

EURL Training Course: Introduction to validation of Diagnostic methods for Aquatic animal diseases



Copenhagen, 7th – 9th of October 2024

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

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General introduction

The training course titled “Introduction to Validation of Diagnostic Methods for Aquatic Animal Diseases” was organized by the EURL for Fish and Crustacean Diseases located in at the National Institute of Aquatic Resources, Technical University of Denmark. The course was held from October 7th to October 9th 2024 and included 16 trainees.

The overall purpose of the training course was to provide an opportunity for the employees of NRLs to obtain training in validation of diagnostic methods, as this is a requisite to obtain accreditation for diagnostic methods for listed fish and crustacean diseases. The staff of the EURL and DTU Aqua provided this training together with invited speakers from the scientific community. Knowledge-sharing and discussions between participants and teachers were important parts of the course.

Course: Introduction to Validation of Diagnostic Methods for Aquatic Animal Diseases

The 3-days course in “Introduction to validation of Diagnostic methods for Aquatic animal diseases” was based on a combination of theoretical presentations, practical examples and participant’s presentations with examples of validation protocols used in their laboratories.

Day 1 was dedicated to an overall introduction of the course and the importance of accurate and validated diagnostic methods. In this day much of the focus was on analytical validation.

After a short introduction of the course from the course responsible Argelia Cuenca and Niccolò Vendramin from the EURL, all participants had the opportunity to introduce themselves and explain their experiences and expectations from the course. This was followed by a talk addressing the importance of accurate and reliable diagnostics given by Britt Bang Jensen.

After a coffee break, Niccolò Vendramin provided an overview of the validated diagnostic methods for surveillance and diagnostics of listed fish and crustacean diseases.

In the afternoon, Argelia Cuenca gave two talks the first introducing the World Organization of Animal Health (WOAH) pathway for validation of diagnostic methods, followed by a talk on Assay optimization, standard samples, elaboration of protocols and Analytical Validation.

To conclude the day, the first invited speaker, WOAH expert for VHSV and IHNV from DFO in Canada, Dr. Kyle Garver gave a talk on showing case studies on method optimization and analytical validation of molecular methods.

In the evening, there was a social dinner in Lyngby.

On **day 2** the course started with an online contribution from Dr. Peter Mohr, team leader and diagnostician from CSIRO in Australia. The talk was carried on online and it focused on the opportunities and challenges posed by introducing pooling samples for conducting diagnostic and surveillance in the field of aquatic animal health. Later on, Argelia Cuenca gave an overview on regulatory compliance and quality assurance.

After the coffee break, the invited speaker Nils Toft, a former professor in veterinary epidemiology at DTU, introduced the diagnostic assessments for the diagnostic test, including cut-off values. This covered two sessions across lunch. Finally, Lidye Canier, coordinator for the EURL for mollusc diseases, who also participated in the course, gave a talk on the accreditation process for molecular and histopathological assays for mollusc-listed diseases.

Day 3 started with an online talk by Scientist David Strand from the Norwegian Veterinary Institute addressing the diagnostic of Crayfish plague, including validation and maintenance of validation with the discovery of new variants, and the use of eDNA for supplementing surveillance. This was followed by a talk on Predictive values of diagnostic data by Britt Bang Jensen.

After coffee break, Juliane Sørensen gave a talk on the validation of HT-qPCR for detecting multiple pathogens, followed by an overview of the criteria for accreditation and a description of the process for producing, delivering and reporting the Interlaboratory proficiency test, by Niccolò Vendramin and Teena Klinge.

After lunch, it was time for the participants to actively contribute, and each of them gave a presentation on the validation projects that they had initiated and conducted in each laboratory. During this session, participants were encouraged to pose questions and discuss the results presented.

Finally, to close the course there was a common discussion on how the course had developed, where the participants had the opportunity to give feedback and comments for improvement.

The methods taught were primarily focused on the protocols given in the EU legislation, the WOA (OIE) guidelines from the Manual of Aquatic Animal Diseases and ISO 17025 and 17043 standards.

A get-together, a dinner event for all teachers and participants was held on the first day.

Participant list

Name	Surname	Country	Affiliation
Amina Jazic	Solunovic	Bosnia Herzegovina	NRL for fish diseases, Veterinary faculty in Sarejevo
Andrea	Basso	Italy	NRL for fish, mollusc and crustacean diseases - Istituto Zooprofilattico Sperimentale delle Venezie
Dimitrije	Glisic	Serbia	Institute of veterinary medicine of Serbia
Dorotea	Grbin	Croatia	NRL for diseases for aquatic animals, Croatian Veterinary Institute
Jan Inge	Øvrebø	Norway	Norwegian Veterinary Institute
Juris	Kibilds	Latvia	NRL Latvia for fish and crustacean diseases - Institute of Food Safety, Animal Health and Environment – BIOR
Kilin	Ákos	Hungary	National food chain safety office, Food chain safety laboratory directorate, National reference department of Parasitology, Fish and bee diseases
Leticia	Hernández Martínez	Spain	NRL of fish pathology of Spain
Lydie	Canier	France	Ifremer
Marjukka	Rask	Finland	Finnish Food Authority - Laboratory and research division, Wild and Aquatic Pathology section
Sofija	Šolaja	Serbia	Institute of Veterinary Medicine of Serbia
Suzana S.	Arvelius	Sweden	Division of fish NLR at the department of animal health and antimicrobial strategies, Swedish Veterinary Agency in Uppsala
Valentina	Paolini	Italy	Istituto Zooprofilattico delle Venezie - NRL for crustacean diseases – Legnaro
William	Leigh	Scotland	Marine directorate scottish government
Christina B.	Ladime	Denmark	DTU Aqua
Anna Luiza	Farias Alencar	Denmark	DTU Aqua
Valentina Laura	Donati	Denmark	DTU Aqua
Juliane	Sørensen	Denmark	DTU Aqua

Course Description: Introduction to validation of Diagnostic methods for Aquatic animal diseases

Topics covered in the course

This 3-day course is primarily a theoretical course based on relevant guidelines for the validation of diagnostic methods. The topics addressed here were:

1. **Importance of accurate diagnostics:** understanding the importance of diagnosing diseases in aquatic animals, and how diagnostic tests should be fit for purpose (surveillance, eradication, confirmation diagnostics, etc)
2. **Diagnostic Methods Overview:** a comprehensive look at various diagnostic techniques, including molecular, serological, and cell-based methods. This is based on the EURL manual for listed fish and crustacean diseases. ‘
3. **Regulatory Standards:** Overview of international and national standards for diagnostic test validation, including guidelines from organizations like the WHOA (World Organisation for Animal Health)
4. **Principles of Validation:** Key concepts in validating diagnostic tests, such as analytical sensitivity, analytical specificity, accuracy, limit of detection and precision.
5. **Sample Collection and Handling:** Best practices for collecting and handling samples to ensure the reliability of diagnostic results.
6. **Molecular Diagnostics:** Detailed study of optimization and analytical validation for PCR, qPCR, and other molecular techniques used in diagnosing aquatic animal diseases.
7. **Pooling in molecular diagnostics:** assessing the pooling of samples and its effect in analytical and diagnostic validation
8. **Quality Control and Assurance:** Ensuring the quality and reliability of diagnostic tests through proper quality control measures
9. **Diagnostic assessment of tests:** how to assess false positive and false negative rates and samples to include
10. **Cutt-off values;** how to select cut-off values for diagnostic tests, and how this relates with the purpose of the specific test
11. **Emerging Technologies:** Exploration of new and emerging diagnostic technologies (HT-qPCR) and their potential applications.
12. **Case studies:** case studies provided by invited speakers addressing optimization of molecular methods, analytical validation of qPCR, use of eDNA and validation of molecular and histological methods.
13. **Student cases:** case studies provided by the course participants, including either different methodologies for validation of diagnostic methods or examples of current validation projects are provided.

Course Program

Day 1	Day 2	Day
Monday 7 th October	Tuesday 8 th October	Wednesday 9 th October
9:00-9:30 Introduction and presentations of participants Argelia Cuenca Niccolò Vendramin	9:00-9:30 Pooling of samples & Case studies crustacean Peter Mohr	9:00-9:45 Diagnostics of crayfish plague David Strand
9:30-10:30 The importance of accurate and reliable diagnostics Intended purpose of diagnostic assay Britt B. Jensen	9:30-10:20 Regulatory compliance and quality assurance Argelia Cuenca	9:45-10:30 Predictive values of diagnostic data Britt Bang Jensen
Coffee break 10:30-10:50	Coffee break 10:20-10:45	Coffee break 10:30-10:50
10:50-11:45 EURL Diagnostic manuals for listed fish and crustacean diseases Niccolò Vendramin	10:45-12:00 Diagnostic validation Nils Toft	10:50-11:20 Novel technologies for aquatic animal diagnostics Juliane Sørensen
11:45-12:00 The validation pathway WOA Argelia Cuenca		11:20-12:00 Proficiency tests Niccolò Vendramin
Lunch 12:00-13:00	Lunch 12:00-12:45	Lunch 12:00-13:00
13:00-14:15 Stage1 & 2 WOA path for validation Analytical validation Argelia Cuenca	12:45-14:15 Diagnostic validation and exercises - Nils Toft	Practical exercises from students and discussion/1 Tutor Panel
Coffee break 14:15-14:45	Coffee break 14:15-14:45	Coffee break 14:15-14:45
14:45-16:00 Analytical validation Kyle Garver Case studies	14:45-16:00 Case studies: Diagnosis of mollusc diseases Validation of PCR & histological tests Lydie Canier	14:45-16:00 Practical exercises from students and discussion/2 Tutors Panel Course evaluation and conclusions

Intended learning outcomes

The student should be able to

1. Apply the basic principles of validation.
2. Explain how to conduct and interpret validation studies for various diagnostic methods.
3. Reflect on validation as a dynamic process that needs to be maintained and corroborated.
4. Interpret the differences between the analytical and diagnostic attributes of the tests.
5. Be aware of the basic requirements to ensure compliance with regulatory standards.
6. Explain how adequate control and standard samples should be used and implemented for the maintenance of reliable diagnostics.
7. Be exposed to the requirements of maintaining

The course material

The course material, reading chapters and presentations are distributed by email to course participants.

Assessment

Each participant should provide a short presentation that will be discussed in the plenum. The participants will be able to provide feedback on the last day of the course and time is allocated for that. During this discussion, the participants provide a generally favourable assessment of the course. Some of the points for improvement were:

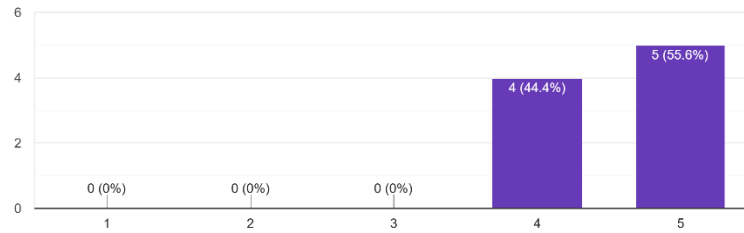
1. More hands-on exercises, particularly covering the evaluation of the diagnostic properties of tests (false positive and false negative rates).
2. Computer exercises using some of the software introduced in the course.
3. Possibility to send the course material in advance.

Finally, a questionnaire (google form) will be distributed by email to the participants for course evaluation. The answers to this form remain anonymous, and the participant's answers can be seen below.

Evaluation: Introduction to validation of Diagnostic methods for Aquatic animal diseases

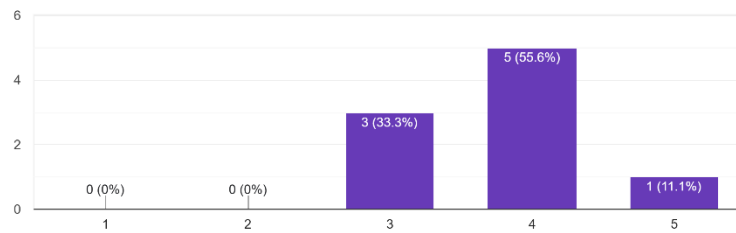
Did the course cover the topics that you were expecting to learn about?

9 responses



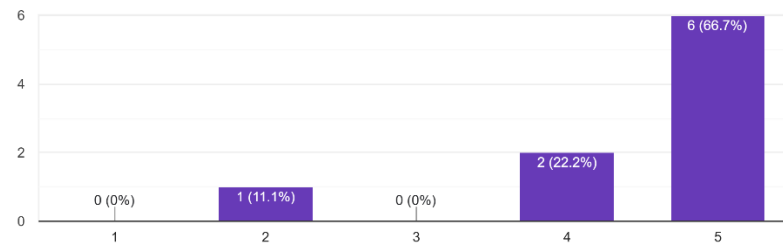
Did you find a good balance between theoretical and practical sessions (case studies)?

9 responses



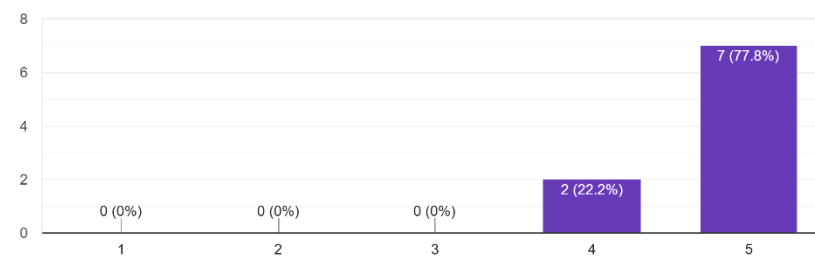
Were the case studies shown by the invited speakers (Kyle Garver, Peter Mohr, Lydie Canier, David Strand) useful for you?

9 responses



Were the theoretical classes useful for you?

9 responses



Was there any topic that you think was not correctly covered, or that you would like that it was covered in more detail/extent?

5 responses

- More practical examples (e.g. calculations of diagnostic sensitivity/specificity using a relevant example)
- can't think of any
- Validation of other methods, like cell culture, ELISA, IFAT
- I missed more information about cell cultures and more practical sessions
- I'd have liked to have get more detailed information about the pooling of samples

Is there any topic that you think should be removed from the course? Or explained in less detail?

3 responses

- can't think of any
- Perhaps the overlap of theory on the first day.
- Sometimes the analytical and diagnostic validation were repetitive

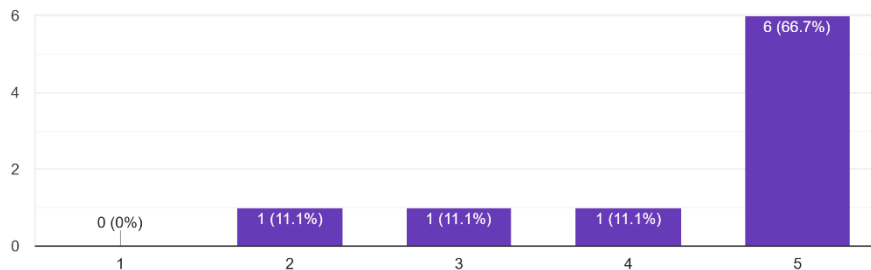
Is there any topic that you would like to have in a course like this that has not been included at all?

4 responses

- There was a lot on qPCR - it would be nice with other methods as well.
- can't think of any
- Epidemiology and fish pathology (cell culture, histology...)
- No

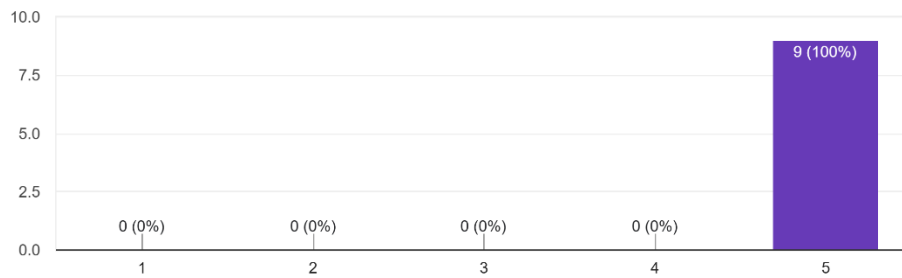
Did you find the student session useful?

9 responses



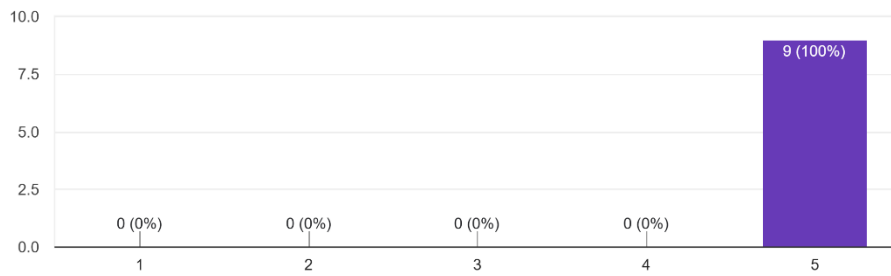
Were the teachers well prepared for their lectures?

9 responses



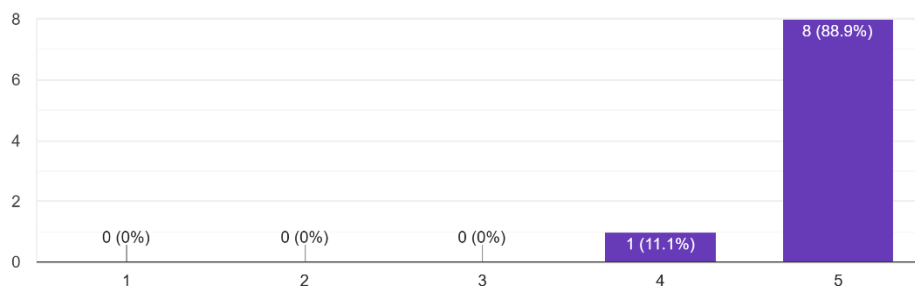
Were the teachers accessible to solve questions during their lectures?

9 responses



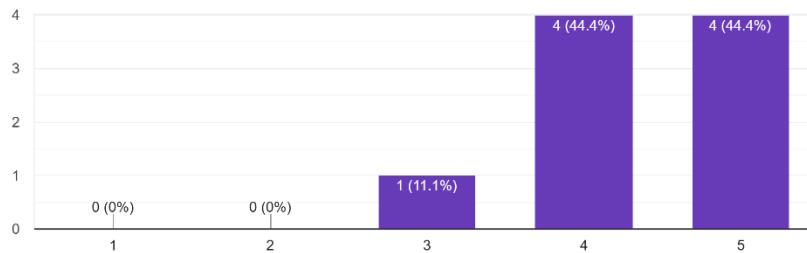
Were the invited speakers well prepared for their lectures?

9 responses



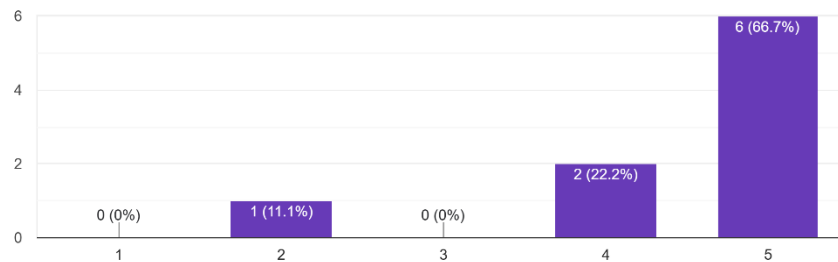
Do you think the course is appropriate for new staff in the NRL

9 responses



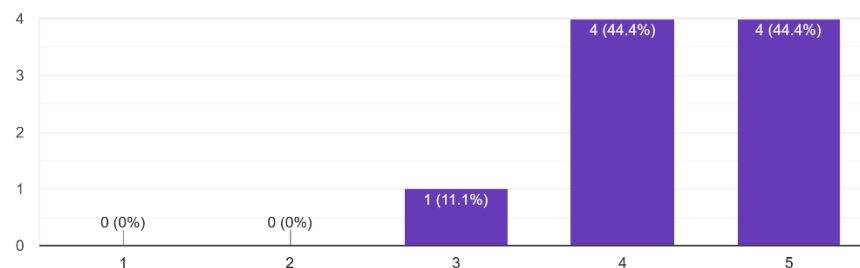
Would you recommend this course to other members of your lab?

9 responses



Were your expectations for this course met?

9 responses



Please provide general suggestions on how to improve the course for subsequent editions:

2 responses

- At the ending of the course it was suggested that adding some homework reading could be beneficial. I think that maybe it could be a list of 'recommended reading' or at least not very long mandatory reading because there will always be some participants who will not have time to read a lot before the course.
- More practical sessions, including online tools that were shown during the lectures. Maybe more team work

Closing remarks

The EURL training course 2024 was - based on the feedback from the participants - considered a success. The evaluation schemes enabled the participants to evaluate each day and topic in the course. The majority of the participants evaluate the courses with the highest marks.

The possibility of providing financial support to participants made it possible to offer training to laboratories where the lack of funding usually makes it hard to find the resources to participate in such activities. This way of funding the training courses, therefore, holds the possibility to increase the expertise in all National Reference Laboratories within the EU.

In this year's course on "Introduction to Validation of Diagnostic Methods for Aquatic Animal Diseases" we have included five invited international speakers who provided an added value to the course.

Finally, all laboratory technicians and scientists in the Section for fish and shellfish diseases at DTU Aqua are deeply acknowledged for delivering excellent teaching and help with practical issues.

Copenhagen, Tuesday, 10 December 2024

Niccoló Vendramin, Argelia Cuenca, Britt Bang Jensen
EURL for Fish and Crustacean Diseases