.30-10.00** **Coffee break** **10.00-12.00** **Mycoplasma staining and reading** Demonstration of staining. Positive and negative slides will be inspected in the microscope.	**8.30-12.00** **IFAT** Fixation of plates. IFAT staining and reading. Discussion of results. **Coffee break when it fits**	**8.30 – 9.30** **Immunohistochemistry** Demonstration of IHC procedure. **9.30-10.00** Coffee break **10.00 – 12.00** **Immunohistochemistry** Self study of tissue slides. **Virus on cells** Self study of 24 well trays inoculated with virus and titration plates.
12.00 - 13.00 Lunch	**12.00 - 13.00 Lunch**	**12.00 - 13.00 Lunch**	**12.00 - 13.00 Lunch**	**12.00 - 13.00 Lunch**
13.00 – 14.45 **ELISA** ELISA theory. In lab: Bench	**13.00-15:00** **ELISA** Washing, blocking and inoculation of	**13.00 – 16.00** **Titer calculation** Titer calculation will be	**13.00 – 15.00** **Immunohistochemistry** Theory of IHC design and optimization of	**13.00 - 14.30** **Evaluation** **Last minutes questions and Good Byes**

work. Design and start ELISA for ID of content in each of four ampoules (coating of trays done beforehand). **14.45-15.15** **Coffee break** **15.15-17.00** **IFAT** IFAT Theory. Bench design and preparation of test. Demonstration and study of ISA-, KHV-, RANAV- and other relevant IFAT staining properties IFAT	following layers. Finishing staining and reading **15.00 - 15.30** **Coffee break** **15.30-17.00** **Virus titration** Virus titration will be demonstrated. Each person will titrate their own virus. Inoculation of content in ampoules onto cell cultures for IFAT. **Virus CPE (VHSV, IHNV, IPNV, EHNV)** Plates infected with different viruses will be provided. The plates will be inspected and the different kinds of CPE will be discussed.	demonstrated. Each person will be given titration results for own calculation of titer. **Media for cell cultivation** The production of media will be shown by a PowerPoint presentation. **Cell susceptibility testing** **and serum testing** Theoretical presentation of how we perform cell susceptibility tests and test of serum used for cell culture. **Coffee break** **when it fits**	procedures. **15.00-15.30** **Coffee break** **15.30 – 16.30** **Immunohistochemistry** Tour at the histology lab and pre-treatment of tissue slides.	Wrapping up of the course and questionnaire fill out (coffee at the tables).

Evaluation of General Virology

Evaluation scheme for the IMMUNOFLUORESCENCE course / %

	Very low	Low	Average	Good	Very good
Teachers expertises					100
Teachers preparedness				50	50
Course relevance for you				38	63
Increase of your knowledge				25	75
Overall opinion of course				13	88

Evaluation scheme for IMMUNOHISTOCHEMISTRY course

	Very low	Low	Average	Good	Very good
Teachers expertises				0	100
Teachers preparedness			10	38	63
Course relevance for you			24	38	38
Increase of your knowledge				25	75
Overall opinion of course				25	75

Evaluation scheme for ELISA course

	Very low	Low	Average	Good	Very good
Teachers expertises				0	100
Teachers preparedness			13	13	75
Course relevance for you				25	75
Increase of your knowledge				25	75
Overall opinion of course				13	88

Evaluation scheme for CELL course

	Very low	Low	Avarage	Good	Very good
Teachers expertises					100
Teachers prepardness					100
Course relevance for you:					
Basic cell culture techiques			14	29	57
Freezing/thawing of cells			14	29	57
Mycoplasma testing			0	0	100
Inoculation and subcultivation procedures			0	29	71
Virus titration			0	43	57

Reading of plates (CPE, toxic effect etc.)			14	14	71
Production of cell culture medium			43	29	29
Cell susceptibility test and serum test			14	29	57

What did you find good about the course:

-very good expertice of the teachers.
-very rich : a lot of methods described
-an interesting practical part
-a lot of interaction between teachers and participants.
-very friendly
-many useful advices for my job.

Suggestions for improvements:

-maybe training materials in electronic version before starting the course.
-more time for some of the methods, especially for CPE reading and IFAT reading.

Closing remarks

The EURL training course 2012 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. Unfortunately the courses were not funded specifically and we therefore reduced the cost by withdrawing daily allowances and by only reimbursing flight tickets and we thereby managed to keep the cost within our budget to the EURL Fish Diseases.

The European Union Commission is acknowledged for their financial contribution and technical support to the training courses.
DTU-Vet is acknowledged for offering training course facilities for free.
Secretary Eva Haarup Sørensen, DTU-Vet is acknowledged for her excellent help with all financial and many practical issues.
All laboratory engineers and scientists in the 2 fish diseases units of DTU VET are deeply acknowledged for delivering excellent teaching and training.

Aarhus, 10.01.2012
Niels Jørgen Olesen
EURL Fish Diseases