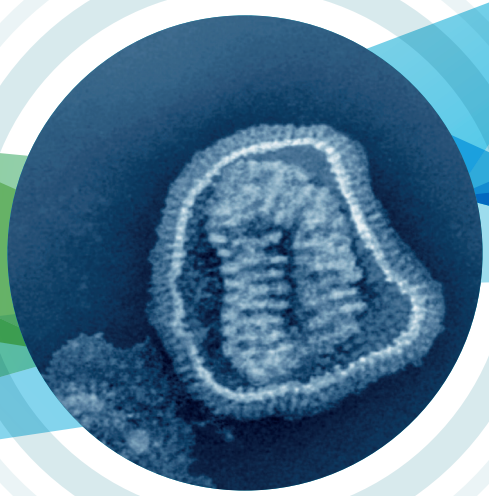
ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

World Health Organization
European Region



European External Influenza Virus Quality Assessment Programme

2023

INTERAGENCY ASSESSMENT

European External Quality Assessment Programme, Influenza Virus

2023

This report was jointly commissioned by European Centre for Disease Prevention and Control (ECDC) (framework contract ECDC/2022/001, project AURORAE) coordinated by Angeliki Melidou and Eeva Broberg (ECDC) and the World Health Organization (WHO) Regional Office for Europe (Agreement for Performance of Work; Reference 2023/1352979-0) for the countries in the WHO European Region outside EU/EEA), coordinated by Karen Nahapetyan (WHO). It was produced by Dr. Adam Meijer, National Institute for Public Health (RIVM), the Netherlands on behalf of participants in the European Reference Laboratory Network for Human Influenza (ERLI-Net) in the European Union/European Economic Area (EU/EEA), Western Balkans and Türkiye and in the network of National Influenza Centres (NICs) from countries outside EU/EEA in the WHO European Region.

Acknowledgements

Contributing authors: Kamelia Stanoeva, Wanda Han and Adam Meijer (RIVM) performed the analysis and prepared the report. Ingmar Visser analysed the additional questionnaire on virus isolation methods.

Panel preparation: Gabriel Goderski, Sharon van den Brink, Lisa Wijsman, Mariam Bagheri, John Sluimer and Chantal Herrebrugh (RIVM) contributed to the design and planning of the exercise and the production of the panel at the Respiratory and Enteric Viruses Department, Centre for Infectious Diseases Research, Diagnostics and Laboratory Surveillance, RIVM, Bilthoven, The Netherlands. Prof. Dr. Ron Fouchier, Mark Pronk and Pascal Lexmond (Erasmus Medical Centre, Department Viroscience, Rotterdam, The Netherlands) contributed to the design of the panel with antigen characterisation data. We are grateful to the Worldwide Influenza Centre (WHO Collaborating Centre) at the Francis Crick Institute, London, UK, for additional antigenic characterisation data.

The panel, with specimens labelled in random order, was pre-tested at Erasmus Medical Centre, Department Viroscience, Rotterdam, The Netherlands, Centre National de Référence Virus des Infections Respiratoire, Laboratoire de Virologie, Institut des Agents Infectieux, Lyon, France, and Institut Pasteur, Paris, France.

Panel distribution and data services were provided by Quality Control for Molecular Diagnostics (QCMD), Glasgow, UK, with special thanks to Elaine McCulloch, Alan Flish and Mateusz Zoldak.

Contributing experts, ECDC: Angeliki Melidou, Eeva Broberg. *Contributing experts, WHO*: Karen Nahapetyan, Iris Hasibra, Alina Guseinova.

ECDC exclusively provided the content, conclusions and recommendations for EU and EEA countries, Western Balkan countries (Albania, Bosnia and Herzegovina, Montenegro, North Macedonia, Serbia) and Türkiye. WHO exclusively provided the content, conclusions and recommendations for Armenia, Azerbaijan, Belarus, Israel, Kyrgyzstan, Republic of Moldova, Russian Federation, Switzerland, Ukraine, and the United Kingdom (UK).

Suggested citation: European External Influenza Virus Quality Assessment Programme 2023. Stockholm: European Centre for Disease Prevention and Control (ECDC) and World Health Organization (WHO) Regional Office for Europe; 2025.

Stockholm, October 2025

ISBN 978-92-9498-810-2 (ECDC) doi: 10.2900/3006976 Catalogue number TQ-01-25-040-EN-N
ISBN 9789289062428 (WHO)

Contents

Figures

Tables

Related documents

Annexes – For Annex 1 and 2, please see the separate downloadable document available on ECDC's website.

Abbreviations

AANI	No amino acid substitution previously associated with (highly) reduced inhibition
AAHRI	Amino acid substitution previously associated with highly reduced inhibition
AARI	Amino acid substitution previously associated with reduced inhibition
BXM	Baloxavir marboxil
CDC	Centers for Disease Control and Prevention, Atlanta, USA
CPE	Cytopathic effect
Ct	Cycle threshold
ECDC	European Centre for Disease Prevention and Control
EEIQAP	European External (Influenza) Quality Assessment Programme for Influenza
EISN	European Influenza Surveillance Network
EISNINF_MD23	Programme in EEIQAP 2023 comprising molecular detection, typing, type A H- and N-subtyping and type B lineage determination
EISNINF_VI23	Programme in EEIQAP 2023 comprising virus isolation and antigenic and genetic characterisation
EISNINF_VS23	Programme in EEIQAP 2023 comprising antiviral (neuraminidase inhibitors) susceptibility determination
EISS	European Influenza Surveillance Scheme
ELISA	Enzyme linked immunosorbent assay
EQA	External quality assessment
EQAP	External quality assessment project
Erasmus MC	Erasmus Medical Centre, Rotterdam, the Netherlands
ERLI-Net	European Reference Laboratory Network for Human Influenza
EU/EEA	European Union/European Economic Area
FRA	Focus reduction assay
GISAID	Global Initiative on Sharing All Influenza Data
GISRS	Global Influenza Surveillance and Response System
HA	Haemagglutinin
hCK	Humanized Canine Kidney cell line (a Madin-Darby canine kidney (MDCK) cell line that expresses high levels of human influenza virus receptors and low levels of avian virus receptors)
HI	Haemagglutination inhibition
HINT	High-content imaging-based neutralisation test
HRI	Highly reduced inhibition
IC_{50}	50% Inhibitory Concentration
INF23	Influenza virus detection, typing, subtyping and lineage determination, virus isolation and genetic and antigenic characterisation and antiviral susceptibility determination specimens of the EEIQAP 2023 panel
IRINA	Influenza Replication Inhibition Neuraminidase-based Assay
ISO	International Organization for Standardization
ITEMS	QCMD Information Technology EQA Management System
MDCK	Madin-Darby Canine Kidney
MUNANA	20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid
N/A	Not applicable
NA	Neuraminidase
NAAT	Nucleic acid amplification technologies
NGS	Next generation sequencing
NI	Normal inhibition
NIC	National Influenza Centre
nM	Nanomolar
QCMD	Quality Control for Molecular Diagnostics, Glasgow, UK
RBC	Red blood cells
RI	Reduced inhibition
RIVM	National Institute for Public Health and the Environment, Bilthoven, the Netherlands
RS	Reduced susceptibility
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription polymerase chain reaction

SIAT	Human alpha-2,6-sialyltransferase
SNP	Single nucleotide polymorphism
$TCID_{50}$	50% tissue culture infectious dose
TESSy	The European Surveillance System
VN	Virus neutralisation
VTM	Virus transport medium
WHO	World Health Organization
WHO CC	WHO Collaborating Centre
WHO CC London	WHO CC at the Francis Crick Institute Worldwide Influenza Centre, London, UK.

Executive summary

Influenza epidemics occur every winter with high impact on disease burden, hospitalisations and excess mortality in the countries of Europe. To understand the characteristics of circulating influenza viruses during seasonal epidemics, virological influenza surveillance is performed, and detected viruses are further characterised at national influenza centres (NICs) that are part of the wider network of the WHO Global Influenza Surveillance and Response System (GISRS). External quality assessment (EQA) is an important instrument in assessing the quality of the generated data that are reported nationally and internationally through The European Surveillance System (TESSy) and presented in The European Respiratory Virus Surveillance Summary (ERVISS), the joint European Centre for Disease Prevention and Control (ECDC) and World Health Organization (WHO) Regional Office for Europe weekly update on respiratory virus infections (https://erviss.org/).

From March to July 2023, a European External Influenza (virus) Quality Assessment Programme exercise (EEIQAP) was held for NICs and other national influenza reference laboratories in the European Union/European Economic Area (EU/EEA) and other countries in the WHO European Region. The exercise covered influenza virus molecular detection, isolation, strain genetic and antigenic characterisation and antiviral susceptibility testing. This was the eighth detection, isolation and strain characterisation panel and the sixth antiviral susceptibility testing panel since the start of the scheme in 2000.

Since EEIQAP 2010, all exercises have been organised with the support of the European Centre for Disease Prevention and Control (ECDC) and since EEIQAP 2018, with the additional support of the WHO Regional Office for Europe. The EEIQAP 2023 was organised by the contractor, the National Institute for Public Health and the Environment (RIVM) in Bilthoven, the Netherlands, for the European Reference Laboratory Network for Human Influenza (ERLI-Net). Participation of laboratories from the EU/EEA, Western Balkan countries and Türkiye was supported by ECDC. Participation in EEIQAP 2023 by laboratories in additional European countries was supported by the WHO Regional Office for Europe. For the third time, results are jointly presented for the EU/EEA together with other participating laboratories in the WHO European Region.

The objectives of the exercise were to collect information on the capacity and capability of the network regarding i) rapid molecular influenza virus detection, A/B typing, type A H- and N-subtyping and B lineage determination by reverse transcription polymerase chain reaction (RT-PCR), ii) influenza virus isolation and strain characterisation using antigenic and/or genetic techniques, and iii) antiviral susceptibility testing using genetic and/or phenotypic techniques, within a defined reporting timeframe of seven working days for i) and 36 working days for ii) and iii). This exercise aimed to provide the participants with an independent assessment of their own laboratories' performance and a comparison with other reference laboratories for influenza in the European Region. In addition, performance in the EEIQAP 2023 provided a validity check of the data reported to TESSy. For the first time in 2023, the programme included genetic testing for reduced susceptibility markers to the antiviral drug baloxavir marboxil (BXM). Reporting of the sequence data obtained for the hemagglutinin (HA), neuraminidase (NA) and acidic polymerase (PA) coding genome segments was included in conjunction with reported genetic characterisation data.

A total of 53 laboratories in 44 of the 53 countries in the WHO European Region participated in the EEIQAP 2023 Molecular Detection challenge. Nineteen laboratories participated in the full programme, including the Characterization and Antiviral Susceptibility challenge. The panel consisted of seven simulated clinical specimens containing variable amounts of live virus, one simulated clinical specimen that did not contain virus, and three inactivated specimens containing viruses with neuraminidase inhibitor-reduced inhibition or baloxavir-reduced susceptibility.

Overall, the performance of the network in molecular detection and simultaneous typing and type A H-subtyping of seasonal influenza viruses can be rated as excellent (52 of 53 laboratories were able to determine the type and 50 of 51 determined the A H-subtype correctly). Those laboratories determining the N-subtype of type A influenza viruses and lineage of type B viruses demonstrated satisfactory to excellent performance (10 of 16 laboratories were able to determine the N-subtype and 41 of 43 laboratories correctly determined the B lineage). Surveillance data reported weekly to TESSy that derive from molecular testing can therefore be considered accurate. However, an increase in the number of laboratories with capability to N-subtype and to determine the B-lineage would be desirable. Similar to the EEIQAP 2018 and EEIQAP 2020, the EEIQAP 2023 showed that the issue with the performance of the network in virus isolation still persists (10 of 31 laboratories that participated in this challenge were able to isolate virus from all influenza virus-containing specimens). Not being able to isolate a virus has an immediate impact on the number of isolates available for antigenic characterisation and phenotypic antiviral susceptibility testing, as well as subsequent reporting to TESSy. Although there was satisfactory-to-good concordance among laboratories in the reported antigenic and genetic characterisation categories for the EEIQAP 2023 panel specimens, the results also indicate that subtle antigenic differences between viruses, and differences in amino acid changes defining genetic categories are not picked up accurately (only 4 out of 23 laboratories provided completely correct results for reported specimens). Therefore, the weekly analysis and interpretation of genetic and antigenic characterisation data reported to TESSy in the predefined categories should be interpreted with some caution. Genetic and phenotypic testing of antiviral susceptibility varied in accuracy from satisfactory to

excellent when looking at the sequence and IC_{50} (50% Inhibitory Concentration) data obtained. However, interpretation of less common amino acid change data and phenotypic testing of such viruses was inaccurate for a relatively high number of laboratories (7 of 23 laboratories provided fully correct amino acid substitutions results for reported specimens and 4 of 17 laboratories provided fully correct phenotypic results for reported specimens). This complicates the analysis of antiviral susceptibility data reported to TESSy.

While the overall performance of the participating laboratories was satisfactory, some laboratories are encouraged to enhance their testing performance by evaluating the sensitivity and specificity of the assays in place and to apply necessary updates accordingly. Other issues (e.g. incorrect translation into TESSy categories) will be addressed jointly by ECDC and the WHO Regional Office for Europe through training or by adapting validation and analysis of data captured in TESSy (e.g. by making better use of reported HA and NA sequences, with a particular focus on sequence analysis, interpretation and reporting).

1 Introduction

Influenza viruses cause a highly contagious acute respiratory disease that can spread rapidly, causing considerable morbidity and mortality in Europe. Influenza viruses evolve rapidly from season to season through point mutations, leading to genetic drift that sometimes results in antigenic drift. The segmented nature of the influenza genome also makes genomic reassortment an important mechanism for producing genetic diversity, which may lead to antigenic shift if new haemagglutinin (H)- and neuraminidase (N)-subtypes of influenza A viruses are introduced to which humans are naive. This process is particularly important in influenza A virus because of its potential to generate new pandemic strains [1,2].

Early detection and characterisation of circulating influenza viruses is therefore of great importance for timely risk assessment, treatment recommendations, and vaccine formulation. The laboratory network responsible for the virological surveillance of influenza in the WHO European Region is part of the WHO Global Influenza Surveillance and Response System (GISRS) [3]. In total, 47 of 53 WHO European Region Member States have National Influenza Centres (NICs) recognised by WHO [4], and laboratories from all 27 EU and two EEA Member States participate in the European Reference Laboratory Network for Human Influenza (ERLI-Net), coordinated by ECDC [5]. The European Influenza Surveillance Network (EISN), which includes the European Reference Laboratory Network for Human Influenza (ERLI-Net), is a dedicated network for the epidemiological and virological surveillance of influenza in the EU/EEA.

The introduction of nucleic acid amplification technologies (NAAT) that can rapidly detect influenza viruses with high sensitivity and specificity has led to the replacement of less sensitive rapid antigen detection assays by molecular methods. NAAT allow simultaneous identification of the type of virus (A, B), the haemagglutinin (H)- and neuraminidase (N)-subtype of influenza A viruses (H1N1, H3N2, H7N9, etc.), and the genetic lineage of influenza B viruses (B/Victoria/2/87-like, in short Victoria and in B/Yamagata/16/88-like, in short Yamagata). As a result, these tests are considered to be the gold standard for diagnosing individual patients and in surveillance.

Using phylogenetic and amino acid substitution analysis, it is possible to genetically characterise the haemagglutinin genome segment of influenza viruses and categorise circulating viruses in genetic (sub)clades or (sub)groups. This provides data on the evolution and possible emergence of variants that might escape from (vaccine-induced) immunity (i.e. the matching of vaccine strains with circulating strains and to known markers for increased virulence). Similarly, genetic characterisation of the NA and polymerase acidic subunit (PA) genome segments provide useful information on known markers for (highly) reduced inhibition by NA inhibitors (oseltamivir and zanamivir) and reduced susceptibility for BXM (polymerase inhibitor), respectively. However, the ability to accurately determine the antigenic profile of an influenza virus still requires the ability to isolate the virus in cell culture or embryonated eggs and carry out serological tests (haemagglutination inhibition (HI) or virus neutralisation (VN) assays). Moreover, virus isolates are necessary to determine the phenotypic antiviral susceptibility profile (e.g. through NA enzyme activity inhibition assays or plaque reduction assay) by measuring their level of susceptibility to NA inhibitors and other types of antivirals.

It is essential to assess the performance of such technologies to ensure the reliability and comparability of results reported to physicians and to disease surveillance systems, both nationally and at regional level [6]. External quality assessment (EQA) provides a means of carrying out independent and objective laboratory performance evaluation. The influenza laboratory network in Europe has performed EQA studies on all aspects of laboratory influenza surveillance, as described above (the first of which was performed in 2000, with antiviral susceptibility testing added in 2010, and covering the whole WHO European Region since the 2018 EEIQAP programme) [7-9].

The European influenza EQA programme was the first of its kind to include virus isolation, strain characterisation and antiviral susceptibility determination. Molecular EQA has been covered by the WHO's global External Quality Assessment Programme (EQAP) for NICs and national influenza laboratories since 2007. The Programme was designed for the detection and subtyping of seasonal, and potentially pandemic zoonotic avian influenza viruses [10]. When the WHO programme began, the European Influenza Surveillance Scheme (EISS) was already showing that European reference laboratories were capable of detecting and subtyping zoonotic avian influenza virus subtypes through participation in another international EQA programme, arranged in 2006 [11]. Antiviral susceptibility determination has been added on an optional basis to the WHO EQAP since 2013, initially only targeting A(H1N1)pdm09 NA-H275Y [10]. There is no international EQA programme available for virus isolation and antigenic and genetic strain identification, although virus isolation EQA studies from WHO's South East Asia and Western Pacific Regions have been reported [12,13]. However, that EQA programme did not include strain identification through antigenic or genetic characterisation. Therefore, the European External Influenza Quality Assessment Programme (EEIQAP) is still filling a gap by integrating all aspects of laboratory influenza surveillance in one EQA panel: molecular detection, typing, type A H- and N-subtyping and type B lineage determination, virus isolation, antigenic and genetic strain characterisation and antiviral susceptibility determination. All of these aspects are part of the routine influenza surveillance data published in The European Respiratory Virus Surveillance Summary (ERVISS), the joint ECDC/WHO Regional Office for Europe weekly online update on influenza, COVID-19 and respiratory syncytial virus (RSV) infection (https://erviss.org). EEIQAP studies evaluate the reliability and comparability of results reported to TESSy, and subsequently to ERVISS, and help identify where improvement is needed in laboratory influenza surveillance and diagnostic capability.

In 2022, a new framework contract (following a previous ECDC framework contract for the period 2017–2021) was put in place with ECDC for laboratory support of national influenza laboratories in the EU/EEA, Western Balkans and Türkiye for the period 2022–2026, which includes wetlab EQA studies for influenza virus. This EQA, conducted in 2023, was the first to take place under the new contract. Agreements on performance of work with the WHO Regional Office for Europe have ensured the participation of NICs in countries within the WHO European Region that are not part of the EU/EEA in EEIQAP 2018, EEIQAP 2020 and the current EEIQAP 2023. The EEIQAP 2018 results, which were jointly presented for the WHO European Region as a whole for the first time, and the EEIQAP 2020 results were published in reports available on ECDC's website [14]. Part of the EEIQAP 2023 study aimed to address issues identified in the EEIQAP 2018 and EEIQAP 2020 studies.

Objectives

The goal of ECDC-supported EQAs is to appraise the proficiency of public health microbiology laboratories in using microbiological test methods that underpin capabilities in the areas set out below, defined in ECDC's EQA strategy [6].

- Diagnostic confirmation of disease for reporting to TESSy, in accordance with EU case definitions for 52 notifiable diseases and antimicrobial resistance for EU/EEA countries;
- Outbreak detection, investigation and response;
- Control of communicable diseases (e.g. tuberculosis isolation and treatment);
- Preparedness (e.g. avian influenza viruses).

The operational public health objectives of ECDC-supported EQAs are [6]:

- Assessment of the quality and comparability of surveillance data reported by EU/EEA Member States;
- Support of threat detection capabilities for emerging diseases, epidemic diseases, and drug resistance.

These objectives are in line with the laboratory and public health objectives of EQAs outlined in the WHO laboratory quality management system handbook [15].

Translated into operational procedures in the laboratory, the main purposes of EQAs, as also intended for the current EEIQAP 2023, include:

- Comparison of performance and results across different test sites;
- Provision of early warning for systematic problems associated with kits or operations;
- Provision of objective evidence of testing quality;
- Identification of areas that need improvement;
- Identification of training needs.

This report presents the results of the EEIQAP 2023 for influenza reference laboratories in Europe, designed and prepared by the contractor and funded by ECDC and WHO Regional Office for Europe. The results of laboratories located in EU/EEA countries and non-EU/EEA countries within the WHO European Region are summarised. The main objective of the EEIQAP 2023 was to assess the performance of individual influenza reference laboratories in the areas set out below.

- Rapid detection by Reverse Transcription Polymerase Chain Reaction (RT-PCR) or other NAAT including typing, type A H- and N-subtyping and type B lineage determination, within a defined reporting timeframe of seven working days.
- Virus isolation, including follow-up strain characterisation by HI-assay or virus neutralisation (VN) and/or sequencing within a defined reporting timeframe of 36 working days.
- Determination of susceptibility to the neuraminidase inhibitors oseltamivir, zanamivir and the polymerase inhibitor BXM by genotypic and/or phenotypic methods within a defined reporting timeframe of 36 working days.

2 Study design

2.1 Organisation

The EEIQAP panel was designed by staff from the contractor, RIVM, and the final composition was agreed with ECDC. With the exception of two viruses making up the antiviral component of the panel, all viruses were taken from the repository at RIVM. One virus for the antiviral component of the panel was kindly provided by Prof Dr Maria Zambon and Dr Angie Lackenby, UK Health Security Agency, National Influenza Centre, Colindale, UK for the purposes of this EQA only. Another virus for the antiviral component was kindly provided by Dr. Larisa Gubareva, CDC, Atlanta, USA. The panel was prepared and quality controlled at the Department of Emerging and Endemic Viruses, Center for Infectious Disease Research, Diagnostics and Laboratory Surveillance (IDS), RIVM, Bilthoven, the Netherlands. Pre-testing was performed by three subcontractors: Erasmus Medical Centre (Erasmus MC), Department of Viroscience, Rotterdam, the Netherlands; National Reference Centre for Respiratory Virus Infections, Virology Laboratory, Institute of Infectious Agents, Lyon, France and Institut Pasteur, Paris, France. The final panel composition was determined based on the pre-testing results of a larger number of potential panel specimens (15 instead of the 11 finally selected). Panels frozen at 80°C were distributed to participants on dry ice by specialist courier (organised by the subcontractor, Quality Control for Molecular Diagnostics (QCMD), Glasgow, UK) between March and July 2023. Participants submitted results to the web-based database Information Technology EQA Management System (ITEMS), which is hosted by QCMD and was adapted to the needs of EEIQAP 2023.

2.2 Panel composition, preparation and validation

The EEIQAP panel consisted of eight simulated clinical specimens, seven of which contained different genetic clades of live seasonal influenza viruses that were circulating in humans at the time of panel preparation, including human influenza viruses A(H1N1)pdm09 and A(H3N2) and the B/Victoria genetic lineage (Table 1). One specimen with no virus completed the live virus part of the panel. In addition, three specimens (of two vials each) specifically designed for antiviral susceptibility testing were included in the panel which contained inactivated virus with amino acid substitutions causing (highly) reduced inhibition (HRI or RI) by the neuraminidase inhibitors oseltamivir and/or zanamivir or reduced susceptibility (AARS) to the polymerase inhibitor BXM (Table 1).

Table 1. Panel composition, European EQA Programme, Influenza, 2023

Specimen code EISN_	Matrix	Strain; genetic clade; antiviral amino acid substitution	Type and subtype/lineage	Cycle threshold (Ct) value[1]	pfu/ml[2]
EISN_INF23-2	A549 cells in VTM	A/Netherlands/10866/2022; 3C.2a1b.2a.2b; none	A(H3N2)	19.6	34500
EISN_INF23-3	A549 cells in VTM	B/Netherlands/10895/2022; V1A.3; none	B/Victoria	25.0	345
EISN_INF23-4	A549 cells in VTM	A/Netherlands/10025/2022; 3C.2a1b.2a.2a.3; none	A(H3N2)	18.1	34500
EISN_INF23-5	A549 cells in VTM	No virus	N/A	N/A	N/A
EISN_INF23-6	A549 cells in VTM	A/Netherlands/10219/2021; 6B.1A.5a.1; none	A(H1N1)pdm09	16.2	34500
EISN_INF23-7	A549 cells in VTM	B/Netherlands/11164/2022; V1A.3a.2; none	B/Victoria	20.2	1091
EISN_INF23-8	A549 cells in VTM	A/Netherlands/10206/2021; 3C.2a1b.1a; none	A(H3N2)	18.1	10910
EISN_AV23-1	VTM; Triton X-100 inactivated	A/West Virginia/20/2022; NA-H275Y[3]	A(H1N1)pdm09 (provided)	N/A	N/A
EISN_AV23-1	VTM; heat inactivated	A/West Virginia/20/2022; NA-H275Y[3]	A(H1N1)pdm09 (provided)	23.2	N/A
EISN_AV23-2	VTM; Triton X-100 inactivated	A/Netherlands/10253/2022; PA-E23G	A(H3N2) (provided)	N/A	N/A
EISN_AV23-2	VTM; heat inactivated	A/Netherlands/10253/2022; PA-E23G	A(H3N2) (provided)	21.3	N/A
EISN_AV23-3	VTM; Triton X-100 inactivated	A/England/74000497/2017; NA-aa245-248deletion[4]	A(H3N2) (provided)	N/A	N/A
EISN_AV23-3	VTM; heat inactivated	A/England/74000497/2017; NA-aa245-248deletion[4]	A(H3N2) (provided)	23.1	N/A

VTM = virus transport medium. N/A = not applicable

[1] For influenza type A viruses derived from generic influenza type A virus detection RT-qPCR (matrix gene) and for influenza type B viruses derived from B/Victoria specific detection RT-qPCR (HA gene).

[2] Plaque forming units (pfu) transposed by multiplying with 0.69 from $TCID_{50}$ (50% tissue culture infectious dose) determined by titration on MDCK-MIX cells (MDCK-I and MDCK-SIAT) in rotating tubes. This way of titrating generates a higher titer than using 96-well microtiter plates and static incubation. End-points were determined by CPE (cytopathic effect) and NA activity for each tube. Titers were determined from a one-time freeze-thawed panel to simulate transport and freeze-thawing by participants. *SIAT = Human alpha-2,6-sialyltransferase.*

[3] Kindly provided by Dr. Larisa Gubareva, CDC, Atlanta, USA.

[4] Kindly provided by Prof. Dr. Maria Zambon and Dr. Angie Lackenby, UKHSA, National Influenza Centre, Colindale, UK for this EQA only.

All viruses were selected based on known antigenic (Figure A1−A3), genetic (Figure A4−A6) and antiviral susceptibility characteristics (Table 1) previously determined at RIVM, Erasmus MC and WHO Collaborating Centre at the Francis Crick Institute Worldwide Influenza Centre, London (WHO CC London). All viruses were grown in monolayers of Madin-Darby Canine Kidney (MDCK)-MIX cells, consisting of equal amounts MDCK-I cells and MDCK cells stably expressing human alpha 2,6-sialyltransferase (MDCK-SIAT) to stocks with a sufficient concentration to prepare the required number of panels for distribution to the intended number of laboratories. The specimens with viruses exhibiting reduced inhibition by NA inhibitors and the specimen with virus exhibiting reduced susceptibility for BXM were inactivated with 1% Triton X-100 for one hour at room temperature, as this procedure preserves NA activity best for phenotypic NA inhibitor susceptibility testing [14,16]. Heat-inactivation was used to preserve RNA integrity for direct sequencing of the viruses exhibiting reduced inhibition by NA inhibitors or reduced susceptibility for BXM [14]. A virus with reduced susceptibility for BXM was included in this panel for the first time.

The live virus specimens were diluted to a concentration high enough for successful virus isolation at RIVM and pre-testing laboratories. The inactivated virus specimens were diluted to an NA enzyme activity high enough for direct use in phenotypic antiviral susceptibility testing or direct sequencing using Sanger or next generation sequencing (NGS) protocols at RIVM and pretesting laboratories. The live virus specimens were prepared in virus transport medium (VTM) with a final concentration of 1 x 10^5/ml adenocarcinomic human alveolar basal epithelial (A549) cells to simulate a real clinical specimen. All specimens in the panel were aliquoted and stored frozen at -80°C until dispatch to QCMD for further distribution. One panel was thawed and pre-tested at RIVM using in-house methods, and panels with random numbering of specimens different from the numbering of the final panel were sent frozen on dry ice from RIVM to the three independent laboratories for pre-testing. The final panels were shipped frozen on dry ice to the participating laboratories between March and July 2023. Expected results for all specimens in the panel are listed in Tables 2 and 3. For a final check on the viability of the live viruses in the panel, one panel, stored frozen at -80°C, was thawed and the specimens were cultured on MDCK-MIX cells at RIVM. All specimens with live virus became positive within seven calendar days after one passage, confirming viability after long storage time (several months) simulating the expected freeze-thaw cycle that would be undertaken in the participating laboratories.

2.3 Participation

Participation in EQA is one of the key tasks of ECDC ERLI-Net laboratories [5] and plays a key role in strengthening the WHO GISRS diagnostic capacity and preparedness to effectively respond to influenza outbreaks [3]. Participation in the components of the EEIQAP 2023, for which NICs and national influenza reference laboratories in the WHO European Region routinely report data to TESSy, was strongly recommended. All laboratory contact points of ERLI-Net and NICs outside the EU/EEA were notified of the EEIQAP 2023 exercise in advance, jointly by ECDC and WHO Regional Office for Europe in English and, where appropriate, Russian. Laboratories were asked to sign up for participation in any of the four areas: molecular detection, virus isolation, virus characterisation and antiviral susceptibility determination. Where no response was received, follow-up was undertaken with the laboratories. A final list of the laboratories that participated in the EEIQAP 2023 can be found in Table A9.

2.4 Testing

All participating laboratories were expected to perform the molecular detection, typing, type A H- and N-subtyping and type B lineage determination component of the EEIQAP 2023 using the tests routinely applied in the laboratory, the weekly results of which are reported to TESSy. In addition, if the usual laboratory procedures included virus isolation and strain characterisation and/or antiviral susceptibility testing and the resulting data were reported to TESSy, the laboratories were strongly recommended to also complete the corresponding parts of the EEIQAP 2023. For any tests used, the laboratories were asked to provide detailed information (e.g. assay target, type of assay, reagents used, references, use of controls and duplicates) to be able to put the reported results into context.

The laboratories were instructed to test the following:

- EISNINF_MD23 programme – Molecular detection, typing, type A H- and N-subtyping and type B lineage determination: specimens EISN_INF23-1 through -8) using RT-PCR or other NAAT.
- EISNINF_VI23 programme – Virus isolation and antigenic and/or genetic characterisation: specimens EISN_INF23-1 through -8. Virus isolation was to be performed in cell culture and/or embryonated eggs. For antigenic characterisation, the viruses had to be isolated and propagated first, followed by HI-assay or VN. For genetic characterisation, the simulated clinical specimen or virus isolate had to be sequenced by Sanger or NGS techniques.
- EISNINF_AV23 programme – Antiviral (NA- and PA-inhibitors) susceptibility determination: specimens EISN_AV23-1 through -3 and specimens EISN_INF23-1 through 8 by available phenotypic (neuraminidase enzyme activity inhibition assay) and/or genotypic (single nucleotide polymorphism (SNP) RT-PCR, Sanger sequencing or NGS or pyrosequencing) antiviral susceptibility determination methods. EISN_AV23 tubes labelled 'phenotypic' contained virus inactivated by Triton X-100 and could be used directly for phenotypic NA-inhibitor susceptibility testing. The EISN_AV23 tubes labelled 'sequencing' contained virus inactivated by heat and could be used directly for genotypic antiviral susceptibility testing, including one-step full segment Sanger or NGS. EISN_INF23-1 through -8 specimens were intended to be isolated and propagated first before phenotypic antiviral susceptibility testing was performed.

The instructions for testing were made available to the participants through QCMD ITEMS in English and Russian.

2.5 Data reporting

The deadline for reporting molecular detection and typing, type A H- and N-subtyping and type B lineage determination results was within seven working days of receiving the panel. The deadline for reporting virus isolation and strain characterisation results and for antiviral susceptibility testing results was within 36 working days of receiving the panel. Data were reported in the web-based QCMD-owned database ITEMS, adapted for collating EEIQAP 2023 data, similar to previous EEIQAP rounds [19-21]. Expected results for each programme are displayed in Tables 2 and 3.

For the molecular detection programme of EEIQAP 2023, the laboratories were asked to report type (A/B), H- and N-subtype for influenza A viruses and lineage for influenza B viruses using drop-down lists. We also asked for details of tests used.

For virus isolation, participants were asked to report whether the virus was isolated and using which method via drop-down lists. They identified the methods used to confirm virus isolation using predefined categories, including the option 'Other'.

For strain characterisation, the participants were asked to report the result of antigenic and/or genetic characterisation, using drop-down lists with categories reflecting the TESSy categories for the seasons 2021/22 and 2022/23. For the purposes of genetic characterisation, they were also asked to report the clade name, as defined by the WHO (generated by e.g. Nextclade). In addition, the laboratories were asked to provide details on the methods used for antigenic and/or genetic characterisation and to upload their obtained HA gene sequences for evaluation in the context of reported genetic characterisation results.

For genotypic antiviral susceptibility testing, participants were asked to report the relevant amino acid changes associated with a change in susceptibility to oseltamivir, zanamivir and/or BXM, and to upload the NA and PA gene sequences obtained for evaluation in the context of reported genetic antiviral susceptibility testing results.

Table 2. Expected results of the panel, molecular detection, virus isolation and antigenic and genetic characterisation, EQA programme, influenza 2023

Specimen code	Programme EISNINF_MD23	Programme EISNINF_VI23				
	Type and subtype/ lineage	Virus isolation	Type and subtype/ lineage	Antigenic category[1]	Genetic category [2]	WHO clade[2]
EISN_INF23-1	A(H1N1)pdm09	Positive	A(H1N1)pdm09	A(H1)pdm09 A/Victoria/2570/2019 (H1N1)-like (IVR-215VICT19) but also close to A(H1)pdm09 A/Sydney/5/2021 (H1N1)-like within <4-fold distance.	genAH1/Norway/25089/2022, because in addition to the A/Victoria/2570/2019 group the virus has HA1 K54Q, P137S, K142R, A186T, Q189E, E224A, R259K, D260E, T277A and K308R substitutions.	6B.1A.5a.2a.1
EISN_INF23-2	A(H3N2)	Positive	A(H3N2)	A(H3) not attributed to category. Closest but at >4-fold distance to old vaccine strain A(H3) A/South Australia/34/2019 (SA19) as well as to the more recent vaccine strains A(H3) A/Cambodia/e0826360/2020 (CAMB20) and A(H3) A/Darwin/9/2021 (DARW21).	genAH3/Bangladesh/4005/2020, because in addition to 3C.2a1b.2a the virus has HA1 K83E, Y94N, Y159N, T160I, L164Q, G186D, D190N, F193S and Y195F substitutions and HA2 I193M substitution. However, A/Bangladesh/4005/2020 is not a reassortant and the A/Netherlands/10866/2022 virus also has HA1 E50K, F79V, I140K and S262N. Similarly, the more closely-related WHO CC reference strain A/Norway/29511/2021 has HA1 E50K, but otherwise only HA1 R269K.	3C.2a1b.2a.2b NA: 3C.2a1b.1a
EISN_INF23-3	B/Victoria	Positive	B/Victoria	B(Vic) lineage not attributed to category. Closest to B/Washington/02/2019-like (B/Victoria/2/87 (del162-164)-lineage) (WASH19) but at just >4-fold distance.	genBVicB/Netherlands/11267/2022, because it has characteristic amino acid substitutions of HA1 K75E, E128K, R133G, T155A, G184R and G230N compared to B/Washington/02/2019.	V1A.3
EISN_INF23-4	A(H3N2)	Positive	A(H3N2)	Closest to A(H3) A/Cambodia/e0826360/2020-like (CAMB20) and A(H3) A/Darwin/9/2021-like (DARW21) but at just >4-fold distance.	genAH3/Darwin/9/2021, because in addition to the A/Bangladesh/4005/2020 group the virus has HA1 H156S, but not HA1 D53G. Instead it has HA1 D53N and other amino acid substitutions HA1 N96S and HA1 I192F, similar to the more closely-related WHO CC London reference strain A/England/214191723/2021.	3C.2a1b.2a.2a.3
EISN_INF23-5	N/A	Negative	N/A	N/A	N/A	N/A
EISN_INF23-6	A(H1N1)pdm09	Positive	A(H1N1)pdm09	Similar to A(H1)pdm09 A/Guangdong-Maonan/SWL1536/2019 (H1N1)-like (CINC1909-GD19) within <4-fold distance, but also within <4-fold distance to A/Brisbane/02/2018 (IVR-190BRISB18) and A/Michigan/45/2015 (MICH15).	genAH1/Guangdong-Maonan/SWL1536/2019 because the virus has the same amino acid substitutions as the A/Norway/3433/2018 group but with additional HA1 D187V/A and Q189E substitutions. However, phylogenetically this virus is distant from genAH1/Guangdong-Maonan/SWL1536/2019 with additional amino acid changes HA2 I183T and HA1 S326P common with others. Therefore this virus could also be allocated to the TESSy category genAH1SubgroupNotListed within clade 6B.1A.5a.1.	6B.1A.5a.1
EISN_INF23-7	B/Victoria	Positive	B/Victoria	Similar to B/Austria/1359417/2021-like (Bvictoria/2/87 (del162-164B)-lineage) (AUST21) within <4-fold distance.	genBVicB/Austria/1359417/2021, because it has characteristic amino acid substitutions A127T, R133G, P144L, N150K, G184E, N197D, K203R and R279K compared to B/Washington/02/2019.	V1A.3a.2
EISN_INF23-8	A(H3N2)	Positive	A(H3N2)	A(H3) not attributed to category. Closest but at >>4-fold distance to vaccine viruses genetically positioned in the 3C.2a1b.1b clade A/Hong-Kong/2671/2019 (HK19) as well as the 3C.2a1b.2a.2 clade A/South Australia/34/2019 (SA19).	genAH3/Denmark/3264/2019, because in addition to group 3C.2a the virus has HA1 E62G, K92R, T128A (loss of a glycosylation site), HA1 T135K (loss of a glycosylation site), HA1 R142G and H311Q substitutions and specific substitutions of HA1 G186D, D190N and S198P. However, the A/Netherlands/10206/2021 virus also has HA1 I192F, T131I, K207R and I58V.	3C.2a1b.1a

[1] *For details see Figures A1–A3.*
[2] *For details see Figures A4–A6.*

Table 3. Expected results of the panel, antiviral susceptibility, EQA Programme Influenza, 2023

Specimen code	Programme EISNINF_AV23					
	Phenotypic		Genotypic (expected result when full NA/PA is sequenced)			
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Baloxavir	Accession number NA in GISAID*
EISN_INF23-1	NI	NI	AANI	AANI	AANS	EPI2192963/EPI2192960
EISN_INF23-2	NI	NI	AANI	AANI	AANS	EPI2024524/EPI2024521
EISN_INF23-3	NI	NI	AANI	AANI	AANS	EPI2017933/EPI2017930
EISN_INF23-4	NI	NI	AANI	AANI	AANS	EPI1967354/EPI1967353
EISN_INF23-5	N/A	N/A	N/A	N/A	N/A	N/A
EISN_INF23-6	NI	NI	AANI	AANI	AANS	EPI1956842/EPI1956841
EISN_INF23-7	NI	NI	AANI	AANI	AANS	EPI2027589/EPI2027586
EISN_INF23-8	NI	NI	AANI	AANI	AANS	EPI1950934/ EPI1950933
EISN_AV23-1	HRI[a]	NI[a]	AAHRI NA-H275Y[b]	AANI NA-H275Y[b]	AANS	EPI2067492/EPI2067489
EISN_AV23-2	NI[c]	NI[c]	AANI	AANI	AARS PA-E23G[d]	EPI1996485/EPI1996482
EISN_AV23-3	HRI[e]	RI[e]	AAHRI NA- aa del245-248; AAHRI based on phenotypic data virus isolate and extrapolation from published data[e,f]	AARI NA- aa del245-248; AARI based on phenotypic data virus isolate and extrapolation from published data[e,f]	AANS	Sequences available on request

*Neuraminidase inhibitors interpretation: NI = normal inhibited (fold-change IC_{50}; A viruses <10; B viruses <5); RI = reduced inhibited (fold-change IC_{50}; A ≥10 & ≤100; B A ≥5 & ≤50); HRI = highly reduced inhibited (fold-change IC_{50}; A >100; B >50); AANI = has no amino acid substitutions previously associated with RI or HRI; AARI = has amino acid substitutions previously associated with RI; AAHRI = has amino acid substitution previously associated with HRI. *GISAID = Global Initiative on Sharing All Influenza Data.*

Baloxavir marboxil (BXM) interpretation: AANS = has no amino acid substitutions previously associated with reduced susceptibility (RS) (highest fold-change in range ≤ 3 in WHO Table); AARS = has amino acid substitutions previously associated with RS (fold-change > 3 in WHO Table). N/A = not applicable.

[a] Fold-change compared to median IC_{50} of recent Dutch A(H1N1)pdm09 viruses for oseltamivir determined at 561 (HRI) and for zanamivir at 1.6 (NI) at the Dutch National Influenza Centre (NIC) location National Institute for Public Health and the Environment (RIVM).

[b] According to the WHO reference table for neuraminidase inhibitors for A(H1N1)pdm09 viruses available from: https://www.who.int/publications/m/item/summary-of-neuraminidase-(na)-amino-acid-substitutions-associated-with-reduced-inhibition-by-neuraminidase-inhibitors-(nais); accessed 29 May 2023.

[c] Fold-change compared to median IC_{50} of recent Dutch A(H3N2) viruses for oseltamivir determined at 0.8 (NI) and for zanamivir at 1.1 (NI) at the Dutch NIC location National Institute for Public Health and the Environment (RIVM).

[d] According to the WHO reference table for BXM for A(H3N2) viruses available from: https://www.who.int/publications/m/item/summary-of-polymerase-acidic-(pa)-protein-amino-acid-substitutions-analysed-for-their-effects-on-baloxavir-susceptibility; accessed 29 May 2023. The fold-change to PA sequence matched wild-type for baloxavir marboxil for A/Netherlands/10253/2022 PA-E23G has been determined at 4.50-5.13 fold at WHO CC Tokyo and Atlanta using three different methods (focus reduction assay (FRA), HINT (high-content imaging-based neutralisation test) and influenza replication inhibition neuraminidase-based assay (IRINA)), well above the provisional threshold of 3-fold for reduced susceptibility.

[e] Fold-change compared to median IC_{50} of recent Dutch A(H3N2) viruses for oseltamivir determined at 109 (HRI) and for zanamivir at 19 (RI) at the Dutch NIC location National Institute for Public Health and the Environment (RIVM).

[f] According to the WHO reference table for neuraminidase inhibitors for A(H3N2) viruses available from: https://www.who.int/publications/m/item/summary-of-neuraminidase-(na)-amino-acid-substitutions-associated-with-reduced-inhibition-by-neuraminidase-inhibitors-(nais); accessed 29 May 2023.

Participants were also asked to provide an interpretation of the generated results via drop-down lists with the categories used for reporting to TESSy, taking into account the level of testing (e.g. SNP test versus full length neuraminidase (NA) genome segment sequencing). For phenotypic antiviral susceptibility testing, participants were asked to report IC_{50} values for oseltamivir and zanamivir and to provide an interpretation of their results via drop-down lists with the categories used for reporting to TESSy. For both methodologies, we asked for details in order to put the reported results in the context of the methodologies used. Additional data were also collected on the accreditation status of laboratories.

2.6 Data analysis

All the challenges involved in EEIQAP 2023 were considered 'educational' and therefore no pass/fail criteria were defined. In this report, overall performance in the range 90–100% was described as excellent, 80–89% as good, 60–79% as satisfactory and 0–59% as poor. A scoring system was used in which a correct result for a specimen was scored 0 (for 0 errors). Penalty points were given depending on the level of detail reported and the complexity of the challenge. For molecular detection, the maximum penalty per specimen was three points. Mistakes in determining A or B type were penalised with one point; mistakes in H- and N-subtyping were given one penalty point each; mistakes in B-lineage determination were given two penalty points; a false positive of the negative specimen was penalised with three points. For virus isolation and genetic characterisation, the maximum penalty per specimen was one point (unsuccessful isolation of virus-containing specimen, or incorrect TESSy genetic category/WHO clade assignment). For antiviral susceptibility, the maximum penalty per specimen was two points and partial correct answers were given one point. Each laboratory received a cumulative performance score for each challenge which was the sum of the individual specimen scores. For network performance, the percentages of laboratories with a specific cumulative performance score were calculated and plotted in overview bar graphs. The detailed scoring system used for each challenge is provided in the footnotes to these graphs and the individual laboratory result appear in the tables in the annexes. In addition, where appropriate, explanations of the judgements to conclude that an error was made are provided for individual specimens in the footnotes of the individual laboratory results tables in the annex.

If a laboratory did not perform a particular test because it was not available at that laboratory, it was not counted as an error. Therefore, the individual and network cumulative scoring is not a reflection of the capability of the individual laboratory or the network to perform a specific test. Therefore, in the results overview tables, the number of laboratories (or datasets) that have performed a specific test is shown as a denominator, overall or for individual panel specimens, as applicable. As the same panel was used in the molecular detection challenge and the virus isolation challenge, laboratories had already determined the type/subtype or lineage of the viruses. The EEIQAP 2023 sought to find clear proof of the type/subtype or lineage of isolated viruses being confirmed by adapting QCMD ITEMS. These reported results were separately analysed. Uploaded consensus HA, NA and PA genome segment sequences were aligned with the sequences generated at RIVM for all panel specimens using BioEdit software (version 7.2.5). Reported sequences were evaluated for completeness of the coding region, presence of mismatched nucleotides, insertion and deletions, and presence of stretches of Ns (indicating regions with missing nucleotides where the sequence remained unknown) using the following definitions:

- Complete = from ATG through stop codon
- Nearly complete = small stretches missing at 3' and/or 5' end
- Incomplete = large part of the sequence missing
- Identical = less than six mismatches with the original throughout the sequence
- Poor = many stretches of Ns and/or many mismatches with original, including insertions and deletions.

Each sequence was subsequently allocated to one of the categories:

- No sequence reported
- Complete; identical to original
- Nearly complete; identical to original
- Incomplete; identical to original (for HA at least (part) of HA1)
- (Nearly) complete; poor
- Incomplete; poor
- More incomplete; poorer.

All sequences were analysed using BioEdit, FluSurver (https://flusurver.bii.a-star.edu.sg/) and Nextclade (https://clades.nextstrain.org) for nucleotide and amino acid composition in conjunction with reported interpretation of the phylogenetic analysis of the HA sequences (allocation to TESSy genetic categories and determination of WHO clade) and antiviral susceptibility marker analysis of the NA and PA sequences (genetic assessment of antiviral susceptibility).

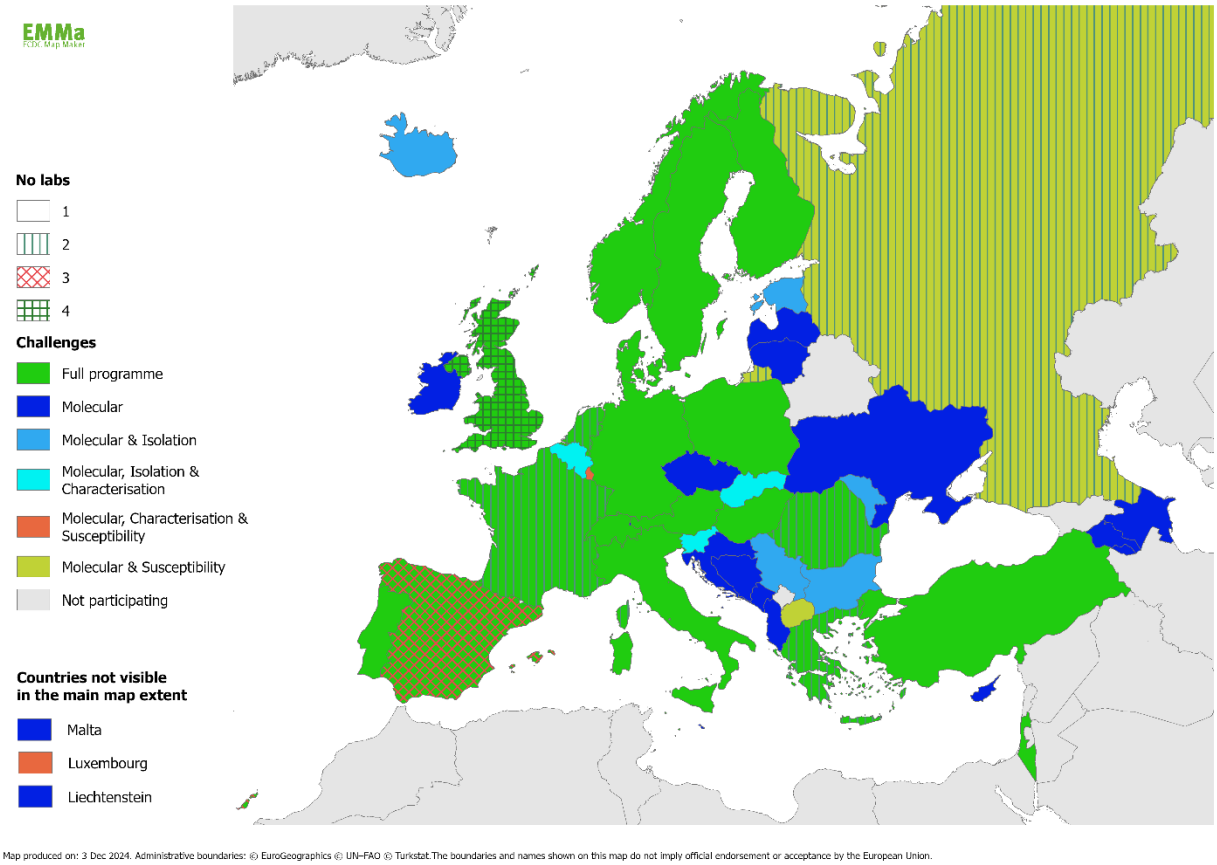
An 'Expected results' document for self-evaluation was shared with each participant in November 2023 through QCMD ITEMS with an email notification from QCMD after the last participant had submitted their results. Participants were offered contact information if they had any questions on their results. One question on virus isolation was received and answered.

3 Results

3.1 Participating laboratories

A total of 54 laboratories in 44 of the 53 countries in the WHO European Region participated in the EEIQAP 2023. Of these, 33 laboratories were located in the 30 EU/EEA countries and the other 21 were located in 14 countries outside of the EU/EEA. Among the 59 laboratories initially registered for EEIQAP 2023, five laboratories (located in countries outside the EU/EEA) did not participate for logistical reasons. An aggregated breakdown of participating laboratories by challenge type is shown in Table 4, and by participating laboratory in Table A10. Only 43% of laboratories participated in the full programme covering molecular virus detection, virus isolation, antigenic and/or genetic characterisation and antiviral susceptibility profiling. An overview by country is represented in Figure 1 (for countries with more than one participating laboratory, the one covering most challenges was taken into account).

Figure 1. Overview of participation per country, European EQA Programme, Influenza 2023*



**Colours indicate the challenges that the laboratories have registered for: full programme (green); molecular only (dark blue), isolation, characterisation and antiviral susceptibility challenges, or combinations thereof (shades of blue, red, olive green) (see Table A10 for details). Where multiple laboratories participated in one country, the laboratory performing most challenges is the representative. Grid lines indicate the number of laboratories per country (no grid: one laboratory, vertical grid lines: two laboratories, tilted square grid lines: three laboratories per country, square grid lines: four laboratories, one in each nation of the UK).*

Table 4. Breakdown of the number of participants by challenge type, EQA Programme Influenza, 2023

Region	Number of participants for each challenge, n (%)																	
	Molecular detection		Virus isolation		Characterisation						Antiviral susceptibility testing						Full programme[1]	
					Antigenic only		Genetic only		Both		Genetic only		Phenotypic only		Both			
	n	(%)	n	(%)													n	%
ECDC-funded[2] (N=42)	42	(100%)	26	(61.9%)	2	(4.8%)	10	(23.8%)	10	(23.8%)	7	(16.7%)	3	(7.1%)	11	(26.2%)	20	(47.6%)
WHO-funded[2] (N=12)	11	(91.7%)	5	(41.7%)	0	(0%)	2	(16.7%)	1	(8.3%)	3	(25%)	1	(8.3%)	2	(16.7%)	3	(25%)
Total (N=54)	**53**	**(98.1%)**	**31**	**(57.4%)**	**2**	**(3.7%)**	**12**	**(22.2%)**	**11**	**(20.4%)**	**10**	**(18.5%)**	**4**	**(7.4%)**	**13**	**(24.1%)**	**23**	**(42.6%)**

[1] *See Table A10 for details of full programme.*

[2] *Participation of laboratories located in EU/EEA countries, Western Balkans and Türkiye was supported by ECDC. Participation of additional laboratories in the WHO European Region was supported by the WHO Regional Office for Europe.*

3.2 Molecular detection

A total of 53 laboratories from 44 countries participated in the molecular detection, typing, type A H- and N-subtype and type B lineage determination challenges. An aggregated breakdown of the results is shown in Table 5 by specimen code, and in Table A11 by participating laboratory. The methodologies used are listed by laboratory in Table A11. An overview of the percentage of laboratories by cumulative performance score for detection, A/B typing and type A H-subtyping is shown in Figure 2 and by participating laboratory in Table A11.

Almost all participating laboratories (52/53, 98%) identified the influenza virus type in all panel specimens. Most of the laboratories tested for and determined the A H-subtype correctly (50/51, 94% of all 53 participants). Only around one third of the laboratories tried to identify the A N-subtype and 63% of them succeeded for all panel specimens (10/16, 19% of all participants). Many laboratories did not report on type A N-subtyping (35/53, 66% for N1pdm09 and 37/53, 70% for N2) and B lineage determination (10/53, 19%), probably because such assays were not available in the laboratory (Table 5 and Table A11). A total of 43 laboratories determined the B lineage, most of them successfully for all samples (41/43, 77% of all participants). Overall, 26% of all laboratories (14/53) tested the panel completely by performing typing, type A H- and N-subtyping and type B lineage determination, and only nine laboratories reported a fully correct result (9/14, 17% of all participants) (Figure 2). Some laboratories made reporting errors by reporting 'negative' instead of 'not tested' for subtyping or lineage (Figure 2). No incorrect subtyping or B lineage determination results were reported, only false negatives: one laboratory with A H-subtype false negative (1/53, 2%), six laboratories with A N-subtype false negative (6/16, 11% of all participants) and two laboratories with B lineage false negative (2/43, 4% of all participants). Most laboratories (44/53; 83%) with a cumulative performance score of 0 correctly identified the influenza virus type: 42 laboratories correctly identified the type A H-subtype (42/44, 79% of all participants) and 36 of them correctly determined the B lineage (36/44, 68% of all participants)(Table A11).

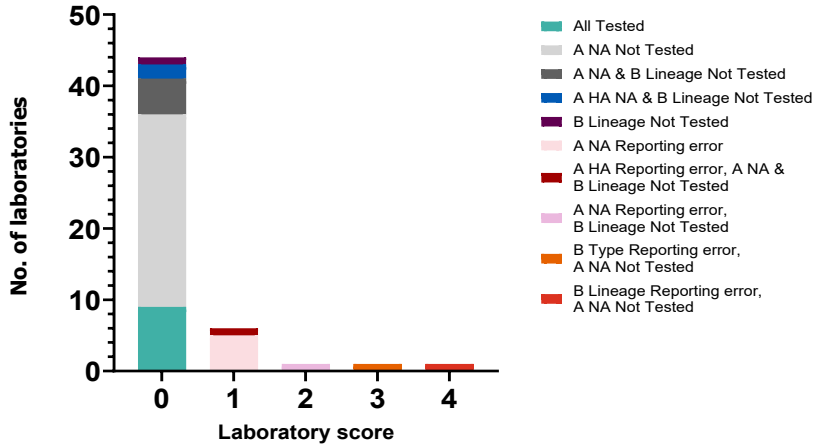
The high performance of laboratories with reported datasets using a wide variety of assays for the specific challenge types (Table A11) indicates that there were no specific issues with any of these assays.

Table 5. Overview of molecular detection, typing, type A H- and N-subtype and type B lineage determination results by specimen code, European EQA Programme, Influenza, 2023

Specimen code	Expected results	Cycle threshold (Ct) value by contractor laboratory[1]	Molecular detection by type, A H- and N-subtyping and B lineage determination (N=53)[2]										
			Type		A H-subtype			A N-subtype			B lineage			All correct n (%)
			Correct n (%)	Incorrect n (%)	Correct n (%)	Incorrect n (%)	Not tested n (%)	Correct n (%)	Incorrect n (%)	Not tested n (%)	Correct n (%)	Incorrect n (%)	Not tested n (%)	
EISN_INF23-01	A(H1N1)pdm09	18.4	53 (100%)	0 (0%)	51 (96.2%)	0 (0%)	2 (3.8%)	17 (32.1%)	1 (1.9%)	35 (66.0%)	n/a	n/a	n/a	17 (32.1%)
EISN_INF23-02	A(H3N2)	19.6	53 (100%)	0 (0%)	50 (94.3%)	1 (1.9%)	2 (3.8%)	15 (28.3%)	1 (1.9%)	37 (69.8%)	n/a	n/a	n/a	15 (28.3%)
EISN_INF23-03	B/Victoria	25.0	52 (98.1%)	1 (1.9%)	N/A	N/A	N/A	N/A	N/A	N/A	41 (77.4%)	2 (3.8%)	10 (18.9%)	41 (77.4%)
EISN_INF23-04	A(H3N2)	18.1	53 (100%)	0 (0%)	51 (96.2%)	0 (0%)	2 (3.8%)	16 (30.2%)	0 (0%)	37 (69.8%)	N/A	N/A	N/A	16 (30.2%)
EISN_INF23-05	Negative	n/a	52 (98.1%)	1 (1.9%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	52 (98.1%)
EISN_INF23-06	A(H1N1)pdm09	16.2	53 (100%)	0 (0%)	51 (96.2%)	0 (0%)	2 (3.8%)	17 (32.1%)	1 (1.9%)	35 (66.0%)	N/A	N/A	N/A	17 (32.1%)
EISN_INF23-07	B/Victoria	20.2	53 (100%)	0 (0%)	N/A	N/A	N/A	N/A	N/A	N/A	42 (79.2%)	1 (1.9%)	10 (18.9%)	42 (79.2%)
EISN_INF23-08	A(H3N2)	18.1	53 (100%)	0 (0%)	51 (96.2%)	0 (0%)	2 (3.8%)	12 (22.6%)	4 (7.6%)	37 (69.8%)	N/A	N/A	N/A	12 (22.6%)

[1] For influenza type A viruses derived from generic influenza type A virus detection RT-qPCR (matrix gene) and for influenza type B viruses derived from B/Victoria specific detection RT-qPCR (HA gene).
[2] Methodologies are shown in Table A11.

Figure 2. Overview of cumulative performance scores for molecular detection, typing (A/B), type A H-and N-subtyping and type B lineage determination, European EQA Programme, Influenza 2023



Scoring system used:

- *A viruses: incorrect type, 1; incorrect H-subtype or reporting error (negative), 1; incorrect N-subtype or reporting error (negative), 1; Laboratories not testing for A H- and/or N-subtype were not scored for the respective part.*
- *B viruses: incorrect type, 1; incorrect lineage or reporting error (negative), 2; Laboratories not testing for B lineage were not scored for the respective part.*
- *Negative specimen: Negative 0; all other results, 3*

3.3 Virus isolation and antigenic and genetic characterisation

A total of 31 of the 54 laboratories participated in the virus isolation challenge. For the antigenic and genetic characterisation challenge, 25 laboratories participated. Of these, 11 reported both antigenic and genetic characterisation results, 12 genetic characterisation results only and two antigenic characterisation results only.

3.3.1 Virus isolation

An aggregated breakdown of the virus isolation results of the 31 laboratories that returned results is shown in Table 6 and the details by participating laboratory and cumulative performance score in Table A12. The methodologies used are listed by laboratory in Table A12. An overview of laboratories by cumulative performance score is shown in Figure 3.

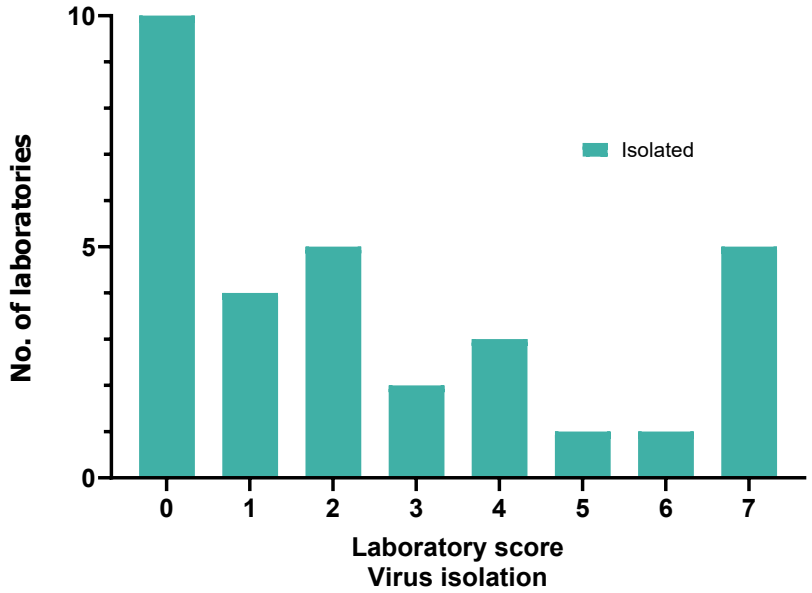
Table 6. Overview of virus isolation results by specimen code, European External Influenza Quality Assessment Programme, 2023

Specimen code	Virus	Pfu/ml; Ct value [1]	Expected results	Viral isolation results (n=31)			
				Correct Results		Incorrect results	
				Result	n (%)	Result	n (%)
EISN_INF23-01	A(H1N1)pdm09	345000; 18.4	Positive	Positive	19 (61.3%)	Negative	12 (38.7%)
EISN_INF23-02	A(H3N2)	34500; 19.6	Positive	Positive	23 (74.2%)	Negative	8 (25.8%)
EISN_INF23-03	B/Victoria	345; 25.0	Positive	Positive	13 (41.9%)	Negative	18 (58.1%)
EISN_INF23-04	A(H3N2)	34500; 18.1	Positive	Positive	22 (71.0%)	Negative	9 (29.0%)
EISN_INF23-05	No virus	Not applicable	Negative	Negative/not attempted[2]	30 (96.8%)	Positive	1 (3.2%)
EISN_INF23-06	A(H1N1)pdm09	34500; 16.2	Positive	Positive	18 (58.1%)	Negative	13 (41.9%)
EISN_INF23-07	B/Victoria	1091; 20.2	Positive	Positive	24 (77.4%)	Negative	7 (22.6%)
EISN_INF23-08	A(H3N2)	10910; 18.1	Positive	Positive	21 (67.7%)	Negative	10 (32.3%)

[1] Plaque forming units (pfu) transposed by multiplying with 0.69 from $TCID_{50}$ determined by titration on MDCK-MIX cells (MDCK-I and MDCK-SIAT) in rotating tubes. This way of titrating generates a higher titer than using 96-well microtiter plates and static incubation. End-points were determined by CPE (cytopathic effect) and NA activity for each tube. For influenza type A viruses Ct values derived from generic influenza type A virus detection RT-qPCR (matrix gene) and for influenza type B viruses Ct values derived from B/Victoria specific detection RT-qPCR (HA gene).

[2] 'Not attempted' indicates that the laboratory did not attempt virus isolation because molecular testing was negative and the laboratories' algorithms probably only include virus-positive specimens in virus isolation. Therefore these results are considered correct.

Figure 3. Overview of cumulative performance scores for virus isolation, European EQA Programme, Influenza, 2023



Scoring system used:

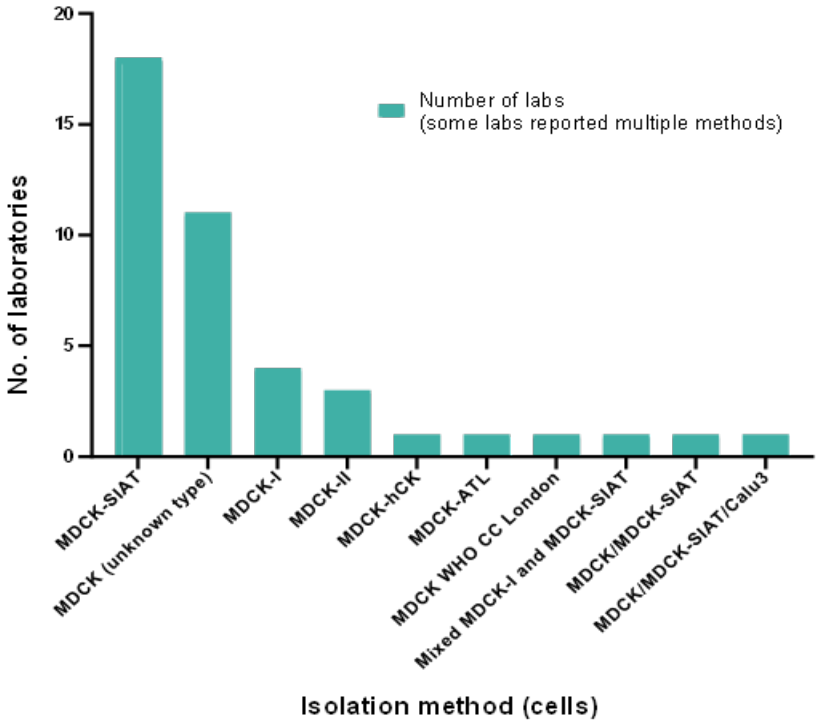
- *Virus containing specimens: Positive and Not Attempted (because of negative result in molecular detection), 0; Negative and all other results, 1.*
- *Specimen without virus: Negative and Not Attempted (because of negative result in molecular detection), 0; all other results, 1.*

A third of the laboratories (10/31; 32%) had completely correct results (cumulative performance score of 0) reporting all influenza virus-containing specimens with the correct isolated virus and the specimen not containing a virus as negative (Figure 3). Of the 31 laboratories, four laboratories failed to isolate virus from all virus-containing specimens. Nine laboratories failed to isolate virus from both A(H1N1)pdm09 containing specimens, while six laboratories failed to isolate all three A(H3N2)-containing specimens and five laboratories failed to isolate virus from both B/Victoria-containing specimens. One laboratory reported having isolated virus from the negative specimen, while this laboratory reported the expected result (no result) for antigenic and genetic characterisation for the negative specimen, suggesting a reporting error rather than a technical virus isolation error. Overall, laboratories performed less well on the B/Victoria-containing specimens, with lower viral load than higher viral load and on A(H1N1)pdm09-containing specimens than the A(H3N2)-containing specimens (Table 7).

All laboratories used some variant of MDCK cells for virus isolation, MDCK-SIAT was most frequently reported although a wide variety of other variants of MDCK cell lines were also reported (or an unknown variant or source was reported) (Figure 4 and Table A12). None of the laboratories reported the use of eggs for virus isolation. Failure to isolate certain virus types/subtypes/lineages was not clearly linked to the use of specific variants of MDCK cells. The number of freeze/thaw cycles before inoculation (median 1, range 1–4 cycles) and the volume of the original panel specimen inoculated (median 0.2 ml, range 0.02–1 ml) was also not clearly correlated with failure to isolate (details not shown in Table A12).

The majority of laboratories used haemagglutination (22/31 laboratories, 71%), mainly combined with cytopathic effect (CPE) for detection and confirmation of virus growth (19/22 laboratories, 61% of all participants) (Table A12). In total, 25 laboratories (25/31, 81%) checked for CPE. In addition, RT-PCR (16/31 laboratories, 52%), immunofluorescence (7/31 laboratories, 23%) and NA activity assay (5/31 laboratories, 16%) were used to detect and/or confirm growth of virus. For haemagglutination, a wide variety of red blood cell (RBC) types was used. Several laboratories used a combination of RBC types or a specific RBC type, depending on the influenza virus type and type A H-subtype (Table A12).

Figure 4. Overview of reported cells used for virus isolation, European EQA Programme, Influenza, 2023



Due to the relatively high number of laboratories reporting difficulties in isolating viruses, an additional questionnaire was developed and distributed to the network to get insight into possible causes for failure to isolate. Of the 31 laboratories that participated in the virus isolation challenge, seven responded to the additional questionnaire. There was considerable variation in the reported isolation methods, however no correlation could be identified between specific factors and failure in virus isolation (Annex 1).

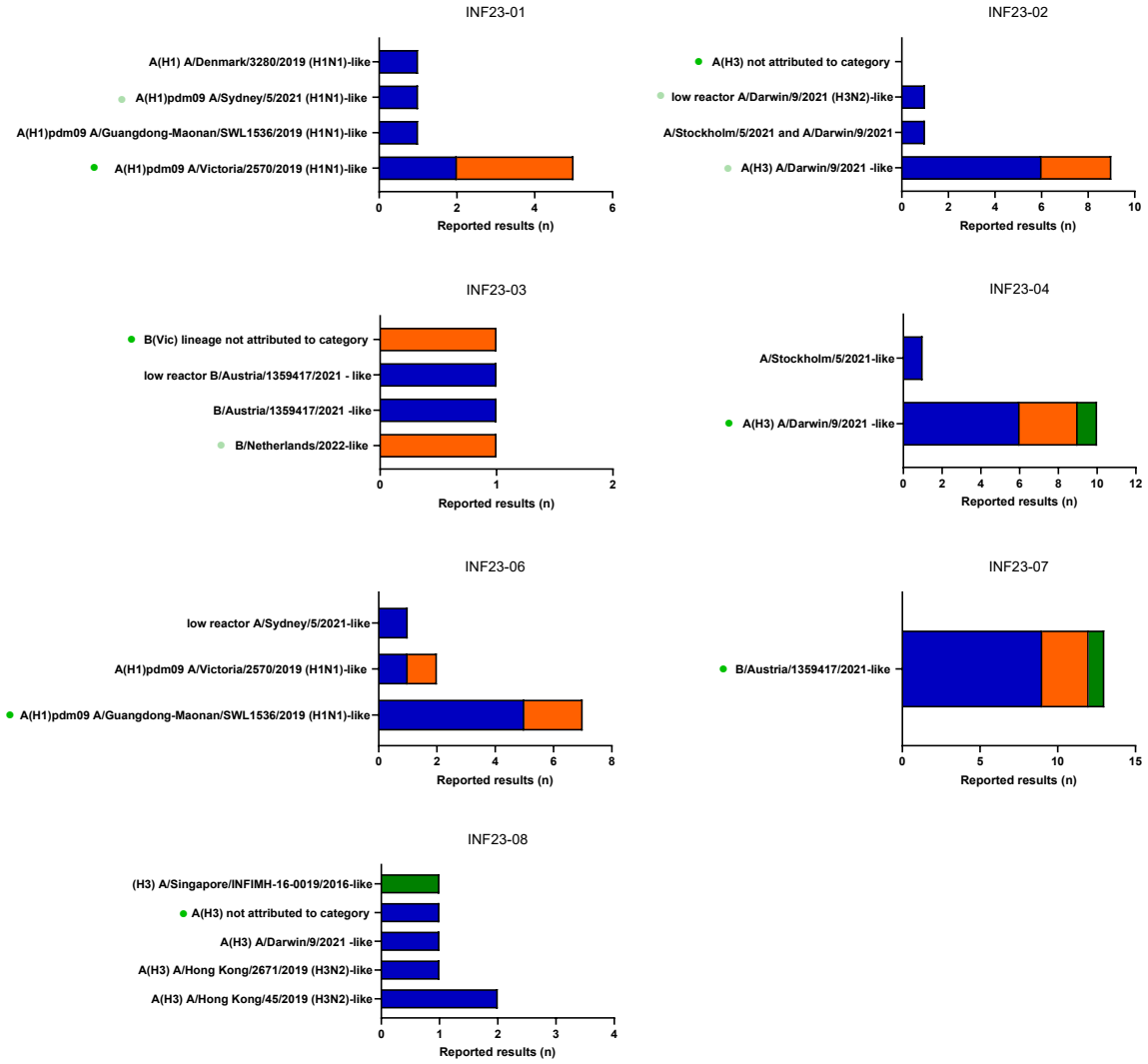
3.3.2 Antigenic characterisation

Thirteen laboratories reported antigenic characterisation results. Evaluation of the reported antigenic characterisation results in comparison with the expected results was challenging. The antigenic characterisation category returned, as pre-set in the TESSy reporting system, largely depends on the number and specificity of the antisera used in HI-assays and the characteristics of the used assays. For example, this can be the type of RBCs used in HI-assay or the deployment of oseltamivir in the HI-assay for viruses with NA-related haemagglutination (although with current A(H3N2) influenza viruses NA-related haemagglutination does not seem to be a serious problem anymore). To summarise the antigenic characterisation results and address these subjectivities and the wide range of reported categories, the reported results, in combination with the source of antisera, are depicted per specimen in Figure 5 and by participating laboratory in Table A13 in the annexes. The methodologies used are listed by laboratory in Table A13 in the annexes. All 13 laboratories reporting on antigenic characterisation used HI-assay.

The type of sera used in HI-assay was primarily ferret sera provided by WHO CC London. Sera from ferrets raised in-house was also used and one lab used WHO CC London ferret sera in combination with in-house generated rooster sera (Table A13). Most laboratories used guinea pig RBC or turkey RBC, alone or together (Table A13). To get more insight into the background of the reported characterisation categories, participating laboratories were asked to report the exact strain specificities of the reference sera and viruses used (shown in Table A14). The overall median number of three different sera/viruses used per panel specimen is relatively low for precise characterisation, and for three specimens one or two laboratories did not use sera/viruses that corresponded with the reference viruses mentioned in the expected results.

The overall use of sera with a narrower or wider spectrum of specificities certainly influenced the characterisation results. This reflects the variety of results that can be reported to TESSy for the same virus, depending on the laboratory that performed the analysis, using different sets of reference sera (Table A14).

Figure 5. Overview summarising the reported antigenic characterisation categories by specimen code and source reference antisera, European EQA Programme, Influenza 2023



Expected result categories are indicated with a green dot next to the category name; a light green dot was used for results that were considered as close to the expected result.

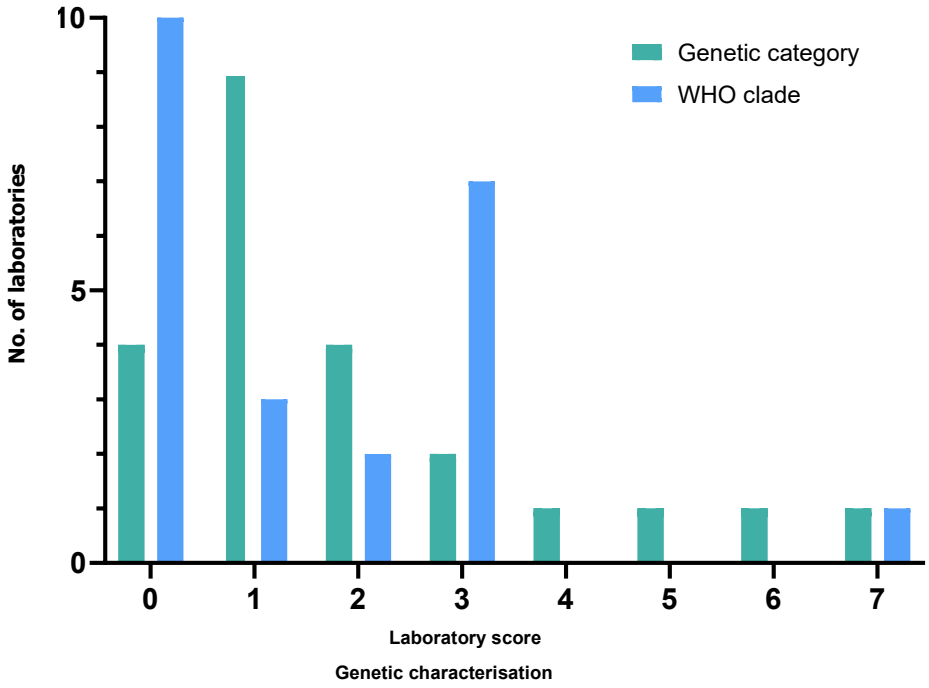
The origin of the serum panel used for the characterisation is indicated by colours in the bars: WHO CC London ferret sera (blue); in-house generated ferret sera (orange); WHO CC London ferret sera and in-house generated rooster sera (green).

3.3.3 Genetic characterisation

A total of 23 laboratories performed genetic characterisation, however, four laboratories did not report for all panel specimens (Figure 6 below and Table A15 in the annexes). An overview of the percentage of laboratories by cumulative performance score is shown in Figure 6. Participants reported the genetic characterisation by TESSy genetic category assignment (e.g. genAH3/Darwin/9/2021) and by WHO clade assignment (e.g. 3C.2a1b.2a.2a.3) following phylogenetic analysis. For genetic category assignment, reported strain names in the same clade as the expected genetic category were considered correct (Table 7 below, green). Reported strain names that were not allocated to the correct clade, are depicted in light orange in Table 7 when they were in an approximately common root/clade to the expected genetic category (Figure A4-6 in the annexes). Reported strain names that were allocated to an incorrect clade are depicted in dark orange in Table 7.

Four laboratories (4/23; 17%) had fully correct results (cumulative performance score of 0) for reported specimens (Figure 6 below). Correctly reported results ranged from 57–91% (13/23 – 21/23) for the individual panel specimens (Table 7). The A(H3N2) viruses of specimen EISN_INF23-02, EISN_INF23-04 and EISN_INF23-08 were misassigned by six, four and three laboratories, respectively, to a genetic group with a reference strain in a different genetic clade in the phylogenetic tree topology (Table 7 below and Figure A5 in the annexes). For the EISN_INF23-01 A(H1N1)pdm09 virus specimen, seven laboratories mis-assigned the virus to an approximately common clade/root. When genetic characterisation was reported by WHO clade assignment, the percentage of correctly reported results improved, compared to TESSy genetic category assignment (Table 8 below, and Table A16 in the annexes). Ten laboratories (10/23; 44%) had fully correct results (cumulative performance score of 0) for reported specimens (Figure 6 below), and correctly reported results for the individual panel specimens ranged from 61–96% (14/2 3 –22/23) (Table 8 below). The methodologies used are listed by laboratory in Table A15 in the annexes.

Figure 6. Overview of cumulative performance scores for genetic characterisation by genetic category and WHO clade assignment, European EQA Programme, Influenza, 2023



Scoring system used: Correct: 0; Incorrect: 1 (for detailed arguments on allocating results to 'Correct' or 'Incorrect', see footnote to Table A15).

Table 7. Overview summarising the reported genetic characterisation using (TESSy) genetic category assignment by specimen code, European EQA Programme, Influenza, 2023

Specimen code	Expected Results Genetic category [1]	Participants with results n	Genetic category results					
			Correct			Incorrect (dark orange = wrong category; light orange = close to correct category)		
			Result	n (%)	Total n (%)	Result	n (%)	Total n (%)
EISN_INF23-01	genAH1/Norway/25089/2022	23	**genAH1/Norway/25089/2022**	13 (56.5%)	13 (56.5%)	A/Guangdong-Maonan/SWL1536/2019	1 (4.3%)	10 (43.5%)
						A(H1)pdm09 not attributed to clade	1 (4.3%)	
						A/Victoria/2570/2019	3 (13.0%)	
						A(H1) A/Sydney/5/2021	4 (17.4%)	
						Failed	1 (4.3%)	
EISN_INF23-02	genAH3/Bangladesh/4005/2020	23	**A/Bangladesh/4005/2020**	14 (60.9%)	14 (60.9%)	A/Darwin/9/2021	6 (26.1%)	9 (39.1%)
						A(H3) not attributed to clade	1 (4.3%)	
						A(H3) other: attributed to recognised group in current guidance but not listed here	2 (8.7%)	
EISN_INF23-03	genBVicB/Netherlands/11267/2022	21	**B/Netherlands/11267/2022**	15 (71.4%)	15 (71.4%)	B/Austria/1359417/2021	1 (4.8%)	6 (28.6%)
						B(Vic) lineage not attributed to clade	1 (4.8%)	
						B/Washington/02/2019	3 (14.3%)	
						Failed	1 (4.8%)	
EISN_INF23-04	genAH3/Darwin/9/2021	23	**A/Darwin/9/2021**	13 (56.5%)	13 (56.5%)	A/Bangladesh/4005/2020	4 (17.4%)	10 (43.5%)
						A(H3) not attributed to clade	1 (4.3%)	
						A(H3) other: attributed to recognised group in current guidance but not listed here	5 (21.7%)	
EISN_INF23-05	N/A							
EISN_INF23-06	genAH1/Guangdong-Maonan/SWL1536/2019. This virus could also be allocated to the TESSy category genAH1SubgroupNotListed within clade 6B.1A.5a.1.	23	**A/Guangdong-Maonan/SWL1536/2019**	20 (90.0%)	21 (91.3%)	A(H1)pdm09 not attributed to clade	1 (4.3%)	2 (8.7%)
			A/Belgium/S1111/2022	1 (4.3%)		Failed	1 (4.3%)	
EISN_INF23-07	genBVicB/Austria/1359417/2021	21	**B/Austria/1359417/2021**	17 (81.0%)	18 (85.7%)	B/Washington/02/2019	1 (4.8%)	3 (14.3%)
			B(Vic)-lineage B/Bishkek/11/2022	1 (4.8%)		B(Vic) lineage not attributed to clade	1 (4.8%)	
						Failed	1 (4.8%)	
EISN_INF23-08	genAH3/Denmark/3264/2019	23	**A/Denmark/3264/2019**	19 (82.6%)	19 (82.6%)	A/Hong Kong/2671/2019	3 (13.0%)	4 (17.4%)
						A(H3) not attributed to clade	1 (4.3%)	

[1] *Details in Table 2.*

Table 8. Overview summarising the reported genetic characterisation using WHO clade assignment by specimen code, European EQA Programme, Influenza, 2023

Sample code	Number tested	Expected results	Result correct	WHO clade correct, n(%)	Result incorrect	WHO clade incorrect, n(%)
EISN_INF23-01	23	6B.1A.5a.2a.1	6B.1A.5a.2a.1	14 (60.9%)	6B.1A.5a.2 (5); 6B.1A.5a.2a (1); 6B.1A.5a.1 (1); FAILED (2)	9 (39.1%)
EISN_INF23-02	23	3C.2a1b.2a.2b	3C.2a1b.2a.2b	15 (65.2%)	3C.2a1b.2a.2 (7); FAILED (1)	8 (34.8%)
EISN_INF23-03	21	V1A.3	V1A.3	18 (85.7%)	V1A.3a.2 (1) FAILED (2)	3 (14.3%)
EISN_INF23-04	23	3C.2a1b.2a.2a.3	3C.2a1b.2a.2a.3	15 (65.2%)	3C.2a1b.2a.2 (6); 3C.2a1b.2a.2b (1); FAILED (1)	8 (34.8%)
EISN_INF23-05	N/A	N/A	N/A	N/A	N/A	N/A
EISN_INF23-06	23	6B.1A.5a.1	6B.1A.5a.1	21 (91.3%)	FAILED (2)	2 (8.7%)
EISN_INF23-07	21	V1A.3a.2	V1A.3a.2	18 (85.7%)	V1A.3 (1); FAILED (2)	3 (14.3%)
EISN_INF23-08	23	3C.2a1b.1a	3C.2a1b.1a	22 (95.7%)	FAILED (1)	1 (4.3%)

N/A – Not applicable

Twenty-three laboratories uploaded 151 obtained HA sequences, of which 17 laboratories (81%) uploaded for all virus-containing specimens (Table A15 in the annexes). The majority contained the full coding region. Six laboratories reported incomplete sequences or poor sequences with stretches of Ns (no nucleotide assignment) and/or nucleotide mismatches with the sequences of the original viruses in the specimens (details in Table A15). On two occasions this led to an incorrect WHO clade assignment when all sequences were subjected to analysis in Nextclade (https://clades.nextstrain.org/). For all other sequences, the expected WHO clade assignment was returned when subjected to analysis in Nextclade. Therefore, those laboratories that uploaded sequences and reported a not fully correct WHO clade name (Table 8 above and Table A16 in the annexes) did generate sequences that were suitable for the WHO clade assignment, as expected. This suggests that WHO clade assignment can be easily improved (e.g. by using publicly available tools such as Nextclade).

3.4 Antiviral susceptibility

Twenty-seven laboratories participated in the antiviral susceptibility challenge, of which 13 performed genetic and phenotypic testing, 10 genetic testing only and four phenotypic testing only. In general, results for the three specimens specifically designed for antiviral susceptibility testing (EISN_AV23-1 to EISN_AV23-3) were reported by almost all participating laboratories (Table A17 and A18 in the annexes). Meanwhile, for samples EISN_INF23-01 to EISN_INF23-08 reporting of results was often incomplete in terms of inhibitors and/or samples tested. One of the reasons could be unsuccessful isolation in the virus isolation challenge as a prerequisite for especially phenotypic testing, although in some cases with successful virus isolation, antiviral susceptibility results were still not reported. The level of participation was therefore quite diverse, with a number of laboratories that tested a limited number of specimens and/or inhibitors (Table A17 and A18).

3.4.1 Genetic testing

A total of 23 laboratories performed genetic testing for antiviral susceptibility. An overview of the percentage of laboratories by cumulative performance score is shown in Figure 7. Seven laboratories (7/23, 30%) had fully correct results (cumulative performance score of 0) for reported specimens. Incorrect results consisted of genotype (misidentifying mutations/deletions) and interpretation errors. Genetic testing results are shown in Table 9 below with an aggregated breakdown of the identified amino acid substitutions and Table 10 below with an aggregated breakdown of the reported interpretations. Results on amino acid substitution identification and interpretation by participating laboratory and cumulative performance score are shown in Table A17 in the annexes.

While all 23 laboratories reported results for oseltamivir and zanamivir susceptibility (for some or all specimens), 17 laboratories reported results for baloxavir susceptibility. Only five laboratories tested all virus-containing specimens for both NA inhibitors (oseltamivir and zanamivir) and the PA-inhibitor baloxavir (Table A17 in the annexes). The level of testing depended on the type of tests available and deployed.

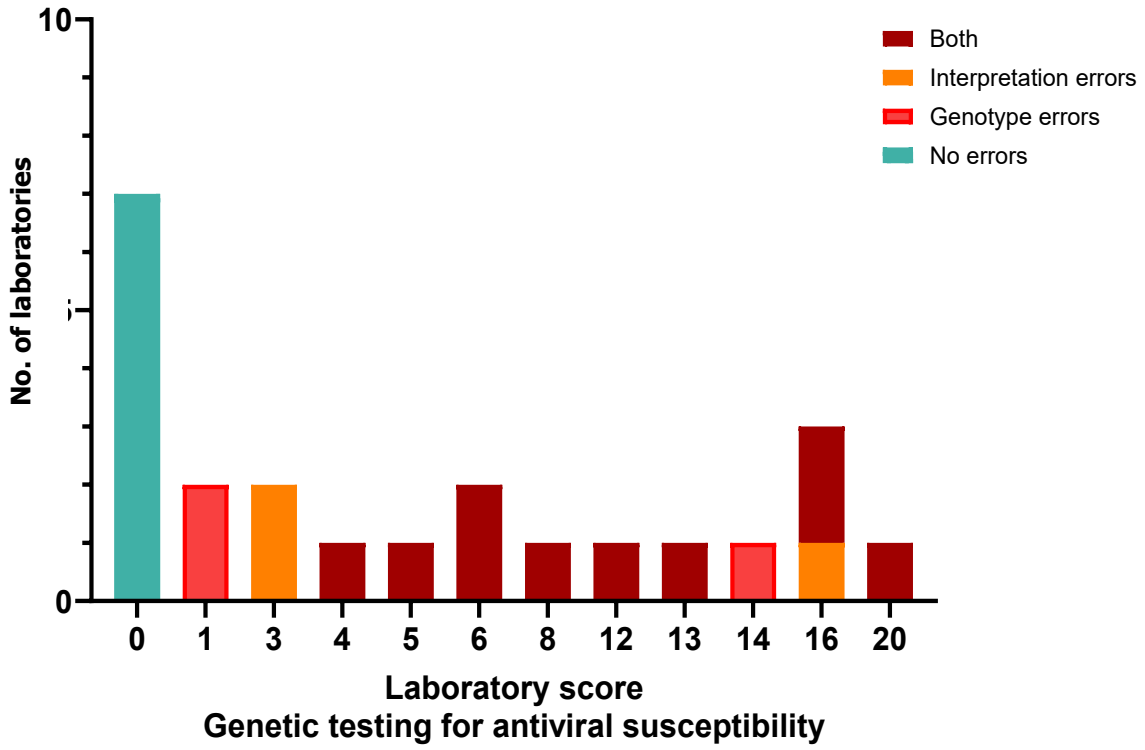
Twenty of 21 (95%) laboratories identified the NA-H275Y amino acid substitution in specimen EISN_AV23-01 (Table 9 below), of which a correct NA-inhibitor susceptibility interpretation was reported by 19 and 20 laboratories for oseltamivir (AAHRI) and zanamivir (AANI), respectively (Table 10 below). The laboratory that did not identify H275Y used full-length sequencing, but no NA sequence was uploaded for evaluation.

Fifteen of 16 (94%) laboratories identified the PA-E23G amino acid substitution in specimen EISN_AV23-02 (Table 9 below), 10 of which reported a correct interpretation for baloxavir (AARS) (Table 10 below). The laboratory that did not report the PA-E23G amino acid substitution uploaded a PA sequence containing the substitution.

Twelve of 21 (57%) laboratories correctly identified the 245-248 amino acid deletion in specimen AV23-03 (Table 9), 10 of which reported a correct interpretation for oseltamivir (AAHRI) and zanamivir (AARI) (Table 10). Six laboratories reported partially correct amino acid substitutions (e.g. Del244-247 instead of Del 245-248), four of which reported a correct interpretation for oseltamivir (AAHRI) and three for zanamivir (AARI). Three laboratories incorrectly reported no amino acid substitutions associated with reduced antiviral inhibition, while the expected 245-248 amino acid deletion was present in the uploaded NA sequences.

The majority of laboratories correctly identified genetic antiviral susceptibility in cases where the specimens contained no amino acid substitutions associated with reduced inhibition (Tables 10 and 11 below, Table A17 in the annexes). Three laboratories reported an NA-amino acid substitution for one or two specimens. One laboratory reported S150R and R150S with interpretation AAHRI for oseltamivir for A(H3N2)-containing specimens EISN_INF23-04 and EISN_INF23-08, respectively. Another laboratory reported N329S for A(H3N2)-containing specimens EISN_INF23-04 and EISN_INF23-08. Although the reported codons were present in the sequences uploaded by these laboratories, these are natural polymorphisms that do not affect antiviral susceptibility, as they are not mentioned in the WHO table [22].

Figure 7. Overview of cumulative performance scores for genetic antiviral susceptibility testing, European EQA Programme, Influenza 2023



Scoring system used:

EISN_AV23-01 substitutions – NA-H275Y found, 0; any other, 1. Not tested, not scored.
EISN_AV23-01 interpretation oseltamivir: AAHRI, 0; AARI, 1; rest, 2. Interpretation zanamivir: AANI, 0; rest, 2. Interpretation baloxavir: AANS, 0; rest, 2. Not tested, not scored.
EISN_AV23-02 substitutions: PA-E23G found, 0; rest, 2; Not tested, not scored.
EISN_AV23-02 interpretation oseltamivir: AANI, 0; rest, 2. Interpretation zanamivir: AANI, 0; rest, 2. Interpretation baloxavir: AARS, 0; rest, 2. Not tested, not scored.
EISN_AV23-03 substitutions: NA-Del245-248 found, 0; partial deletions, 1; rest, 2; Not tested, not scored.
EISN_AV23-03 interpretation oseltamivir: AAHRI, 0; AARI, 1; rest, 2. Interpretation zanamivir: AARI, 0; AAHRI, 1; rest, 2. Interpretation baloxavir: AANS, 0; rest, 2. Not tested, not scored.
EISN_INF23-01 – 08 (except 05) substitution: none found, 0, any other, 2; Not tested, not scored.
EISN_INF23-01 – 08 (except 05) interpretation oseltamivir and zanamivir: AANI, 0; any other, 2; Not tested, not scored.

Table 9. Overview summarising the reported identified amino acid substitutions associated with reduced antiviral susceptibility by specimen code, European EQA Programme, Influenza, 2023

Sample code	Identification of NA amino acid subtitutions [3]							Identification of PA amino acid subtitutions [3]				
	Number of labs tested, n (%) [1]	Expected results	Correct, n (%)	Partial correct results (n)	Partially correct, n (%)	Incorrect results (n)	Incorrect, n (%)	Number of labs tested, n (%) [1]	Expected results	Correct, n (%)	Incorrect results	Incorrect, n (%)
EISN_AV23-01	21 (100%)	H275Y	20 (95.2%)		0 (0.0%)	None (1)	1 (4.8%)	15 (100%)	None	14 (93.3%)	L28P (1)	1 (6.7%)
EISN_AV23-02	21 (100%)	None	17 (81.0%)		0 (0.0%)	N329S (3), R292K (1)	4 (19.0%)	16 (100%)	E23G	15 (93.8%)	None (1)	1 (6.2%)
EISN_AV23-03	21 (100%)	Del245-248	12 (57.1%)	Del244-247 (4), A246del (1), del245-248; R292K; N294S (1)	6 (28.6%)	None (3)	3 (14.3%)	17 (100%)	None	17 (100%)		0 (0.%)
EISN_INF23-01	18 (100%)	None	17 (94.4%)		0 (0.0%)	no sequence (1)	1 (5.6%)	13 (100%)	None	13 (100%)		0 (0%)
EISN_INF23-02	20 (100%)	None	15 (75.0%)		0 (0.0%)	N329S (3), H347Q (1), no sequence (1)	5 (25.0%)	14 (100%)	None	14 (100%)		0 (0%)
EISN_INF23-03	18 (100%)	None	16 (88.9%)		0 (0.0%)	A395T (1), no sequence (1)	2 (11.1%)	9 (100%)	None	8 (88.9%)	E194X (1)	1 (11.1%)
EISN_INF23-04	20 (100%)	None	17 (85.0%)		0 (0.0%)	S150R (2), no sequence (1)	3 (15.0%)	15 (100%)	None	15 (100%)		0 (0%)
EISN_INF23-05												
EISN_INF23-06	19 (100%)	None	18 (94.7%)		0 (0.0%)	no sequence (1)	1 (5.3%)	13 (100%)	None	13 (100%)		0 (0%)
EISN_INF23-07	18 (100%)	None	16 (88.9%)		0 (0.0%)	A395V (1), no sequence (1)	2 (11.1%)	11 (100%)	None	11 (100%)		0 (0%)
EISN_INF23-08	20 (100%)	None	15 (75.0%)		0 (0.0%)	R150S (2), N329S (2), no sequence (1)	5 (25.0%)	14 (100%)	None	14 (100%)		0 (0%)

[1] A number of laboratories did not perform genotypic antiviral susceptibility testing for all specimens, depending on available tests.
[2] No substitutions = no amino acid substitutions associated with a reduction in neuraminidase inhibitor susceptibility following full NA segment sequencing.
[3] The reported result has been translated from amino acid profiles reported and judged as correct or incorrect, regardless of whether the NA segment was fully or partially sequenced or whether only SNP detection assay was used.

Table 10. Overview summarising the reported interpretation of amino acid substitution identification associated with reduced antiviral susceptibility by specimen code, European EQA Programme, Influenza, 2023

Sample code	Oseltamivir interpretation							Zanamivir interpretation							Baloxavir interpretation				
	Number of labs tested, n (%) [1]	Expected results [2]	Correct, n (%)	Partial correct results (n)	Partially correct, n (%)	Incorrect results (n)	Incorrect, n (%)	Number of labs tested, n (%)	Expected results [2]	Correct, n (%)	Partial correct results (n)	Partially correct, n (%)	Incorrect results (n)	Incorrect, n (%)	Number of labs tested, n (%)	Expected results [2]	Correct, n (%)	Incorrect results (n)	Incorrect n (%)
EISN_AV23-01	21 (100.0%)	AAHRI	19 (90.5%)		0 (0%)	AARI (1), NO (1)	2 (9.5%)	21 (100.0%)	AANI	20 (95.2%)		0 (0%)	NO (1)	1 (4.8%)	15 (100%)	AANS	13 (86.7%)	AARS (1), NO (1)	2 (13.3%)
EISN_AV23-02	21 (100.0%)	AANI	19 (90.5%)		0 (0%)	AAHRI (1), NO (1)	2 (9.5%)	21 (100.0%)	AANI	19 (90.5%)		0 (0%)	NO (2)	2 (9.5%)	16 (100%)	AARS	13 (81.2%)	AANS (2), NO (1)	3 (18.8%)
EISN_AV23-03	21 (100.0%)	AAHRI	14 (66.7%)	AARI (3)	3 (14.3%)	AANI (3), NO (1)	4 (19.0%)	21 (100.0%)	AARI	12 (57.1%)	AAHRI (1)	1 (4.8%)	AANI (5), NO (3)	8 (38.1%)	17 (100%)	AANS	16 (94.1%)	NO (1)	1 (5.9%)
EISN_INF23-01	18 (100.0%)	AANI	18 (100%)		0 (0%)		0 (0%)	18 (100.0%)	AANI	18 (100.0%)		0 (0%)		0 (0%)	13 (100%)	AANS	12 (92.3%)	NO (1)	1 (7.7%)
EISN_INF23-02	20 (100.0%)	AANI	19 (95.0%)		0 (0%)	NO (1)	1 (5.0%)	20 (100.0%)	AANI	19 (95.0%)		0 (0%)	NO (1)	1 (5.0%)	14 (100%)	AANS	13 (92.9%)	NO (1)	1 (7.1%)
EISN_INF23-03	18 (100.0%)	AANI	18 (100%)		0 (0%)		0 (0%)	18 (100.0%)	AANI	18 (100.0%)		0 (0%)		0 (0%)	9 (100%)	AANS	8 (88.9%)	AARS (1)	1 (11.1%)
EISN_INF23-04	20 (100.0%)	AANI	19 (95.0%)		0 (0%)	AAHRI (1)	1 (5.0%)	20 (100.0%)	AANI	20 (100.0%)		0 (0%)		0 (0%)	15 (100%)	AANS	14 (93.3%)	NO (1)	1 (6.7%)
EISN_INF23-05																			
EISN_INF23-06	19 (100.0%)	AANI	19 (100%)		0 (0%)		0 (0%)	19 (100.0%)	AANI	19 (100.0%)		0 (0%)		0 (0%)	13 (100%)	AANS	12 (92.3%)	NO (1)	1 (7.7%)
EISN_INF23-07	18 (100.0%)	AANI	18 (100%)		0 (0%)		0 (0%)	18 (100.0%)	AANI	18 (100.0%)		0 (0%)		0 (0%)	11 (100%)	AANS	11 (100%)		0 (0%)
EISN_INF23-08	20 (100.0%)	AANI	18 (90.0%)		0 (0%)	AAHRI (1), NO (1)	2 (10.0%)	20 (100.0%)	AANI	19 (95.0%)		0 (0%)	NO (1)	1 (5.0%)	14 (100%)	AANS	13 (92.9%)	NO (1)	1 (7.1%)

[1] A number of laboratories did not perform genotypic antiviral susceptibility testing for all specimens, depending on available tests.
[2] Expected result when the full NA and PA segment has been sequenced or specific amino acid substitution has been identified with any test; AANI = no amino acid substitution previously associated with (highly) reduced inhibition; AARI = amino acid substitution previously associated with reduced inhibition; AAHRI = amino acid substitution previously associated with highly reduced inhibition; 100% due to partial NA segment information (SNP RT-PCR, partial- or pyrosequencing).

3.4.2 Phenotypic testing

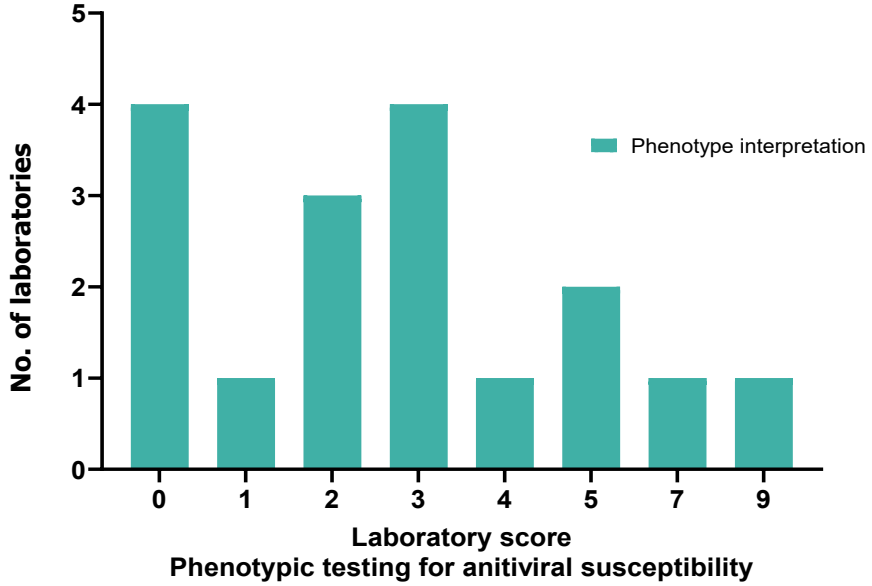
A total of 17 participants performed phenotypic testing for antiviral susceptibility. However, not all specimens were tested by all laboratories and not all were tested for oseltamivir and zanamivir (Table 11 below). An overview of laboratories by cumulative performance score is shown in Figure 8. Four laboratories (24%) had fully correct results (cumulative performance score of 0) for reported specimens. Table A18 in the annexes shows a breakdown of the interpretations of the reported IC_{50} values, and by participating laboratory with cumulative performance score and used assay to determine IC_{50} values. The methodologies used are also listed in aggregated form in Table 12 below.

The overall performance of the laboratories in phenotypic testing was excellent: for the EISN_AV23-01 specimen (NA-H275Y mutation)16/17 (94%) and 17/17 (100%) laboratories reported the correct oseltamivir and zanamivir susceptibility, respectively (Table 11 below). For the EISN_AV23-02 specimen (PA-E23G mutation), 17/17 (100%) laboratories reported the correct oseltamivir and zanamivir susceptibility. The EISN_AV23-03 specimen (NA-aa245-248 deletion) was more challenging as only 8/17 (47%) and 7/17 (41%) reported the correct oseltamivir and zanamivir susceptibility, respectively. In the specimens with wildtype virus (EISN_INF23-01 – 08, except EISN_INF23-05), phenotypic antiviral susceptibility was correctly reported in all instances (if tested), except for EISN_INF23-07. Based on IC_{50} obtained for the latter, four laboratories reported RI (n=2) or HRI (n=2) for oseltamivir and one laboratory also reported RI for zanamivir (Table A18 in the annexes).

IC_{50} fold-changes were calculated for the EISN_AV23-01 and EISN_AV23-03 specimens using the mean of those available for wildtype A(H1N1pdm09 (EISN_INF23-01 and 06) and A(H3N2) (EISN_INF23-02, 04, 08) viruses as a reference. Fold-change data were plotted and compared with the interpretation reported by the laboratories (Figure 9). For specimen EISN_AV23-01, IC_{50} fold-change values are well above 100 for oseltamivir and below 10 for zanamivir, as reflected by correct oseltamivir (HRI) and zanamivir (NI) susceptibility interpretation by the laboratories. For specimen EISN_AV23-03, many of the IC_{50} fold-change values are around 100 for oseltamivir and around 10 for zanamivir, partly explaining the more variable oseltamivir and zanamivir susceptibility interpretation by the laboratories. The differences in interpretation between our calculation and that of the laboratories probably depended on what was used as a reference for the calculation of the fold-changes. We used the IC_{50} mean value of the wildtype virus EISN_INF23 specimens, when laboratories used either the median (n=7) or mean (n=3) of seasonal influenza viruses routinely tested in the laboratory, or a wild-type reference virus as control (n=7) (Table 12 below). The ECDC guidance for reporting antiviral susceptibility data in accordance with the WHO antiviral working group guidelines recommends using the median IC_{50} value of the (sub)type previous season after removal of obvious outliers, or the floating median of the current season after removal of obvious outliers, if IC_{50} values for ≥15 viruses are available.

A variety of techniques and approaches were used for IC_{50} measurement and calculations of fold-changes needed for interpretation of IC_{50} data (Table 12 below). However, the majority of laboratories used an in-house MUNANA (20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid) substrate-based assay with pre-titration of NA-activity and an 'HPA Excel template' for IC_{50} calculation, reflecting the training activities performed in the past at the UK Health Security Agency (Table 12 below and Table A18 in the annexes).

Figure 8. Overview of cumulative performance scores for phenotypic antiviral susceptibility testing, European EQA Programme, Influenza, 2023



Scoring system used:

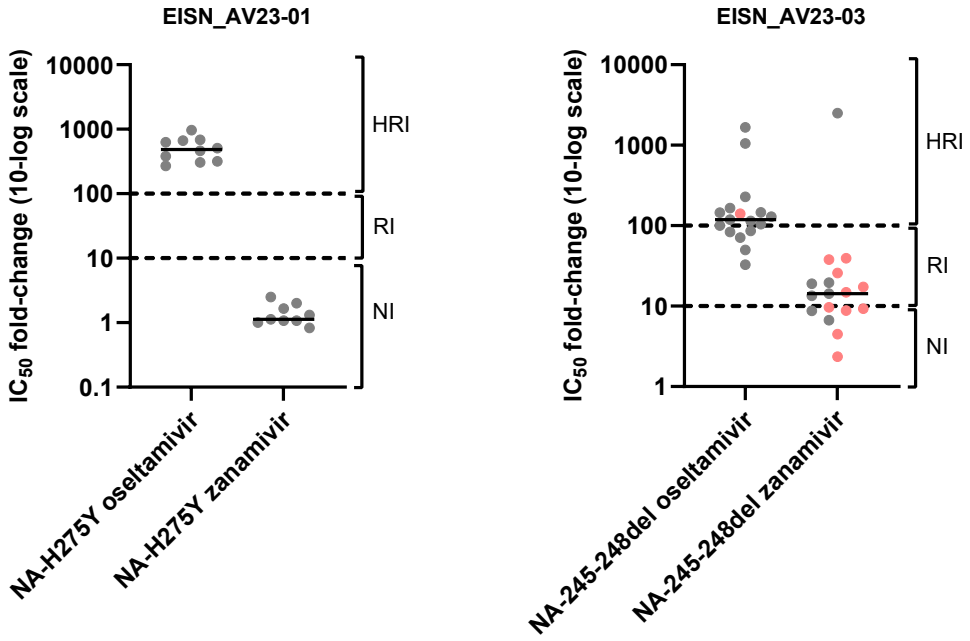
EISN_AV23-01: oseltamivir: Highly Reduced Inhibition (HRI), 0; Resistant, 1; other, 2; zanamivir: NI, 0; other, 2.
EISN_AV23-02: oseltamivir: NI, 0; other, 2; zanamivir: NI, 0; other, 2.
EISN_AV23-03: oseltamivir: HRI, 0; other, 2; zanamivir: NI, 0; other, 2.
EISN_I–F23-01 - 08 (except 05): oseltamivir and zanamivir: NI, 0; Sensitive No IC_{50}, 1; other, 1.
Score only for the specimens tested.

Table 11. Overview of phenotypic antiviral susceptibility testing results by specimen code, European External Influenza Quality Assessment Programme, 2023

Sample code	Oseltamivir phenotypic testing							Zanamivir phenotypic testing						
	Number of labs tested, n (%)	Expected results	Correct, n (%)	Partial correct results (n)	Partially correct, n (%)	Incorrect results (n)	Incorrect, n (%)	Number of labs tested, n (%)	Expected results	Correct, n (%)	Partial correct results (n)	Partially correct, n (%)	Incorrect results (n)	Incorrect, n (%)
EISN_AV23-01	17 (100%)	HRI[a]	16 (94.1%)	RESISTANT (1)	1 (5.9%)		0 (0%)	17 (100.0%)	NI[a]	17 (100%)		0 (0%)		0 (0%)
EISN_AV23-02	17 (100%)	NI[b]	17 (100%)		0 (0%)		0 (0%)	17 (100.0%)	NI[b]	17 (100%)		0 (0%)		0 (0%)
EISN_AV23-03	17 (100%)	HRI[c]	8 (47.1%)	RI (8)	8 (47.1%)	NI (1)	1 (5.9%)	17 (100.0%)	RI[c]	7 (41.2%)		0 (0%)	NI (10)	10 (58.8%)
EISN_INF23-01	8 (100%)	NI	7 (87.5%)	SENSITIVE_NO_IC50 (1)	1 (12.5%)		0 (0%)	7 (100.0%)	NI	6 (85.7%)	SENSITIVE_NO_IC50 (1)	1 (14.3%)		0 (0%)
EISN_INF23-02	9 (100%)	NI	8 (88.9%)	SENSITIVE_NO_IC50 (1)	1 (11.1%)		0 (0%)	9 (100.0%)	NI	8 (88.9%)	SENSITIVE_NO_IC50 (1)	1 (11.1%)		0 (0%)
EISN_INF23-03	5 (100%)	NI	5 (100%)		0 (0%)		0 (0%)	5 (100.0%)	NI	5 (100%)		0 (0%)		0 (0%)
EISN_INF23-04	11 (100%)	NI	10 (90.9%)	SENSITIVE_NO_IC50 (1)	1 (9.1%)		0 (0%)	10 (100.0%)	NI	9 (90%)	SENSITIVE_NO_IC50 (1)	1 (10.0%)		0 (0%)
EISN_INF23-05		-							-					
EISN_INF23-06	8 (100%)	NI	8 (100%)		0 (0%)		0 (0%)	8 (100.0%)	NI	8 (100%)		0 (0%)		0 (0%)
EISN_INF23-07	12 (100%)	NI	8 (66.7%)		0 (0%)	RI (2), HRI (2)	4 (33.3%)	12 (100.0%)	NI	11 (91.7%)		0 (0%)	RI (1)	1 (8.3%)
EISN_INF23-08	10 (100%)	NI	9 (90.0%)	SENSITIVE_NO_IC50 (1)	1 (10.0%)		0 (0%)	10 (100.0%)	NI	9 (90.0%)	SENSITIVE_NO_IC50 (1)	1 (10.0%)		0 (0%)

[1] A number of laboratories that participated in the antiviral susceptibility challenge did not perform any phenotypic testing; some laboratories tested only a limited number of specimens and not always for both oseltamivir and zanamivir; not testing is mainly due to not having isolated a virus.
[2] NI = normal inhibited (fold-change IC_{50}; A <10; B <5); RI = reduced inhibited (fold-change IC_{50}; A ≥10 & ≤100; B A ≥5 & ≤50); HRI = highly reduced inhibited (fold-change IC_{50}; A >100; B >50).
[a] Fold-change compared to median IC_{50} of recent Dutch A(H1N1)pdm09 viruses for oseltamivir determined at 561 (HRI) and for zanamivir at 1.6 (NI) at the Dutch NIC location National Institute for Public Health and the Environment.
[b] Fold-change compared to median IC_{50} of recent Dutch A(H3N2) viruses for oseltamivir determined at 0.8 (NI) and for zanamivir at 1.1 (NI) at the Dutch NIC location National Institute for Public Health and the Environment.
[c] Fold-change compared to median IC_{50} of recent Dutch A(H3N2) viruses for oseltamivir determined at 109 (HRI) and for zanamivir at 19 (RI) at the Dutch NIC location National Institute for Public Health and the Environment.

Figure 9. Overview of calculated IC_{50} fold-change values for the EISN_AV23-01 and -03 specimens, European EQA Programme, Influenza, 2023*



**Fold change IC_{50} was calculated for those laboratories that reported also IC_{50} values for the wild type viruses of the same subtype or lineage included in the EISN_INF23 specimens (for A(H1N1pdm09; mean EISN_INF23-01 and 06; for H2N3; mean EISN_INF23-02, 04, 08). IC_{50} fold-change categories definitions, for type A viruses: NI = IC_{50} fold-change <10; RI=IC_{50} fold-change ≥10 – ≤100; HRI=IC_{50} fold change >100. Results in red indicate when phenotype interpretation 'Normal inhibition', as reported by the participant laboratory, was not concordant with the expected result HRI for oseltamivir or RI for zanamivir.*

Table 12. Methodologies used by laboratories to determine and evaluate IC_{50} values, European EQA Programme, Influenza, 2023

Method	Number of laboratories	
Assay type [1]		
MUNANA In-house	12	
NA Fluor Kit	3	
NA-STAR Kit	1	
NA XTD Kit	1	
Drug range nanomolar (nM) tested	Lowest concentration	Highest concentration
Oseltamivir Minimum	0.001	1
Oseltamivir Maximum	1000	31 250
Zanamivir Minimum	0.001	1
Zanamivir Maximum	1000	31 250
Pre-titration NA-activity		
Yes	14	
No	3	
Measurements		
Duplicate	14	
Single	3	
Control viruses [2]		
In-house	6	
CDC	3	
ISIRV	2	
ISIRV; CDC	1	
ISIRV; In-house	2	
ISIRV; CDC; In-house	1	
Other	2	
Evaluation of IC_{50} against		
known wild-type virus of the same subtype	7	
median of previous season data (after removal of obvious outliers) [4]	5	

Method	Number of laboratories
mean of previous season data (after removal of obvious outliers)	2
median of current season data (after removal of obvious outliers)	2
mean of current season data (after removal of obvious outliers)	1
Software to calculate IC_{50} [3]	
HPA Excel template	9
GraphPad Prism	4
Excel template	1
SigmaPlot	1
Kaleidagraph	1
MS Office Toolpack	1

[1] *MUNANA = 20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid substrate producing a fluorescent product after neuraminidase cleavage; NA = neuraminidase*
[2] *ISIRV = International Society for Influenza and other Respiratory Virus Diseases antiviral working group (anno 2018 the ISIRV-AVG has stopped providing reference viruses); CDC = Centers for Disease Control and Prevention, Atlanta, USA, International Reagent Resource.*
[3] *HPA Excel template = Excel template file provided by Health Protection Agency, currently Public Health England, London, UK.*
[4] *In addition one lab responded: 'When 20 datapoints were not available from previous season, datapoints from before the previous season (complete seasons) were included (outliers were calculated from the whole datasets, not season-wise, and removed.'*

3.5 Accreditation requirements

Participation in EQA programmes is an important element for accreditation of laboratories. Table 13 below outlines the accreditation status of the laboratories.

Table 13. Summary of survey on laboratory accreditation, European EQA Programme, Influenza, 2023

Question and response	Further specification and numbers of laboratories
Is your laboratory accredited?	n
Yes	**36**
ISO[1] 15189	22
ISO 17025	6
ISO 15189 & ISO 17025	2
ISO 9001	2
WHO[2]	3
State Metrological Agency of Ukraine	1
No, in the process of obtaining accreditation	**5**
ISO 15189	5
No, and not in the process of obtaining accreditation	**10**
No response	2

[1] *ISO = International Organization for Standardization*

[2] *WHO = World Health Organization, however WHO does not perform accreditations. Response, although not always explicitly indicated, assumed to refer to participation in the External Quality Assessment Project (EQAP).*

Data on accreditation were reported by 51 laboratories. Of the 51 laboratories, 36 (71%) were accredited: 24 (47%) by ISO 15189 (medical laboratories), two of these also by ISO 17025 (testing and calibration laboratories); eight (16%) by ISO 17025; two (4%) by ISO 9001 (certification for quality management and, as such, not an accreditation); and four (8%) by other organisations (not specified for what and not an ISO norm). A further 5/51 (10 %) laboratories, one located in an EU/EEA country and four in non-EU/EEA countries, were in the process of obtaining ISO 15189 accreditation. Furthermore, 10 (20%) laboratories, eight located in EU/EEA countries and two in non-EU/EEA countries, reported that they were not accredited and were not in the process of obtaining accreditation. Being ISO accredited and having all techniques within the scope of accreditation might be beneficial, as this should include clear procedures to solve issues involving these techniques.

4 Discussion

The EEIQAP 2023 provides a comprehensive overview of the capabilities and performance of laboratories in the WHO European Region and WHO GISRS and ECDC ERLI-Net influenza reference laboratories for the different aspects of influenza surveillance in the Region. The laboratories performed very well in applying molecular testing to influenza virus detection, A/B typing and type A H-subtyping of the current seasonal circulating A(H1N1)pdm09, A(H3N2) and B/Victoria influenza viruses. Overall, 44/53 (83%) laboratories had correct results for all specimens within their level of reporting, reconfirming the correct and reliable reporting of surveillance detection data by the network laboratories to TESSy. Comparison of performance with previous EQAs is difficult, since the panels contained other viruses and other concentrations. The percentage of laboratories with fully correct molecular detection results was good to excellent in previous EQA studies (95% and 96% in the 2018 and 2020 EEIQAP study, respectively; 90% for 38 laboratories in EU/EEA countries in the 2015 ECDC EQA study; and 80% for 45 NICs in the WHO European Region in the 2020 WHO EQAP study, which also included avian type A subtypes) [14,17,18].

Similar to the 2018 and 2020 studies, the EEIQAP 2023 challenged participating laboratories to determine the influenza A virus N-subtype and influenza B virus lineage of detected viruses. Once again, fewer laboratories performed N-subtyping of type A influenza viruses compared to lineage determination of type B viruses (for N1 34%, 39% and 38% in EEIQAP 2023, 2020 and 2018 respectively; for N2 30%, 41% and 38% in EEIQAP 2023, 2020 and 2018, respectively; for B lineage 81%, 87% and 84% in EEIQAP 2023, 2020 and 2018, respectively), while both are included in the dataset for reporting to TESSy. This suggests that only a few laboratories have extended their capability for A N-subtyping or B-lineage determination over the last five years. Nevertheless, all datasets reporting the N-subtype and/or B-lineage were correct. Both type A N-subtyping and type B lineage determination are important capabilities for influenza reference laboratories. N-subtyping is important for early detection of H and N reassortants. In 2001, identification of the emerging A(H1N2) reassortant virus was delayed because seasonal viruses were not widely N-subtyped [19]. This emergence led to inclusion of the N-subtype in the EISS database (predecessor of TESSy for influenza) to be able to determine its spread [20]. In 2018, one A(H1N2) reassortant virus was rapidly identified in routine surveillance because N-subtyping was included [21]. The B-lineage is important to know in order to detect the (re)emergence and distribution of lineages [22,23], and to assess the lineage match with the strain included in trivalent or quadrivalent vaccines and the differential impact of both lineages (e.g. lineage-specific vaccine effectiveness) [24]. In addition, as natural circulation of the B/Yamagata lineage has not been convincingly detected since March 2020, making a conclusion on the possible extinction of this lineage requires enhanced capacity to determine the lineage of detected influenza B viruses [25,26].

Although the proportion of laboratories with full correct results for virus isolation increased from 21/30 (70%) in the 2010 ERLI-Net EQA to 26/32 (81%) in the 2015 ERLI-Net EQA [17], it fell to 27/44 (61%) in the EEIQAP 2018 [14], 18/28 (64%) in the EEIQAP 2020 and 10/31 (32%) in the EEIQAP 2023. Logical defining factors for the success of virus isolation, such as a correlation with the concentration of virus, the MDCK cell types used, the number of extra freeze/thaw cycles before the specimen was inoculated and the volume inoculated, could not explain the high number of isolation failures. Therefore, it seems that the success of virus isolation is laboratory specific. An alternative explanation is that the current panel specimens had a different correlation between RNA content and infectious virus than before, although the pretesting laboratories and a number of participants were successful in isolating virus from all virus-containing specimens. Conducting an additional survey on virus isolation procedures (see Annex 1) did not reveal a clear pattern of differences in procedures between laboratories that were successful in virus isolation and those that had failures. One laboratory indicated parallel successful isolation of influenza virus from clinical specimens while the panel specimens remained negative. Nevertheless, as virus isolation is required for antigenic characterisation and phenotypic antiviral susceptibility testing, failure to isolate virus from panel specimens had an immediate effect on the number of EEIQAP 2023 specimens that could be included in the phenotypic characterisation analyses. Seen from a broader perspective, failure to isolate influenza virus from clinical specimens reduces the number of antigenic characterisations that can be reported to TESSy and the selection of viruses with specific characteristics to be forwarded to the WHO CC London.

Comparable antigenic characterisation of influenza viruses across laboratories remains a challenging task, even among the WHO CCs. Assays are difficult to standardise and changes in the viruses may prevent them from agglutinating RBCs or cause NA-induced haemagglutination [27,28]. This is also reflected in the struggle by the NICs to generate accurate antigenic characterisation data, as shown in previous EISN EQAs [17], the EEIQAP 2018 and 2020 [14] and the current EEIQAP 2023. The viruses A(H1N1)pdm09 clade 6B.1A.5a (EISN_INF23-01, 06), A(H3N2) clade 3C.2a1b.2a.2a (EISN_INF23-04) and B/Victoria clade V1A.3a.2 (EISN_INF23-07), included in the EEIQAP, were antigenically characterised correctly by most laboratories. More difficult to characterise antigenically were the viruses A(H3N2) clade 3C.2a1b.2a.2b (EISN_INF23-02), clade 3C.2a1b.1a (EISN_INF23-08) and B/Victoria clade VIA.3 (EISN_INF23-03). The variable results in EEIQAP 2023 were similar to EEIQAP 2018 and 2020, possibly reflecting the reference sera and viruses used. The variability in source, number and specificity of reference sera/viruses used (reference viruses isolated from 2008 to 2019, with corresponding sera) was high for A(H1N1)pdm09, A(H3N2) and B/Victoria (Table A14 in the annexes). The inventories and analyses in EEIQAP 2018, 2020 and 2023 underpin the conclusion that categorised antigenic characterisation data reported to TESSy should be interpreted with some caution.

Genetic characterisation also remains challenging, as was also the case for the EEIQAP 2020 and 2018 and the EQA 2015 [14,17]. Correct interpretation of the sequences and allocating the panel viruses to the appropriate category by phylogenetic analysis using the reference viruses and detailed category criteria, as provided by WHO European Regional Office in collaboration with the WHO CC London (Table 2), is necessary [19,20]. The main errors made with A(H3N2) viruses were allocating them to a more recent or older category of the clade. This was probably a result of not fully taking into account the phylogenetic position and amino acid substitution characteristics for a specific category. Allocation errors in data reported to TESSy may lead to flawed estimates when analysing the emergence and spread of variants, and laboratories should carry out appropriate analysis to avoid such errors. This could be complemented by timely automated analysis of available sequence data to validate the categories reported to TESSy, especially if they concern emerging variants which will have an impact on vaccine strain match. More correct results were obtained when reporting the WHO clade without categorisation to a reference strain. However, despite obtaining correct HA sequences, some panel viruses were still assigned to the wrong clade. Using publicly available tools, such as Nextclade, could have helped to avoid this.

For correct genetic antiviral susceptibility profiling, good sequence data are essential. Overall, the quality of the uploaded sequences by the laboratories was good, with (nearly) complete and identical sequences to original. However, some laboratories submitted incomplete and poor (many unknown nucleotides (stretches of Ns) and/or many mismatches with original) sequences. Furthermore, even though most uploaded sequences were identical to the original, only a minority of the laboratories (4/23; 17%) reported correct results for all virus-positive specimens (cumulative performance score of 0) (Figure 6). Identifying unusual amino acid changes, such as the A(H3N2) NA-245-248 deletion, and interpreting these correctly seemed to be the most challenging aspect, similar to observations with previous panels. However, in most cases, the sequences uploaded by these laboratories were identical to those of the panel virus and those uploaded by all other laboratories, and therefore the laboratories should have been capable of allocating the panel viruses to the appropriate category.

Phenotypic testing for antiviral susceptibility and interpretation of IC_{50} values were accurate for most specimens. The results reported for two specimens, EISN_AV23-03 with A(H3N2) Del245-248 and EISN_INF23-07 with B/Victoria, were more variable. In the 2015 ERLI-Net EQA [17] and the EEIQAP 2018 and 2020 [14], the majority of incorrect results were obtained using commercial kit-based fluorescent or chemiluminescent assays. In the EEIQAP 2023, most laboratories used in-house MUNANA and no clear correlation was identified between correct interpretation and methodology used.

A relatively high number of laboratories in the network have obtained ISO 15189 or ISO 17025 accreditation or ISO 9001 certification for medical laboratories including quality management, for testing or calibrating laboratories, or for quality management, respectively. A few laboratories reported working towards obtaining ISO 15189 accreditation, similar to the situation reported in EEIQAP 2020. Nevertheless, 10 laboratories (eight located in EU/EEA countries and two in non-EU/EEA countries) indicated that they were not accredited and were not in the process of obtaining accreditation. Accreditation is important to help systematically address issues such as those discovered through EQA, as well as to improve the quality of delivered results. It will also improve the accuracy of data reported to TESSy.

5 Conclusions

Overall, the performance of the network in molecular detection and simultaneous typing and type A H-subtyping of seasonal influenza viruses can be rated as excellent. Those laboratories determining the N-subtype of type A influenza viruses and lineage of type B viruses demonstrated satisfactory and excellent performance respectively. Surveillance data that are reported on a weekly basis to TESSy that derive from molecular testing can therefore be considered accurate. However, an increase in the number of laboratories with capability to N-subtype and to determine the B-lineage would be desirable.

Similar to the EEIQAPs 2018 and 2020, the EEIQAP 2023 has clearly identified an issue with the performance of the network in virus isolation. This is possibly linked to lack of sensitivity of the procedures used and not the use of a particular type of MDCK cell. Not being able to isolate virus has an immediate impact on the number of isolates available for antigenic characterisation and phenotypic antiviral susceptibility testing, as well as subsequent reporting to TESSy. It also limits the provision of viruses to the WHO CC London for further analysis with the aim to inform the WHO vaccine composition recommendation meetings. Having national capacity would also be beneficial in the context of pandemic preparedness to be able to locally characterise viruses antigenically when vaccines become available.

Although there is relatively good concordance among laboratories in the reported antigenic characterisation categories for the EEIQAP 2023 panel specimens, the results also indicate that subtle antigenic differences between viruses are not picked up accurately. This is probably caused partly by the use of a limited and highly diverse set of reference sera and viruses. Antigenic characterisation results reported to TESSy in the predefined categories should therefore be interpreted with some caution. Genetic characterisation of the HA of seasonal influenza viruses by the network is of high accuracy. However, certain (3–7) laboratories had difficulties with allocating some viruses to the correct predefined genetic categories. Therefore, genetic categories reported to TESSy should also be analysed and interpreted with some caution. Reporting of the WHO clade without categorisation to specific reference strains was more accurate. This accuracy can easily be improved by using publicly available tools, such as Nextclade. In addition, this approach can keep better track of the evolution of the influenza viruses than reporting in predefined TESSy reporting categories which are usually updated only once a year.

Interpretation of amino acid change and IC_{50} data for genetic and phenotypic testing of antiviral susceptibility is challenging for the network, since a relatively high number of laboratories reported inaccurate results. This complicates the analysis of antiviral susceptibility data reported to TESSy.

A high number of laboratories have obtained ISO 15189 or ISO 17025 accreditation or are in the process of applying for it, suggesting that performance of tests for laboratory surveillance of influenza should be of a high standard if included in the scope. The high quality of molecular detection and typing, A-subtyping and B-lineage determination, is evident in the EEIQAP 2018, 2020 and 2023 results.

6 Recommendations

Molecular detection, typing, type A H- and N-subtyping and type B lineage determination

- Laboratories currently not performing type A N-subtyping and/or type B lineage determination should consider adding this capability to their assay repertoire.
- Seasonal review of primers and probes (as to whether they are still fit for purpose for currently circulating viruses) should be part of the routine of preparing for the upcoming season. For in-house tests, this is the responsibility of the laboratory. For commercial or non-commercial assays obtained from another laboratory, this is the responsibility of the provider, if sequences are not released.

Virus isolation

- A review of all relevant standard operating procedures is recommended for laboratories that have issues with virus isolation, especially if more than one specimen failed, as these errors are usually due to reagent, cell or sensitivity issues. Success rate with clinical specimens should also be taken into account, to identify necessity and target actions.
- Continued training for virus isolation is important to maintain this capability within Europe. This is particularly relevant for countries with decreasing capacity, especially with the increased use of direct sequence analysis from clinical specimens putting antigenic characterisation and phenotypic antiviral susceptibility testing capabilities of laboratories under pressure.

Antigenic characterisation

In the EEIQAP 2023, it appeared difficult for laboratories to allocate the A(H3N2) viruses to one category, probably due to the limited number of reference sera/viruses used. Standardisation of assays and the reagents used and testing with an increased number of reference sera/viruses covering strains seen in multiple seasons might be an approach to increase accuracy of antigenic characterisation. One suggestion would be to define a minimum set for use (e.g. based on what is provided by WHO CC London). However, distribution of high volumes and high numbers of ferret sera to all network laboratories is probably impossible due to production limitations and limited comparability of multiple batches of ferret sera raised against the same strain. Appropriate use of the seasonal guidelines on influenza virus characterisation provided by WHO Regional Office for Europe and collaboration with WHO CC, London, might provide further direction.

Genetic characterisation

- Errors made in allocating some viruses to the correct TESSy genetic categories were possibly due to incorrect interpretation of the results after phylogenetic and amino acid substitution analysis, since the vast majority of laboratories uploaded correct sequences. Therefore, training should be organised in these types of analysis and appropriate use of the seasonal guidelines on influenza virus characterisation provided by WHO's Regional Office for Europe, in collaboration with WHO CC London (ECDC/WHO CC London for 2021–2022 and WHO Regional Office for Europe/WHO CC London for 2022–2023) used for development of the EEIQAP 2023) and of online tools, such as Nextclade [29].
- Reporting of correct data to TESSy is the responsibility of the submitting laboratory. However, as laboratories are also asked to report Global Initiative on Sharing All Influenza Data (GISAID) accession numbers for HA sequences to TESSy, interpretation of genetic strain characterisation data reported to TESSy is backed up by the possibility for ECDC and WHO Regional Office for Europe to perform additional sequence analysis and to validate suspicious entries (e.g. when preparing ERVISS).
- As reporting of the WHO clade was more accurate and can easily be done using publicly available tools, such as Nextclade, inclusion by ECDC of a WHO clade reporting variable in the TESSy INFLANTIVIR record type is recommended. Whether this should include subclade reporting, as has recently been used by WHO in communication, is a point for discussion.

Genetic antiviral susceptibility testing

- Errors have been made in the identification of relatively uncommon amino acid changes associated with reduced susceptibility, despite the uploading of sequences that contained these changes, or in the interpretation of amino acid changes associated with reduced susceptibility for NA inhibitors. Standard operating procedures used for antiviral susceptibility profiling for appropriate inclusion in ECDC guidelines on interpretation and reporting of antiviral susceptibility data and of FluSurver can be reviewed by ECDC. Together with the use of the lists provided by the WHO working group on surveillance of influenza antiviral susceptibility, laboratories should be able to solve these issues.
- Similar to genetic strain characterisation, reporting correct data is the responsibility of the submitting laboratory. However, laboratories are also asked to report GISAID accession numbers for NA sequences to TESSy. Therefore, interpretation of genetic antiviral susceptibility data reported to TESSy is backed up by the possibility for ECDC and WHO Regional Office for Europe to perform additional sequence analysis to validate suspicious results.
- In order to increase the accuracy of data submitted to TESSy, laboratories would benefit from training on interpretation and reporting of genetic antiviral susceptibility data.

Phenotypic antiviral susceptibility testing

Laboratories using commercial kit-based NA activity inhibition assays should consider switching to an in-house MUNANA assay for routine use. In order to increase the accuracy of data submitted to TESSy, laboratories would benefit from training on the use of the in-house MUNANA assay.

Accreditation

ISO 15189 and ISO 17025 require EQA for self-evaluation and addressing test issues in a systematic way. Laboratories could probably make better use of this methodology to address issues, as provided by interpretations of the ISO 15189 and ISO 17025 accreditation [32]. Laboratories would also benefit from inclusion of all tests used in the routine laboratory surveillance of influenza in the scope of ISO 15189 and ISO 17025 accreditation. Laboratories that have no ISO accreditation are encouraged to apply for one of the above standards, or the equivalent national standards, where available.

Training and resources available

Recordings of previous webinars and instruction documents on a number of the above-mentioned topics are available on the ECDC Learning Portal (https://learning.ecdc.europa.eu/).

References

1. Wu A, Su C, Wang D, Peng Y, Liu M, Hua S, et al. Sequential reassortments underlie diverse influenza H7N9 genotypes in China. Cell Host Microbe. 2013;14(4):446-52. Epub 20130919. doi: 10.1016/j.chom.2013.09.001. PubMed PMID: 24055604.
2. Westgeest KB, Russell CA, Lin X, Spronken MI, Bestebroer TM, Bahl J, et al. Genome-wide analysis of reassortment and evolution of human influenza A(H3N2) viruses circulating between 1968 and 2011. J Virol. 2014;88(5):2844-57. Epub 20131226. doi: 10.1128/JVI.02163-13. PubMed PMID: 24371052; PubMed Central PMCID: PMCPMC3958060.
3. World Health Organization (WHO). Global Influenza Surveillance and Response System (GISRS). Available from: https://www.who.int/initiatives/global-influenza-surveillance-and-response-system
4. World Health Organization (WHO). National Influenza Centres, WHO European Region. Available from: https://www.who.int/initiatives/global-influenza-surveillance-and-response-system/national-influenza-centres#euro
5. European Centre for Disease Prevention and Control (ECDC). European Reference Laboratory Network for Human Influenza (ERLI-Net). Available from: https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/erlinet
6. European Centre for Disease Prevention and Control (ECDC). Strategy for the external quality assessment of public health microbiology laboratories. Available from: https://www.ecdc.europa.eu/en/publications-data/strategy-external-quality-assessment-public-health-microbiology-laboratories.
7. Valette M, Aymard M. Quality control assessment of influenza and RSV testing in Europe: 2000-01 season. Euro Surveill. 2002;7(11):161-5. doi: 10.2807/esm.07.11.00375-en. PubMed PMID: 12631989.
8. Meijer A, Valette M, Manuguerra JC, Perez-Brena P, Paget J, Brown C, et al. Implementation of the community network of reference laboratories for human influenza in Europe. J Clin Virol. 2005;34(2):87-96. doi: 10.1016/j.jcv.2005.02.005. PubMed PMID: 16157259.
9. Thompson CI, Lackenby A, Daniels RS, McCauley JW, Pereyaslov D, Broberg EK, et al. Evaluation of influenza virus antiviral susceptibility testing in Europe: results from the first external quality assessment exercise. J Clin Virol. 2013;56(3):212-8. Epub 20121130. doi: 10.1016/j.jcv.2012.11.005. PubMed PMID: 23201459.
10. World Health Organization (WHO). External Quality Assessment Project. Available from: https://www.who.int/teams/global-influenza-programme/laboratory-network/eqa-project
11. Mackay WG, van Loon AM, Niedrig M, Meijer A, Lina B, Niesters HG. Molecular detection and typing of influenza viruses: are we ready for an influenza pandemic? J Clin Virol. 2008;42(2):194-7. Epub 20080324. doi: 10.1016/j.jcv.2008.02.005. PubMed PMID: 18359662.
12. Reading PC, Leung VK, Buettner I, Gillespie L, Deng YM, Shaw R, et al. The first external quality assessment of isolation and identification of influenza viruses in cell culture in the Asia Pacific region, 2016. J Clin Virol. 2017;97:54-8. Epub 20171102. doi: 10.1016/j.jcv.2017.10.018. PubMed PMID: 29127947.
13. Leung VKY, Deng YM, Todd A, Peck H, Buettner I, Zakis T, et al. A second external quality assessment of isolation and identification of influenza viruses in cell culture in the Asia Pacific region highlights improved performance by participating laboratories. J Clin Virol. 2021;142:104907. Epub 20210707. doi: 10.1016/j.jcv.2021.104907. PubMed PMID: 34274614.
14. European Centre for Disease Prevention and Control (ECDC)/World Health Organization (WHO) Regional Office for Europe. European external quality assessment programme for influenza virus – 2018. Stockholm and Copenhagen: ECDC and WHO; 2019. Available from: https://www.ecdc.europa.eu/en/publications-data/european-external-quality-assessment-programme-influenza-virus-2018
15. World Health Organization (WHO). Laboratory quality management system: handbook 2011. Available from: https://www.who.int/publications/i/item/9789241548274
16. Jonges M, Liu WM, van der Vries E, Jacobi R, Pronk I, Boog C, et al. Influenza virus inactivation for studies of antigenicity and phenotypic neuraminidase inhibitor resistance profiling. J Clin Microbiol. 2010;48(3):928-40. Epub 20100120. doi: 10.1128/JCM.02045-09. PubMed PMID: 20089763; PubMed Central PMCID: PMCPMC2832438.
17. European Centre for Disease Prevention and Control (ECDC). External quality assessment scheme for detection, isolation and characterisation of influenza viruses for the European Reference Laboratory Network for Human Influenza – 2015. Stockholm: ECDC, 2016. Available from: https://www.ecdc.europa.eu/en/publications-data/external-quality-assessment-scheme-detection-isolation-and-characterisation
18. World Health Organization (WHO). Detection of influenza viruses by reverse transcription polymerase chain reaction: WHO external quality assessment programme summary analysis, 2020. Weekly epidemiological record. 2021;96(4):21-32.
19. Gregory V, Bennett M, Orkhan MH, Al Hajjar S, Varsano N, Mendelson E, et al. Emergence of influenza A H1N2 reassortant viruses in the human population during 2001. Virology. 2002;300(1):1-7. doi: 10.1006/viro.2002.1513. PubMed PMID: 12202200.
20. Paget WJ, Meerhoff TJ, Goddard NL, EISS. Mild to moderate influenza activity in Europe and the detection of novel A(H1N2) and B viruses during the winter of 2001-02. Euro Surveill. 2002;7(11):147-57. doi: 10.2807/esm.07.11.00373-en. PubMed PMID: 12631987.

21. Meijer A, Swaan CM, Voerknecht M, Jusic E, van den Brink S, Wijsman LA, et al. Case of seasonal reassortant A(H1N2) influenza virus infection, the Netherlands, March 2018. Euro Surveill. 2018;23(15). doi: 10.2807/1560-7917.ES.2018.23.15.18-00160. PubMed PMID: 29667576; PubMed Central PMCID: PMCPMC6836195.
22. Caini S, Kusznierz G, Garate VV, Wangchuk S, Thapa B, de Paula Junior FJ, et al. The epidemiological signature of influenza B virus and its B/Victoria and B/Yamagata lineages in the 21st century. PLoS One. 2019;14(9):e0222381. Epub 20190912. doi: 10.1371/journal.pone.0222381. PubMed PMID: 31513690; PubMed Central PMCID: PMCPMC6742362.
23. Langat P, Raghwani J, Dudas G, Bowden TA, Edwards S, Gall A, et al. Genome-wide evolutionary dynamics of influenza B viruses on a global scale. PLoS Pathog. 2017;13(12):e1006749. Epub 20171228. doi: 10.1371/journal.ppat.1006749. PubMed PMID: 29284042; PubMed Central PMCID: PMCPMC5790164.
24. Kissling E, Valenciano M, Pozo F, Vilcu AM, Reuss A, Rizzo C, et al. 2015/16 I-MOVE/I-MOVE+ multicentre case-control study in Europe: Moderate vaccine effectiveness estimates against influenza A(H1N1)pdm09 and low estimates against lineage-mismatched influenza B among children. Influenza Other Respir Viruses. 2018;12(4):423-37. Epub 20180314. doi: 10.1111/irv.12520. PubMed PMID: 29125681; PubMed Central PMCID: PMCPMC6005601.
25. Paget J, Caini S, Del Riccio M, van Waarden W, Meijer A. Has influenza B/Yamagata become extinct and what implications might this have for quadrivalent influenza vaccines? Euro Surveill. 2022;27(39):2200753. doi: 10.2807/1560-7917.ES.2022.27.39.2200753. PubMed PMID: 36177871; PubMed Central PMCID: PMCPMC9524051.
26. Caini S, Meijer A, Nunes MC, Henaff L, Zounon M, Boudewijns B, et al. Probable extinction of influenza B/Yamagata and its public health implications: a systematic literature review and assessment of global surveillance databases. Lancet Microbe. 2024;5(8):100851. Epub 20240507. doi: 10.1016/S2666-5247(24)00066-1. PubMed PMID: 38729197.
27. Ampofo WK, Azziz-Baumgartner E, Bashir U, Cox NJ, Fasce R, Giovanni M, et al. Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Vaccine. 2015;33(36):4368-82. Epub 20150703. doi: 10.1016/j.vaccine.2015.06.090. PubMed PMID: 26148877.
28. Hampson A, Barr I, Cox N, Donis RO, Siddhivinayak H, Jernigan D, et al. Improving the selection and development of influenza vaccine viruses - Report of a WHO informal consultation on improving influenza vaccine virus selection, Hong Kong SAR, China, 18-20 November 2015. Vaccine. 2017;35(8):1104-9. Epub 20170125. doi: 10.1016/j.vaccine.2017.01.018. PubMed PMID: 28131392; PubMed Central PMCID: PMCPMC5357705.
29. Aksamentov I, Roemer C, Hodcroft E, Neher R. Nextclade: clade assignment, mutation calling and quality control for viral genomes. Journal of Open Source Software. 2021;6(67). doi: 10.21105/joss.03773.

Annexes

For Annex 1 and 2, please see the separate downloadable document available on ECDC's website.

European Centre for Disease Prevention and Control (ECDC)

Gustav III:s Boulevard 40
16973 Solna, Sweden

Tel. +46 858 60 10 00
Fax +46 858 60 10 01
www.ecdc.europa.eu

Contact us
publications@ecdc.europa.eu

Follow us on Twitter
@ECDC_EU

Like our Facebook page
www.facebook.com/ECDC.EU

World Health Organization Regional Office for Europe

UN City, Marmorvej 51, DK-2100 Copenhagen Ø, Denmark

Tel. +45 45 33 70 00
Fax +45 45 33 70 01
www.euro.who.int

Contact us
eucontact@who.int

Follow us on Twitter
@WHO_EUROPE

Like our Facebook page
www.facebook.com/WHOEurope