NEWSLETTER

General introduction

AURORAE activities started in June 2022 and are foreseen to be supported by ECDC for up to four years. These activities can be divided into three components; external quality assessments, centralised laboratory support and training activities.

EQAs under the AURORAE Framework Contract of ECDC include the assessment of workflows in the participating laboratories, including diagnostic assays (e.g. RT-PCR, rapid antigen tests, antibody test), virus isolation and characterisation (e.g. genetic, antigenic, antiviral susceptibility profile), and virus neutralisation tests (VNT). The assessment of bioinformatic workflows including generation of consensus genome sequence from sequence reads, clustering and classification, and prediction of antiviral susceptibility is included in bioinformatics ring trials , even here with a focus on SARS-CoV-2 and influenza viruses.

The scope of the centralised laboratory support for influenza and SARS-CoV-2 viruses is to ensure surveillance and in-depth genetic and antigenic characterisation of circulating influenza and SARS-CoV-2 viruses. All participating laboratories, particularly those currently lacking sufficient capacity or capability, shall get access to virus characterisation services of sufficient quantity and turn-around-time that allows for timely pandemic response.

The training formats include face-to-face training courses (wet lab and dry lab), tailored twinning visits of various durations, and virtual trainings including self-paced online courses, webinars, instructive videos, and instructor-led-courses. Expressions of interest for trainings from the networks as communicated by ECDC to the ERLI-Net and/or ECOVID-LabNet were collected in March 2024. Participation is open for participants from national public health institutes or associated institutes in countries of the EU/EEA, the Western Balkans, and Türkiye.

If you wish to receive more information about these activities, please send an email to **info.aurorae@rivm.nl.** If you would like to receive technical support or would like to have an influenza or SARS-CoV-2 sample characterized, please click one of the links below and fill in the request form. We will contact you as soon as possible!

Technical support request and laboratory protocols upload:
https://ec.europa.eu/eusurvey/runner/RegistrationTechnicalSupport
SARS-CoV-2 characterization requests:
https://ec.europa.eu/eusurvey/runner/RegistrationSARS-CoV-2Samples
Influenza characterization requests:
https://ec.europa.eu/eusurvey/runner/RegistrationInfluenzaSamples

All links and actual information about project AURORAE can be found on our new webpage: https://www.rivm.nl/en/international-projects/aurorae

ECDC/WHO European Region laboratory network teleconference on COVID-19

The ECDC/WHO European Region laboratory network teleconference on COVID-19 of the 6th of December 2023 was dedicated to project AURORAE. The AURORAE consortium was given the opportunity to present updates on their activities and the plans for next year.
Below the highlights of the discussions on the various topics mentioned: The previous and future wetlab EQA's for SARS-CoV-2 and flu were discussed. These were conducted in 2023 and the final report is expected to be published in Q2 2024. The registration for the new EQA, regarding zoonotic flu is now completed and panels are expected to be shipped in April 2024. The upcoming bioinformatics ring trial will focus on SARS-CoV-2 and the preparedness of public health laboratories to detect zoonotic viruses from human specimens. The invitation for this EQA has been sent out.

Bart Haagmans from Erasmus Medical Center (EMC) presented an update on SARS-CoV-2 antigenic diversity. An important question is if we're still protected against new SARS-CoV-2 variants or do we need to update the vaccines? Information from the field is necessary to answer this question and can be gathered by studying:

- The neutralizing capacity of current human sera against new variants.
- The antigenic differences of the new variants.

Therefore, AURORAE consortium together with ECDC has planned to collect SARS-CoV-2 and serum samples from the countries for phenotypic characterisation and you will receive an invitation to participate soon.

Training: past experiences and future programme – connection with other parts of the AURORAE projects (Maria Exindari from Aristotle University, Thessaloniki, Greece).
An overview of past and planned training activities of various formats were presented and it was discussed which laboratories from the targeted for AURORAE training activities?

- Laboratories planning to improve knowledge and skills of their staff.
- Priority for laboratories with EQA results that are not satisfactory.
- Priority for laboratories that have difficulties with viral characterization, drug resistance assessment and/or novel strain detection.

Overview of EQA's

Pathogen	Wetlab or bioinformatic ring trial	Activity Year	Status
Influenza	Wetlab	2023	Final report expected inQ2 2024
SARS-CoV-2	Wetlab	2023	Final report expected in Q2 2024
SARS-CoV-2 and influenza	Bioinformatics ring trial	2023	Finalized – Final reports published
Zoonotic influenza	Wetlab	2024	Registration completed. Panels expected to be shipped in April 2024.
Zoonotic influenza	Drylab	2024	In progress

SARS-CoV-2 EQA

The AURORAE consortium conducted a SARS-CoV-2 EQA in 2023. This was the first EQA conducted by AURORAE, but the third ECDC SARS-CoV-2 EQA overall. We assessed whether European expert laboratories have maintained or improved their SARS-CoV-2 diagnostic capabilities following the COVID-19 pandemic.

We generated and distributed an EQA panel containing 10 samples. Seven were samples with inactivated material from different SARS-CoV-2 lineages (Alpha and three Omicron variants, namely BA.4, BA.5, and BQ1.18), two control samples with inactivated material from endemic human coronaviruses (HCoV-229E and HCoV-OC43), and one negative control sample with phosphate buffered saline (PBS).Two concentrations of BA.4, BA.5, and BQ1.18 were included in the panel to evaluate sensitivity.

These variants were chosen to reflect the circulating variants at that time. B.1.1.7 was present in previous EQAs and was included in this EQA as well to provide continuity.

Participants were asked to score their samples (positive, negative, inconclusive) and to provide an appropriate description. Participating laboratories were also asked to provide information about the extraction method, types of RT-PCRs, and cycle threshold (Ct) values. In addition, as an optional activity, participants could report the determined SARS-CoV-2 variant (by WHO label or by Pango lineage), signature mutations, or both, together with information about the methods used. Results reporting was done via an online platform (hosted on the EU survey platform).

Thirty-eight expert laboratories from 31 countries, covering 26 of the 30 EU/EEA countries and five of the eight EU pre-accession countries, reported results. 82% (31/38) of the participating laboratories identified all 10 samples correctly as positive or negative for SARS-CoV-2; 11% (4/38) reported 9 samples and 8% (3/38) reported 8 or fewer samples correctly. Thirteen of the participating laboratories performed sequencing on a voluntary basis. Of the seven SARS-CoV-2 positive samples, 23% (3/13) reported six samples correctly and 23% (3/13) five samples. In addition to the specific variants, some laboratories only mentioned that the samples were VOCs.

Overall, 27 laboratories from 25 countries have participated in all 3 EQAs. Of these 27 laboratories, 19 had a score <100% in the 2020 EQA. In 2021, 10 labs out of the 27 had a score <100%. In the current EQA only 4 of the 27 laboratories had a score <100%, showing that the overall performance of the laboratories improved over time.

Zoonotic influenza viruses wetlab EQA 2024

During the global outbreak of highly pathogenic influenza H5N1 (clade 2.3.4.4b) type A influenza virus in over a 100 million wild birds and poultry in the past 3 years, an expanding range of mammals have been found infected. Since increased activity of clade 2.3.4.4b viruses from mid-2021 also at least 12 confirmed human cases with this clade have been reported to the WHO, most after direct contact with affected poultry. This amplified the concern about the enzootic circulation of A(H5N1) influenza virus and transmission to humans. There are also concerns about direct transmission of swine influenza viruses to humans and about possible reassortment, of swine influenza viruses with avian and/or human seasonal influenza viruses, increasing for example at mixed poultry and swine farms, the risk that viruses develop with enhanced potential for transmission to and between humans. Current external quality assessments (EQA) organised by WHO and other commercial and not-for-profit EQA providers provide panels for detection of potentially zoonotic avian influenza viruses, but not for (potentially) zoonotic swine influenza viruses. Hemagglutinin and neuraminidase subtyping is often optional, if included in the challenge. Sequencing for genetic characterization (clade assignment) and evaluation of markers for enhanced mammalian transmission and virulence and antiviral resistance are not, or only for antiviral reduced susceptibility, included in these programmes. Therefore, it is hardly known from objective EQAs what the capabilities of the National Influenza Centres

and National Influenza Reference Laboratories in the EU/EEA, Western Balkan and Türkiye are with respect to detection, subtyping and molecular characterization of zoonotic type A influenza viruses. To fill this gap and to prepare for potential human cases of avian or swine influenza viruses, on the request of ECDC, the National Institute for Public Health and the Environment (RIVM) with partners in the AURORAE consortium developed an EQA for potentially zoonotic type A influenza viruses.

This 2024 panel is dedicated to molecular detection and subtyping by PCR or other Nucleic Acid Amplification Test (NAAT) and genetic characterization through sequencing of potential zoonotic influenza viruses. Registration started on the 1st of March 2024 and the panel is planned to be distributed shortly after Western Easter holidays the second week of April 2024.

SARS-CoV-2 and zoonotic influenza virus drylab (bioinformatics) EQA

From 1 March until 1 April 2024, the second European SARS-CoV-2 and Influenza virus Bioinformatics EQA (ESIB-EQA) is held. The EQA is dedicated to sequence analyses primarily conducted through bioinformatic workflows. The influenza virus part of the EQA is held together with the regular European External Influenza virus Quality Assessment Programme (EEIQAP).
This year, the EEIQAP and ESIB-EQA focuses specifically on influenza viruses of zoonotic origin. The SARS-CoV-2 part on the other hand focuses as before on mainly currently circulating viruses.

A total of 27 have registered, the large majority of which for both SARS-CoV-2 and influenza virus components. After completion by 1 April, results will be analysed and reported back to participants. Participants will receive a certificate of participation. The final report will be drafted by 30 June.

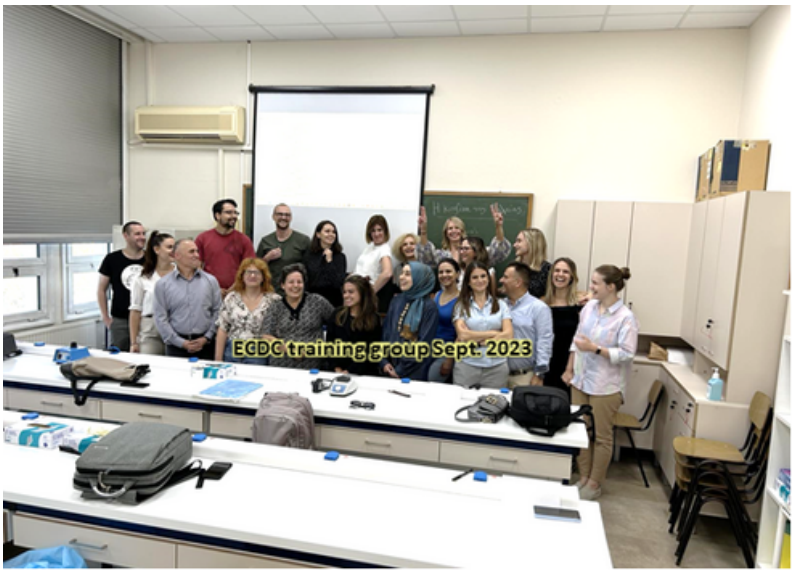
TRAINING ACTIVITIES

Various training activities are again promoted by the ECDC and delivered by the AURORAE Consortium partner laboratories, intending to build and/or strengthen capacity and familiarisation with methods and techniques regarding respiratory pathogen detection, isolation, and investigation, as well as data sharing and reporting. Colleagues from 19 European countries have already made good use of these opportunities.
Up to now, the training plan mostly addresses to laboratories with limited experience on virus isolation, antigenic and molecular techniques, sequencing, bioinformatics, and reporting. Laboratories with difficulties in recent EQAs are particularly invited to use their priority in participating to any type of training courses.
Furthermore, several training courses are planned to include more sophisticated approaches of influenza, SARS-CoV-2, and other respiratory viruses.

Recent

Face-to-face courses (wet labs, dry labs)

1. A wet/dry lab course was organised by the NIC N. Greece, AUTH (Laboratory of Microbiology, School of Medicine, Aristotle University of Thessaloniki) in 25-29 September 2023. The agenda included Influenza laboratory methods (isolation, antigenic approach), sequence analysis, phylogeny (beginner bioinformatics) and reporting.



2. Soon after, a new wet/dry lab course was successfully organised by the Robert Koch Institute, RKI (Public Health Institute of Germany, Berlin, in 9-13 October 2023. This time the agenda included SARS-CoV-2 laboratory methods, variant analysis (beginner bioinformatics), and reporting.
This year the face-to-face courses were attended by colleagues coming from Laboratories of Romania, Hungary, Serbia, Montenegro, Bosnia-Herzegovina, Kosovo, North Macedonia, Estonia, Portugal, Liechtenstein, Lithuania, Croatia, Greece, and Türkiye.

Twinning visits

From September 2023 until February 2024, thirteen 5-days twinning visits regarding influenza, SARS-CoV-2, and/or other respiratory viruses have been organised by EMC (Erasmus Medical Centre), RKI, and/or HPI (Hellenic Pasteur Institute). In total, these visits have welcome one or two participants from Kosovo, Poland, Spain, Romania, Cyprus, Montenegro, Serbia, Türkiye, and Slovenia.

Virtual courses

During the last period of training activities, several online activities have taken place:

- A self-paced online course has been delivered by the Robert Koch Institute (RKI) (March 2023), and another one is being delivered by Aristotle University of Thessaloniki (AUTH) (February 2024), both regarding SARS-CoV-2 sequencing and bioinformatics.

- Two webinars were also organised by RKI and AUTH, each one aiming to cover gaps and questions remaining from the previously provided wet/dry lab courses of September and October 2023.

- Two instructional videos are being finalised to be delivered, one regarding influenza virus isolation in embryonated eggs (AUTH), and the other regarding respiratory pathogens multiplex PCR (Hellenic Pasteur Institute, Athens, Greece).

Participants' experiences

Trainings were overall evaluated very positive and participants gave valuable impulses for further optimizing the trainings and suggesting new courses with direct impact on the training program of this year.

"The knowledge I gained is very important for me and I have passed it on to colleagues I work with."

"We had the opportunity to use the information from the training and better understand the work technique."

" I managed to cover my missing practical skills during these days"

Future courses

Wet labs, dry labs

1. Influenza laboratory methods and reporting
2. Detection and typing of seasonal and zoonotic influenza
3. Seasonal and zoonotic Influenza & respiratory viruses multiplex assays
4. SARS-CoV-2 sequence analysis, variant analysis, and reporting
5. Respiratory virus surveillance summer school

Participation in each course: 10 participants belonging to interest expressing targeted laboratories of the EU/EEA, the Western Balkans and Türkiye, ideally coming from 10 different countries.

Twinning visits

Twinning visits: **5 days**
4 courses: SARS-CoV-2 whole genome sequencing and basic bioinformatics
4 courses: Intensive training, Influenza surveillance laboratory methods and reporting

Twinning visits: **2 days**
4 courses: Focused training, Influenza surveillance laboratory methods and reporting
2 courses: Focused training, bioinformatic analysis of SARS-CoV-2 and/or influenza on the INSaFLU platform or content to be decided according to particular needs or circumstances

Virtual trainings

Instructional videos

1. Setting up an absolute quantification assay
2. Evaluation of molecular testing of respiratory viruses

Webinars

1. Biosafety and quality assurance in a public health laboratory
2. Topics according to the suggestions of face-to-face course participants
3. Introduction to INSaFLU
4. Integrated respiratory virus surveillance
5. Topics according to newly occurring needs or situations