

EURL Training Course on Methods for implementation of surveillance procedures for listed fish diseases

Copenhagen, October 11th - 15th 2021

Hosted by the European Union Reference Laboratory for Fish and Crustacean Diseases

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General introduction

The training course on "Methods for implementation of surveillance procedures for listed fish diseases" was organized by the EURL for Fish and Crustacean Diseases located in Kgs. Lyngby at DTU Aqua, Denmark, from October the 11th to the 15th, 2021. Also this year due to the Covid-19 pandemic and related travel restrictions it was only possible to organize a virtual course based on the Zoom platform. The course hosted 25 participants from 15 different countries. Thanks to the organization of the course on a virtual platform we could allow the participation of almost twice the number of participants compared to when the course is organized physically.

The overall purpose of the training course was to provide an opportunity for the NRLs to train in techniques relevant when working with listed fish diseases. The EURL team at DTU Aqua provided the teaching and teaching material for the course. Knowledge-sharing and discussions between participants and teachers were important parts of the course.

Methods for implementation of surveillance procedures for listed fish diseases

The five-day course in "Methods for implementation of surveillance procedures for listed fish diseases" was divided in 5 sessions. The majority of the teaching was based on presentations and discussion with the participants, but for specific sessions it was possible to practically demonstrate the procedures while working group activities were conducted using the breakout rooms function of zoom.

Day 1 was dedicated to legislation and implementation of surveillance plans.

After a welcome and introduction to the course in its new virtual version, all participants were asked to give a short presentation of themselves. Afterwards prof. Niels Jørgen Olesen gave a presentation of the legislative framework for surveillance and control of listed fish diseases. During the presentation, participants were asked to answer some answers using polls function of zoom.

Afterwards a presentation on the recent outbreak of Infectious Haematopoietic Necrosis occurring in 2021 in Denmark was given.

Day 2 was allocated to sampling procedures for surveying listed fish diseases. In the first part of the session a lecture was given by Niccoló Vendramin (EURL coordinator for fish diseases) on criteria and requirements on how to conduct surveillance for listed fish diseases. This part of the session aimed at being interactive engaging all participants in active discussions to share questions and experiences. After a break, a practical demonstration of fish anatomy was given combining two different webcams to show the sampling procedures from different angles. Later on the laboratory procedures to process fish tissues for virological examinations on cell culture was demonstrated. The practical demonstration was performed with the contribution of Laboratory Technician Christina Flink Desler.

Day 3 This day was allocated to present and discuss the use of classical virological examinations on cell cultures.

Initially a demonstration of the inoculation of samples processed for virological examination on day 2 was given. Later on a presentation on the use of fish cell lines was given, again aiming at engaging all the participants in actual discussion on the topic.

After a break, a practical demonstration of various cytopathic effect on different cell culture lines was presented. A set of 6 fish viruses comprising VHSV; IHNV; IPNV; EHNV; Nodavirus (VNNV) was prepared on different cell culture lines. By coupling the microscope camera on zoom platform and sharing screen it was possible for all participants to visualise the effect of different viruses directly on the monitor of their PC.

Day 4 was tutored by senior scientists Argelia Cuenca and Morten Schiøtt. This day was fully dedicated to qPCR methods and molecular methods for surveying listed fish diseases. A lecture including both general concepts of molecular biology and specific information on the diseases targeted in the surveillance and the methods that shall be applied was given by the tutors. A final session explained how BLAST works and how to interpret BLAST results, including a practical exercise and discussions.

Day 5 the participants were divided into four groups. Each group was located in a separate breakout room and was given a task. The task included a scenario specific for each of the listed diseases, group members were given access to relevant material such as the diagnostic manuals of EU and OIE. Each group had to appoint a rapporteur and a chairperson to steer the discussion, and answer all the questions included in the task.

This first part of the exercise lasted approx. 1 hour. After a short break, all the groups were divided into new ones, in a way that each of these new group included a representative for each disease.

The assignment for this second session was to present the findings to all the other members of the group and engage in discussions. This second session lasted approx. 40 minutes.

In both sessions Niels Jørgen Olesen and Niccoló Vendramin assisted the break out rooms if there were queries etc.

Finally all the participants were gathered in a general assembly and the different questions were discussed and answered to ensure a homogeneous transfer of knowledge to all participants.

Participants were then asked to fill a google form for evaluating the course and provide inputs for improvement.

The methods taught were primarily focused on the protocols given in the EU legislation and on the guidelines from the OIE Manual of Aquatic Animal Diseases, and included how to select proper controls, the typical pitfalls, troubleshooting, etc.



Participant list

| Training course 2021 : Methods for implementation of surveillance procedures for listed diseases | | | | | | | |
|--|--------------------|------------------------------|----------------|---|--|--|--|
| Name | Surname | Email | Country | Institution | | | |
| Petya | Stoyanova | pstoyanova05@gmail.com | Bulgaria | National Diagnostic Research Veterinary Institute | | | |
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| Kateřina | Mikulášková | mikulaskova@svujihlava.cz | Czech Republic | Department of Virology, State Veterinary Institute Jihlava | | | |
| Neira | Dedic | <243742@mail.muni.cz> | Czech Republic | Masaryk University | | | |
| Lydie | Canier | lydie.canier@ifremer.fr | France | Ifremer | | | |
| Fiona | Swords | fiona.swords@marine.ie | Ireland | Fish Health Unit, The Marine Institute | | | |
| Thorunn | Soley Bjornsdottir | thorunnsoley@hi.is | Island | The Institute for Experimental Pathology University of Iceland KELDUR | | | |
| Žanete | Zommere | zanete.zommere@bior.lv | Latvia | Institute of Food Safety, Animal Health and Environment "BIOR", Latvia | | | |
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| Marianne | Kraugerud | mariannekraugerud@zoetis.com | Norway | Pharmaq Analytiq | | | |
| William | Leigh | william.leigh@gov.scot | Scotland | Marine Scotland Science | | | |
| Miriam | Macekova | miriam.macekova@svpu.sk | Slovakia | State Veterinary and Food Institute | | | |
| Katarina | Pavlin | katarina.pavlin@vf.uni-lj.si | Slovakia | Faculty of Veterinary Medicine, Institute of Pathology, Wild Animals, Fish and Bees | | | |
| Sabina | Šturm | sabina.sturm@vf.uni-lj.si | Slovenia | Institute for Pathology, Wild Animals, Fish and Bees, Veterinary faculty of the University of Ljubljana | | | |
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| | | | | Republic of Turkey Ministry of Agriculture and | |
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| | | | | Directorate of İzmir - Bornova Veterinary Control | |
| | | | | Institute / Virology Department / Fisheries | |
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| | | | | Izmir/Bornova Veterinary Control Institute | |
| | | | | (Virology Department, . Viral Fish Diseases National | |
| Kemal | Pekmez | kemalpekmez07@hotmail.com | Turkey | Referans Lab | |
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| Murat | Kaplan | kaplanmurat10@gmail.com | Turkey | Izmir/Bornova Veterinary Control Institute | |
| | | | | Bornova Veterinary Control Institute (İzmir/Turkey) | |
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Course Announcement: Methods for implementation of surveillance procedures for listed fish diseases

Lyngby 13.07.2021

Dear colleagues,

As announced at the last Annual workshop for national reference laboratories for fish and crustacean diseases, the travelling uncertainties related to the Covid-19 pandemic have led us to convert the EURL training courses in week 41 and 42 as online courses. This year we will organize two courses "Methods for implementation of surveillance procedures for listed fish diseases" and will be held in week 41 from Monday the 11th to Friday the 15th of October 2021

"Introduction to histopathology in fish and crustacean diseases" and will be held in week 42 from Monday the 18th to Friday the 22nd of October 2021

Programme

The preliminary course programs foresee daily sessions, each session will include lectures, as well as quiz, tasks and work in small groups organized in separate online rooms.

The course contents will reflect the structure of the live courses helded in 2019, with the necessary updates for the changes occurred in the legislative framework (new animal health law implemented on 21st of april 2021) and with the changes necessary to held the courses online.

For the course in week 41, the topics of the 5 sessions will cover the topics normally included in the program for the training course in week 41:

Listed fish disease in Europe: Legislation and implementation of surveillance plans. Sampling procedures for surveying listed fish diseases The use of cell culture for surveying listed fish diseases qPCR methods, sequencing and bioinformatics for surveying listed fish diseases Wrap up session, evaluation of the course and general recommendations

For the course in week 42, the topics of the sessions will cover the topics normally included in the program for the training course in week 42:

principles of normal anatomy of fish and crustaceans pathology and histopathology development and assessment of protocols for special staining (IHC and ISH) show and tell of diagnostic cases of fish and crustacean diseases from invited speakers.

By attending the entire course, participants will receive a course certificate.

Teaching platform and course material

The course will be organized by zoom platform.

Personal invitation to the training course will be send to participants via Outlook calendar, the link should not be shared with others; it is unique to each participant.

A compendium covering the topic of the course will be made available to the participants prior to the course start.

Course fee

The course is for free for NRL representatives in EU member states as well for members of governmental institutions, such as universities and veterinary institutes.

Employees of private companies are welcome to attend the course as well, for them a participating fee of 200 euros is requested.

Please note for all participants, there is cancellation fee of 100 euros if a participation is cancelled less than a week before the course begins.

Registration

Please use the registration form attached to this email.

The registration form has to be followed by a letter of motivation, where your background and working experience are briefly described.

The use of online platform allow us to expand the number of participants to the course to 20.

To properly plan and organize the course online, you will be asked to apply to participate to the course, by sending an email to Lis Vinther Elmsted lvi@aqua.dtu.dk; Linda Stuhr Christensen lschr@aqua.dtu.dk. The information obtained will solely be used to evaluate participants according to the foreseen activities.

Deadline for application is September 6th .

By September 21st we will communicate to you if the application has been accepted.

We look forward to receive your application, do not hesitate to contact us for further information.

Best regards The EURL team



Programme: Methods for implementation of surveillance procedures for listed fish diseases

| Day 1 | Day 2 | Day 3 | Day 3 | Day 5 |
|--|---|---|--|--|
| Section 1 Legislation and implementation of surveillance plans. | Section 2 Sampling procedures for surveying listed fish diseases | Section 3 The use of cell culture for surveying listed fish diseases | Section 4 qPCR methods, sequencing and bioinformatics for surveying listed fish diseases | Section 5 Wrap up session, evaluation of the course and general recommendations |
| 13-17 | 13-17 | 13-17 | 9.30-14.30 | 9:30-13:30 |
| 13.00-13.15 Course introduction 13.15-13.45 Participant presentation 13.45-14:00 Coffee Break 14:00-15:30 The legislative basis for fish health management and the animal health law in EU 15:30-15:45 Coffee break | 13-14:30 Diagnostic flow, theoretical introduction to sample preparation, cell cultivation, virus ID and qPCR for surveillance programs for the non-exotic listed fish disease in Europe 14:30-14:45 Coffee break 14:45-15:15 Practical demonstration of sampling procedures, discussion on pros and cons of different sampling strategies etc | 13:00-14:00 Use of cell culture for implementing surveillance programs for listed fish disease 14:00-14:15 Coffee break 14:15-14:45 Demonstration of cell culture passaging, and viral titration procedures on cell lines 14:45-16:45 Demonstration of cytopathic effect | 9.30 - 10.30 PCR and real time PCR theory and troubleshooting. The diagnostic laboratory - PCR flow. 10.30 - 10.45 Coffee break 10.45 - 11.45 Analysis of results. DNA Sequencing. Blast analysis. 11.45 - 12.30 Lunch break | 9:30-9:45 Exercise introduction and groupwork set up 9:45-11:00 First group round 11:00-11:15 break 11:15-12:15 12:15-12:30 break 12:30-13:00 Wrap up conclusions |
| 15:45-16:30 The outbreak of IHN in Denmark. 16:30-17:00 Wrap up and discussion | 15:15-16:30 Diagnostic manuals | | 12.30 – 14.30 IHNV outbreak example. Practical exercise. | 13:00-13:30 General discussion and Course evaluation |

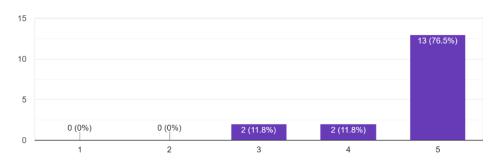


Evaluation: Methods for implementation of surveillance procedures for listed fish diseases

Participant satisfaction level for each respective section was assessed, each aspect of each session was scored on a scale from 1 to 5. Score 1 corresponded to very low, 2 to low, 3 to average, 4 to good, and 5 to excellent. The calculations are based on returned evaluation schemes from 21 participants.

Course evaluation by session

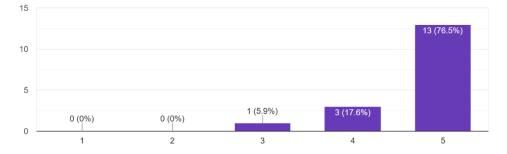
More than 90% of the students scored each of the session as excellent (70% in average) or good (20% in average).



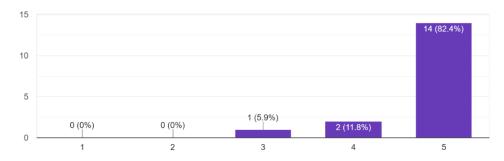
Day 1 introduction to legislative background for listed fish diseases - overall score 17 responses

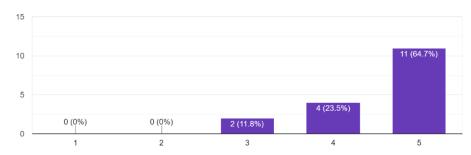
Day 2 general criteria for implementing surveillance of listed fish disease, sampling and sample preparation- overall score

17 responses



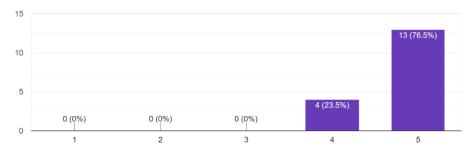
Day 3- use of cell culture for surveillance of listed fish diseases- overall score 17 responses





Dat 4 - molecular methods for implementation of surveillance of listed fish diseases- overall score 17 responses

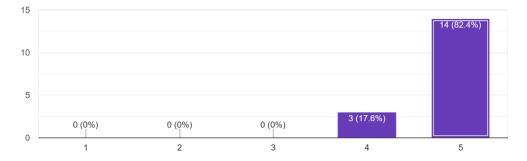
Day 5 - Assignment, discussion and conclusions - overall score 17 responses



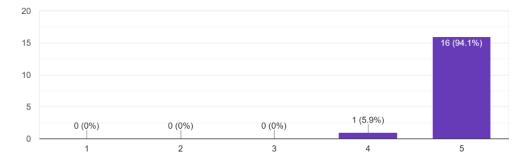
Detailed course evaluation per session

Course evaluation of Day 1

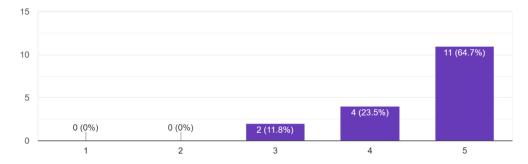
Day 1 introduction to legislative background for listed fish diseases - teacher's expertise 17 responses

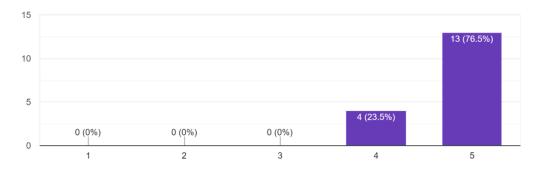


Day 1 introduction to legislative background for listed fish diseases - teacher's preparedness ¹⁷ responses



Day 1 introduction to legislative background for listed fish diseases - relevance for you 17 responses





Day 1 introduction to legislative background for listed fish diseases - increase of your knowledge 17 responses

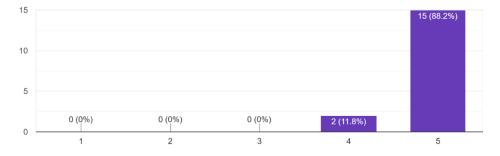
Day 1 introduction to legislative background for listed fish diseases - general comment

- Very useful to me.
- I wish there was education on bacterial diseases
- Include a small practical exercise on legislation like the last day but more focused on legislation. Hard to present and hard to digest.
- it was a positive, nice and useful day
- Not so exciting section, but well presented. Nice to get background to the work I do in the lab.
- very good and complex information and excellent explanation of the topic
- Very clear presentation of the legislation and how all the different parts fit together and how to find the information you need.
- It was very well presented, easy to follow.
- it wasn't so boring
- The lectures were informative and the legislative background explained nicely.
- Excellent!!!
- All was new, so I can't really say anything :)
- I got more understanding about work we are doing: why we get particular amount of samples, when samples have to be taken and why we investigate particular fish diseases.
- it was good and informative
- I would love to see this lecture because I missed it. If possible, please!
- helped us understand a difficult subject
- Material was prepared to shortly introduce with most important things in legislation. I was relieved because that legislation is a beast. Thank you for this way of increasing my knowledge without that much pain.

Course evaluation of Day 2

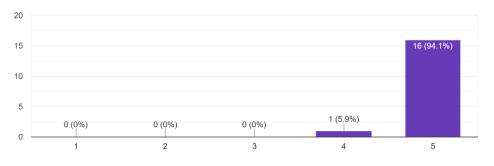
Day 2 general criteria for implementing surveillance of listed fish disease, sampling and sample preparation- teacher's expertise

17 responses



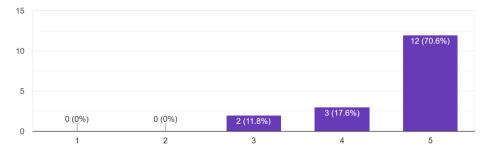
Day 2 general criteria for implementing surveillance of listed fish disease, sampling and sample preparation- teacher's preparedness

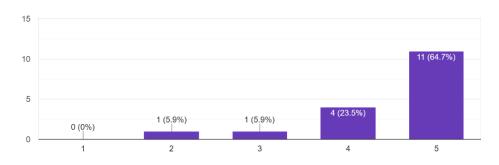
17 responses



Day 2 general criteria for implementing surveillance of listed fish disease, sampling and sample preparation- relevance for you







Day 2 general criteria for implementing surveillance of listed fish disease, sampling and sample preparation- increase of your knowledge 17 responses

Day 2 general criteria for implementing surveillance of listed fish disease, sampling and sample

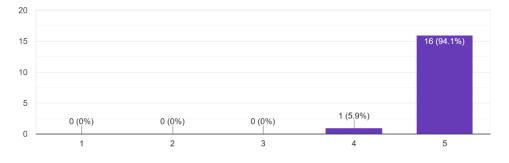
preparation- general comment

- Very usefull to me.
- it was efficient
- Practical demonstration not really working through Zoom, better to use that time for something else.
- it was a positive, nice and useful day
- Very nice demonstration, considering it was over zoom. Well presented and very informative.
- I obtained a lot of new information.
- very good
- Good to learn.
- usefull practical demostration
- Live video course was great.
- Excellent!!!
- Nice to see that
- Information about viruses was valuable and in some parts new and I learned some techniques how to take samples, what is more important and what is less, which I willable to use in our laboratory. All was clear, just would be great practise by ourselves as well.
- it was good and informative
- I would love to see this lecture because I missed it. If possible, please!
- helped me to improve my technique
- Short but relevant way to get though listed diseases. I was satisfied with everything.

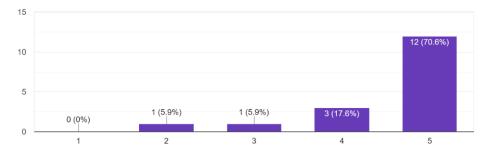
Course evaluation of Day 3

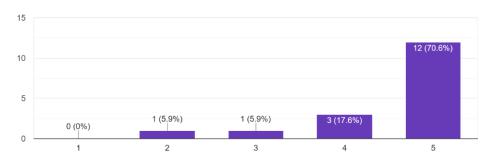
Day 3- use of cell culture for surveillance of listed fish diseases- teacher's expertise 17 responses

Day 3- use of cell culture for surveillance of listed fish diseases- teacher's preparedness $^{\rm 17\,responses}$



Day 3- use of cell culture for surveillance of listed fish diseases- relevance for you 17 responses





Day 3- use of cell culture for surveillance of listed fish diseases- increase of your knowledge 17 responses

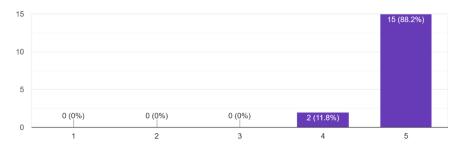
Day 3- use of cell culture for surveillance of listed fish diseases- general comment

- Very usefull to me.
- Since I work on bacterial fish diseases, I am not very familiar with cell culture.
- Demonstration was good, but not that relevant for me.
- it was a positive, nice and useful day
- Useful to me as I am soon to start working in virology lab so nice to get some training before I start.
- It was good to compare your procedure with our.
- I think it would be difficult for me to begin cell culture work in my lab without more in person training
- It was very useful to see the CPE with Niccolo. So far I have never seen CPE in our cells.
- I find very interesting that we could try read if CPE is + or -
- Live microscopy session was great.
- Excellent!!!
- We don't do cell culture, so it was new for me, but nive to here that
- This was very valuable part, because we don't have so much examples of infected fishes and cells examination can be sometimes hard. Practical part let to understand better.
- it was good and informative
- In my opinion, it was well enough explained what our approach should be when choosing an environment. The prepared photo material was a very useful experience.
- I updated my knowledge on cell culture
- This was my day as I work with cell cultures in my everyday work. Pretty much knew everything but it is always nice to refresh your knowledge and learn little features that makes your work easier

Course evaluation of Day 4

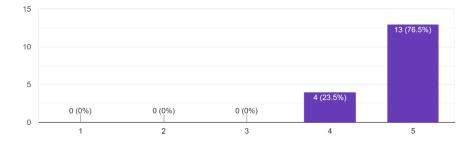
Dat 4 - molecular methods for implementation of surveillance of listed fish diseases- teacher's expertise

17 responses



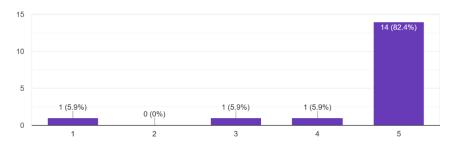
Dat 4 - molecular methods for implementation of surveillance of listed fish diseases- teacher's preparedness

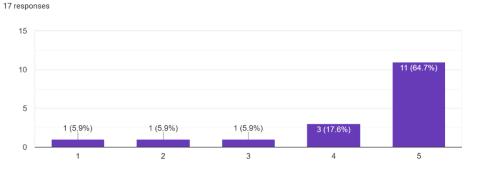
17 responses



Dat 4 - molecular methods for implementation of surveillance of listed fish diseases- relevance for you

17 responses





Dat 4 - molecular methods for implementation of surveillance of listed fish diseases- increase of your knowledge

Dat 4 - molecular methods for implementation of surveillance of listed fish diseases- general

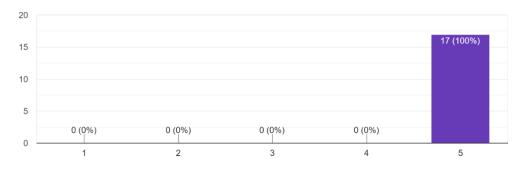
comment

- Very usefull to me.
- I would love to have bacterial fish diseases education. :D
- Good introduction, would like a bit more in depth knowledge. Practical example was very good. Maybe a bit more info about limitations of PCR and troubleshooting when it is not working.
- it was a positive, nice and useful day
- Good module. As I have been doing qPCR/PCR for fish diseases for some years now it was not the most useful module for me personally, but I think benefited many others.
- more difficult part of the course, but good challenge for the future work
- I think there should be more specific information provided for method of genotype identification for each virus when it is required, more of a step by step demonstration and SOP for how exactly you find all the information you need to complete the analysis.
- I did not have all the computer programs, and it was difficult to download free programs and figure out how they work with so little time. I got a bit hectic and lost there. This was the most difficult part of the week.
- sequencing was for me dificult to understand
- The molecular diagnostics exercise was very practical.
- Excellent!!!
- Was nice and interessting for me beacuse we do this methods
- I got some new information, but i think this part could be little bit longer. I think was just not enouth time to practise and we could get some more information about pcr, mix preparation, maybe program settings depending on virus and sequencing. Also some practical exercises about validation would be great to make. Some parts we just touched so would be nice to go little deeper maybe, pcr now become very important in fast detection of diseases so would be great to give more time for it, maybe full day or even 2.
- it was good and informative, just practical part was not so clear
- For me, this lecture was a completely new and unknown subject. This is ahead of me, in my work.

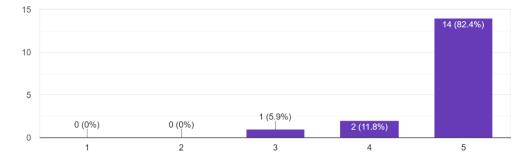
- It was the most challenging part for me
- It was difficult for me to understand the day's material without minimal knowledge about PCR. So I'm not the best person to rate this day.

Course evaluation of Day 5

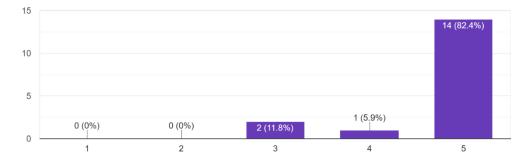
Day 5 - Assignment, discussion and conclusions - teachers' expertise 17 responses

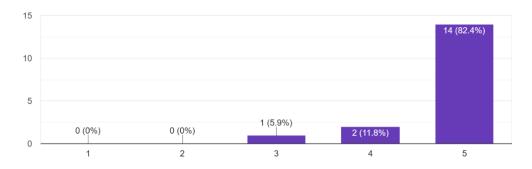


Day 5 - Assignment, discussion and conclusions - teachers' preparedness 17 responses



Day 5 - Assignment, discussion and conclusions - relevance for you 17 responses





Day 5 - Assignment, discussion and conclusions - increase of your knowledge ¹⁷ responses

Day 5 - Assignment, discussion and conclusions - general comment

- Very usefull to me.
- Thank you for everything.
- In general, good, especially given the limitations of Zoom. The use of the second half was questionable, better to use two different scenarios. The second one could be more speculative, like how you would troubleshoot a diagnosis not working, or when you encounter something new.
- it was a positive, nice and useful day
- I think this section went on a little long. Although was useful background information to the lab work I do.
- good idea to work in groups and discuss about tasks together
- it was a very useful exercise, but it was a little difficult in the break out rooms to get any discussion or collaboration going, people just don't want to talk much in online training and everyone is reluctant to take charge
- Great to have the group work, it felt more relaxed to be in a smaller unit and feel we are just all learning this. Some discussion might have gotten lost in translation. Over all good experience.
- very goog idea to work with colleagues and fix the informations
- The assignment was practical and summarized the whole course. I very much appreciate the effort of all of the lecturers to encourage us to participate and discuss in the course during the whole week.
- Excellent!!!
- It was a bit sad that the people don't speak together, but I think this is because of zoom...
- This part maybe didn't give more information, but let the previous information to settle down. We cannot remember all but we need and have to know where to find. I think this day was more about this :)
- It was nice to be in touch and worked at groups.
- The opportunity to meet and exchange opinions with colleagues on the tasks set, to discuss a relevant case this is an indispensable experience. In my opinion, the tasks were chosen correctly and covered all the details of the lectures in the course. I don't think the issue has remained untouched.

- I have been very happy with the assignment part, I was not confident at first but it was really fun. Thank you.
- There were moments when nobody was sure what we were supposed to do (mostly after being added to breakout rooms). Would be nice to have some kind of instructions that we would be able to see at all times. It would lessen the confusion.

Closing remarks

The EURL training course 2021 was - based on the very positive feedback from the participants - considered a success. The evaluation schemes enabled the participants to evaluate each day and topic on the course. A large majority of the participants evaluated the course as "very good".

Due to the Covid-19 pandemics and the restrictions in travelling and gathering, this year the course has been organized virtually again. The virtual form of the course allowed us to increase the number of participants at the course significantly as well as to expand the accessibility of countries located far from Denmark, and thereby providing the possibility to increase the expertise in National Reference Laboratories in EU and abroad.

All participants were registered on a daily basis, and all participated in the whole course. A signed certificate of participation was issued and send individually to the participant at the end of the course.

DTU-Aqua is acknowledged for offering training course facilities for free.

Copenhagen, Thursday, 11 November 2021

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EURL for Fish and Crustacean Diseases