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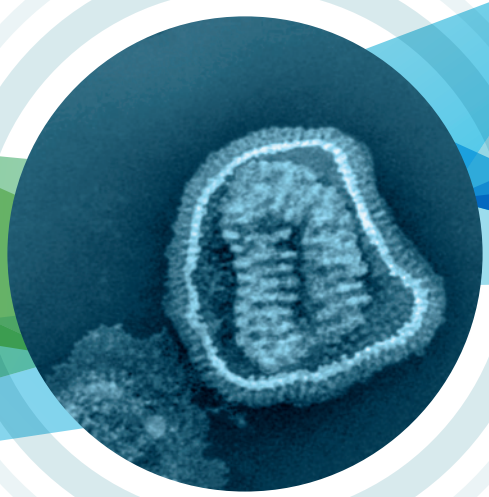

European External Influenza Virus Quality Assessment Programme

2022

2020 data

TECHNICAL REPORT

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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
AV20	Influenza antiviral susceptibility determination specific specimens of the EEIQAP 2020 panel
CDC	Centers for Disease Control and Prevention, Atlanta, United States
CPE	Cytopathic effect
Ct	Cycle threshold
ECDC	European Centre for Disease Prevention and Control
EEIQAP	European External Influenza Quality Assessment Programme
EISN	European Influenza Surveillance Network
EISNINF_MD20	Programme in EEIQAP 2020 comprising molecular detection, typing, type A H- and N-subtyping and type B lineage determination
EISNINF_VI20	Programme in EEIQAP 2020 comprising virus isolation and antigenic and genetic characterisation
EISNINF_VS20	Programme in EEIQAP 2020 comprising antiviral (neuraminidase inhibitors) susceptibility determination
EISS	European Influenza Surveillance Scheme
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
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
HPA	Health Protection Agency, United Kingdom
HRI	Highly reduced inhibition
IC_{50}	50% inhibitory concentration
IF	Immunofluorescence
INF20	Influenza virus detection, typing, subtyping and lineage determination, virus isolation and genetic and antigenic characterisation and antiviral susceptibility determination specimens of the EEIQAP 2020 panel
ISO	International Organization for Standardization
ITEMS	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
NIP	No interpretation possible due to partial neuraminidase segment information
nM	Nanomolar
NP	Not performed
PA	Polymerase acidic subunit
PCR	Polymerase chain reaction
PHE	Public Health England, London, United Kingdom
QCMD	Quality Control for Molecular Diagnostics, Glasgow, United Kingdom
RBC	Red blood cells
RI	Reduced inhibition
RIVM	National Institute for Public Health and the Environment, Bilthoven, the Netherlands
RT-PCR	Reverse transcription polymerase chain reaction
SIAT	Human alpha-2,6-sialyltransferase
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
TCID50	50% tissue culture infectious dose
TESSy	The European Surveillance System
VN	Virus neutralisation
VTM	Virus transport medium
WHO	World Health Organization, Geneva, Switzerland
WHO CC	WHO Collaborating Centre for Reference and Research on Influenza
WHO CC London	WHO CC at the Francis Crick Institute Worldwide Influenza Centre, London, United Kingdom

Executive summary

Influenza epidemics occur every winter with high impact on disease burden, hospitalisations and excess mortality in countries in the World Health Organization (WHO) European Region. 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, 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 Flu News Europe, the joint European Centre for Disease Prevention and Control (ECDC)-WHO Regional Office for Europe weekly influenza update (http://flunewseurope.org/).

From March to June 2020, a European External Influenza Quality Assessment Programme (EEIQAP) exercise was held for NICs and other national influenza reference laboratories in the WHO European Region. The exercise covered influenza virus molecular detection, isolation, strain genetic and antigenic characterisation and antiviral susceptibility testing. It was the sixth detection, isolation and strain characterisation panel and the fifth antiviral susceptibility testing panel, all of which have been organised with the support of the European Union (EU) and ECDC. The EEIQAP 2020 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; previously called CNRL) with the support of ECDC. Participation in EEIQAP 2020 by laboratories in countries outside the European Union/European Economic Area (EU/EEA) was supported by the WHO Regional Office for Europe. For the second time, results are jointly presented for the WHO European Region as a whole. 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. 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 Region. Additionally, performance in the EEIQAP 2020 provided a validity check of the data reported to TESSy. In total, 45 laboratories in 40 of the countries/nations with at least one reference laboratory for influenza participated in at least one of the three components of the study. There were fewer participants than in the EEIQAP 2018, as the COVID-19 pandemic that commenced in early 2020 reduced capacity in laboratories and created difficulties in shipping the panels. The panel consisted of seven simulated clinical specimens containing variable amounts of live virus (one A(H1N1)pdm09 clade 6B.1A5A; three A(H3N2) clade 3C.2a1, 3C.2a1b + 131K and 3C.3a; two B/Victoria clade 1A and 1A (del162-163); and one B/Yamagata clade 3), one negative simulated clinical specimen and two inactivated specimens (one containing A(H1N1)pdm09 N1-D199E showing reduced inhibition (RI) by oseltamivir and normal inhibition (NI) by zanamivir and one containing A(H3N2) E119V + del245-248 showing highly reduced inhibition (HRI) by oseltamivir and HRI by zanamivir).

As with previous panels, the performance for rapid molecular detection, A/B typing and type A H-subtyping was of high accuracy. Equally, the performance of the limited number of laboratories reporting on the influenza virus type A N subtype and the influenza virus type B lineage determination was of high accuracy. Although two false positives were reported, the same laboratories also reported two false negatives, suggesting a switch of specimens or reporting error. These results highlight the wide capability to perform molecular diagnostics across the Region and the high quality of data generated by the national reference laboratories and reported to TESSy. Similar to the EEIQAP 2018, fewer laboratories were able to isolate virus from the virus-containing specimens than for molecular detection. In addition, three laboratories reported false positive virus isolation from the specimen that did not contain virus. There was no particular reason (type of MDCK cell line used, number of freeze/thaw cycles before inoculation, inoculation volume or viral load) that explained the failure to isolate any of the A(H1N1)pdm09-, A(H3N2)-, B/Victoria- or B/Yamagata-containing specimens. The lower performance of a number of laboratories should likely be interpretated taking into consideration the whole process. The lower number of isolated viruses limited the number of viruses that could be antigenically characterised and tested phenotypically for antiviral susceptibility. The antigenic strain characterisations reported were fairly concordant across laboratories for the more recent viruses. The reporting of the antigenic group for A(H3N2) viruses caused some uncertainties as to which group the viruses belonged. Similarly, the B/Victoria viruses that differ because of the two amino acid deletions in the HA1 of one of them, posed a challenge for appropriate allocation to the correct antigenic group. An inventory of the reference sera and viruses used clearly indicated that the use of a limited number of reference sera and viruses with diverse specificities is one of the reasons for variation in results, as well as for allocation to the same antigenic group for recent panel viruses if only one or two reference sera/viruses are used. Genetic strain characterisation by sequencing of the haemagglutinin (HA) genome segment was more straightforward and of relatively high accuracy. However, similar to antigenic characterisation, some of the A(H3N2) viruses were more difficult to allocate to the appropriate genetic category, indicating some difficulty in interpreting phylogenetic and amino acid substitution data. Similarly, incorrectly allocating the B/Victoria viruses with and without the two amino acid deletions at 162-163 indicated inappropriate analysis of available sequence data. This conclusion could be firmly drawn, as the vast majority of laboratories that reported obtained HA sequences uploaded a sequence similar to the sequence of the virus included in the panel samples, including for the viruses that were allocated to

an incorrect category. The results for antigenic and genetic characterisation indicate that the weekly analysis and interpretation of these data during the season requires some caution. However, accession numbers for HA sequences can also be reported to TESSy. They offer an opportunity for additional analysis and validation of the genetic category data reported. The results for detection of amino acid substitutions associated with (highly) reduced inhibition (HRI or RI) by the neuraminidase (NA) inhibitors oseltamivir and zanamivir and the results for IC_{50} determination of wild type viruses and viruses with (H)RI amino acid substitutions were broadly encouraging. For wildtype viruses, a few laboratories reported amino acid changes that were actually wildtype, but only one interpreted that observation incorrectly with reduced susceptibility conclusion. The included panel members with reduced susceptibility were more challenging. Despite identification of amino acid changes in the NA, this did frequently lead to an incorrect interpretation for A(H1N1)pdm09 N1-D199E and oseltamivir susceptibility and for A(H3N2) N2-E119V + del245-248 and zanamivir susceptibility. In addition, even though the uploaded, obtained sequences contained the amino acid changes, both the N1-D199E and the del245-248 changes were frequently not reported as being associated with reduced susceptibility and, therefore, an incorrect interpretation was reported. Therefore, NA amino acid change data in TESSy need to be interpreted with some caution. As accession numbers for NA sequences can also be reported to TESSy, these offer an opportunity for additional analysis and validation of the amino acid composition data reported. Phenotypic antiviral susceptibility results were generally highly accurate for laboratories using in-house 20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid (MUNANA) based assays. Similar to in the EEIQAP 2018 study, the majority of deviant results were obtained by laboratories using commercial kit-based NA inhibition assays, leading to the incorrect conclusion NI instead of RI for A(H1N1)pdm09 N1-D199E by oseltamivir or NI or just RI instead of HRI for A(H3N2) E119V + del245-248 and zanamivir. Surprisingly, some of these laboratories also reported incorrect RI or even HRI instead of NI for the same three wildtype influenza B/Victoria- and B/Yamagata-containing specimens. Therefore, it is advisable that laboratories using the commercial kit-based NA inhibition assays switch to MUNANA in-house based assays to increase comparability of NA inhibition data between laboratories, thereby improving interpretation of these data reported to TESSy. A relatively high number of laboratories in the network have obtained ISO 15189, ISO 17025 or ISO 9001 accreditation for medical laboratories, for testing or calibrating laboratories, or for quality management, respectively. A few reported working towards obtaining ISO 15189 or ISO 17025. Still, several laboratories indicated that they were not accredited or in the process of obtaining accreditation. Not being accredited or not including all tests that are being used in the scope of the laboratory's accreditation might have a correlation with the reporting of correct data to TESSy and influence the capacity to forward strains of interest to the WHO CC for further analysis. Accredited laboratories are required to have an appropriate procedure for traceable internal quality control and for addressing deviant results obtained in an EQA. For example, appropriately addressing issues with virus isolation and characterisation will increase the number of reports to TESSy with correct characterisation and the number of strains of interest for submitting to the WHO CC.

In conclusion, the overall performance of the participating laboratories was good. 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. with incorrect translation into TESSy categories) will be addressed jointly with 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, especially focusing on sequence analysis, interpretation and reporting).

1. Introduction

Influenza viruses cause a highly contagious acute respiratory disease that can spread rapidly, causing important 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 might lead to antigenic shift if new H- and/or N-subtypes that humans are susceptible to are introduced. 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 of great importance for timely risk assessment, treatment recommendations, and vaccine formulation. The laboratory network responsible for the virologic surveillance of influenza in the WHO European Region is part of the WHO Global Influenza Surveillance and Response System (GISRS) [3]. The network consists of national influenza laboratories in 50 countries of the Region; a WHO Collaborating Centre for Reference and Research on Influenza at the Francis Crick Institute Worldwide Influenza Centre, London, United Kingdom (WHO CC London); a WHO Essential Regulatory Laboratory (ERL) at the National Institute for Biological Standards and Control, Potters Bar, United Kingdom (UK); a WHO Collaborating Centre for Studies on Influenza at the Animal-human Interface at the Federal Budgetary Research Institution, State Research Center of Virology and Biotechnology 'VECTOR', Koltosov, Novosibirsk region, Russia [4]; and three WHO H5 reference laboratories in France, Russia and the UK [5]. As of October 2019, there are 56 national influenza laboratories in 46 Member States of the WHO European Region that are recognised by WHO as national influenza centres (NICs) [6], and laboratories in 30 countries of the EU/EEA that participate in the European Reference Laboratory Network for Human Influenza (ERLI-Net), coordinated by ECDC [7]. 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. These new techniques 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 assuming great practical relevance in diagnosing individual patients and in surveillance.

By 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 match of vaccine strains with circulating strains and on known markers for increased virulence). Similarly, genetic characterisation of the NA genome segment provides useful information on known markers for (highly) reduced inhibition by NA inhibitors (oseltamivir and zanamivir) and other registered antivirals like baloxavir marboxyl (polymerase inhibitor; authorised to be used in the EU as of 7 January, 2021), for which reduced susceptibility markers are found in the polymerase acidic subunit (PA) segment of the genome. However, the ability to accurately determine the antigenic profile of an influenza virus still requires the ability to isolate virus in cell culture or embryonated eggs and carry out serological tests (haemagglutination inhibition (HI) or virus neutralisation (VN) assays). Likewise, virus isolates are necessary to determine the phenotypic antiviral susceptibility profile (e.g. by 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 such technologies through effective quality control to ensure the reliability and comparability of results reported to physicians and to disease surveillance systems nationally and at the European level [8]. An integral part of quality control is external quality assessment (EQA), which provides a means of 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 [9-11]). 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 external quality assessment programme (EQAP) for NICs, which was specifically designed for the detection and subtyping of potentially pandemic zoonotic avian influenza viruses, since 2007 [12]. When this 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 [13]. Antiviral susceptibility determination has been added on an optional basis to the WHO EQAP since 2013, initially only targeting A(H1N1)pdm09 NA-H275Y [12]. There is no international EQA programme available for virus isolation and antigenic and genetic strain identification, although virus isolation EQA studies from the WHO Asia Pacific Region have recently been reported [14, 15]. However, that EQA programme did not include strain identification through antigenic or genetic characterisation. Hence, the European External Influenza Quality Assessment Programme (EEIQAP) is still filling a gap 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. These comprise all

aspects of routine influenza surveillance data published in Flu News Europe, the joint ECDC-WHO Regional Office for Europe weekly influenza online update (www.flunewseurope.org).

In 2018, a framework contract with ECDC was put in place for an EEIQAP covering the period 2017 to 2021 to ensure the reliability and comparability of results reported to TESSy and to identify needs for improvement in laboratory influenza surveillance and diagnostic capability. Agreements for Performance of Work with the WHO Regional Office for Europe ensured EEIQAP 2018 and EEIQAP 2020 participation of NICs in countries outside the EU/EEA in the WHO European Region. The EEIQAP 2018 results, which were jointly presented for the WHO European Region as a whole for the first time, have been published in a report available from the ECDC website [16]. Part of the EEIQAP 2020 study aimed at addressing issues identified in the EEIQAP 2018 study.

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 following areas [8]:

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

Operational public health objectives of ECDC-supported EQAs are [8]:

- 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 consistent with the laboratory and public health objectives of EQAs outlined in the WHO laboratory quality management system handbook [17].

Translated into operational procedures in the laboratory, the main purposes of an EQA, as also intended for the current EEIQAP 2020, include:

- Assessment of the general performance standards;
- Assessment of the effects of analytical procedures (method, principles and techniques);
- Evaluation of individual laboratory performance;
- Identification and justification of problem areas;
- Provision of continuing (self) education (testing against specimens of known status) and comparison with other laboratories;
- Identification of training needs.

This report presents the results of the EEIQAP 2020 for influenza reference laboratories in the WHO European Region, designed and prepared by the contractor and funded by ECDC and WHO Regional Office for Europe.

The major objective of the EEIQAP 2020 was to assess the performance of individual influenza reference laboratories in the following areas:

- Rapid detection by 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 calendar days;
- Virus isolation including follow-up strain characterisation by haemagglutination inhibition (HI)-assay or virus neutralisation (VN) and/or sequencing within a defined reporting timeframe of 36 calendar days;
- Determination of the susceptibility to the NA inhibitors oseltamivir and zanamivir by genotypic and/or phenotypic methods within a defined reporting timeframe of 36 calendar days.

Because of the COVID-19 pandemic, the distribution of the panels was delayed. Delivering the panels delivered to the participating laboratories was also a challenge due to very limited availability of transport by air and additional customs requirements. Furthermore, several laboratories experienced a limited capacity to perform the challenges that they had initially signed up for; therefore, not all challenges were performed and some were delayed. Therefore, the reporting timeframe, as outlined above, was not included in the analysis.

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. Except for the antiviral component of the panel, viruses were taken from the repository at RIVM. Viruses for the antiviral component of the panel were kindly provided by Prof Dr Maria Zambon and Dr Angie Lackenby, Public Health England, National Influenza Centre, Colindale, UK for the purposes of this EQA only. The panel was prepared and tested by the Respiratory Viruses Group of the Department Emerging and Endemic Viruses, Division Virology, Center for Infectious Disease Research, Diagnostics and laboratory Surveillance (IDS), RIVM, Bilthoven, the Netherlands. Further pretesting was performed by subcontractors Erasmus Medical Centre (Erasmus MC), Department Viroscience, Rotterdam, the Netherlands and National Reference Centre for Respiratory Virus Infections, Virology Laboratory, Institute of Infectious Agents, Lyon, France. The final panel composition was determined based on the pretesting results of a larger number of potential panel specimens. The panels were frozen on dry ice and distributed to participants by specialist courier (organised by the subcontractor, Quality Control for Molecular Diagnostics (QCMD), Glasgow, UK) between March and June 2020. 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 2020.

2.2 Panel composition, preparation and validation

The EEIQAP panel consisted of eight simulated clinical specimens containing live influenza viruses of type A subtypes and type B lineages that currently circulate or have recently circulated in humans, including human influenza viruses A(H1N1)pdm09 and A(H3N2) and both genetic lineages of influenza B viruses (Table 1). One negative specimen with no virus completed the live virus part of the panel.

Table 1. Panel composition, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen Code EISN_	Matrix[1]	Strain; antiviral amino acid substitution in NA segment; GISAID isolate accession number[2]	Type and subtype/ lineage	Ct value[3]	pfu /ml[4]	IC_{50} (nM)[5]	
						O	Z
INF20-1	A549 cells in VTM	A/Netherlands/757/2017; none; EPI_ISL_270971[6]	A(H3N2)	16.3	8 728	0.14	0.67
INF20-2	A549 cells in VTM	No virus	N/A	N/A	N/A	N/A	N/A
INF20-3	A549 cells in VTM	A/Netherlands/10003/2019; none; EPI_ISL_335585	A(H1N1)pdm09	18.3	86 940	0.52	0.66
INF20-4	A549 cells in VTM	A/Netherlands/10009/2019; none; EPI_ISL_339371	A(H3N2)	16.5	276 000	0.27	0.82
INF20-5	A549 cells in VTM	B/Netherlands/2423/2017; none; EPI_ISL_255660[6]	B/Victoria	17.9	8 728	26	7.9
INF20-6	A549 cells in VTM	B/Netherlands/2424/2017; none; EPI_ISL_255661[6]	B/Yamagata	17.9	2 760	26	6.1
INF20-7	A549 cells in VTM	A/Netherlands/10002/2019; none; EPI_ISL_335584	A(H3N2)	16.3	27 600	0.18	0.86
INF20-8	A549 cells in VTM	B/Netherlands/00302/2018; none; EPI_ISL_308625	B/Victoria	16.8	86 940	28	11
AV20-1	VTM; Triton X-100 inactivated	A/England/90840593/2019; D199E	A(H1N1)pdm09	N/A	N/A	7.4	5.1
AV20-1	VTM; heat inactivated	A/England/90840593/2019; D199E	A(H1N1)pdm09	18.0	N/A	N/A	N/A
AV20-2	VTM; Triton X-100 inactivated	A/England/74000497/2017; E119V aa245-248deletion	A(H3N2)	N/A	N/A	6,785	181
AV20-2	VTM; heat inactivated	A/England/74000497/2017; E119V aa245-248deletion	A(H3N2)	19.5	N/A	N/A	N/A

[1] VTM = virus transport medium.
[2] For INF panel members, the haemagglutinin (HA) and neuraminidase (NA) segments are available in GISAID. For AV panel members the sequences are available by request.
[3] Matrix gene-based RT-PCR for type A influenza viruses and HA-gene based RT-PCR for type B influenza viruses. N/A = not applicable.
[4] pfu per ml as proxy for the concentration of infectious virus particles is transposed by multiplying with 0.69 from TCID50/ml determined by titration on MDCK-MIX cells (MDCK-I and MDCK-SIAT) in rotating tubes. This way of titrating generates, in general, a higher titre compared to using 96-well microtitre plates and static incubation. End-points were determined by CPE for each tube. N/A = not applicable.
[5] As determined at the National Institute for Public Health (RIVM) using MUNANA- based NA inhibition assay. IC_{50} = 50% Inhibitory Concentration; O = oseltamivir; Z = zanamivir; N/A = not applicable.
[6] These viruses were also included in EEIQAP 2018, although at lower concentration.

In addition, two specimens (of two vials each) specifically designed for antiviral susceptibility testing were included in the panel; these contained inactivated virus with amino acid substitutions causing reduced or highly reduced inhibition (RI or HRI) by the NA inhibitors oseltamivir and/or zanamivir (Table 1). In order to address antigenic and genetic characterisation performance, three viruses were included that were also used in the EEIQAP 2018. Influenza B virus-containing specimens with higher concentrations were also included because of reduced performance in virus isolation with influenza virus type B-containing specimens in the EEIQAP 2018.

All viruses were selected based on known antigenic (Figure A1 and A2), genetic (Figure A3) and antiviral susceptibility characteristics (Table 1) previously determined at RIVM, Erasmus MC and Public Health England. All viruses were grown in monolayers of Madin Darby Canine Kidney (MDCK)-MIX cells, consisting of MDCK-I cells and MDCK cells stable 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 were inactivated with 1% Triton X-100 for one hour at room temperature, as this procedure preserves NA activity best [16,18]. Heat-inactivation was used to preserve integrity of RNA for direct sequencing of the viruses exhibiting reduced inhibition by NA inhibitors [16].

The live virus specimens were diluted to a concentration high enough for successful virus isolation at RIVM and pretesting laboratories. The inactivated virus specimens were diluted to a NA enzyme activity high enough for direct use in phenotypic antiviral susceptibility testing or direct sequencing using Sanger or 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 panel members were aliquoted and stored frozen at -80°C until dispatch to QCMD for further distribution. One panel was thawed and pretested at RIVM using in-house methods, and panels with random numbering of specimens different from the numbering of the final panel were frozen on dry ice and sent to the two independent laboratories for pretesting. Pretesting by these laboratories also included the pretesting of the online reporting system ITEMS at QCMD. The final panels were frozen on dry ice and shipped to the participating laboratories between March and June 2020. Expected results for all panel members are listed in Tables 2 and 3. For a final check on 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 the RIVM. All specimens with live virus became positive within seven calendar days, confirming viability after long storage time and 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 [7] 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 2020 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 and the WHO CC in London were notified of the EEIQAP 2020 exercise in advance on 29 October 2019 jointly by ECDC/WHO in English and Russian. Laboratories were asked to sign up by 18 November 2019 for participation in any of the four areas: molecular detection, virus isolation, virus characterisation and antiviral susceptibility determination. They were actively contacted if no response was received. A final list of the laboratories that participated in the EEIQAP 2020 can be found in Table A1. Not every laboratory that signed up was able to actually participate in the EQA scheme, due to the COVID-19 pandemic. Details about this are given in Section 3.1.

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 2020 using the tests routinely used in the laboratory, weekly results of which are reported to TESSy. If, in addition, usual laboratory procedures included virus isolation and strain characterisation and/or antiviral susceptibility testing and resulting data are reported to TESSy, the laboratories were strongly recommended to also complete the corresponding parts of the EEIQAP 2020. For any tests used, the laboratories were asked to provide detailed information to be able to put the reported results in context.

The laboratories were instructed to test for:

- EISNINF_MD20 programme – Molecular detection, typing, type A H- and N-subtyping and type B lineage determination: specimens EISN_INF20-1 through -8) using RT-PCR or other NAAT;
- EISNINF_VI20 programme – Virus isolation and antigenic and genetic characterisation: specimens EISN_INF20-1 through -8. Virus isolation 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 next generation sequencing (NGS) techniques;
- EISNINF_VS20 programme – Antiviral (NA inhibitors) susceptibility determination: specimens EISN_AV20-1 and -2 and specimens EISN_INF20-1 through -8 by available phenotypic (NA enzyme activity inhibition assay) and/or genotypic (single nucleotide polymorphism [SNP] RT-PCR, Sanger sequencing or NGS or pyrosequencing) antiviral susceptibility determination methods. EISN_AV20 tubes labelled 'phenotypic'

contained virus inactivated by Triton X-100 and could be used directly for phenotypic antiviral susceptibility testing. The EISN_AV20 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_INF20-1 through -8 specimens were intended to be isolated and propagated first before phenotypic antiviral susceptibility testing was performed.

The manual for testing was 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 calendar days of receipt of the panel. The deadline for reporting virus isolation and strain characterisation results and for antiviral susceptibility testing results was within 36 calendar days of receipt of the panel. Data were reported in the web-based QCMD-owned database ITEMS, which had been adapted for collating EEIQAP 2020 data. New for EEIQAP 2020 compared to the EEIQAP 2018 was that laboratories were requested to upload the consensus sequences that they retrieved from the panel specimens or isolated viruses. QCMD ITEMS was modified to accommodate this. The documentation for reporting to TESSy for the seasons 2018/19 and 2019/20, and guidance for strain-based reporting of antiviral susceptibility and antigenic and genetic characterisation data, were used for adapting ITEMS [19-21]. Expected results for each programme are displayed in Tables 2 and 3.

For the molecular detection programme of EEIQAP 2020, 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 pick lists. We also asked for details on the tests used.

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

For strain characterisation, the participants were asked to report the results of antigenic and/or genetic characterisation, using drop-down pick lists with categories reflecting the TESSy categories for the seasons 2018/19 and 2019/20. They were also asked to upload obtained hemagglutinin (HA) gene sequences and to provide details on the methods used for antigenic and/or genetic characterisation. Unlike in the EEIQAP 2018, participants were asked to provide details on the strain specificities of reference sera and viruses used for antigenic characterisation.

For genotypic antiviral susceptibility testing, participants were asked to report the relevant amino acid changes associated with a change in susceptibility to oseltamivir and/or zanamivir and to upload obtained NA gene sequences.

Table 2. Expected results panel, molecular detection, virus isolation and antigenic and genetic characterisation, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen code	Programme EISNINF_MD20	Programme EISNINF_VI20			
	type and subtype/ lineage	Virus isolation	Type and subtype/ lineage	Antigenic category[1]	Genetic category[2]
EISN_INF20-1	A(H3N2)	Positive	A(H3N2)	A(H3) A/Hong Kong/4801/2014 (H3N2)-like (has NA induced HA)	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup because has N171K and N121K in HA1 and I77V and G155E in HA2 (EPI1030659)
EISN_INF20-2	N/A	Negative	N/A	N/A	N/A
EISN_INF20-3	A(H1N1)pdm09	Positive	A(H1N1)pdm09	A(H1)pdm09 A/Brisbane/02/2018-like but also very similar to A/Michigan/45/2015	A(H1)pdm09 clade 6B.1A5A representative A/Norway/3433/2018 because has S183P and N260D and additionally N129D and T185A (EPI1342624)
EISN_INF20-4	A(H3N2)	Positive	A(H3N2)	A(H3) A/South Australia/34/2019-like (did not agglutinate RBC at WHO CC)	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup because has E62G, R142G and H311Q in HA1, with additional amino acid substitutions HA1 T131K and HA2 V200I (EPI1360084)
EISN_INF20-5	B/Victoria	Positive	B/Victoria	B/Vic lineage not attributed to category; low reactor B/Brisbane/60/2008-like	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008 because has N75K, N165K and S172P and, additionally, I117V, N129D and V146I (EPI957662)
EISN_INF20-6	B/Yamagata	Positive	B/Yamagata	Bit further away from the most recent vaccine strain B/Phuket/3073/2013, but still considered B/Yam B/Phuket/3073/2013-like	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013 because has S150I, N165Y and G229D with additionally N116K, N202S, K298E and E312K and L172Q and M251V characteristic for recent Clade 3 viruses (EPI957663)
EISN_INF20-7	A(H3N2)	Positive	A(H3N2)	A(H3) A/Kansas/14/2017-like; similar to A/England/538/2018, which is a representative of recent 3C.3a viruses similar to A/Kansas/14/2017 (WHO CC data)	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup because has S91N, N144K and F193S in HA1 and D160N in HA2 (EPI1342623)
EISN_INF20-8	B/Victoria	Positive	B/Victoria	B/Vic B/Colorado/06/2017-like	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017 because has double deletion of HA1 residues 162 and 163 with amino acid substitutions of D129G and I180V, and HA2 R151K (EPI1223306)

N/A = not applicable.
[1] For details see Figure A1 and A2.
[2] For details see Figure A3.

Participants were also asked to provide an interpretation of the generated results using drop-down pick lists with the categories used for reporting to TESSy, taking into account the level of testing, e.g. single nucleotide polymorphism (SNP) test versus full length NA genome segment sequencing (or length at least covering the positions known to be associated with reduced inhibition). One category that does not appear in TESSy was added: 'No interpretation for this drug possible from testing performed' due to incomplete analysis of the NA segment. 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 using drop-down pick lists with the categories used for reporting to TESSy. For both methodologies, details were requested so that the reported results could be put in the context of the methodologies used.

Table 3. Expected results panel, antiviral susceptibility, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen code	Programme EISNINF_AV20				
	Phenotypic[1]		Genotypic (expected result when full NA is sequenced)[2]		
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Accession number NA in GISAID
EISN_INF20-1	NI	NI	AANI	AANI	EPI1030658
EISN_INF20-2	N/A	N/A	N/A	N/A	N/A
EISN_INF20-3	NI	NI	AANI	AANI	EPI1342710
EISN_INF20-4	NI	NI	AANI	AANI	EPI1360083
EISN_INF20-5	NI	NI	AANI	AANI	EPI975564
EISN_INF20-6	NI	NI	AANI	AANI	EPI991483
EISN_INF20-7	NI	NI	AANI	AANI	EPI1342709
EISN_INF20-8	NI	NI	AANI	AANI	EPI1223307
EISN_AV20-1	RI[3]	NI[3]	AARI NA-D199E[4]	AANI[4]	Sequence available on request
EISN_AV20-2	HRI[5]	HRI[5]	AAHRI NA-E119V + aa del245-248; AAHRI based on phenotypic data virus isolate and extrapolation from published data[4,5]	AAHRI NA-E119V + aa del245-248; AAHRI based on phenotypic data virus isolate and extrapolation from published data[4,5]	Sequence available on request

[1] NI = normal inhibition (fold-change IC_{50}; type A <10; type B <5); RI = reduced inhibition (fold-change IC_{50}; type A ≥10 & ≤100; type B ≥5 & ≤50); HRI = highly reduced inhibition (fold-change IC_{50}; type A >100; type B >50); N/A = not applicable.
[2] 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; N/A = not applicable.
[3] Fold-change compared to median IC_{50} of recent Dutch A(H1N1)pdm09 viruses for oseltamivir determined at 14.2 (RI) and for zanamivir at 7.7 (NI) at the National Institute for Public Health (RIVM).
[4] According to the WHO reference table [22].
[5] Fold-change compared to median IC_{50} of recent Dutch A(H3N2) viruses for oseltamivir, determined at 34 500 (HRI) and for zanamivir at 231 (HRI) at the RIVM.

2.6 Data analysis

All challenges of EEIQAP 2020 were considered 'educational' and, therefore, no pass/fail criteria were defined. A scoring system was used in which a correct result for a specimen was scored 0 (for 0 errors). Depending on the level of testing, a specimen with an error reported obtained a score of 1, 2 or 3, with the maximum score for an error kept equal for different aspects within one challenge. For each challenge, each laboratory received a cumulative performance score by summing up the individual specimen scores. For network performance, the percentages of laboratories (or reported datasets if one or more laboratories reported more than one dataset) 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 tables in the Annex. In addition, where appropriate, explanations of the judgements to conclude an error 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 is not available in 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. To that end, in the overview results 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 2020 sought to find clear proof of confirmation of the type/subtype or lineage of isolated viruses by adapting QCMD ITEMS. These reported results were separately analysed. Uploaded HA and NA segment sequences were aligned with the sequences generated at RIVM for all panel specimens using BioEdit software (version 7.2.5) and analysed for nucleotide and amino acid composition in conjunction with reported interpretation of phylogenetic

analysis of the HA sequences (allocation to TESSy genetic categories) and antiviral susceptibility marker analysis of the NA sequences (genetic assessment of antiviral susceptibility).

An 'Expected results' document for self-evaluation was shared with each participant in September 2020 through QCMD ITEMS with email notification from QCMD neutral office after the last participant had submitted their results. The 'Expected results' document was made available in English and Russian.

3. Results

3.1 Participating laboratories

A total of 61 laboratories were invited to participate in the study, of which 56 signed up, similar to the EEIQAP 2018. Thirty-seven of these 56 laboratories were located in the EU/EEA and 29 in other parts of the WHO European Region. Due to the COVID-19 pandemic, 33/37 (89%) laboratories in the EU/EEA and 12/19 (63%) laboratories outside of the EU/EEA returned results. In total, 45/56 (80%) laboratories that signed up returned results. Of the 11 laboratories that signed up but did not return results, four could not do so because the panel was unable to be delivered, five received the panel but did not return results for unknown reasons, and two specifically asked QCMD not to deliver the panel due to COVID-19-related issues. An aggregated breakdown of participating laboratories by challenge type is shown in Table 4 and by participating laboratory in Table A2. Only 14/45 (31%) laboratories participated in the full programme. Some laboratories indicated participation in a reduced number of challenges due to limited capacity in the laboratory because of COVID-19 response demands. Overall, the COVID-19 pandemic caused difficulties in distribution, due to the limited availability of transport by air and stricter requirements at customs, and affected the capacity of laboratories to perform testing of the panel.

Table 4. Breakdown of number of participants by challenge type, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Region[1]	Number of participants for each challenge (invited N=38 EU/EEA; total 61)[2]															
	Molecular detection		Virus isolation		Characterisation					Antiviral susceptibility testing					Full programme	
	Yes	No	Yes	No	Any	Antigenic only	Genetic only	Both	None	Any	Genetic only	Phenotypic only	Both	None	Yes	No
Signed up EU/EEA	37	1	32	6	33	N/A	N/A	N/A	5	28	N/A	N/A	N/A	10	26	12
Signed up total	56	5	50	11	46	N/A	N/A	N/A	15	36	N/A	N/A	N/A	25	34	27
Results EU/EEA	33	4	22	10	20	3	6	11	13	20	5	1	14	8	16	10
Results total	45	11	28	22	24	3	7	14	22	25	7	2	16	11	20	14

[1] *EU/EEA = Participating laboratories from the European Union (EU) and European Economic Area (EEA).*
[2] *N/A = not applicable; for the Results rows, the denominator is the number of laboratories that signed up.*

3.2 Molecular detection

In total, 45 of the 56 laboratories that signed up returned results for molecular detection, typing, and type A H-subtype determination. A limited number performed molecular type B lineage and an even lower number type A N-subtype determination. The 45 laboratories returned 46 datasets.

An aggregated breakdown of the results is shown in Table 5 by specimen code, in Table 6 by challenge type (detection, typing, H- and N-subtyping and lineage determination) and in Table A3 by participating laboratory. The methodologies used are listed in aggregated form in Figure A4 and by laboratory in Table A3. An overview of percentage of datasets by cumulative performance score for detection, A/B typing, type A H-subtyping and, separately, for the full challenge is shown in Figures 1 and 2, respectively, and by participating laboratory in Table A3.

Table 5. Overview of molecular detection, typing, type A H- and N-subtype and type B lineage determination results by specimen code, European External Influenza Quality Assessment Programme, WHO European Region, 2020

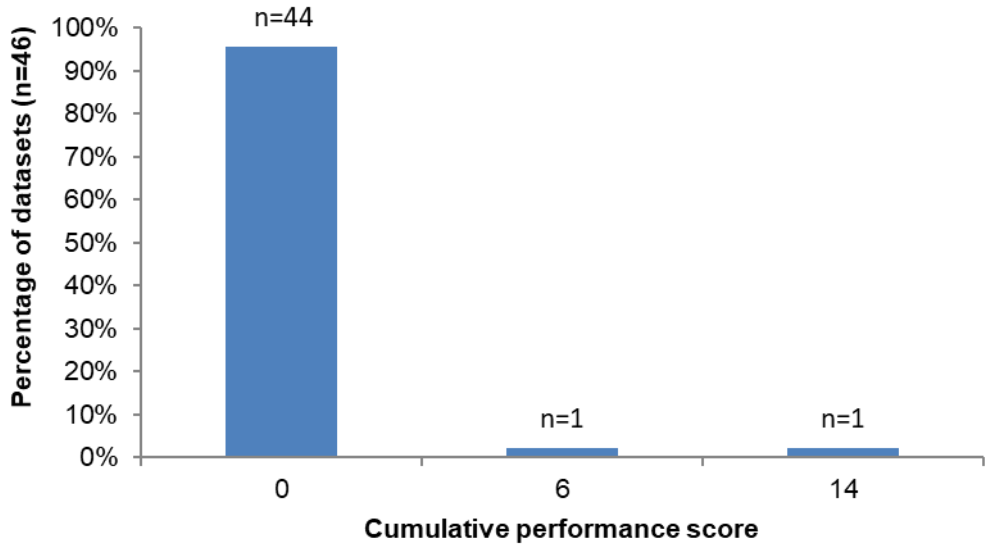
Specimen code (EISN_)	Expected results	Ct value at RIVM	Molecular detection by type, A H- and N-subtyping and B lineage determination (N = 46)[1]								
			Correct type and A H-subtype			Correct type and A H- & N-subtype/B-lineage			Incorrect Result[2]		
			Result	n	%	Result	n	%	Result	n	%
INF20-1	A(H3N2)	16.3	A + H3	45	97.8	A(H3N2)	19	41.3	Negative	1	2.2
INF20-2	No virus	None	Negative	44	95.7	Negative	44	95.7	A(H1)pdm09; B	2	4.3
INF20-3	A(H1N1)pdm09	18.3	A + H1pdm09	45	97.8	A(H1N1)pdm09	18	39.1	A(H3)	1	2.2
INF20-4	A(H3N2)	16.5	A + H3	45	97.8	A(H3N2)	19	41.3	B/Victoria	1	2.2
INF20-5	B/Victoria	17.9	B	46	100	B/Victoria	39	84.8	B/Yamagata	1	2.2
INF20-6	B/Yamagata	17.9	B	45	97.8	B/Yamagata	39	84.8	A(H3)	1	2.2
INF20-7	A(H3N2)	16.3	A + H3	46	100	A(H3N2)	19	41.3	A(H3)+B	1	2.2
INF20-8	B/Victoria	16.8	B	45	97.8	B/Victoria	40	87.0	Negative	1	2.2

[1] Forty-six datasets have been reported by 45 laboratories. Methodologies used are shown in Figure A4. Denominator for all percentage calculations is 46 datasets. A breakdown by level of testing, e.g. whether N-subtyping is performed, is shown in Table 6.
[2] One laboratory reported a number of results that were most likely in the incorrect order. However, results have been processed as they have been reported.

Most datasets (44/46; 95.7% with a cumulative performance score of 0) correctly identified influenza virus type and type A H-subtype in all eight panel specimens (Figure 1). Errors made are summarised in Table 6. One laboratory reported results most likely in an incorrect order and therefore had false positive, false negative and incorrect type/H-subtype for a number of specimens (Table A3). Another laboratory reported a false positive and a false negative result (Table A3). Only 16/46 (34.8%) datasets reported a fully correct result (cumulative performance score of 0) when the reported identification of the type A N-subtype and type B lineage were included (Figure 2). Many datasets did not report on type A N-subtyping (28/46; 60.9% for N1pdm09 and 27/46; 58.7% for N2) and 6/46 (13.0%) did not report on type B lineage determination, likely because such assays were not available in the laboratory (Table 6, Table A3 and Figure A4). Of all datasets that included type A N-subtyping, all identified the correct N-subtype of the four type A influenza viruses included in the panel (Table 6).

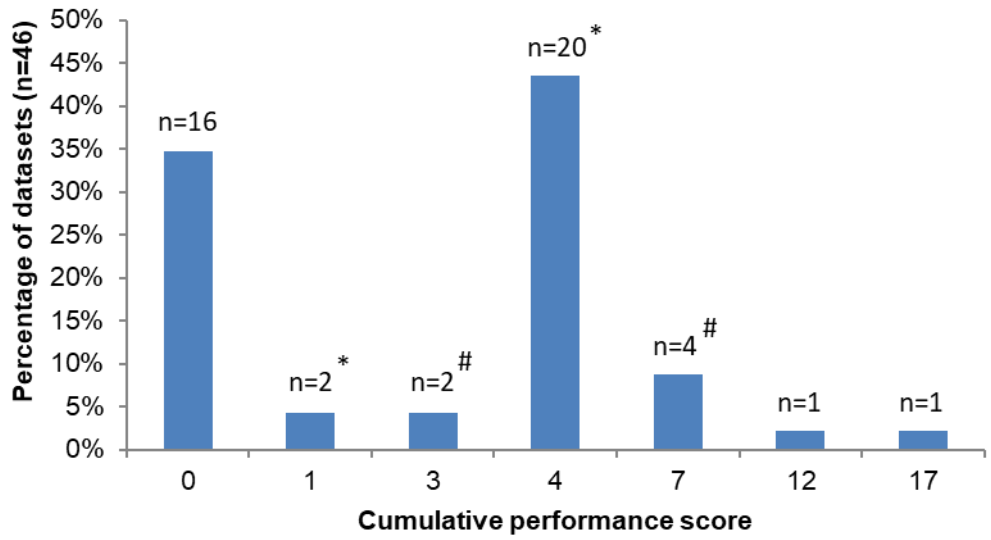
The high performance of laboratories with datasets using a wide variety of assays for the specific challenge types (Table A3, Figure A4 and Table 6) indicates that none of these assays had any specific issues.

Figure 1. Overview of cumulative performance scores for molecular detection, typing (A/B) and type A H-subtyping, European External Influenza Quality Assessment Programme, WHO European Region, 2020



Scoring system used:
Type A viruses: correct type and H-subtype, 0; correct type, 1; all other results, 3;
Type B viruses: correct type, 0; all other results, 3;
Negative specimen: Negative, 0; all other results, 3.

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 External Influenza Quality Assessment Programme, WHO European Region, 2020



Scoring system used:
Type A viruses: correct type and H- and N-subtype, 0; correct type and H-subtype, 1; correct type, 2; all other results, 3;
Type B viruses: correct type and lineage, 0; correct type, 1; all other results, 3;
Negative specimen: Negative, 0; all other results, 3.
** Missing N-subtype of type A influenza viruses (n = 20) or N1pdm09 only (n = 2).*
 # Missing N-subtype of type A influenza viruses and lineage of type B influenza viruses (n = 4) or N2 and lineage of type B influenza viruses (n = 2).

Table 6. Overview of molecular detection, typing, type A H- and N-subtype and type B lineage determination results by challenge type, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Evaluation	Performance for all 55 participating laboratories					Performance for laboratories that used the assays appropriate for the indicated challenge type (column one)					
Challenge type (number of specimens with this challenge)	% of datasets with fully correct result	Number of datasets with error or missing data	% of all specimens tested in challenge with correct result	Number of specimens with error or missing data	Type of error (number of specimens)[1]	Number of datasets included in evaluation	% of datasets with fully correct result	Number of datasets with error	% of all specimens tested in challenge with correct result	Number of specimens with error	Type of error (number of specimens)[1]
positive/negative (8)	95.7	2	98.9	4	false positive (2); false negative (2)	46	95.7	2	98.9	4	false positive (2); false negative (2)
type A/B (7)	95.7	2	98.8	4	false negative (2); incorrect type (2)	46	95.7	2	98.8	4	false negative (2); incorrect type (2)
H1pdm09 subtype (1)	97.8	1	97.8	1	incorrect subtype (1)	46	97.8	1	97.8	1	incorrect subtype
H3 subtype (3)	95.7	2	98.6	2	false negative (1); incorrect type (1)	46	95.7	2	98.6	2	false negative (1); incorrect type (1)
N1pdm09 subtype (1)	39.1	28	39.1	28	N-subtype missing (27); incorrect H-subtype (1)	18[2]	100	0	100	0	N/A
N2 subtype (3)	41.3	27	41.3	81	N-subtype missing (79); false negative (1); incorrect type (1)	19[2]	100	0	100	0	N/A
Victoria lineage (2)	84.8	7	85.9	13	B-lineage missing (11); false negative (1); incorrect lineage (1)	40[3]	97.5	1	98.8	1	incorrect lineage (1)
Yamagata lineage (1)	84.8	7	84.8	7	B-lineage missing (6); incorrect type (1)	40[3]	97.5	1	97.5	1	incorrect type (1)

[1] The vast majority of incorrect results are from the one laboratory that reported a number of results that were most likely in the incorrect order; N/A = not applicable.
[2] Laboratories that did not report on type A N1pdm09 and/or N2 subtyping were excluded.
[3] Laboratories that did not report on type B lineage determination were excluded.

3.3 Virus isolation and antigenic and genetic characterisation

A total of 28 of the 50 laboratories that signed up for participation in the virus isolation challenge returned results. For the antigenic and/or genetic characterisation challenge, this was 24 of 46 laboratories. Of these 24, 14 reported both antigenic and genetic characterisation results, seven genetic characterisation results only and three antigenic characterisation results only. Four laboratories that performed virus isolation did not perform characterisations.

3.3.1 Virus isolation

An aggregated breakdown of the virus isolation results of the 28 laboratories that returned results is shown in Table 7 and the details by participating laboratory and cumulative performance score in Table A4. The methodologies used are listed in aggregated form in Figure A5 and by laboratory in Table A4. An overview of percentage of laboratories by cumulative performance score is shown in Figure 3.

Table 7. Overview of virus isolation results by specimen code, European External Influenza Quality Assessment Programme, WHO European Region, 2020

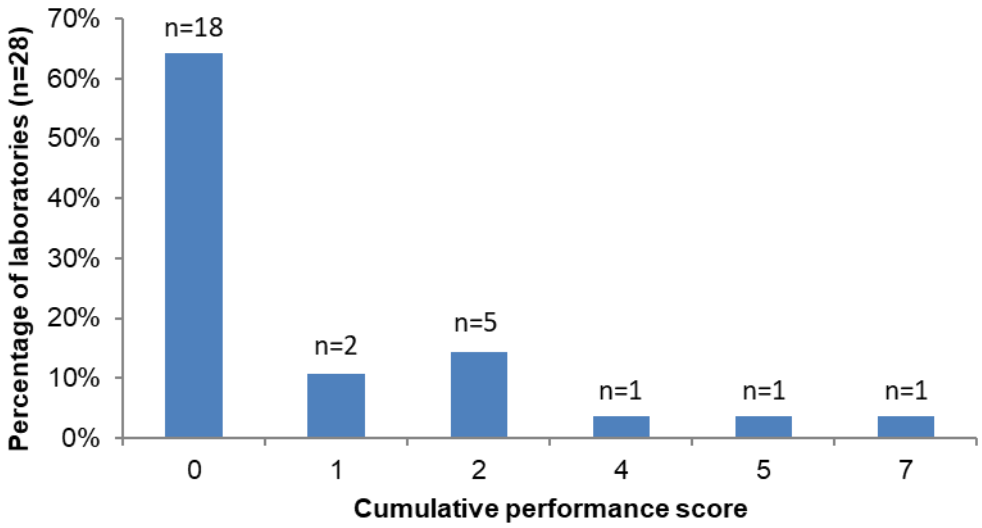
Specimen code	Virus	pfu/ml[1]; Ct value	Expected results	Viral isolation results (n = 28)					
				Correct results			Incorrect results		
				Result	n	%	Result	n	%
EISN_INF20-1	A(H3N2)[2]	8728; 16.3	Positive	Positive	22	78.6	Negative	6	21.4
EISN_INF20-2	No virus	N/A	Negative	Negative/Not attempted[3]	25	89.3	Positive	3	10.7
EISN_INF20-3	A(H1N1)pdm09	86940; 18.3	Positive	Positive	25	89.3	Negative	3	10.7
EISN_INF20-4	A(H3N2)	276000; 16.5	Positive	Positive	25	89.3	Negative	3	10.7
EISN_INF20-5	B/Victoria[2]	8728; 17.9	Positive	Positive	26	92.9	Negative	2	7.1
EISN_INF20-6	B/Yamagata[2]	2760; 17.9	Positive	Positive	25	89.3	Negative	3	10.7
EISN_INF20-7	A(H3N2)	27600; 16.3	Positive	Positive	25	89.3	Negative	3	10.7
EISN_INF20-8	B/Victoria	86940; 16.8	Positive	Positive	24	85.7	Negative	4	14.3

[1] pfu transposed by multiplying with 0.69 from TCID50 determined by titration on MDCK-MIX cells (MDCK-I and MDCK-SIAT) in rotating tubes. This way of titrating generates, in general, a higher titre compared to using 96-well microtitre plates and static incubation. End-points were determined by CPE for each tube.
[2] These viruses were also included in EEIQAP 2018.
[3] 'Not attempted' indicates that the laboratory did not attempt virus isolation because molecular testing was negative and likely the laboratories' algorithms include only virus-positive specimens in virus isolation. Therefore, these results are considered correct.

Well over 60% of the laboratories (18/28; 64.3%) had fully correct results (cumulative performance score of 0) reporting all influenza virus-containing specimens with the correct isolated virus and the negative specimen as negative (Figure 3). Of all 28 laboratories, one failed to isolate virus from all virus-containing specimens, whereas this laboratory indicated the control viruses grew well. Two laboratories failed to isolate all three A(H3N2)-containing specimens, of which one also failed to isolate the A(H1N1)pdm09-containing specimen. A further three laboratories failed to isolate the lowest viral load A(H3N2)-containing specimen EISN_INF20-1, of which one also failed to isolate the A(H1N1)pdm09-containing specimen. In addition to the laboratory not isolating any of the viruses, one did not isolate the B/Yamagata- and one of the two B/Victoria-containing specimens. A further four did not isolate the B/Yamagata-containing specimen (n = 1) or one or the other B/Victoria-containing specimen (n = 3). Overall, laboratories performed less well on the A(H3N2)- and B/Victoria-containing specimens with lower viral load compared to those with higher viral load (Table 7). Three laboratories reported false positives for the no virus-containing specimen. Two of which reported not being able to type/characterise it (Table A5) and one of which detected by molecular method a type B virus in the original specimen (Table A3). However, this laboratory did not detect the B/Victoria EISN_INF20-8 by molecular method and did not isolate this specimen either.

All laboratories used MDCK cells for virus isolation, although the type or types of MDCK cell lines used varied widely (Figure A5.A-C). MDCK-SIAT cells alone or in combination with other MDCK types were used more often for isolation of A(H3N2) influenza viruses (16/28; 57.1%) than for isolation of A(H1N1)pdm09 (10/28; 35.7%) and type B influenza viruses (11/28; 39.3%). Failure to isolate certain virus types/subtypes/lineages was not clearly linked to the use of specific types of MDCK cells. Also, the number of freeze/thaw cycles before specimens were subjected to virus isolation (median 1; range 0-3) and the volume of specimen inoculated (median 0.2 ml; range 0.1-1 ml) did not correlate with success of virus isolation (Table A4).

Figure 3. Overview of cumulative performance scores for virus isolation, European External Influenza Quality Assessment Programme, WHO European Region, 2020



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.

The majority of laboratories used haemagglutination (22/28; 78.6%), mainly combined with cytopathic effect (CPE) (20/28; 71.4%), for detection and confirmation of virus growth (Figure A5.D). In addition, RT-PCR (14/28; 50.0%) and NA activity assay (7/28; 25.0%) were most often used to detect and/or confirm growth of virus. For haemagglutination, a wide variety of red blood cell (RBC) types was used (Figure A5.E). Several laboratories used a combination of type of RBCs (Table A4) or a specific type of RBC dependent on the influenza virus type and type A H-subtype (details not shown). Also, for confirmation of the type and subtype or lineage of the isolated viruses, a wide variety of techniques was used, although the majority used RT-PCR (14/28; 50.0%) and HI-assay (9/28; 32.1%) using the same RBCs as used for haemagglutination for confirmation of virus growth (Figure A5.F). For HI-assay, WHO CC London ferret sera (8/9; 88.9%) were used most often (Figure A5.F).

3.3.2 Antigenic characterisation

Seventeen laboratories reported antigenic characterisation results. The evaluation of the reported antigenic characterisation results in comparison with the expected results was challenging. The returned antigenic characterisation category as pre-set in the TESSy reporting system largely depends on the specificity of the antisera used in HI-assays and the characteristics of the used assays, like the type of RBCs used in HI-assay or the deployment of oseltamivir in the HI-assay for viruses with NA-related haemagglutination (e.g. many of the current A(H3N2) influenza viruses). Individual specimen radar graphs were chosen to summarise the antigenic characterisation results and address these subjectivities and the wide range of reported categories per specimen (aggregated breakdown of the reported results in Figure 4 and by participating laboratory in Table A5).

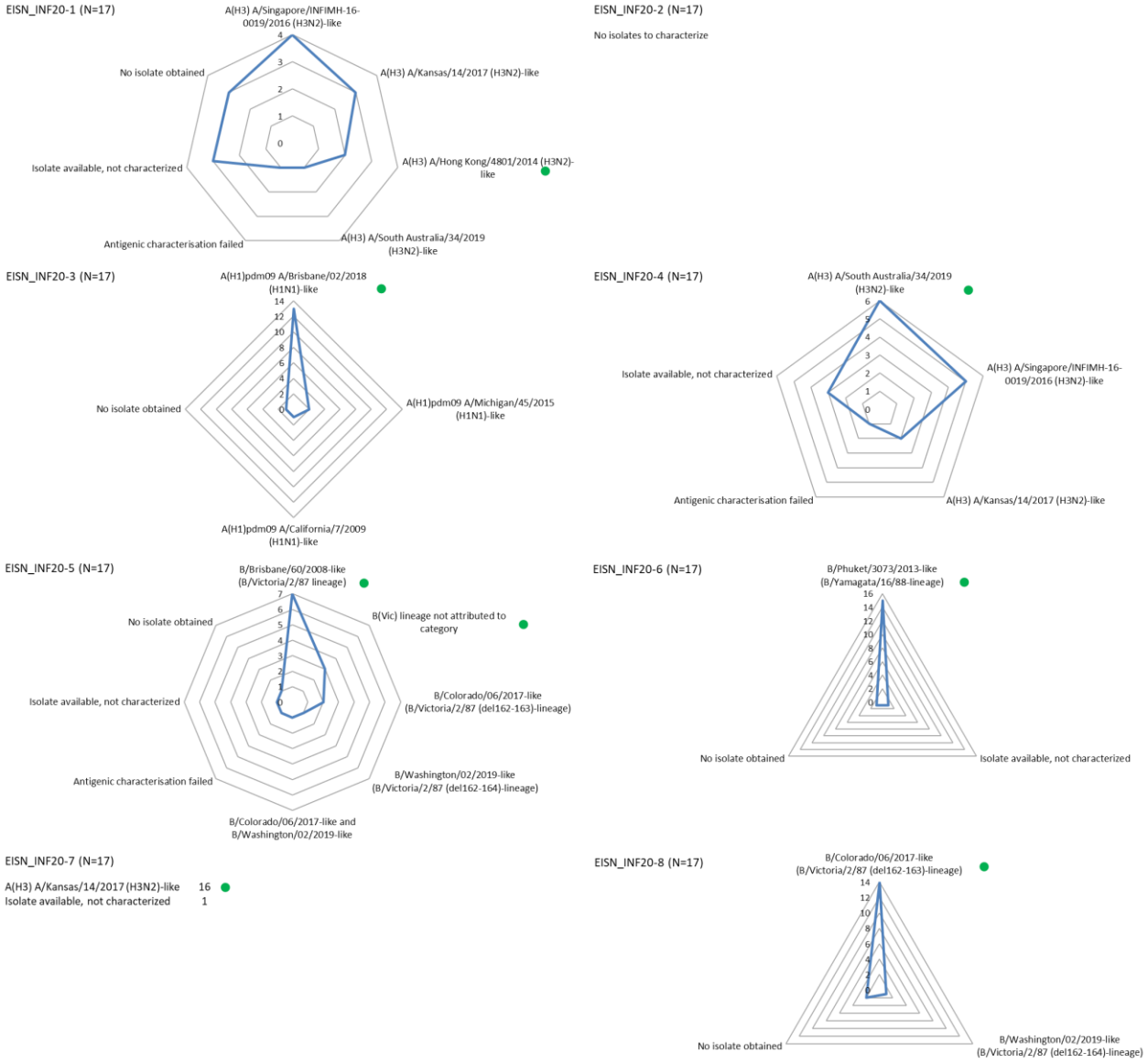
The methodologies used are listed by laboratory in Table A5. All 17 laboratories reporting on antigenic characterisation used HI-assay, of which five used oseltamivir with the A(H3N2) viruses and one with all viruses.

In Figure A6, an aggregated breakdown of type of sera used in HI-assay is shown. WHO CC (London or Atlanta) or in-house ferret sera were used alone or in combination with other sera by all laboratories except one that used in-house generated rat sera. Most laboratories used guinea pig RBC or turkey RBC alone or in combination with each other. 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 (Figure A7). Most laboratories (up to 87.5%) used at least two sera to characterise A(H1N1)pdm09, A(H3N2) and B/Victoria viruses (Figure A7.E). For B/Yamagata, 50% of laboratories that characterised the B/Yamagata-containing specimen used only one serum that was raised against B/Phuket/3073/2013. The selection of this serum was highly likely because for many years B/Yamagata viruses appear to be all antigenically B/Phuket/3073/2013-like. The percentage of laboratories that used three or more sera was highest for A(H3N2) (68.8%) followed by B/Victoria (62.5%) and A(H1N1)pdm09 (56.3%). The percentage of laboratories using four to seven different sera was highest for A(H3N2) virus (7/16; 43.8%). The majority of laboratories used at least sera that corresponded with the expected characterisation of the included A(H1N1)pdm09, B/Victoria and B/Yamagata viruses (Figure A7.A, A7.C and A7.D, respectively). However, less laboratories included B/Brisbane/60/2008 serum (12/17; 70.6%), corresponding with the older B/Victoria strain included in specimen EISN_INF20-5, than B/Colorado/06/2017 serum (15/17; 88.2%), corresponding with the more recent B/Victoria strain included in specimen EISN_INF20-8. Similarly, for the A(H3N2) viruses, the number of laboratories was highest for including sera corresponding with the more recent strains of A(H3N2) selected for inclusion in the

Northern (A/Kansas/14/2017, 16/17; 94.1% and A/Singapore/INFIMH-16-0019/2016, 11/17; 64.7%) or Southern Hemisphere (A/South Australia/34/2029, 9/17; 52.9%) seasons (Figure A7.B). Serum corresponding to the included A/Hong-Kong/4801/2014-like strain was only included by 6/17 (35.3%) of the laboratories.

The overall use of sera with a narrower or wider spectrum of specificities surely has influenced the characterisation results. Therefore, the one or two categories per specimen reported with the highest counts (Figure 4) can be considered correct. At the same time, however, 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 sera. The A(H1N1)pdm09 virus was mainly categorised as A/Brisbane/02/2018-like (13/17; 76.5%) (TESSy category for the 2019/2020 season), and only in a minority as A/Michigan/45/2015-like (2/17; 11.8%) (TESSy category for the 2018/2019 season). Nevertheless, both sera were used most often, by 16 and 13 laboratories, respectively, and these two strains do not greatly differ antigenically (within 4-fold HI titre difference; see Figure A1.A). The A(H3N2) viruses were more difficult to characterise. Except for one occasion, laboratories attributed the oldest strain included, A/Hong-Kong/4801/2014-like (EISN_INF20-1), to either A/Singapore/INFIMH-16-0019/2016 (n = 4), A/Kansas/14/2017 (n = 3) or, correctly, A/Hong-Kong/4801/2014 (n = 2). This is more or less a logical outcome given the range of sera being used to characterise the A(H3N2) panel viruses (Figure A7.B), in which only roughly a third included a serum against A/Hong-Kong/4801/2014. The A/South Australia/34/2019-like (EISN_INF20-4) virus included was almost equally attributed by most laboratories to either A/South Australia/34/2019 (n = 6) or A/Singapore/INFIMH-16-0019/2016 (n = 5), both clade 3C.2a1 viruses. Three of the five laboratories that characterised this virus as A/Singapore/INFIMH-16-0019/2016-like did not use a serum against A/South Australia/34/2019. The two laboratories attributing both EISN_INF20-1 and EISN_INF20-4 A(H3N2) viruses to A/Kansas/14/2017-like reported that they only used a serum against this strain. All 16 laboratories that characterised the included A/Kansas/14/2017-like (EISN_INF20-7) virus characterised it correctly, as such. This reflects the fact that all these laboratories included at least one serum against this strain in their A(H3N2) characterisation efforts; actually, 14/16 laboratories included two or more sera and 11/16 three or more sera against recent A(H3N2) reference strains. The included older B/Victoria virus was a low reactor B/Brisbane/60/2008-like (EISN_INF20-5) and was indeed most often reported as B/Brisbane/60/2008-like (n = 7) or not attributed to a category (n = 3). However, only one of the more recent three was tested with a serum against B/Brisbane/60/2008, whereas all three included a serum against B/Colorado/06/2017. Opposingly, the three laboratories that characterised this specimen as A/Colorado/06/2017-like (n = 2) or A/Washington/02/2019-like (n = 1) all included a serum against B/Brisbane/60/2008. Clearly, this older B/Victoria panel virus was more difficult to characterise than the more recent B/Victoria B/Colorado/06/2017-like (EISN_INF20-8) panel virus. That panel virus was characterised as B/Colorado/06/2017-like by the vast majority of laboratories (14/16), of which 13 laboratories included two or more sera and ten laboratories three or more sera, 12 of which included a serum against B/Brisbane/60/2008. The B/Yamagata B/Phuket/3073/2013-like (EISN_INF20-6) panel virus was characterised as such by all 15 laboratories that performed characterisation of this specimen. Of 14 of these laboratories, only seven used two or more sera, of which six included serum against the previous vaccine strain B/Massachusetts/2/2012.

Figure 4. Overview summarising the reported antigenic characterisation categories by specimen code, European External Influenza Quality Assessment Programme, WHO European Region, 2020

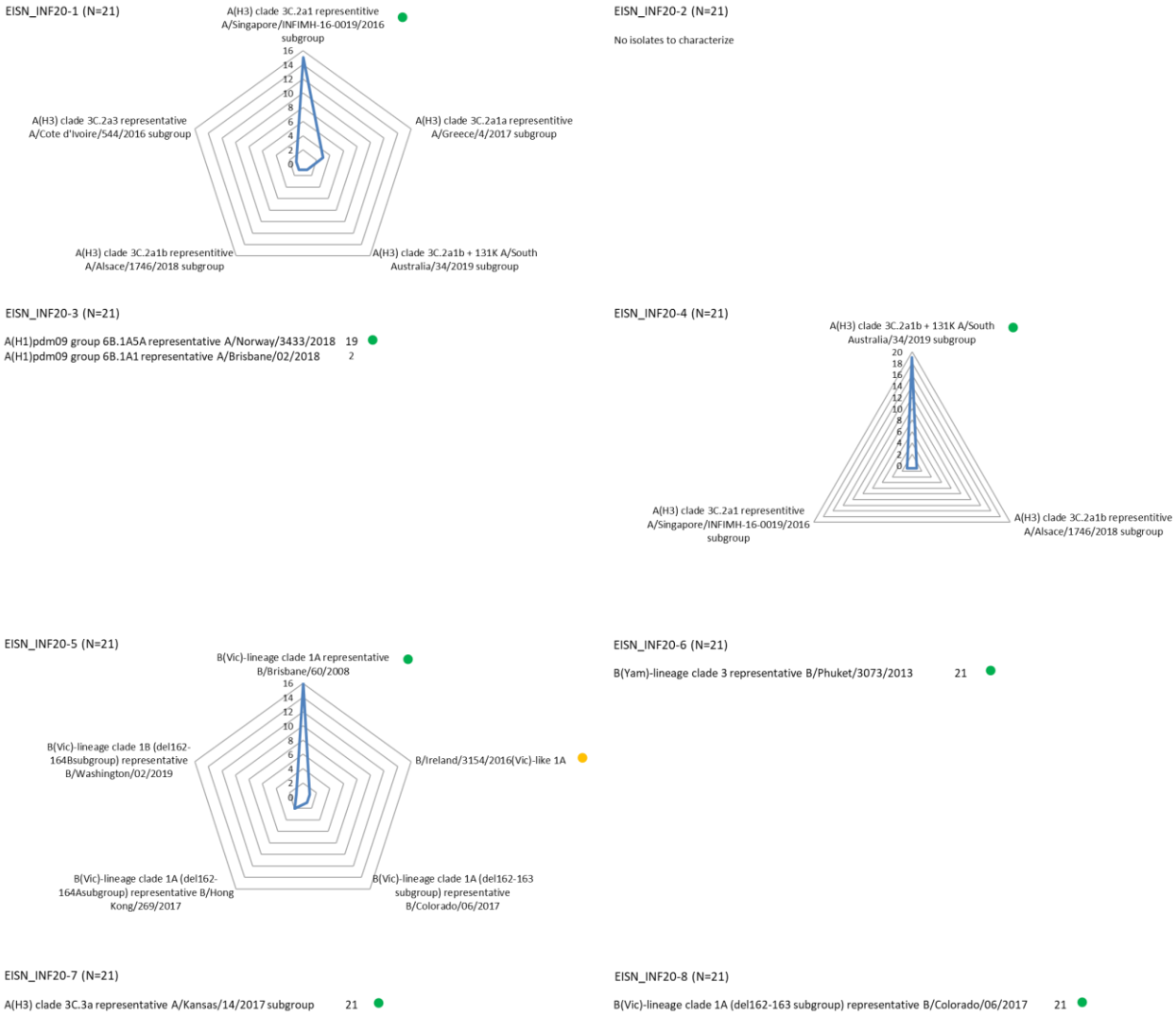


Expected result categories are indicated with a green dot (for details, see Table 2).

3.3.3 Genetic characterisation

A total of 21 laboratories performed genetic characterisation (aggregated breakdown of reported results in Figure 5 and by participating laboratory and overall performance score in Table A6). An overview of the percentage of laboratories by cumulative performance score is shown in Figure 6. The methodologies used are listed by laboratory in Table A6. Of the 21 laboratories, 14 performed sequencing on the simulated clinical specimen, two on the simulated clinical specimen or the virus isolate, two on both and three on the virus isolate only. Twelve of the laboratories used Sanger sequencing and nine NGS. Of the 21 laboratories, 18 laboratories submitted full or partial HA coding region sequences for evaluation. Of those submitting partial sequences, three submitted HA1 for all viruses, one HA1 for the B/Yamagata virus only and one HA2 for the B/Victoria and B/Yamagata viruses. The vast majority of sequences were fully identical or identical, except for a few nucleotides to the sequences obtained at RIVM. One laboratory uploaded a sequence for EISN_INF20-1 that was identical to the correct EISN_INF20-4, whereas another laboratory uploaded a sequence for EISN_INF20-1 that was almost identical to the correct EISN_INF20-4. One laboratory uploaded a sequence for EISN_INF20-5 with many nucleotide mutations compared to the original and another uploaded EISN_INF20-5 sequences. One laboratory uploaded a sequence for EISN_INF20-3 with several nucleotide mutations compared to those for the original and another uploaded EISN_INF20-3 sequences. Finally, one laboratory uploaded for EISN_INF20-5 B/Victoria a sequence with a three amino acid deletion at 162-164, while the panel virus did not harbour this deletion and such a virus was not included in the panel.

Figure 5. Overview summarising the reported genetic characterisation categories by specimen code, European External Influenza Quality Assessment Programme, WHO European Region, 2020

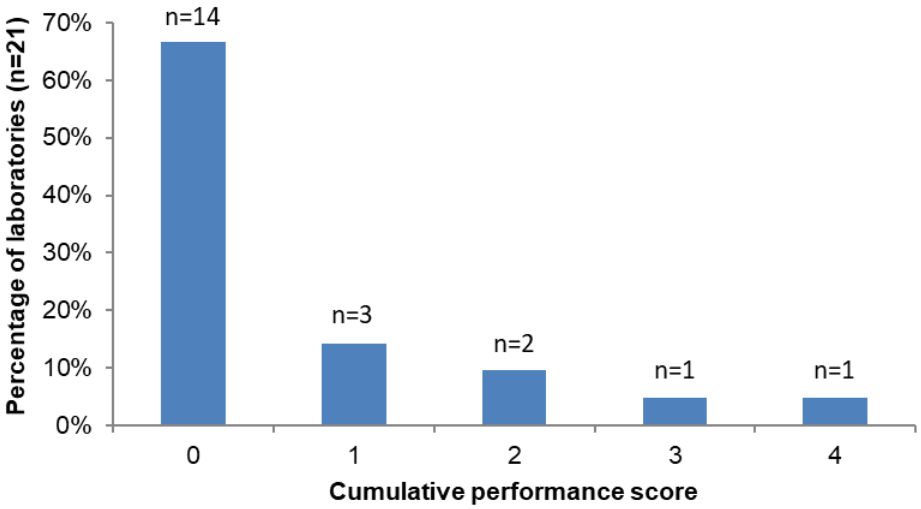


Expected result categories are indicated with a green dot and closely related categories with an orange dot (for details, see Table 2).

Based on the uploaded sequences, about 90% of laboratories should have been capable of allocating the panel viruses to the appropriate category by phylogenetic analysis using the reference viruses and detailed category criteria as provided by ECDC/WHO CC London (Table 2) [19,20]. However, less laboratories (14/21; 66.7%) reported correct results for all virus-positive specimens (cumulative performance score of 0) (Figures 5 and 6). The A(H1N1)pdm09 virus of specimen EISN_INF20-3 was misassigned by two laboratories to the genetic group 6B.1A1 further down the evolutionary tree (Figure 5 and Figure A3.A). However, the sequences uploaded by these two laboratories were identical to that of the panel virus and to those uploaded by all other laboratories. For the three A(H3N2) viruses included in the panel, only EISN_INF20-7 clade 3C.3a was assigned correctly by all laboratories; all sequences that were uploaded by 18 laboratories for this virus were identical to the panel virus. For the two other viruses, EISN_INF20-1 (3C.2a1) and EISN_INF 20-4 (3C.2a1b +131K), 6/21 (28.6%) and 2/21 (9.5%) laboratories, respectively, misassigned the viruses to clades higher up or further down in the evolutionary tree (Figure 5 and Figure A3.B). Of the sequences for EISN_INF20-1 uploaded by 18 laboratories, 16 (88.9%) were identical to that of the panel virus. The other two laboratories uploaded a sequence that had higher identity with EISN_INF20-4 and EISN_INF20-7, respectively, and these laboratories misassigned the virus. Of the other four laboratories that misassigned EISN_INF20-1, two uploaded a sequence. These sequences were identical to the panel virus and to those uploaded by the other laboratories that assigned this virus correctly. Of the two laboratories that misassigned EISN_INF20-4, one uploaded a sequence. That sequence was identical to that of the panel virus and to those uploaded by the other laboratories that assigned this virus correctly. The B/Victoria virus of specimen EISN_INF20-5 was assigned by four laboratories to genetic groups within clade 1A with deletion of amino acids 162-163 or 162-164 in HA1 (Figure 5). However, this virus did not have these deletions and, therefore, should have been assigned to the root 1A clade with B/Brisbane/60/2008 representative (Table 2 and Figure A3.C). Of these four laboratories, three uploaded sequences. One laboratory submitted only HA2 and, therefore, could not have identified the presence or absence of the clade-defining amino acid deletions. Two laboratories uploaded

sequences, of which one had no amino acid deletions and the other had amino acid deletions 162-164 (which seems to be the reason for misassignment). The EISN_INF20-6 B/Yamagata virus was assigned correctly by all 21 laboratories; all sequences that were uploaded by 18 laboratories for this virus were identical to that of the panel virus.

Figure 6. Overview of cumulative performance scores for genetic characterisation, European External Influenza Quality Assessment Programme, WHO European Region, 2020



Scoring system used: correct: 0; incorrect: 1. For details, see Table A6.

3.4 Antiviral susceptibility

Twenty-five laboratories participated in the antiviral susceptibility challenge; 16 performed genetic and phenotypic testing, seven genetic testing only and two phenotypic testing only. For phenotypic testing, the level of participations was more divers with a number of laboratories that only tested for oseltamivir susceptibility or tested a limited number of specimens (Table A8).

3.4.1 Genetic testing

A total of 23 laboratories performed genetic testing for antiviral susceptibility. Genetic testing results are shown in two tables, Table 8 with an aggregated breakdown of the identified amino acid substitutions and Table 9 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 A7. The methodologies used are listed in aggregated form in Figure A8 and by laboratory in Table A7. An overview of the percentage of laboratories by cumulative performance score is shown in Figure 7.

Two laboratories tested eight of the nine available specimens and two laboratories tested only one or two of the specific antiviral susceptibility testing panel specimens and none of the other virus-containing specimens (Table A7). The level of testing depended on the type of tests available and deployed. One laboratory used only RT-PCR for N1-H275Y and only tested the A(H1N1)pdm09-containing specific antiviral susceptible testing specimen EISN_AV20-1. All other laboratories used full length and/or partial sequencing, sometimes combined with pyrosequencing for nucleotide mutations associated with specific amino acids (Figure A8).

Fourteen of twenty-two (63.6%) laboratories identified the D199E amino acid substitution in specimen EISN_AV20-1, of which 12 reported correctly AARI for oseltamivir and AANI for zanamivir. The other two laboratories reported AANI for oseltamivir and zanamivir. Of the eight laboratories that did not identify D199E, one used only N1-H275Y SNP RT-PCR. Of the seven other laboratories, five uploaded sequences in which the codon for the D199E amino acid substitution was present and therefore could have been identified. Seventeen of twenty-two (77.3%) laboratories identified the E119V amino acid substitution and deletion of amino acids 245-248 correctly and another four identified only E119V correctly. One laboratory did not identify E119V nor the 245-248 deletion; the uploaded sequence was too short to identify E119V, but contained the deletion. Of the four laboratories that identified E119V, three uploaded sequences. Two of them contained the deletion and one had amino acids SASG at this position (unclear where these came from).

Table 8. Overview summarising the reported identified amino acid substitutions associated with reduced antiviral susceptibility by specimen code, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen code (EISN_)	Number tested[1]	Expected results[2]	Identification of amino acid substitutions[3]								
			Correct			Partial correct			Incorrect		
			Result	n	%	Result	n	%	Result	n	%
AV20-1	22	D199E	D199E	14	63.6	–	–	–	None[4]	8	36.4
AV20-2	22	E119V, 245-248del	E119V, 245-248del[5]	17	77.3	E119V[6]	4	18.2	None[7]	1	4.5
INF20-1	21	No substitutions	No substitutions	20	95.2	–	–	–	D161N[8]	1	4.8
INF20-2	0	No virus	–	–	–	–	–	–	–	–	–
INF20-3	20	No substitutions	No substitutions	20	100	–	–	–	–	–	–
INF20-4	21	No substitutions	No substitutions	19	90.5	–	–	–	D161N or N329S[8]	2	9.5
INF20-5	21	No substitutions	No substitutions	21	100	–	–	–	–	–	–
INF20-6	21	No substitutions	No substitutions	20	95.2	–	–	–	K360R[8]	1	4.8
INF20-7	21	No substitutions	No substitutions	21	100	–	–	–	–	–	–
INF20-8	21	No substitutions	No substitutions	21	100	–	–	–	–	–	–

[1] A number of laboratories did not perform genotypic antiviral susceptibility testing for all specimens depending on available tests.
[2] No substitutions indicates that no amino acid substitutions were associated with a reduction in neuraminidase (NA) inhibitor susceptibility following full NA gene 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; in the scoring of the interpretation of these results, the level of testing has been taken into account (Table 10; for details see footnote to Table A4).
[4] One laboratory performed only SNP H275Y assay. Sequences uploaded by five other laboratories contained the D199E mutations, but were not identified as associated with reduced susceptibility for NA inhibition.
[5] 245-248del was also reported as 244-247del (n = 5), 246-248del (n = 1) and 247-250del (n = 1); for those that uploaded sequences (n = 5), 245-248del could be clearly identified.
[6] Of those that uploaded sequences (n = 3), two contained the deletion 245-248 and one contained amino acids SASG at this position.
[7] Reported sequence was too short for identification of E119V, but contained the 245-248del.
[8] All reported sequences and the original determined sequences contained these amino acid substitutions; the laboratories reporting D161N or N329S added a comment that these positions were associated with reduced inhibition in other viruses (but reported interpretation AANI for oseltamivir and zanamivir).

For the EISN_INF specimens, the majority of laboratories did correctly identify no amino acid substitutions associated with reduced inhibition for the majority of specimens. Three laboratories reported an amino acid substitution for one or two specimens. One laboratory reported D161N with interpretation AANI for oseltamivir and zanamivir for A(H3N2)-containing specimens EISN_INF20-1 and EISN_INF20-4. Although 161N was present in the uploaded sequences by this laboratory, this is the natural amino acid present in these two viruses. In addition, this position is not mentioned in the WHO table [22]. For EISN_INF20-4, another laboratory reported N329S with interpretation AANI for oseltamivir and zanamivir; this laboratory uploaded a sequence with a codon for 329S. However, also for this virus, 329S is the naturally occurring amino acid present at this position. Although the 329 position is mentioned in the WHO table, only the N329K (-CHO) variant causes reduced inhibition. The EISN_INF20-1, -4 and -7 A(H3N2) containing specimens show the natural wildtype polymorphism at this position: 329N, 329S and 329T, respectively. Another laboratory reported the amino acid substitution K360R with interpretation AARI for oseltamivir and AANI for zanamivir for the B/Yamagata-containing specimen EISN_INF20-6; this laboratory uploaded a sequence with a codon for 360R. However, for this virus, 360R is the naturally occurring amino acid present at this position. Although position 360 is mentioned in the WHO table [22], only the K360E variant causes reduced inhibition and for peramivir only.

Table 9. Overview summarising the reported interpretation of amino acid substitution identification associated with reduced antiviral susceptibility against the expected interpretation by specimen code, European External Influenza Quality Assessment Programme, WHO European Region, 2020.

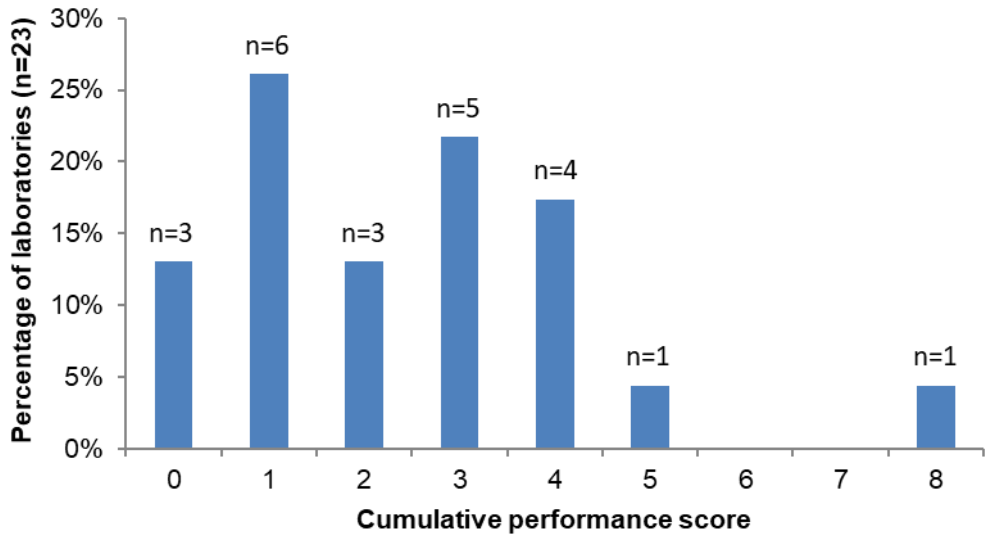
Specimen code (EISN_)	Number tested[1]	Expected interpretation for oseltamivir[2]	Results interpretation for oseltamivir[3]						Expected interpretation for zanamivir[2]	Results interpretation for zanamivir[3]					
			Correct			Incorrect				Correct			Incorrect		
			Result	n	%	Result	n	%		Result	n	%	Result	n	%
AV20-1	22	AARI	AARI	12	54.5	AANI[4]	10	45.5	AANI	AANI	21	95.5	NIP[5]	1	4.5
AV20-2	22	AAHRI	AAHRI, AARI[6]	21	95.5	AANI	1	4.5	AAHRI	AAHRI,AARI[6,7]	15	68.2	AANI, NIP[7]	7	31.8
INF20-1	21	AANI	AANI	21	100	–	–	–	AANI	AANI	21	100			
INF20-2	0	no virus	–	–	–	–	–	–	no virus	–	–	–	–	–	–
INF20-3	20	AANI	AANI	20	100	–	–	–	AANI	AANI	20	100	–	–	–
INF20-4	21	AANI	AANI	21	100	–	–	–	AANI	AANI	21	100	–	–	–
INF20-5	21	AANI	AANI	21	100	–	–	–	AANI	AANI	21	100	–	–	–
INF20-6	21	AANI	AANI	20	95.2	AARI	1	4.8	AANI	AANI	21	100	–	–	–
INF20-7	21	AANI	AANI	21	100	–	–	–	AANI	AANI	21	100	–	–	–
INF20-8	21	AANI	AANI	21	100	–	–	–	AANI	AANI	21	100	–	–	–

[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 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; NIP = no interpretation possible due to partial NA segment information (single nucleotide polymorphism (SNP) RT-PCR, partial- or pyrosequencing).
[3] Judged as correct or incorrect after comparison with the expected result, taking into account the level of testing. For viruses without amino acid substitution, AANI is correct if at least the full part of the NA segment has been sequenced that harbours positions previously associated with reduced antiviral susceptibility and no substitutions have been identified, otherwise NIP should have been reported. For details see Table A7.
[4] AANI is incorrect against the expected result and therefore labelled with an error score 1 in Table A7; however, 8/10 were reported AANI because these laboratories did not identify D199E.
[5] NIP was incorrect against the expected results, but considered correct in Table A7 because the laboratory only screened the specimen with H275Y SNP RT-PCR.
[6] AARI was generally considered correct against the expected result, taking into account variability in IC_{50} data reported and calculated fold-change data (see Figures 8 and 10).
[7] For zanamivir, a number of reported results allocated to correct or incorrect against the expected result were differently rated for a score in Table A7 when the reported identification of amino acid changes was taken into account (e.g. AAHRI was considered incorrect and AANI was correct when only E119V was identified).

There were few specimens with interpretation errors when the assessment was based on the identified and reported presence or absence of amino acid substitutions previously associated with (highly) reduced inhibition (Table A7). Two laboratories interpreted incorrectly the detected presence of D199E as AANI for oseltamivir for EISN_AV20-1. EISN_AV20-2 was more challenging, with one well-known amino acid substitution E119V and the less well-known amino acids deletion 245-248 present. All laboratories that reported E119V only (4/22; 18.2%) or E119V and deletion of amino acids 245-248 (or 244-247 or 246-248 or 247-250) (17/21; 81.0%) correctly interpreted for oseltamivir AAHRI (n = 20) or AARI (n = 1). However, for zanamivir, 2/4 (50%) laboratories that detected E119V only reported the incorrect interpretation AARI or AAHRI. For the 17 laboratories that detected both mutations, one (5.9%) laboratory incorrectly interpreted NIP and three (17.7%) AANI. For the EISN_INF specimens, one laboratory incorrectly interpreted the detection of K360R as AARI for oseltamivir. When comparing reported interpretations against the expected results ignoring the reported detected mutations, more incorrect interpretations were reported (Table 9; n = 20), especially for EISN_AV20-1 and oseltamivir (AANI instead of AARI; 10/22, 45.5%) and EISN_AV20-2 and zanamivir (AANI instead of AAHRI/RI; 6/21, 28.6%). These observations highlight the importance of correct detection of amino acid changes and interpretation of detected changes to arrive at a correct interpretation of the antiviral susceptibility profile.

Figure 7 shows the cumulative performance score for the amino acid substitution analysis and the interpretation. Only 3/23 (13.0%) laboratories had fully correct results (cumulative performance score of 0; specimens not tested were not scored). This low percentage of laboratories with fully correct results is obviously caused by failing to detect the D199E in EISN_AV20-1 or, when detected incorrectly, interpreting as AANI for oseltamivir and/or failing to detect the 245-248 amino acid deletion in EISN_AV20-2 or interpreting as AANI for zanamivir, as outlined above (Table 9 and Table A7).

Figure 7. Overview of cumulative performance scores for genetic antiviral susceptibility determination, amino acid substitution analysis and interpretation of this analysis, European External Influenza Quality Assessment Programme, WHO European Region, 2020



Scoring system used (only those specimens scored for which a result was reported):
EISN_AV20-1 substitutions: D199E found, 0; any other, 1; not tested, not scored.
EISN_AV20-1 interpretation oseltamivir: D199E AND any test AND AARI, 0; any other, 1; not tested, not scored.
EISN_AV20-1 interpretation zanamivir: NA sequenced covering known RI/HRI amino acid substitutions AND AANI, 0; SNP OR partial sequenced AND no interpretation possible (NIP), 0; any other, 1; not tested, not scored.
EISN_AV20-2 substitutions: E119V and 245-248 del (or del at approximate location but with different numbering) found, 0; only E119V found, 1; none found, 2; not tested, not scored.
EISN_AV20-2 interpretation oseltamivir: E119V AND 245-248 del AND any test AND AAHRI, 0; E119V AND 245-248 del AND any test AND AARI, 1; E119V only AND any test AND AAHRI or AARI, 0; any other, 2; not tested, not scored.
EISN_AV20-2 interpretation zanamivir: E119V AND 245-248 del AND any test AND AAHRI, 0; E119V AND 245-248 del AND any test AND AARI, 1; E119V only AND any test AND AANI, 0; any other, 2; not tested, not scored.
EISN_INF20-01 – 08 (except 02) substitution: none found, 0; any other, 1; not tested, not scored.
EISN_INF20-01 – 08 (except 02) interpretation oseltamivir and zanamivir: NA sequenced covering known RI/HRI amino acid substitutions AND AANI, 0; SNP OR partial sequenced AND no interpretation possible (NIP), 0; any other, 1; not tested, not scored.

3.4.2 Phenotypic testing

A total of 18 participants performed phenotypic testing for antiviral susceptibility. However, not all specimens were tested by all laboratories and not all were tested for both oseltamivir and zanamivir (Table 10). Although for specimens EISN_INF20-1 to -8 the virus first had to be isolated, four laboratories used one or more of the specimens without preceding virus isolation. Two that tested all specimens without virus isolation used NA-XTD or NA-Fluor kits. The other two used MUMANA in-house assays and tested each specimen directly because no virus isolate was obtained. Figures 8 and 9 show an aggregated overview of the IC_{50} values reported and Figure 10 an aggregated overview of IC_{50} fold-changes of EISN_AV20-1 and -2 calculated using the IC_{50} values reported for wild type EISN_INF20-3 and wild type

EISN_INF20-1, -4 and -7, respectively (by participating laboratory in Figures A9 and A10). Table 10 shows an aggregated breakdown of the interpretations of the reported IC_{50} values and by participating laboratory with cumulative performance score in Table A8. An overview of the percentage of laboratories by cumulative performance score is shown in Figure 11. The methodologies used are listed in aggregated form in Table 11 and by laboratory for the type of NA inhibition assay used in Table A8.

Table 10. Overview of phenotypic antiviral susceptibility testing results by specimen code, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen code (EISN_)	Oseltamivir phenotypic testing								Zanamivir phenotypic testing							
	Number tested[1]	Expected results[2]	Results phenotypic testing						Number tested[1]	Expected results[2]	Results phenotypic testing					
			Correct			Incorrect					Correct			Incorrect		
			Result	n	%	Result	n	%			Result	n	%	Result	n	%
AV20-1	18	RI	RI	12	66.7	NI	6	33.3	16	NI	NI	14	87.5	RI	2	12.5
AV20-2	18	HRI	HRI or RI	18	100	-	-		16	HRI	HRI or RI	15	93.8	NI	1	6.3
INF20-1	15	NI	NI	14	93.3	No isolate	1	6.7	13	NI	NI	12	92.3	No isolate	1	7.7
INF20-2	–	no virus	–	–	–	–	–	–	–	no virus	–	–	–	–	–	–
INF20-3	17	NI	NI	17	100	–	–	–	14	NI	NI	14	100	–	–	–
INF20-4	15	NI	NI	14	93.3	No isolate	1	6.7	14	NI	NI	13	92.9	No isolate	1	7.1
INF20-5	16	NI	NI	16	100	–	–	–	13	NI	NI	13	100	–	–	–
INF20-6	15	NI	NI	12	80.0	RI or HRI	3	20.0	13	NI	NI	13	100	–	–	–
INF20-7	16	NI	NI	15	93.8	No isolate	1	6.3	14	NI	NI	13	92.9	No isolate	1	7.1
INF20-8	15	NI	NI	11	73.3	RI or no isolate	4	26.7	13	NI	NI	12	92.3	No isolate	1	7.7

[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.

[2] Type A viruses: normal inhibition (NI) = IC_{50} fold-change < 10; reduced inhibition (RI) = IC_{50} fold-change ≥ 10 – ≤ 100; highly reduced inhibition (HRI) = IC_{50} fold change > 100. Type B viruses: NI = IC_{50} fold-change < 5; RI = IC_{50} fold-change ≥ 5 – ≤ 50; HRI = IC_{50} fold-change > 50.

The overall performance of the laboratories in phenotypic testing was relatively good; 8/18 (44%) laboratories had all specimens correct and the other 10/18 (56%) reported an incorrect result for only one or up to four specimens (Figure 11 and Table A8). A variety of techniques and approaches for IC_{50} measurement and calculations were used (Table 11). However, the majority of laboratories used an in-house MUNANA substrate-based assay with pre-titration of NA-activity and an 'HPA Excel template' or GraphPad Prism software for IC_{50} calculation, reflecting the training activities performed in the past at the UK Health Security Agency (formerly called Public Health England and the Health Protection Agency (HPA)). Of the ten laboratories with incorrect results, six used a commercial kit-based assay (Table A8).

The EISN_AV20-1 specimen with A(H1N1)pdm09 N1-D199E was correctly determined as RI by 12/18 (66.7%) laboratories for oseltamivir and as NI by 14/16 (87.5%) laboratories for zanamivir (Table 10). Of the seven deviant results (NI instead of RI by oseltamivir and RI instead of NI by zanamivir), six were obtained using commercial kit-based assays (Table A8). However, when comparing reported IC_{50} values with those available for wildtype EISN_INF20-3, two laboratories reporting RI by oseltamivir had normal fold-change (8.8 and 9.8) (Figure 10 and Figure A10). In addition, of five laboratories reporting NI by oseltamivir, three had RI fold-change (13.8, 26.0 and 29.0) and the other two had an NI fold-change (4.5 and 5.1) (Figure 10 and Figure A10). For zanamivir, five laboratories that reported NI had fold-changes (10.0-101.8) indicating RI-HRI, whereas the two laboratories reporting RI had fold-changes (5.5) indicative for NI and fold-changes (12.5) indicative for RI (Figure 10 and Figure A10). The notified differences in interpretation of IC_{50} values likely depended on what was used as a reference for the calculation of the fold-changes (Table 12) and a result for the wildtype virus EISN_INF20-3 that is less representative for median or mean IC_{50} values obtained in routine seasonal analyses.

Table 11. Methodologies used by laboratories to determine and evaluate IC_{50} values, European External Influenza Quality Assessment Programme, WHO European Region, 2020

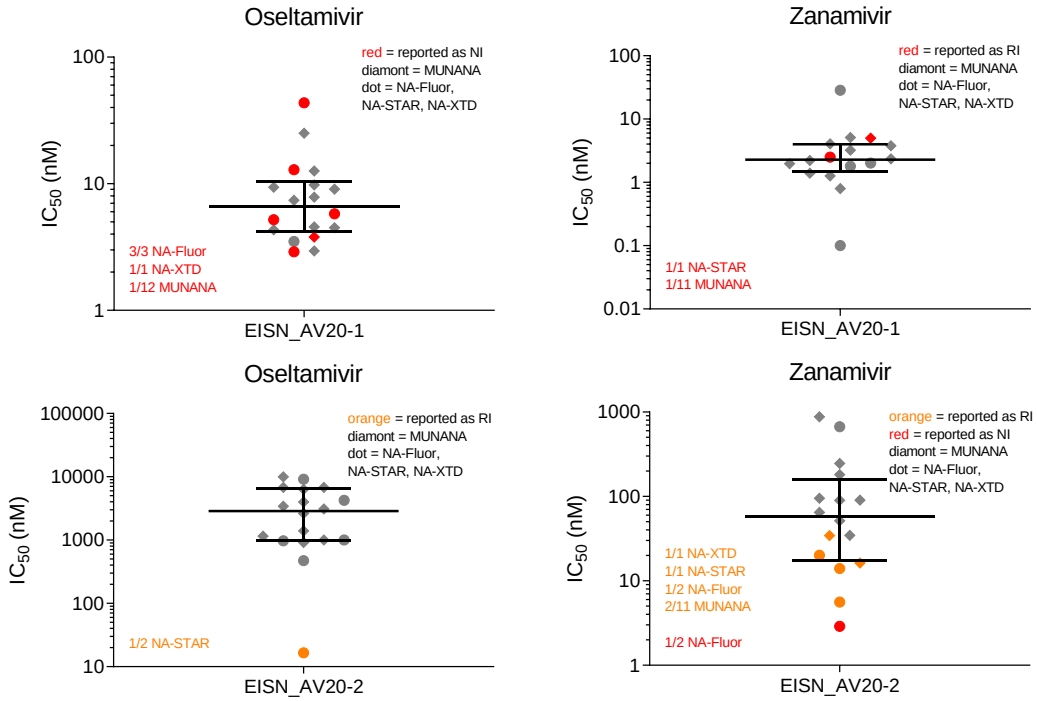
Method	Number of laboratories	
Assay type[1]		
MUNANA In-house	12	
NA Fluor Kit	3	
NA-STAR Kit	2	
NA XTD Kit	1	
Drug range (nM) tested	**Lowest concentration**	**Highest concentration**
Median	0.01	4 000
Mean	0.16	6 734
Minimum	0.001	250
Maximum	1	31 250
Pre-titration NA-activity		
Yes	15	
No	3	
Measurements		
Duplicate	13	
Single	5	
Control viruses[2]		
In-house	6	
ISIRV	5	
CDC	3	
ISIRV; In-house	2	
ISIRV only sensitive	1	
In-house; WHO virus	1	
Software to calculate IC_{50}[3]		
HPA Excel template	9	
GraphPad Prism (https://www.graphpad.com/scientific-software/prism/)	5	
Excel template	1	
IC_{50} Calculator | AAT Bioquest (https://www.aatbio.com/tools/ic50-calculator)	1	
Origin (https://www.originlab.com)	1	
SigmaPlot (http://www.sigmaplot.co.uk/products/sigmaplot/)	1	
Evaluation of IC_{50} against		
Wildtype virus	7	
Median previous season	3	
Median current season	2	
Mean previous season	2	
Mean current season	1	
Mean previous season; wildtype	1	
Median previous; median current season	1	
Median previous or more seasons	1	

[1] MUNANA = 20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid substrate producing a fluorescent product after NA 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; WHO = World Health Organisation.
[3] The HPA Excel template was a file provided by the Health Protection Agency, currently Public Health England, London, UK.

The EISN_AV20-2 specimen with A(H3N2) N2-E119V and deletion of amino acids 245-248 was correctly reported as HRI by oseltamivir by 17/18 (94.4%) laboratories (Table 10). One laboratory reported RI, which was considered correct as the fold-change obtained was 61.9 when IC_{50} was compared with mean IC_{50} wildtype EISN_INF20-1, -4 and -7 A(H3N2) viruses from this laboratory (Figure 10 and Figure A10). This laboratory used an NA-STAR commercial kit. In general, 4/6 (66.7%) laboratories that used commercial kits and for which fold-changes could be calculated obtained lower fold-changes (median: 716; range: 61.9-1 743) than those that used in-house MUNANA-based assays (median: 20 298; range 4 458-34 500), although two laboratories using NA-Fluor kit or NA-XTD kit obtained fold-changes of 68 828 and 4 735, respectively (Figure 10 and Figure A10).

For zanamivir, the results with the EISN_AV20-2 specimen with A(H3N2) N2-E119V and deletion of amino acids 245-248 were more variable. For oseltamivir, 10/16 (62.5%) laboratories correctly reported HRI, 5/16 (31.3%) reported RI and 1/16 (6.3%) reported incorrect NI (Table A8). The reported RI were considered correct given the fold-changes calculated based on mean IC_{50} values obtained with wildtype EISN_INF20-1, -4 and -7 A(H3N2) viruses by four of these laboratories (Figure 10 and Figure A10); the median fold-change obtained was 56.9 (range: 14.1-96.4). The median fold-change for laboratories reporting HRI was 215 (range: 132-454). Surprisingly, the laboratory reporting NI for zanamivir had a calculated fold-change of 290 well in the range of others reporting HRI, suggesting an error in reporting the interpretation NI (Figure 10 and Figure A10).

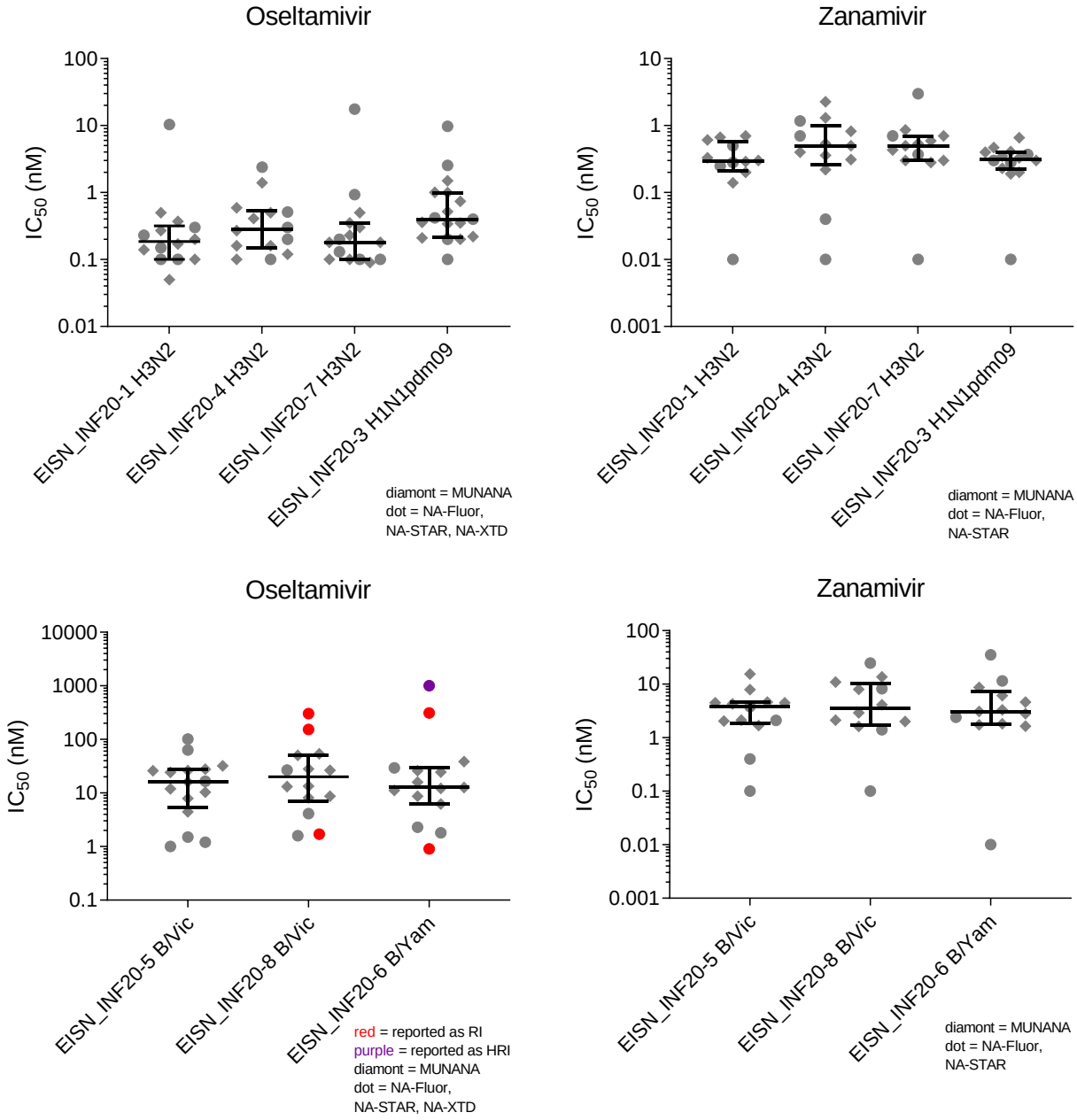
Figure 8. Overview of reported IC_{50} values by code EISN_AV specimens, European External Influenza Quality Assessment Programme, WHO European Region, 2020



Red markers indicate specimens with incorrect result, IC_{50} level not as expected and/or interpretation not as expected. Orange markers indicate not fully correct result, IC_{50} level not as expected and/or interpretation not as expected (RI instead of expected HRI). For details by laboratory, see Table A8 and Figure A9. Large horizontal bars represent the median and whiskers represent the interquartile range.

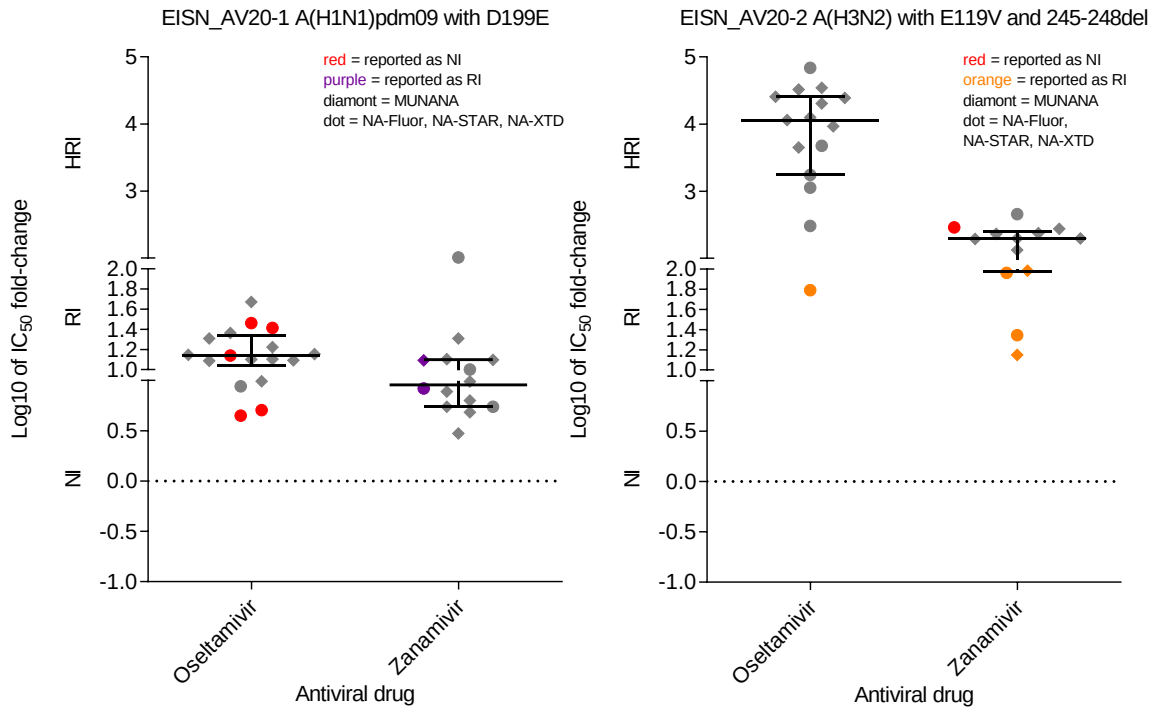
For the EISN-INF specimens, the majority of results were correctly reported as RI by oseltamivir and zanamivir (Table A8). However, three laboratories incorrectly reported the same two viruses EISN_INF20-6 (B/Yamagata) and EISN_INF20-8 (one of two B/Victoria in the panel) incorrectly as RI or HRI by oseltamivir (Table A8). Two of these laboratories reported much higher IC_{50} values for both specimens (EISN_INF20-6 312 and 1003 nM, respectively, and EISN_INF20-8 303 and 153 nM, respectively) than the other laboratories (EISN_INF20-6 median: 12.2 nM; range: 0.9-38.5 nM and EISN_INF20-8 median: 13.2 nM; range: 1.7-53.8 nM) (Figure 9 and Figure A9). The other laboratory reporting RI measured normal IC_{50} values for both specimens, 0.9 and 1.7 nM respectively (Figure 9 and Figure A9). All three laboratories reported NI by oseltamivir for the other B/Victoria specimen EISN_INF20-5, providing IC_{50} values of 102, 63 and 1 nM, respectively (same order as above; Figure 9 and Figure A9). The other laboratories reported median IC_{50} of 14.0 nM (range: 1.2-27.8 nM) for oseltamivir for this specimen (Figure 9 and Figure A9). Two of the above three laboratories reported IC_{50} values for zanamivir for the three influenza B virus specimens with values (range: 0.01-0.1 nM and range: 3.8-11.5 nM) lower or higher than the range of IC_{50} values reported by the other laboratories (range: 0.4-35.4 nM) (Figure 9 and Figure A9), suggesting that it is not the method itself generating deviant results with oseltamivir and influenza B viruses for these three laboratories, but rather a combination of method measuring IC_{50}, calculation to achieve fold-change data, interpretation and reporting.

Figure 9. Overview of reported IC_{50} values by code EISN_INF specimens, European External Influenza Quality Assessment Programme, WHO European Region, 2020



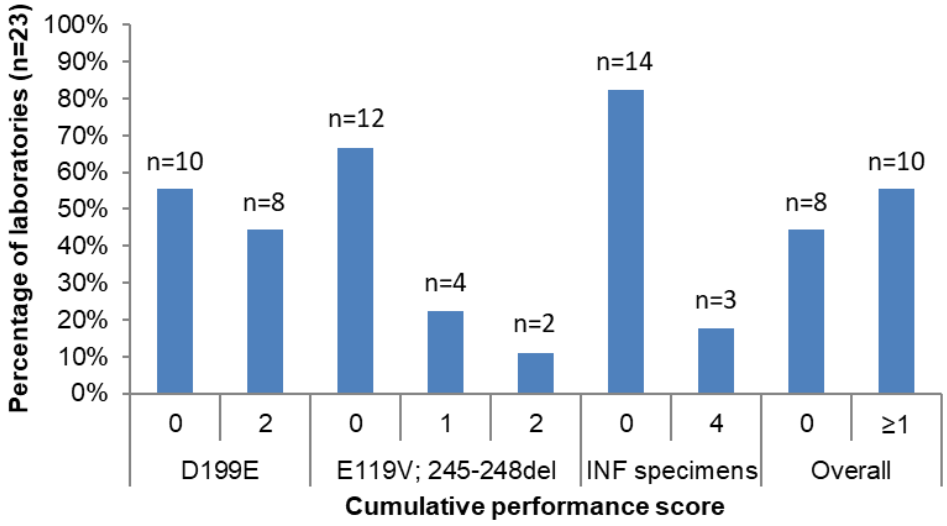
Red or purple markers indicate specimens with incorrect result, IC_{50} level not as expected and/or interpretation not as expected. For details by laboratory, see Table A8 and Figure A9. Large horizontal bars represent the median and whiskers represent the interquartile range.

Figure 10. Overview of calculated IC_{50} fold-change values for the EISN_AV20-1 and -2 specimens, European External Influenza Quality Assessment Programme, WHO European Region, 2020



Laboratory-specific fold-change values were calculated for those laboratories that also reported IC_{50} values for the wild type viruses of the same subtype or lineage included in the EISN_INF20 specimens, EISN_INF20-3 and mean EISN_INF20-1, -4 and -7 respectively. Large horizontal bars represent the median and whiskers represent the interquartile range. 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; fir type B viruses: NI = IC_{50} fold-change < 5, RI = IC_{50} fold-change ≥ 5 – ≤ 50, HRI = IC_{50} fold change > 50. Red markers indicate specimens with incorrect result; IC_{50} level not as expected and/or interpretation not as expected. Purple markers indicate laboratory reported RI whereas NI was expected. Orange markers indicate laboratory reported RI whereas HRI was expected. For details by laboratory see Table A8 and Figure A10.

Figure 11. Overview of cumulative performance scores for phenotypic antiviral susceptibility determination, European External Influenza Quality Assessment Programme, WHO European Region, 2020



Scoring system used (specimens were only scored if a result was reported):
EISN_AV20-1: oseltamivir: RI, 0; other, 1; zanamivir: NI, 0; other, 2; not tested or no isolate, not scored.
EISN_AV20-2: oseltamivir: HRI, 0; RI, 1; other, 2; zanamivir: HRI, 0; RI, 1; other, 2; not tested or no isolate, not scored.
EISN_INF20-1 - 8 (except -2): oseltamivir and zanamivir: NI, 0; other, 2; not tested or no isolate, not scored.

3.5 Accreditation

Participation in EQA programmes is an important element in accreditation of laboratories. Table 12 outlines the accreditation status of the laboratories.

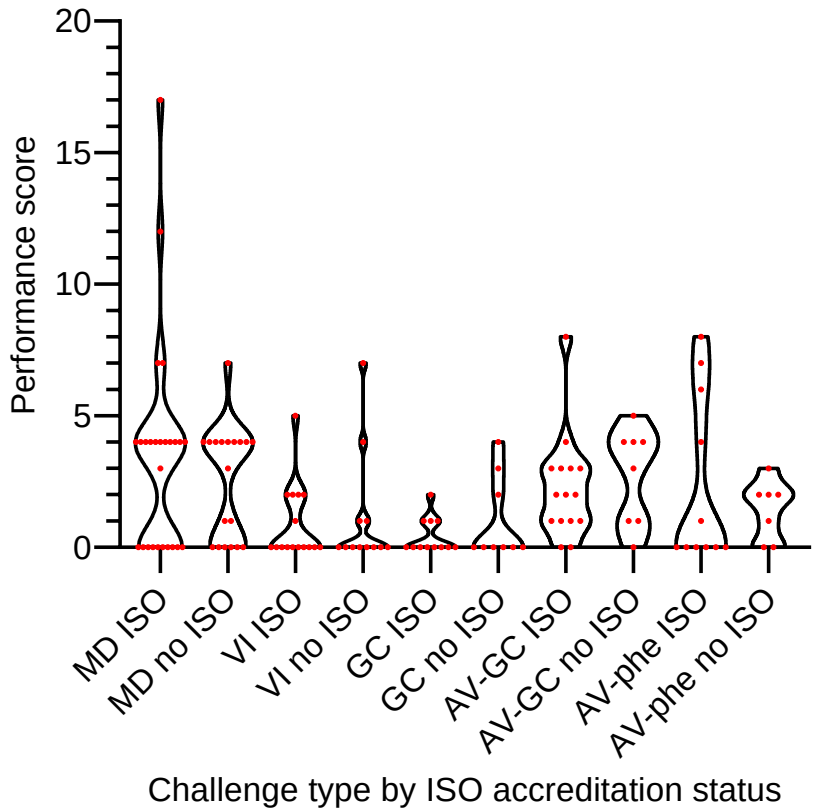
Table 12. Summary of survey on laboratory accreditation, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Question and response[1]	Number of laboratories (N = 45)
Is your laboratory accredited?	**n**
Yes	
ISO 15189	17
ISO 15189, 17025	2
ISO 15189, WHO[1] (not specified)	1
ISO 17025	4
ISO 17025/2005	1
ISO 17025/2006	1
ISO 9001[2]	2
National, Ministry of Health	1
WHO[1] (not specified)	2
WHO[1] recognised National Influenza Centre[3]	1
Subtotal	32
No, in the process of obtaining accreditation	
ISO 15189	3
ISO 17025-2017	1
Subtotal	4
No, and not in the process of obtaining accreditation	9
Total	**45**

[1] *WHO = World Health Organization.*
[2] *ISO 9001 is a certification rather than accreditation.*
[3] *National Influenza Centre recognition is not an accreditation but compliance to Terms of Reference.*

Data on accreditation were reported by 45 laboratories. Of the 45 laboratories, 32 (71%) were accredited: 20 (44%) by ISO 15189 (medical laboratories), of which one also by ISO 17025 (testing and calibration laboratories) and one also by WHO (not specified for what and is not an ISO norm); six (13%) by ISO 17025; two (4.4%) by ISO 9001 (certification for quality management and, as such, not an accreditation); and four (8.9%) by National Accreditation or WHO (not specified for what and is not an ISO norm). A further 4/45 (8.9%) laboratories were in the process of obtaining ISO 15189 or ISO 17025 accreditation and nine (20%) laboratories reported that they were not accredited and were not in the process of obtaining accreditation. Figure 12 shows a summary of performance scores by ISO status for all challenges except antigenic characterisation, as calculation of a performance score for that challenge was doubtful. ISO 15189 and ISO 17025 were taken together, as these ISO norms describe procedures to ensure quality of performance of assays as defined in the scope by the laboratory. All other categories in Table 12 were considered 'No ISO'. No clear relationship between performance and being ISO accredited was observed. Being ISO accredited and having all techniques in the scope might still be beneficial, as this should include clear procedures to solve issues with these techniques.

Figure 12. Violin plot with individual values for the assessment of dependency of performance scores for each challenge except antigenic characterisation on ISO accreditation status of a laboratory, European External Influenza Quality Assessment Programme, WHO European Region, 2020



ISO = ISO 18159 and/or 17025 accredited; no ISO = not ISO 15189 and/or 17025 accredited; MD = molecular diagnostics; VI = virus isolation; GC = genetic characterisation; AV-GC = antiviral genetic characterisation; AV-phe = antiviral phenotypic characterisation.

4. Discussion

There was high participation in the EEIQAP 2020 among the 54 WHO European Region countries/nations (the four nations in the UK were counted separately; Andorra, Monaco and San Marino were excluded because they do not have an influenza reference laboratory; Liechtenstein was excluded because they are not a member of WHO and don't have an influenza reference laboratory) and the 61 laboratories in this Region. Forty-nine countries/nations and 56 laboratories participated. However, actual participation was compromised by the COVID-19 pandemic and only 45 laboratories (46 datasets for molecular detection, as one laboratory reported two datasets) in 40 countries/nations reported data. Therefore, compared to the EEIQAP 2018, in which 56 laboratories in 49 countries/nations participated [16], the EEIQAP 2020 provides a less comprehensive overview of the capabilities and performance of laboratories in the WHO European Region and WHO GISRS and ECDC EISN ERLI-Net influenza reference laboratories for the different aspects of influenza surveillance in the Region.

The 45 laboratories with 46 datasets 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), B/Victoria and B/Yamagata influenza viruses. Overall, 44/46 (95.7%) datasets reported correct results for all specimens, reconfirming the correct and reliable reporting of surveillance detection data by the network laboratories to TESSy. This percentage of laboratories with correct results was similar to that for the 56 laboratories in the 2018 EEIQAP study (95%) and higher than that reported for 38 ERLI-Net laboratories in EU/EEA countries in the 2015 EQA study (90%) and for 45 NICs in the WHO European Region in the 2020 WHO EQAP study (80%), which also included avian type A subtypes [16,23,24]. Similar to the 2018 study, the EEIQAP 2020 challenged participating laboratories to determine the influenza A virus N-subtype and influenza B virus lineage of detected viruses. Again, fewer laboratories performed N-subtyping of type A influenza viruses (18/46; 39% for N1 and 19/46; 41% for N2 compared to 21/55; 38% in EEIQAP 2018) than lineage determination of type B viruses (40/46; 87% compared to 46/55; 84% in EEIQAP 2018), both included in the dataset for reporting to TESSy. This suggests that few laboratories have extended their capability for A N-subtyping or B-lineage determination. Nevertheless, all datasets that reported the N-subtype and/or B-lineage did so correctly (after one laboratory's incorrect results were excluded, as it was highly likely that these results were reported in the incorrect order). 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, the emergence of H1N2 reassortant virus was detected late because seasonal viruses were not widely N-subtyped [25]. 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 [26]. In 2018, one H1N2 reassortant virus was rapidly identified in routine surveillance because N-subtyping was included [27]. B-lineage is important to know in order to detect the (re)emergence and distribution of lineages [25], 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) [28].

After 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 [23], it dropped to 27/44 (61%) in the EEIQAP 2018 [16] and 18/28 (64%) in the EEIQAP 2020. For the EEIQAP 2018, this was explained by the high number of isolation failures for influenza virus type B, especially for the specimens with the lowest concentration of virus, which was also observed in the first virus isolation EQA in the Asia Pacific region [14]. Therefore, in the EEIQAP 2020 panel, specimens with a higher concentration of virus were included. However, this did not increase the percentage of correct virus isolation results. Of the 10 laboratories with incorrect results, one laboratory failed to isolate virus from all virus-containing specimens and four failed to isolate virus from two to five specimens containing A(H1N1)pdm09, A(H3N2), B/Victoria or B/Yamagata virus. Nevertheless, the same three laboratories failed to isolate virus from the three concentrations of A(H3N2) virus-containing specimens and an additional three failed to isolate from the lowest concentration A(H3N2) specimen only. Therefore, there seems to be – to some extent – a concentration effect for A(H3N2) virus. Two concentrations of B/Victoria were included and the concentration of B/Yamagata was lower than the lowest concentration for B/Victoria, but without a correlation of concentration with virus isolation success. The highest concentration specimen with B/Victoria B/Colorado/06/2017-like virus with deletion 162-163 in HA1 was not isolated by four laboratories, the lower concentration B/Victoria specimen was not isolated by two laboratories and the even lower concentration B/Yamagata specimen was not isolated by three laboratories. Analysing the wide variety of MDCK cell types suitable for influenza virus isolation that were used [29], the number of freeze/thaw cycles used before the specimens were subjected to virus isolation and the volume of the specimen inoculated did not provide any indication why laboratories failed to isolate virus from one or more of the virus-containing specimens. MDCK-SIAT has been developed specifically to support cell-based assays for measuring NA inhibitor susceptibility of human influenza viruses by increased expression of the human variant of the influenza virus receptor containing sialic acid alpha(2,6) linked to galactose [30]. These MDCK-SIAT cells were shown to support the growth of A(H1N1), A(H3N2) and type B influenza viruses better than native MDCK cells [31]. Similarly, a new variant of MDCK cell line has been developed, the humanised MDCK-hCK cell line, which also overexpresses the human receptor for influenza virus and is meant for efficient isolation and propagation of human influenza viruses [32]. Several laboratories that isolated all viruses from the panel used MDCK-SIAT only and one laboratory used only the MDCK-hCK cell line. However, a number of laboratories that used only MDCK-SIAT failed to isolate one or more of the panel viruses, whereas laboratories that

used MDCK-SIAT in combination with other MDCK cell types (or only other MDCK cell types) isolated all panel viruses. Therefore, using MDCK-SIAT is not the only requirement for successful virus isolation. Responses to additional questions regarding the number of extra freeze/thaw cycles before the specimen was inoculated and the volume inoculated (alone or in combination with each other, as well as with the cell lines used) also did not reveal any logical defining factors in the success of virus isolation. Therefore, it seems that the success of virus isolation is laboratory specific. As virus isolation is required for antigenic characterisation and phenotypic antiviral susceptibility testing, failure to isolate virus had an immediate effect on the number of EEIQAP 2020 specimens that could be included in these analyses. In 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 [33, 34]. This is also reflected in the struggle of NICs to generate accurate antigenic characterisation data, as shown in previous EISN EQAs [23], the EEIQAP 2018 [16] and the current EEIQAP 2020. Most recent viruses A(H1N1)pdm09 clade 6B.1A5A, A(H3N2) clade 3C.3a and B/Victoria clade 1A(del 162-163), and the rather antigenically conserved B/Yamagata clade 3 panel virus (also included in EEIQAP 2018), were antigenically characterised correctly by all or most laboratories. More difficult to characterise antigenically were the less current A(H3N2) clade 3C.2a1 and low reactor B/Victoria clade 1A viruses that were also included in EEIQAP 2018. Compared to the results of the EEIQAP 2018 study, the results of the EEIQAP 2020 were even more variable, possibly reflecting the reference sera and viruses used. The A(H3N2) clade 3C.2a1b + 131K panel virus was also more difficult to characterise antigenically, resulting in reporting in largely two categories that are close to each other. Therefore, to get better insight into the background for allocating the viruses to antigenic categories, the EEIQAP 2020 included questions on strain specifications of the reference sera and reference viruses used to characterise each individual panel virus. The variability in source, number and specificity of reference sera/viruses (reference viruses isolated from 2008 to 2019, with corresponding sera) used was high. Depending on A-subtype or B-lineage, two to seven (13-50%) laboratories used just one reference serum/virus and three to four (19-29%) additional laboratories used just two reference sera/viruses. Three reference sera/viruses were used by only one to seven (7-44%) laboratories, a minimum requirement to be able to triangulate when using the antigenic cartography approach [35], as shown in Figures A1 and A2. Furthermore, the limited selection of reference sera/viruses used by most laboratories was often biased towards most recent circulating antigenic categories, making it more difficult to allocate the older panel viruses to the correct antigenic category. This might also explain why the more recent panel viruses were allocated more adequately to the appropriate antigenic categories than the older panel viruses. This extra inventory and analysis in EEIQAP 2020 underpins the conclusion of the EEIQAP 2018 report [16] with relevant data on the reference sera/viruses used and again suggests that antigenic characterisation data reported to TESSy should be interpreted with some caution.

Genetic characterisation of the haemagglutinin segment of the panel viruses after sequencing was more straightforward for the 14/21 (67%) laboratories that reported correct results for all panel specimens. This was, however, less than the 73% in the EEIQAP 2018 and 75% in the EQA 2015 [16, 23]. The main errors made with A(H3N2) viruses in EEIQAP 2020 was allocating them to a more recent (EEIQAP_INF20-1; seven labs with error) or older (EEIQAP_INF20-4; two labs with error) category of clade 3C.2a1 viruses. This was probably a result of not fully taking into account the phylogenetic position and amino acid substitution characteristics for clade 3C.2a1 viruses represented by A/Singapore/INFIMH-16-0019/2016, namely N121K and N171K, and clade 3C.2a1b +131K viruses represented by Australia/34/2019 subgroup, namely E62G, R142G and H311Q in HA1, with additional amino acid substitutions HA1 T131K and HA2 V200I [20]. A particular error made with B/Victoria clade 1A virus in EEIQAP_INF20-4 was allocating it to the subclades of clade 1A, which harbour the deletion in HA at positions 162-163 or 162-164, although the virus included in the panel did not have these amino acid deletions. Similar observations were made in the EEIQAP 2018 programme and, therefore, for the EEIQAP 2020 programme participants were asked to upload obtained sequences for evaluation. The vast majority of uploaded sequences matched the sequences of the panel viruses generated at RIVM, indicating that correct phylogenetic and amino acid substitution analysis should have been possible. However, two laboratories with allocation error for EISN_INF20-1 uploaded for this specimen the same or highly similar sequence as for EISN_INF20-4, suggesting an error somewhere in the workflow from sequencing to reporting results. One of these laboratories allocated EISN_INF20-5 B/Victoria clade 1A to B/Victoria clade 1A (del 162-164) and, indeed, this laboratory uploaded a sequence with this deletion whereas all sequences for this virus uploaded by other laboratories did not contain this deletion. Most laboratories that reported sequences uploaded (near) full-length HA sequences. Only three laboratories uploaded the HA1 sequence that is optimal for clade designation for all viruses and only one laboratory did so for just one virus. However, one laboratory uploaded the HA2 sequence for the B viruses, which is not the most optimal for clade designation. Allocation errors in data reported to TESSy might 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 with impact on vaccine strain match.

Reporting of NA amino acid substitution for antiviral susceptibility profiling as required for TESSy reporting [21] were often not reported accurately in the EEIQAP 2018 [16]. Therefore, for EEIQAP 2020 participants were asked to report the identified amino acid change associated with reduced antiviral susceptibility and to upload the generated NA sequence for evaluation. For the EISN_INF20 specimens containing wild type viruses, the overall results were very good, with only one laboratory reporting for the B/Yamagata containing specimen AARI linked with NA-K360R, although this amino acid change is wild type. Two other laboratories reported for A(H3N2) EEIQAP_INF20-4 amino acid substitutions NA-N329S or NA-D161N, although these are also wildtype changes. Nevertheless, these laboratories interpreted these specimens correctly as AANI. Except for one sequence, all NA sequences uploaded for EIS_INF were identical to the sequences obtained at RIVM for the panel specimens, indicating the starting point for antiviral susceptibility profiling was good. One laboratory uploaded for EISN_INF20-1 the same NA sequence as for EISN-INF20-4, similar to what this laboratory did for the HA sequence of EISN_INF20-1, confirming an error somewhere in the workflow of this laboratory for this specimen. The EISN_AV20 specimens containing viruses with amino acid changes previously associated with reduced susceptibility appeared to be more challenging, as two of them (D199E in N1pdm09 and 245-248 deletion in N2) have been sporadically detected before [36,37]. Such sporadically detected changes included in previous programmes led to very good results: 92% of laboratories correctly identified and interpreted B/Yamagata NA-E105K in the EEIQAP 2018 [16,38] and there was a high accuracy for detection of B/Victoria NA-I221L in the 2015 ERLI-Net EQA [39,40]. All sequences uploaded for A(H1N1)pdm09 EEIQAP_AV20-1 contained the N1-D199E amino acid substitution, confirming the basis for antiviral susceptibility profiling of this specimen was very good. Although N1-D199E was identified by many laboratories, not all of them interpreted this as AARI by oseltamivir, indicating difficulty in finding leads for appropriate interpretation. Furthermore, a relatively high number did not identify N1-D199E as an amino acid substitution previously associated with reduced antiviral susceptibility, indicating difficulty in finding leads for identifying such amino acid changes. The second specimen, EISN_AV20-2 with N2-E119V and 245-248 amino acids deletion, showed clearly that it was easier to identify and interpret correctly the well-known E119V substitution than the sporadically detected 245-248 amino acid deletions. Except for two uploaded sequences for this specimen, all contained the E119V amino acid substitution and the 245-248 amino acid substitutions, again indicating the basis for antiviral susceptibility profiling was very good. For one laboratory, the sequence was too short to identify E119V but contained 245-248del; however, this laboratory did not identify both. The sequence of the other laboratory did contain E119V but not 245-248del, indicating an error somewhere in the sequencing workflow. Nevertheless, except for one laboratory, all interpreted the presence of detected E119V or E119V plus 245-248del correctly as AAHRI (or AARI by one laboratory) by oseltamivir. This was likely because E119V and 245-248del are listed separately with HRI profile for oseltamivir in the WHO guidance table [22]. Even the laboratories that identified a four amino acid deletion (but shifted compared to 245-248del) interpreted their finding correctly as AAHRI by oseltamivir. The interpretation for zanamivir was obviously more challenging, likely because the WHO table does not have the double mutant E119V plus 234-248del included and the separate profiles are listed as NI and NI/RI, respectively. Following from phenotypic HRI-by-zanamivir data obtained by laboratories also reporting genetic profiling data, more laboratories could have drawn the conclusion that both mutations work synergistically and the genetic profile for zanamivir should have been AAHRI. This observation highlights the importance of phenotypic confirmation of new mutations or a new constellation of multiple mutations. It also highlights that it is important in EQA to ask for a final conclusion based on the combined results of genetic and phenotypic antiviral profiling data.

Phenotypic testing for antiviral susceptibility and interpretation of IC_{50} values were highly accurate for all specimens that contained wildtype virus. Only three laboratories reported RI or HRI instead of the expected NI for the same two viruses. For the two reduced susceptible viruses, the results were less accurate with respect to determined IC_{50} and interpretation. The A(H1N1)pdm09 N1-D199E virus was accurately profiled NI for zanamivir, but for oseltamivir a number of laboratories reported NI instead of the expected RI. The A(H3N2) N2-E199V + del245-248 double mutant was accurately profiled HRI for oseltamivir, but for zanamivir profiling was more variable with HRI (as expected) and RI reported by most laboratories. Similar to observations in the 2015 ERLI-Net EQA [39] and the EEIQAP 2018 [16], the majority of deviant results were obtained using commercial kit-based fluorescent or chemiluminescent assays; of the 21 deviant results, 17 (81%) were reported by the six labs that used kits and only four (19%) by four of the 12 labs using MUNANA in-house assays. Although in-house assays are possibly a bit more difficult to perform, they provide more consistent results between laboratories and therefore could be used to harmonise phenotypic assessment of NA inhibitor susceptibility in the region. An additional argument to use the in-house MUNANA is that this assay is more sensitive in detecting mutants with RI profile in the low fold-change range, like the A(H1N1)pdm09 N1-D199E variant, than the kit-based assays. Furthermore, the in-house MUNANA is the standard used by WHO CCs for global analysis of NA inhibitor susceptibility of influenza viruses.

A relatively high number of laboratories in the network have obtained ISO 15189, ISO 17025 or ISO 9001 accreditation for medical laboratories, for testing or calibrating laboratories, or for quality management, respectively. A few reported working towards obtaining ISO 15189 or ISO 17025 accreditation. This is a positive development, as the proportion has increased compared to that in the EEIQAP 2018. Still, nine laboratories indicated that they were not accredited and were not in the process of obtaining accreditation. Accreditation is important to help systematically address issues like 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 very satisfactory. Those laboratories determining the N-subtype of type A influenza viruses and lineage of type B viruses demonstrated excellent performance. Surveillance data derived 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, the EEIQAP 2020 has clearly identified an issue in the performance of the network in virus isolation. This is possibly linked to lack of sensitivity of the procedures used and not to 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 in an even more international context aimed to inform the WHO vaccine composition recommendation meetings.

Although there is relatively good concordance among laboratories in the reported antigenic characterisation categories for the EEIQAP 2020 panel specimens, the results also indicate that subtle antigenic differences between viruses are not picked up accurately. This is likely caused partly by the use of a limited and highly diverse set of reference sera and viruses. 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, some laboratories had difficulties with allocating some viruses to the correct predefined categories. Therefore, genetic categories reported to TESSy should also be analysed and interpreted with some caution.

Genetic and phenotypic testing of antiviral susceptibility by the network is of relatively good accuracy when looking at the sequence and IC_{50} data obtained. However, interpretation of amino acid change data was inaccurate for a relatively high number of laboratories. Furthermore, the use of commercial kit-based assays often generated deviant results in phenotypic testing. 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 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 and 2020 results. However, in the EEIQAP 2018 study, many laboratories indicated that for some of the methods that were subsequently included in the EEIQAP 2020, an EQA was not required for their ISO 15189 accreditation. Therefore, including all tests in the scope of the accreditation might help to further improve the more complex types of analysis.

6. Recommendations

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

- A review of standard operating procedures (SOPs) for reporting of test data should resolve notified issues in reporting results with the correct specimen.
- A review of SOPs for specimen reception and sample handling should resolve notified issues in reporting positive results for the specimen that did not contain virus due to, for example, switching between specimens or contamination. Repeating specimens that contain virus but were reported negative in molecular detection and a review of the primers and probe used should resolve this issue, if not caused by lack of sensitivity of the molecular assays used.
- 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 SOPs 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.
- 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 2020, it appeared difficult to allocate the A(H3N2) viruses to one category, highly likely 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. A minimum set that should be used could be defined, 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 likely impossible due to production limitations and limited comparability of multiple batches of ferret sera raised against the same strain. Appropriate use of the seasonal ECDC guidelines on influenza virus characterisation (e.g. [19, 20], used for the development of EEIQAP 2020) might give further direction.

Genetic characterisation

- Errors made in allocating some viruses to the correct TESSy categories were possibly due to incorrect interpretation of obtained results after phylogenetic and amino acid substitution analysis, especially because the vast majority of laboratories uploaded correct sequences. Therefore, training in these types of analysis and appropriate use of the seasonal ECDC guidelines on influenza virus characterisation (e.g. [19,20], used for development of the EEIQAP 2020) and of online tools like Nextclade [41] should be organised.
- Review of the original sequencing results against the reported category should resolve notified issues in uploading incorrect sequences and reported connected interpretation that could be a result of switching specimens or errors in reporting. In the SOP for specimen processing and reporting, correlation of results across all analyses done on a specimen and validation of reported results by a second person should avoid this type of error.
- Reporting of correct data to TESSy is first 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 to perform additional sequence analysis to validate suspicious entries, e.g. when preparing Flu News Europe.

Genetic antiviral susceptibility testing

- Errors have been made in the identification of amino acid changes despite the uploading of sequences that contained these changes or in the interpretation of amino acid changes associated with reduced susceptibility for NA inhibitors. Review of SOPs used for antiviral susceptibility profiling for appropriate inclusion of the use of ECDC guidelines on interpretation and reporting of antiviral susceptibility data [21] and of FluSurver [42], together with the use of the lists provided by the WHO working group on surveillance of influenza antiviral susceptibility [22], should solve these issues.
- Similar to genetic strain characterisation, reporting correct data is first 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 to perform additional sequence analysis for validation of suspicious results.
- Laboratories and TESSy would benefit from training on interpretation and reporting of genetic antiviral susceptibility data. Recordings of previous webinars and instruction documents are available on the EISN extranet (https://extranet.ecdc.europa.eu/EISN/Pages/default.aspx).

Phenotypic antiviral susceptibility testing

Deviant phenotypic testing results were mainly reported from the use of commercial kit-based NA activity inhibition assays. Laboratories using such assays should consider switching to an in-house MUNANA assay for routine use. Laboratories and TESSy would benefit from training on the use of the in-house MUNANA assay. Recordings of previous webinars and instruction documents are available on the EISN extranet.

Accreditation

ISO 15189 and ISO 17025 recommend EQA for self-evaluation and addressing test issues in a systematic way. Laboratories could probably make better use of the methodology to address issues, as provided by interpretations of the ISO 15189 and ISO 17025 accreditation. 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 should apply for one of the above standards.

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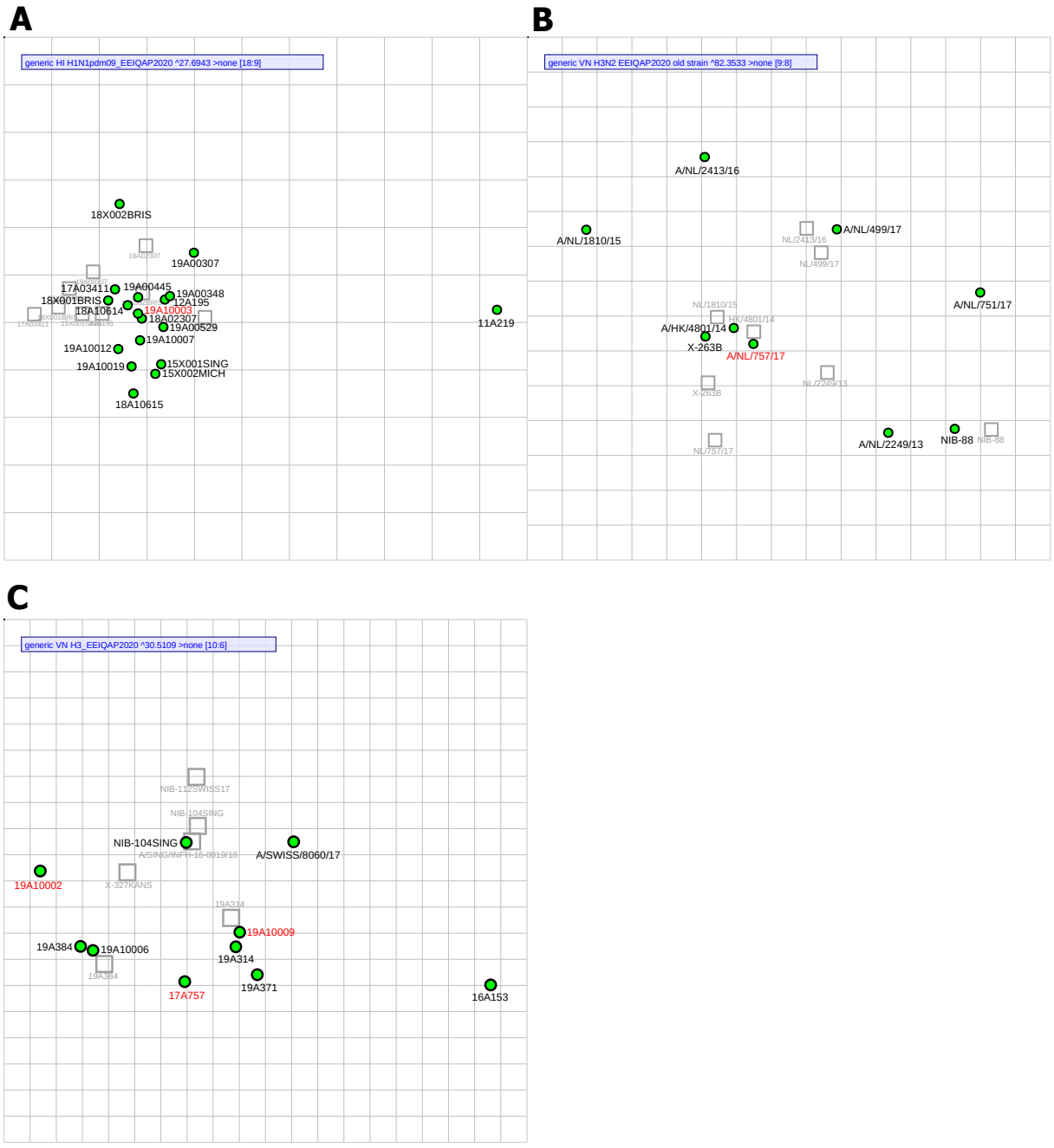
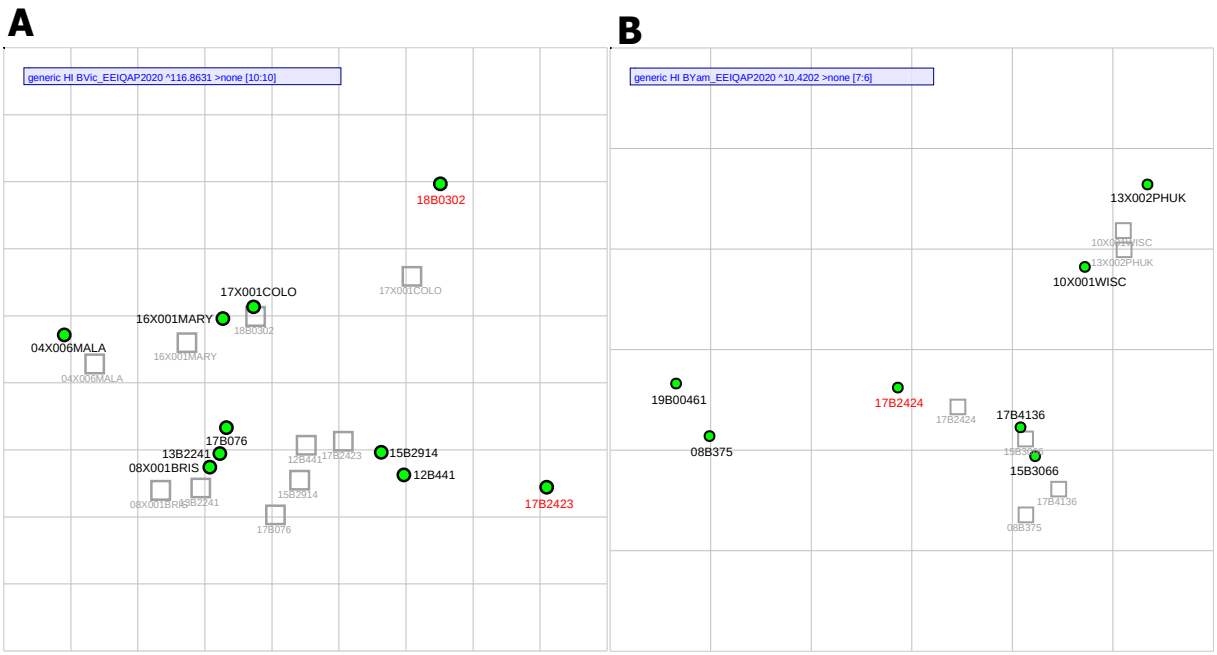
Annexes

Figure A1. Antigenic cartography maps created based on HI-assay data for A(H1N1)pdm09 (A) and on virus neutralisation data for A(H3N2) influenza virus (B, C), generated at the Dutch National Influenza Centre, Erasmus MC, Rotterdam, the Netherlands, European External Influenza Quality Assessment Programme, WHO European Region, 2020



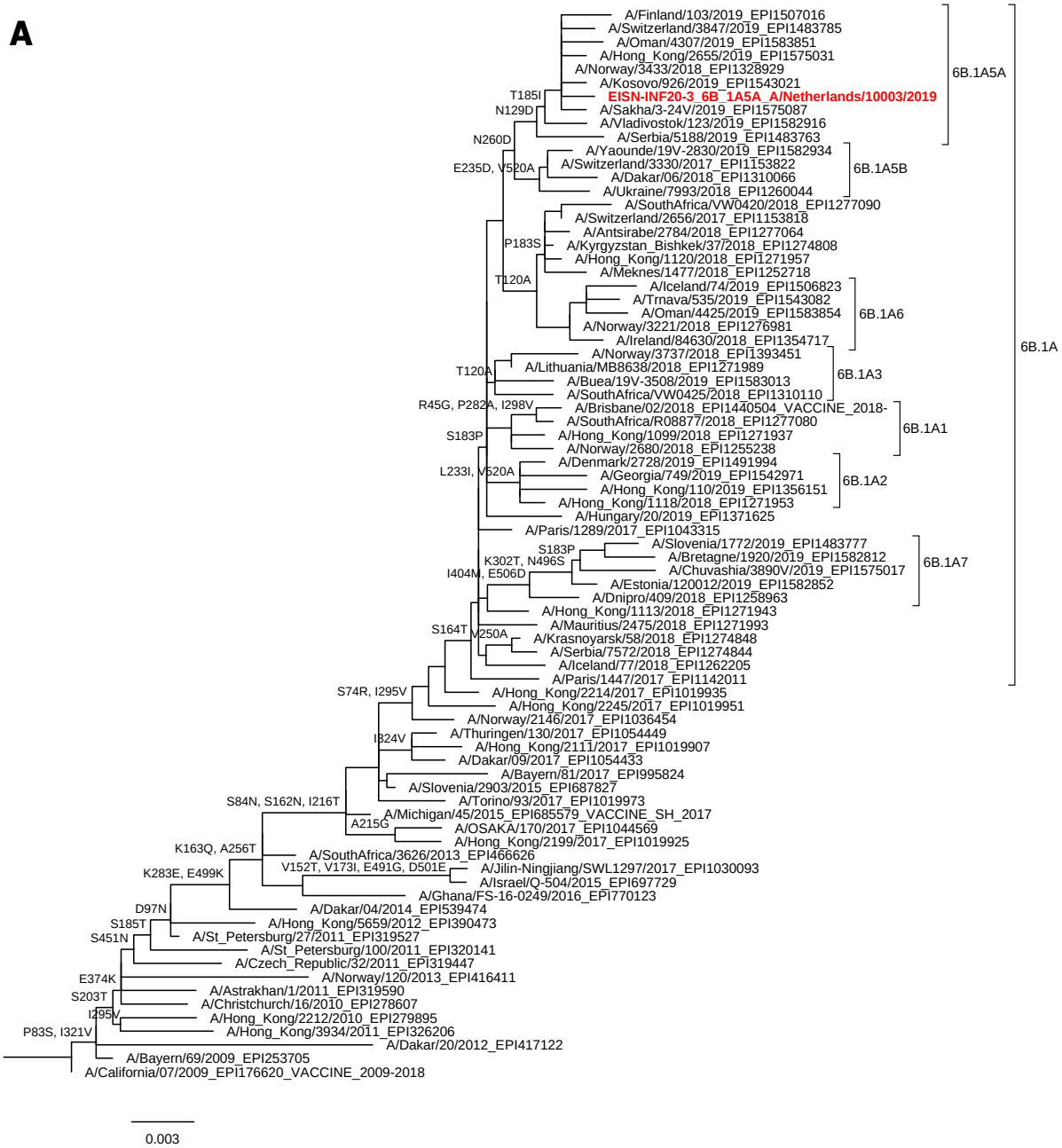
Vaccine viruses are for A(H1N1)pdm09 15X001SING, 15X002MICH and 18X001BRIS. For A(H3N2), the vaccine viruses are A/HK/4801/14 Clade 3C.2a, X-263B (similar to A/HK/4801/14) and NIB-88 (A/Switzerland/9715293/2013) Clade 3C.3a (B) and NIB-104SING Clade 3C.2a1 and A/SWISS/8060/17 Clade 3C.2a2 (C). Selected viruses for the panel are indicated in red. The spacing between grid lines is one unit of antigenic distance, corresponding to a twofold dilution of antiserum in the HI- or VN-assay.

Figure A2. Antigenic cartography maps created based on HI-assay data for B/Victoria (A) and B/Yamagata influenza viruses (B), generated at the Dutch National Influenza Centre, Erasmus MC, Rotterdam, the Netherlands, European External Influenza Quality Assessment Programme, WHO European Region, 2020



Vaccine viruses are for B/Victoria 04X006MALA, 08X001BRIS, 16X001MARY and 17X001COLO. The latter two are HA 2aa-deletion variants. For B/Yamagata, the vaccine viruses are 10X001WISC and 13X002PHUK. Selected viruses for the panel are indicated in red. The spacing between grid lines is one unit of antigenic distance, corresponding to a twofold dilution of antiserum in the HI-assay.

Figure A3.A Phylogenetic tree of full HA of A(H1N1)pdm09 influenza viruses with common amino acid changes for viruses after the indicated branch, European External Influenza Quality Assessment Programme, WHO European Region, 2020

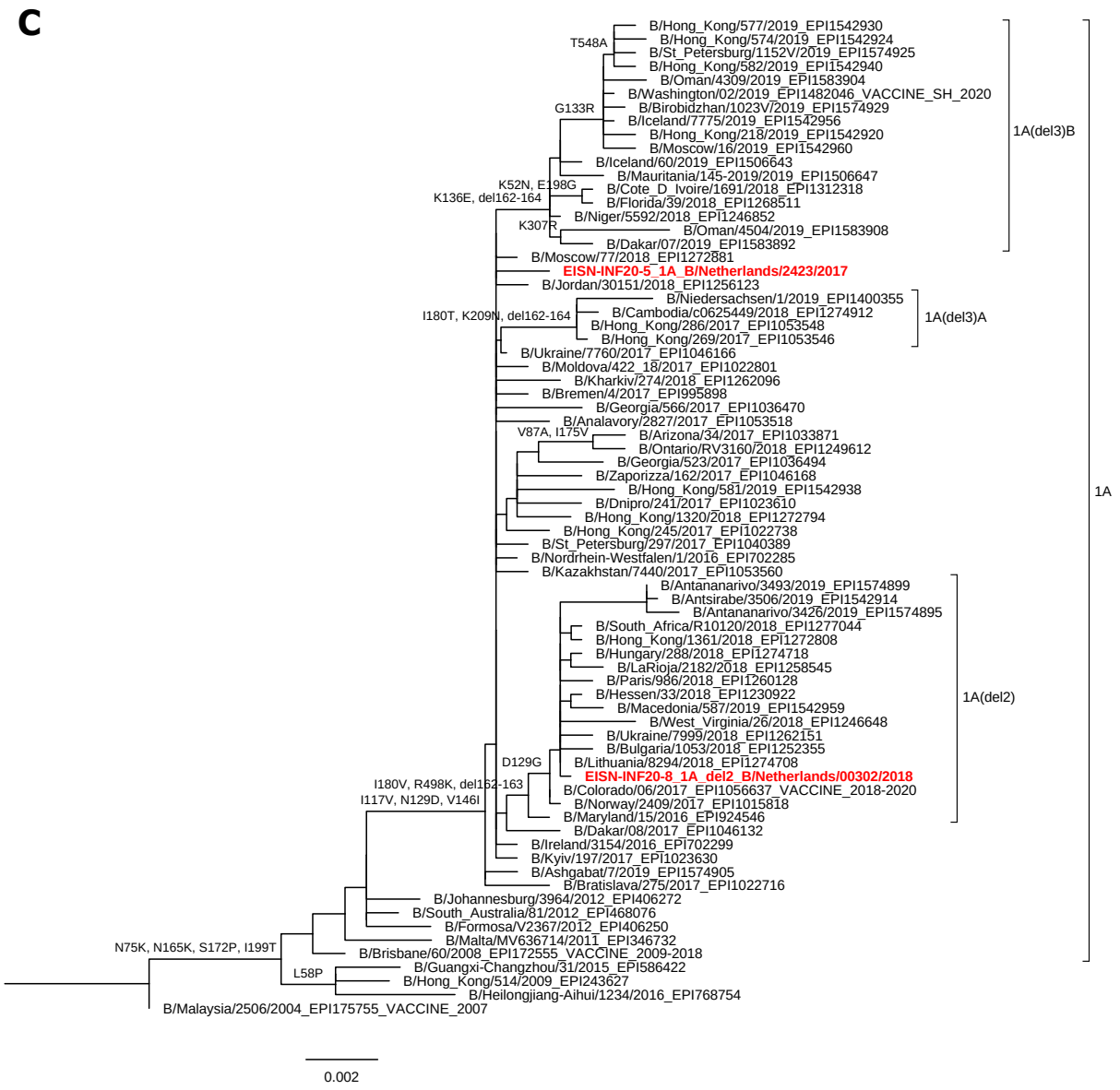


The reference virus set recommended by ECDC for analysis of viruses to be reported to TESSy, supplemented with relevant viruses from the Netherlands, have been used to infer the phylogenetic tree. Vaccine viruses are indicated with the text VACCINE. Viruses that are included in the panel are indicated in red.

Figure A3.B Phylogenetic tree of full HA of A(H3N2) influenza viruses with common amino acid changes for viruses after the indicated branch, European External Influenza Quality Assessment Programme, WHO European Region, 2020

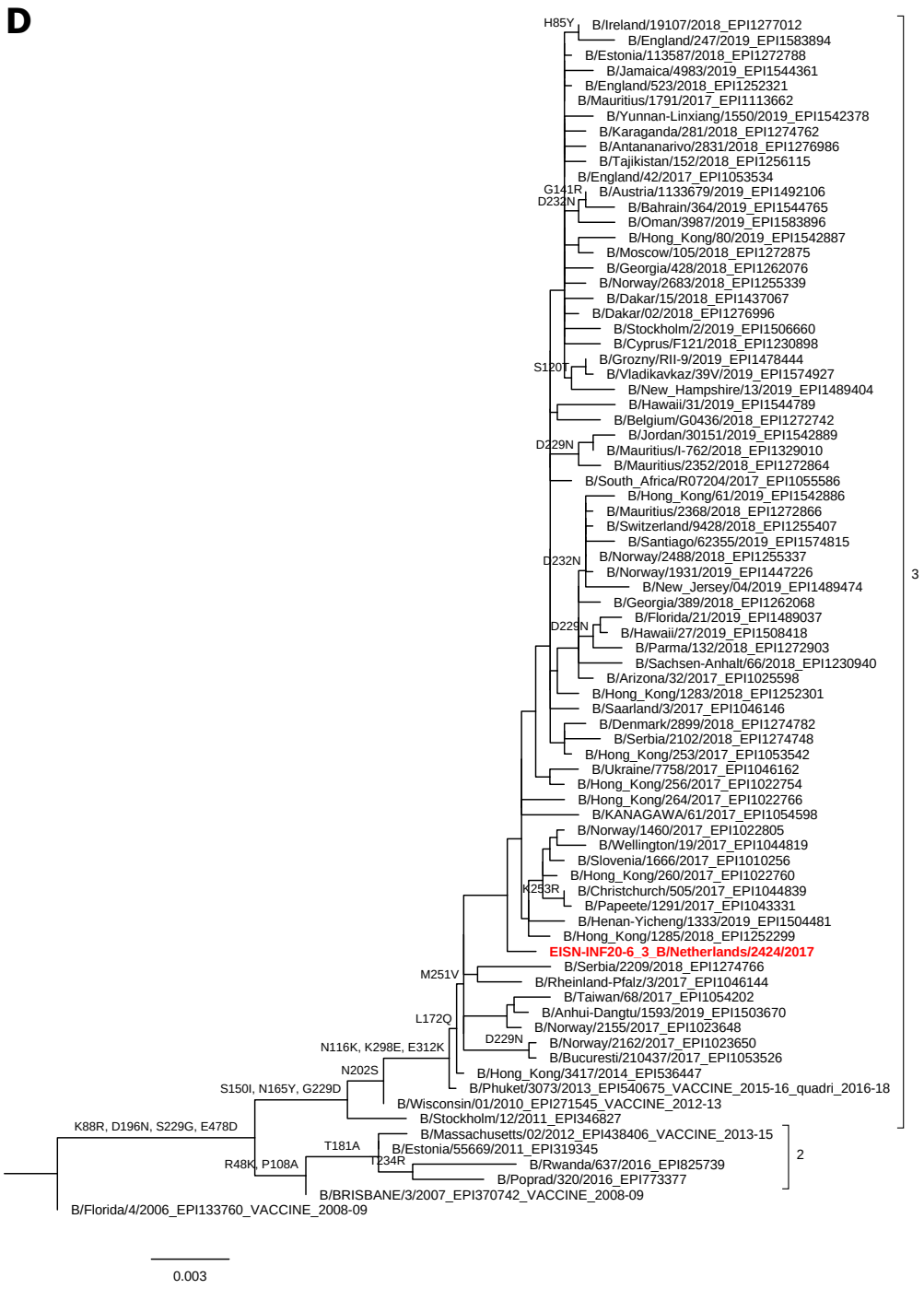
The reference virus set recommended by ECDC for analysis of viruses to be reported to TESSy, supplemented with relevant viruses from the Netherlands, have been used to infer the phylogenetic tree. Vaccine viruses are indicated with the text VACCINE. Viruses that are included in the panel are indicated in red.

Figure A3.C Phylogenetic tree of full HA of B/Victoria influenza viruses with common amino acid changes for viruses after the indicated branch, European External Influenza Quality Assessment Programme, WHO European Region, 2020



The reference virus set recommended by ECDC for analysis of viruses to be reported to TESSy, supplemented with relevant viruses from the Netherlands, have been used to infer the phylogenetic tree. Vaccine viruses are indicated with the text VACCINE. Viruses that are included in the panel are indicated in red.

Figure A3.D Phylogenetic tree of full HA of B/Yamagata influenza viruses with common amino acid changes for viruses after the indicated branch, European External Influenza Quality Assessment Programme, WHO European Region, 2020



The reference virus set recommended by ECDC for analysis of viruses to be reported to TESSy, supplemented with relevant viruses from the Netherlands, have been used to infer the phylogenetic tree. Vaccine viruses are indicated with the text VACCINE. Viruses that are included in the panel are indicated in red.

Table A1. List of participants, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Country	City	Organisation
AUSTRIA	Vienna	Center for Virology, Medical University Vienna
BELGIUM	Brussels	National influenza Centre, Sciensano
BOSNIA and HERZEGOVINA	Sarajevo	Clinical Center University of Sarajevo, OU Clinical microbiology
BOSNIA and HERZEGOVINA, Republic of Srpska	Banjaluka	Public Health Institute of Republic of Srpska
BULGARIA	Sofia	National Centre of Infectious and Parasitic Diseases, Department of Virology
CZECHIA	Prague	National Institute of Public Health, Centre for Epidemiology and Microbiology, Reference Laboratory for Influenza
DENMARK	Copenhagen	Statens Serum Institute
ESTONIA	Tallinn	Health Board Laboratory of Communicable Diseases
FINLAND	Helsinki	Finnish Institute for Health and Welfare (THL), Expert Microbiology Unit/Virology
FRANCE	Lyon	National Reference Centre for Respiratory Virus Infections, IAI – Laboratory for Virology, Centre for Biology North
GERMANY	Berlin	Robert Koch-Institute, NRZ Influenza
GREECE	Athens	Hellenic Pasteur Institute, National Influenza Reference Laboratory for Southern Greece
GREECE	Thessaloniki	National Influenza Centre for Northern Greece, Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki
HUNGARY	Hungary	National Public Health Center
ICELAND	Iceland	National University Hospital
IRELAND	Dublin	UCD National Virus Reference Laboratory, University College Dublin
ISRAEL	Ramat Gan	Central Virology Laboratory, Sheba Medical Center
ITALY	Rome	National Influenza Centre, Department of Infectious Diseases, Italian National Institute of Health
KAZAKSTAN	Almaty	Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring, Reference Laboratory for Viral Infections Control
LATVIA	Riga	Riga East University Hospital, Laboratory service, Latvian Centre of Infectious Diseases, National Microbiology Reference Laboratory, Virology division, NIC of LATVIA
LITHUANIA	Vilnius	National Public Health Surveillance Laboratory
LUXEMBOURG	Luxembourg	National Health Laboratory, Virology and Serology Service
MACEDONIA	Skopje	Institute of Public Health
MOLDOVA	Chisinau	National Center for Public Health, Laboratory of Viral Infections
MONTENEGRO	Podgorica	Institute of Public Health of Montenegro
NETHERLANDS	Bilthoven	Department Emerging and Endemic Viruses, Division Virology, Centre for Infectious Disease Research, Diagnostics and laboratory Surveillance, National Institute for Public Health and the Environment (RIVM)
NETHERLANDS	Rotterdam	Erasmus Medical Centre, ViroScience Department of Virology
NORWAY	Oslo	Section of Influenza, Dept of Virology, Norwegian Institute of Public Health
POLAND	Warsaw	National Institute of Public Health, National Institute of Hygiene
PORTUGAL	Lisbon	National Institute of Health Doctor Ricardo Jorge, National Reference Laboratory for Influenza Virus
ROMANIA	Bucharest	Laboratory for Respiratory Viral Infections, "Cantacuzino" National Military-Medical Institute for Research and Development
RUSSIA	Moscow	FSBI "N.F. Gamaleya NRCEM" Ministry of Health of the Russian Federation
RUSSIA	Novosibirsk	Federal Budgetary Research Institution - State Research Center of Virology and Biotechnology VECTOR, Rospotrebnadzor (FBRI SRC VB VECTOR, Rospotrebnadzor)
RUSSIA	Saint Petersburg	Smorodintsev Research Institute of Influenza
SERBIA	Belgrade	Institute of virology, vaccine and sera, 'Torlak'
SLOVAKIA	Bratislava	National Influenza Center, Public Health Authority of the Slovak Republic

Country	City	Organisation
SLOVENIA	Ljubljana	Laboratory for Public Health Virology, National laboratory for Health, Environment and Food
SPAIN	Barcelona	Laboratory of Microbiology, Hospital Clinic
SPAIN	Valladolid	National Influenza Center of Valladolid, Microbiology Service, University Clinical Hospital of Valladolid
SWEDEN	Solna	The Swedish Public Health Agency
SWITZERLAND	Geneva	National Reference Centre of Influenza, Laboratory of Virology, Geneva University Hospitals
UNITED KINGDOM, ENGLAND	London	Public Health England, Respiratory Virus Unit, Virus Reference Department, National Infection Service, Colindale
UNITED KINGDOM, NORTHERN IRELAND	Belfast	Regional Virus Laboratory, Microbiology Dept, Kelvin Building, Royal Victoria Hospital, Belfast Trust
UNITED KINGDOM, SCOTLAND	Glasgow	West of Scotland Specialist Virology Centre
UNITED KINGDOM, WALES	Cardiff	Wales Specialist Virology Centre, Public Health Wales Microbiology Cardiff, University Hospital of Wales

Table A2. Overview of challenge types that each laboratory participated in, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Participant ID[1]	Challenge type				Full programme
	Molecular detection	Virus isolation	Characterisation (antigenic, genetic)	Antiviral susceptibility testing (genetic, phenotypic)	
95	Yes	Yes	Both	Both	Yes
112	Yes	No	None	Genetic only	No
117	Yes	No	None	None	No
200	Yes	Yes	Both	Both	Yes
1159	Yes	Yes	Genetic only	Both	Yes
2125	Yes	Yes	Both	Both	Yes
2126	Yes	Yes	Both	Both	Yes
2253	Yes	Yes	Antigenic only	None	No
2258	Yes	No	None	None	No
2271	Yes	Yes	Both	Both	Yes
2272	Yes	No	None	None	No
2275	Yes	No	None	None	No
2276	Yes	Yes	Both	Both	Yes
2277	Yes	Yes	Antigenic only	None	No
2278	Yes	Yes	Both	None	No
2820	Yes	Yes	Antigenic only	Genetic only	Yes
3442	Yes	No	None	Both	No
4209	Yes	Yes	None	None	No
10007	Yes	Yes	Both	Genetic only	Yes
10014	Yes	No	None	None	No
10023	Yes	Yes	Genetic only	Both	Yes
10040	Yes	No	None	None	No
10078	Yes	Yes	None	None	No
10080	Yes	Yes	Genetic only	Both	Yes
10104	Yes	Yes	None	Phenotypic only	No
10115	Yes	Yes	Both	Both	Yes
10144	Yes	No	Genetic only	Genetic only	No
10205	Yes	Yes	Genetic only	Genetic only	Yes
10461	Yes	No	None	None	No
10462	Yes	No	None	None	No
10464	Yes	Yes	Both	Both	Yes
10465	Yes	Yes	Genetic only	Both	Yes
10466	Yes	Yes	Both	Both	Yes
1600	Yes	Yes	None	None	No
1991	Yes	No	None	None	No
2814	Yes	No	None	None	No
2817	Yes	Yes	Both	Both	Yes
3558	Yes	Yes	Both	Genetic only	Yes
4344	Yes	No	None	Phenotypic only	No
10053	Yes	Yes	Genetic only	Genetic only	Yes
10142	Yes	No	None	None	No
10248	Yes	Yes	None	None	No
10261	Yes	Yes	Both	Both	Yes
10493	Yes	No	None	None	No
10507	Yes	No	None	None	No

[1] *Cells with orange shading = laboratory located in an EU/EEA country.*

Table A3. Overview of molecular detection and typing and type A H-subtype, type A N-subtype and type B lineage determination results by participant, with performance score and methodology used, European External Influenza Quality Assessment Programme, WHO European Region, 2020

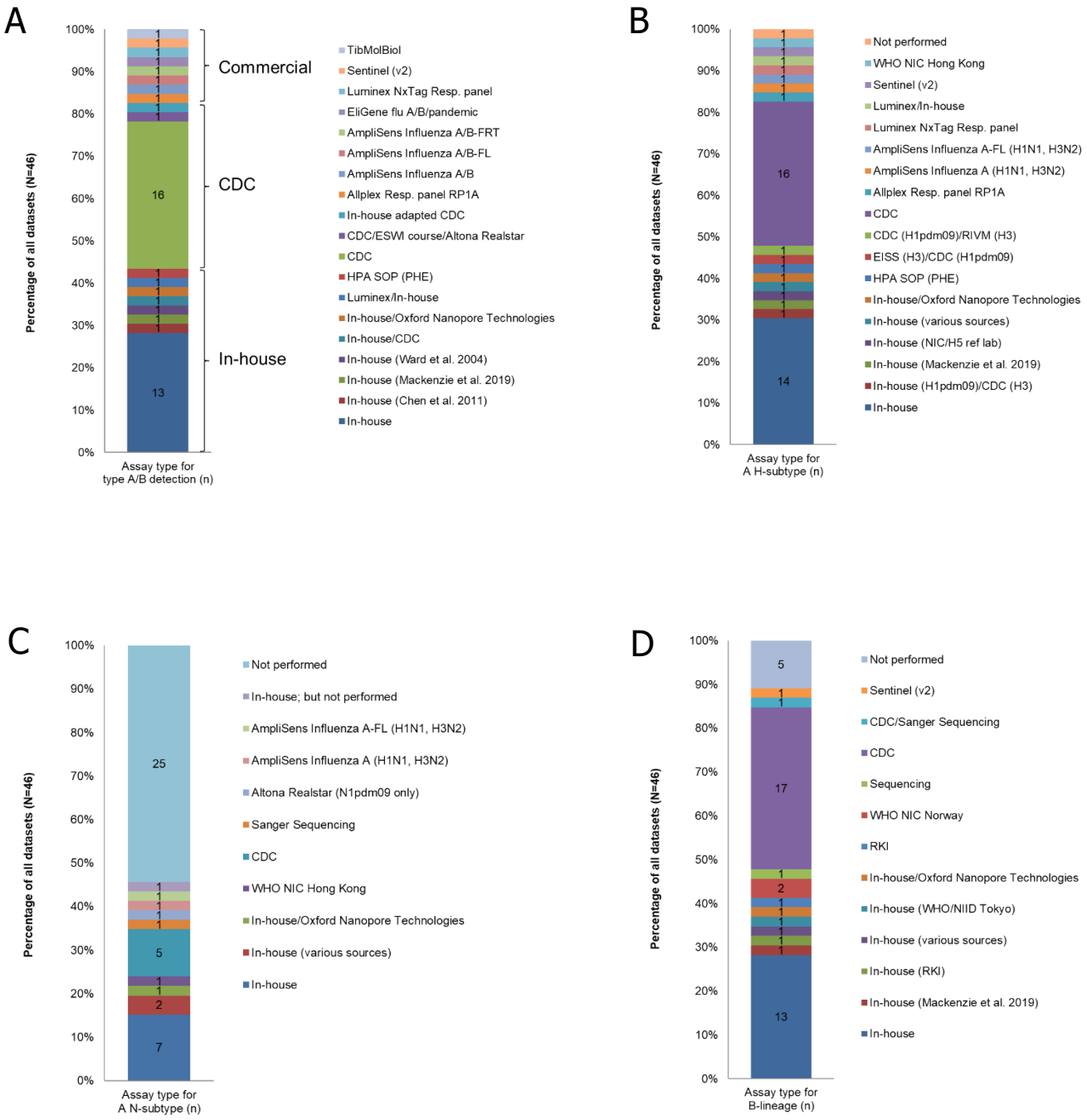
Specimen (EISN_INF20):	1	2	3	4	5	6	7	8	Type and A H-subtype Score[2]	Overall Score[3]	Assay type[4]			
Expected Result:	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic			Type A/B	A H-subtype	A N-subtype	B-lineage
Participant ID[1]														
95	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house/Oxford Nanopore Technologies	In-house/Oxford Nanopore Technologies	In-house/Oxford Nanopore Technologies	In-house/Oxford Nanopore Technologies
112	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	Luminex/ In-house	Luminex/In-house	In-house	Sequencing
117	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B lin. Not Tested	B lin. Not Tested	A(H3) N Not Tested (also positive for B)	B lin. Not Tested	0	7	In-house	In-house	Not performed	Not performed
200	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house	In-house	In-house	In-house
1159	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	TibMolBiol	WHO NIC Hong Kong	WHO NIC Hong Kong	WHO NIC Norway
2125	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house	In-house	In-house	In-house
2126	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	In-house	In-house	Not performed	CDC
2253	A(H3) N Not Tested	Negative	A(H1N1)pdm09	A(H3) N Not tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	3	CDC/ESWI course/Altona Realstar	CDC (H1pdm09)/ RIVM (H3)	Altona Realstar (N1pdm09 only)	CDC
2258[5]	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	A(H3) N Not Tested	B/Vic	14	17	CDC	CDC	Not performed	CDC
2271	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	CDC	CDC	CDC	CDC
2272	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	In-house	In-house	Not performed	In-house
2275	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC
2276	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house (Ward et al. 2004)	In-house (NIC/H5 ref lab)	In-house (various sources)	In-house (WHO/NIID Tokyo)
2277	A(H3) N Not Tested	B lin. Not Tested	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B lin. Not Tested	B lin. Not Tested	A(H3) N Not Tested	Negative	6	12	EliGene flu A/B/pandemic	Not performed	Not performed	not performed
2278	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	In-house (Chen et al. 2011)	In-house (various sources)	Not performed	In-house (RKI)
2820	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC
3442	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	HPA SOP (PHE)	HPA SOP (PHE)	Not performed	In-house (various sources)
4209	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC
10007	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	Sentinel (v2)	Sentinel (v2)	Not performed	Sentinel (v2)
10014[6]	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC
10014[6]	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B lin. Not Tested	B lin. Not Tested	A(H3) N Not Tested	B lin. Not Tested	0	7	Luminex NxTag Resp. panel	Luminex NxTag Resp. panel	Not performed	Not performed
10023	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	In-house	In-house	Not performed	In-house
10040	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B lin. Not Tested	B lin. Not Tested	A(H3) N Not Tested	B lin. Not Tested	0	7	In-house	In-house	Not performed	not performed

Specimen (EISN_INF20):	1	2	3	4	5	6	7	8	Type and A H-subtype Score[2]	Overall Score[3]	Assay type[4]			
Expected Result:	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic			Type A/B	A H-subtype	A N-subtype	B-lineage
Participant ID[1]														
10078	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	In-house
10080	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house (Mackenzie et al. 2019)	In-house (Mackenzie et al. 2019)	In-house (various sources)	In-house (Mackenzie et al. 2019)
10104	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house	In-house	In-house	WHO NIC Norway
10115	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house	In-house	In-house	In-house
10144	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	In-house/CDC	In-house	Not performed	In-house
10205	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B lin. Not Tested	B lin. Not Tested	A(H3N2)	B lin. Not Tested	0	3	In-house	In-house	In-house, but not performed	In-house
10461	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC
10462	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B lin. Not Tested	B lin. Not Tested	A(H3) N Not Tested	B lin. Not Tested	0	7	Allplex Resp. panel RP1A	Allplex Resp. panel RP1A	Not performed	Not performed
10464	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	CDC	CDC	Sanger Sequencing	CDC/Sanger Sequencing
10465	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	EISS (H3)/ CDC (H1pdm09)	Not performed	RKI
10466	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house	In-house	In-house	In-house
1600	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	In-house	In-house	Not performed	In-house
1991	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC
2814	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	CDC	CDC	CDC	CDC
2817	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	AmpliSens Influenza A/B-FL	AmpliSens Influenza A-FL (H1N1, H3N2)	AmpliSens Influenza A-FL (H1N1, H3N2)	In-house
3558	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	In-house adapted CDC	In-house (H1pdm09)/ CDC (H3)	In-house	In-house
4344	A(H3N2)	Negative	A(H1)pdm09 N Not Tested	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	1	AmpliSens Influenza A/B-FRT	CDC	CDC	CDC
10053	A(H3N2)	Negative	A(H1N1)pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	0	CDC	CDC	CDC	CDC
10142	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC
10248	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	CDC	CDC
10261	A(H3N2)	Negative	A(H1)pdm09 N Not Tested	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic	0	1	AmpliSens Influenza A/B	AmpliSens Influenza A (H1N1, H3N2)	AmpliSens Influenza A (H1N1, H3N2)	CDC
10493	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	In-house	In-house	Not performed	In-house
10507	A(H3) N Not Tested	Negative	A(H1)pdm09 N Not Tested	A(H3) N Not Tested	B/Vic	B/Yam	A(H3) N Not Tested	B/Vic	0	4	CDC	CDC	Not performed	CDC

[1] Cells with orange shading = laboratory located in an EU/EEA country.
[2] Scoring for detection with type and type A H-subtyping only:
A viruses: correct type and H-subtype (green or yellow shading), 0; correct type only (shading not applicable), 1; all other results (red shading), 3;
B viruses: correct type (green or yellow shading), 0; all other results (red shading), 3;

Negative specimen: Negative (green shading), 0; all other results (red shading), 3.
[3] Scoring for detection with type, type A H- and N-subtyping and type B lineage determination:
A viruses: correct type and H- and N-subtype (green shading), 0; correct type and H-subtype without N-subtype (yellow shading), 1; correct type only, 2; all other results (red shading), 3;
B viruses: correct type and lineage (green shading), 0; correct type without lineage (yellow shading), 1; all other results (red shading), 3;
Negative specimen: Negative (green shading), 0; all other results (red shading), 3.
[4] In-house = developed in own laboratory or implemented or modified from primers and probes published or personally obtained from elsewhere or if methodology not further specified; CDC = CDC, Atlanta, United States.
[5] The laboratory returned the correct number of virus detections of specific type and subtype/lineage, but likely reported the results in an incorrect order. Results have been analysed as they have been reported.
[6] The laboratory reported two different data sets and both have been included separately in the analysis.

Figure A4. Molecular methodologies reported by 45 laboratories for 46 datasets in the detection of influenza virus types A and B (A), type A H-subtyping (B), type A N-subtyping (C) and type B lineage determination (D), European External Influenza Quality Assessment Programme, WHO European Region, 2020



In-house = developed in own laboratory or implemented or modified from primers and probes published or personally obtained from elsewhere or if methodology not further specified; numbers in the bars indicate the number of datasets. For N-subtyping (C), for a number of reported tests only results for N1pdm09 or N2 subtyping were reported or no N-subtyping results at all (details in Table A3).

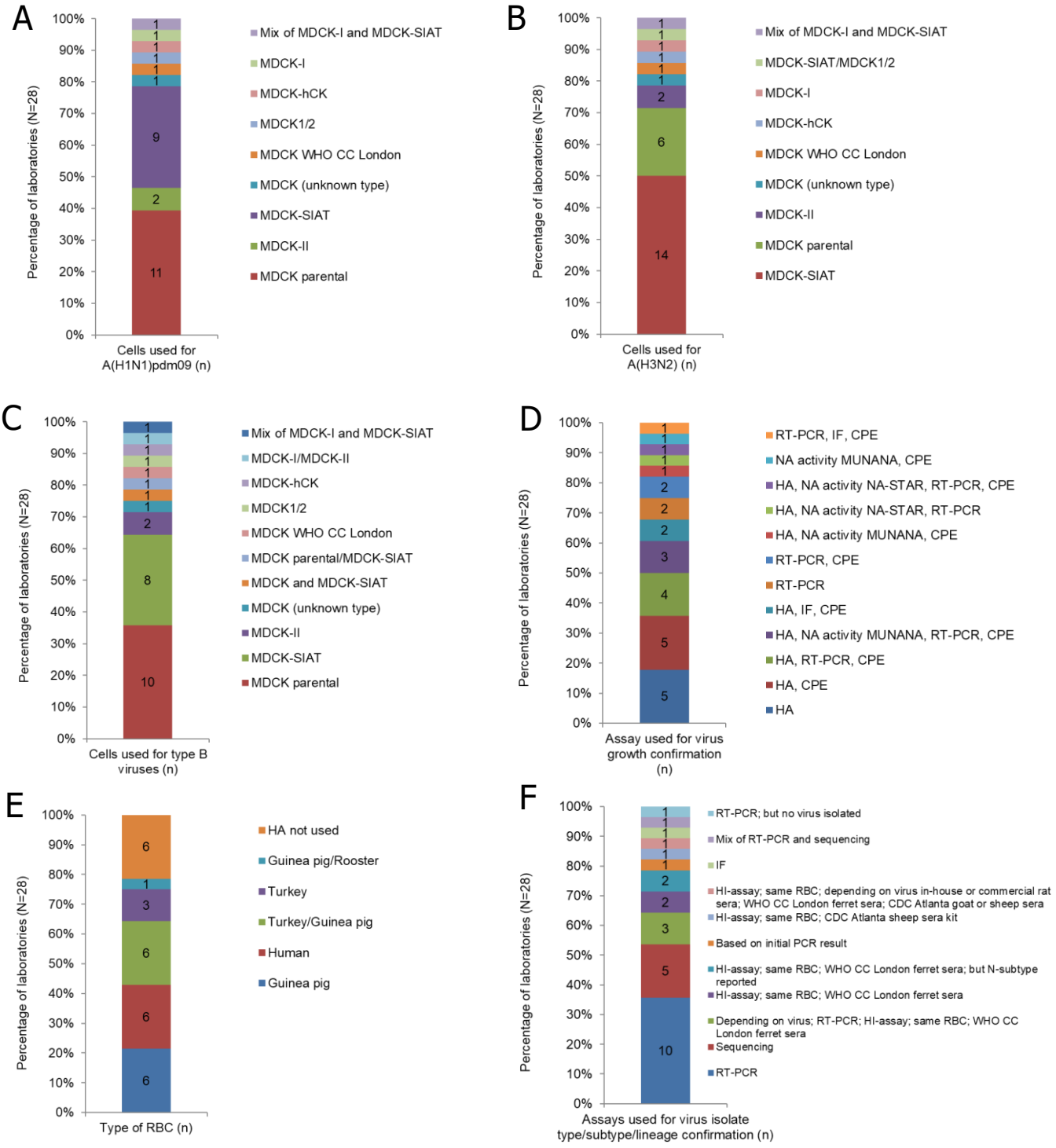
Table A4. Overview of virus isolation results with performance score and methodology used, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen (EISN_INF20):	1	2	3	4	5	6	7	8	Overall score[3]	Isolation method (n specimens)[4,5]	Times thawed before virus isolation[6]	Volume used in virus isolation (ml)	Confirmation method virus isolation (n specimens)[4]					
Subtype/lineage:	A(H3N2)	No virus	A(H1N1) pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic					HA-assay	NA activity		RT-PCR	IF	CPE
Expected Result:	Positive	Negative [2]	Positive	Positive	Positive	Positive	Positive	Positive					RBC species	MUNANA	NA-STAR			
Participant ID[1]																		
95	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-hCK (8)	1	0.1	Turkey (8)	0	7	8	0	0
200	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (3)/ MDCK and MDCK-SIAT (4)	1	0.1	Guinea pig (8)	0	0	0	0	8
1159[7]	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	7	MDCK-SIAT (8)	1	0.2	HA not used	0	0	8	0	0
2125	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK parental (8)	0	0.1	Guinea pig (8)	0	7	8	0	8
2126	Positive	Not attempted	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (7)	1	0.2	Turkey (4)/ Guinea pig (1)	2	0	0	0	7
2253	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Positive	2	MDCK parental (8)	1	0.1	Human (8)	0	0	8	0	7
2271	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	2	MDCK-SIAT (8)	2	0.1	Guinea pig (8)	0	0	0	0	0
2276	Positive	Not attempted	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK WHO CC London (7)	1	0.15	Turkey (7)	0	0	3	0	6
2277	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	2	MDCK-I (7)/ MDCK-II (1)	1	0.8	Human (8)	0	0	7	0	7
2278	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (5)/ MDCK parental (2)	1	0.1	Human (8)	0	0	0	8	8
2820	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	2	MDCK-SIAT (8)	2	0.2	Guinea pig (7)	0	0	8	0	7
4209	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-II (8)	2	0.2	Human (8)	0	0	0	7	8
10007	Positive	Not attempted	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (3)/ MDCK parental (4)	0	0.5	Turkey (4)/ Guinea pig (3)	0	0	0	0	0
10023	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (8)	0	0.5	HA not used	0	0	7	0	8
10078	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive	4	MDCK (unknown type) (8)	1	0.2	HA not used	0	0	8	8	8
10080	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	Mix of MDCK-I and MDCK-SIAT (8)	1	0.25	HA not used	8	0	0	0	8
10104 [8]	Negative	Negative	Positive	Negative	Positive	Negative	Negative	Negative	5	MDCK-SIAT (3)/ MDCK parental (4)	2	0.2	Guinea pig (8)	0	0	0	0	0
10115	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK parental (8)	1	0.2	Turkey (4)/ Guinea pig (5)	0	0	0	0	7
10205	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (8)	1	1	HA not used	0	0	8	0	8
10464	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (3)/ MDCK parental (5)	1	0.15	Turkey (4)/ Guinea pig (3)	7	0	8	0	7
10465	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-II (8)	1	0.5	Guinea pig (8)/ Rooster (8)	8	0	8	0	8
10466 [9]	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (8)	0	0.2	Turkey (7)/ Guinea pig (7)	0	0	0	0	8

Specimen (EISN_INF20):	1	2	3	4	5	6	7	8	Overall score[3]	Isolation method (n specimens)[4,5]	Times thawed before virus isolation[6]	Volume used in virus isolation (ml)	Confirmation method virus isolation (n specimens)[4]					
Subtype/lineage:	A(H3N2)	No virus	A(H1N1) pdm09	A(H3N2)	B/Vic	B/Yam	A(H3N2)	B/Vic					HA-assay	NA activity		RT-PCR	IF	CPE
Expected Result:	Positive	Negative [2]	Positive	Positive	Positive	Positive	Positive	Positive					RBC species	MUNANA	NA-STAR			
Participant ID[1]																		
1600	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK parental (8)	1	0.2	Turkey (8)	0	0	0	0	0
2817	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	1	MDCK parental (8)	1	0.1	Turkey (6)/ Guinea pig (2)	8	0	8	0	8
3558	Positive	Not attempted	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK parental (7)	3	0.2	Guinea pig (7)	0	0	0	0	7
10053	Positive	Not attempted	Positive	Positive	Positive	Positive	Positive	Positive	0	MDCK-SIAT (7)	2	0.2	HA not used	0	0	7	0	0
10248	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	1	MDCK-SIAT (3)/ MDCK1/2 (5)	1	0.25	Human (8)	0	0	0	0	8
10261	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Positive	1	MDCK-SIAT (3)/ MDCK parental (5)	1	0.2	Human (8)	0	0	0	0	0

[1] *Cells with orange shading = laboratory located in an EU/EEA country.*
[2] *Not attempted = considered correct following the widely used algorithm to take into virus isolation only those specimens that are positive in molecular detection. The three laboratories that reported a positive virus isolation result reported type (2820 and 10248) or B/lineage (laboratory 2277) not determined. Laboratory 2277 reported also B/lineage not determined in molecular detection, while the other two laboratories did not detect virus in the specimens in molecular detection (Table A3).*
[3] *Scoring:*
Positive specimens: Positive (green shading), 0; Negative and all other (red shading), 1.
Negative specimens: Negative and Not attempted (because of negative result in molecular detection) (green shading), 0; all other (red shading), 1.
[4] *MDCK = Madin Darby Canin Kidney; SIAT = human alpha 2,6-sialyltransferase; HA = haemagglutination; RBC = Red Blood Cells; NA = neuraminidase; MUNANA = 20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid; RT-PCR = reverse transcription polymerase chain reaction; IF = immunofluorescence; CPE = cytopathic effect observed microscopically.*
[5] *MDCK-SIAT cells were mainly used for A(H3N2) influenza virus only.*
[6] *It is assumed that laboratories that reported 0 have thawed the specimen once before it was inoculated on cells.*
[7] *Laboratory reported: No virus propagation possible, but control samples (reference strains and clinical samples) showed viral growth.*
[8] *Laboratory reported: One passage attempted.*
[9] *Laboratory reported: The samples were diluted 1:2 after arrival, filtrated and stored at 2-8°C before inoculation.*

Figure A5. Summary of methodologies used by 28 laboratories in virus isolation; type of cells for A(H1N1)pdm09 (A), A(H3N2) (B) and type B viruses (C); assay type used for confirmation of virus growth (D), type of red blood cells used in haemagglutination assay (E) and assays used to confirm type/subtype/lineage of the isolated virus (F), European External Influenza Quality Assessment Programme, WHO European Region, 2020



Abbreviations: see footnote in Table A4. Numbers in the bars indicate the number of laboratories.

Table A5. Overview of virus antigenic characterisation results with methodology used, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Individual antigenic characterisation results										Antigenic characterisation method			
Specimen (EISN_INF20):	1	2	3	4	5	6	7	8	Total number of virus isolates subjected to characterisation	RBC used in HI-assay	HI-assay		
Expected Result:	A(H3) A/Hong Kong/4801/2014 (H3N2)-like (has neuraminidase-induced hemagglutination)	No virus	A(H1)pdm09 A/Brisbane/02/2018 -like but also very similar to A/Michigan/45/2015	A(H3) A/South Australia/34/2019-like (did not agglutinate RBC at WHO CC)	B/Vic lineage not attributed to category; low reactor B/Brisbane/60/2008 -like	Bit further away from the most recent vaccine strain B/Phuket/3073/2013, but still considered B/Yam B/Phuket/3073/2013-like	A(H3) A/Kansas/14/2017-like; similar to A/England/538/2018, which is a representative of recent 3C.3a viruses similar to A/Kansas/14/2017 (WHO CC data)	B/Vic B/Colorado/06/2017-like		Species (n viruses)	Oseltamivir used (n viruses)		Sera (Source and species)[2]
											Yes	No	
Participant ID[1]													
95	A(H3) A/Kansas/14/2017 (H3N2)-like	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	7	Turkey (8)	0	8	In-house generated ferret sera
200	Isolate available, not characterised	-	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	Isolate available, not characterised	B/Colorado/06/2017-like and B/Washington/02/2019-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	Isolate available, not characterised	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	4	Guinea pig (8)	0	8	WHO CC London ferret sera
2125	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/South Australia/34/2019 (H3N2)-like	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	7	Guinea pig (8)	0	8	WHO CC Atlanta ferret sera kit/ WHO CC London ferret sera
2126	Isolate available, not characterised	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	Isolate available, not characterised	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	5	Turkey (4)/ Guinea pig (1)	0	5	In-house generated ferret sera
2253	No isolate obtained	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	No isolate obtained	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	5	Human (8)	0	8	WHO CC London ferret sera
2271	No isolate obtained	-	No isolate obtained	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	5	Guinea pig (8)	0	8	WHO CC London ferret sera
2276	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	Antigenic characterisation failed	B/Washington/02/2019-like (B/Victoria/2/87 (del162-164)-lineage)	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	6	Turkey (7)	0	7	WHO CC London ferret sera

Individual antigenic characterisation results										Antigenic characterisation method			
Specimen (EISN_INF20):	1	2	3	4	5	6	7	8	Total number of virus isolates subjected to characterisation	RBC used in HI-assay	HI-assay		
Expected Result:	A(H3) A/Hong Kong/4801/2014 (H3N2)-like (has neuraminidase-induced hemagglutination)	No virus	A(H1)pdm09 A/Brisbane/02/2018 -like but also very similar to A/Michigan/45/2015	A(H3) A/South Australia/34/2019-like (did not agglutinate RBC at WHO CC)	B/Vic lineage not attributed to category; low reactor B/Brisbane/60/2008 -like	Bit further away from the most recent vaccine strain B/Phuket/3073/2013, but still considered B/Yam B/Phuket/3073/2013-like	A(H3) A/Kansas/14/2017-like; similar to A/England/538/2018, which is a representative of recent 3C.3a viruses similar to A/Kansas/14/2017 (WHO CC data)	B/Vic B/Colorado/06/2017-like		Species (n viruses)	Oseltamivir used (n viruses)		Sera (Source and species)[2]
											Yes	No	
Participant ID[1]													
2277	A(H3) A/Kansas/14/2017 (H3N2)-like	Isolate available, not able to characterize	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/Kansas/14/2017 (H3N2)-like	Isolate available, not characterised	Isolate available, not characterised	A(H3) A/Kansas/14/2017 (H3N2)-like	no isolate obtained	4	Human (8)	0	8	WHO CC London ferret sera
2278	Isolate available, not characterised	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/South Australia/34/2019 (H3N2)-like	B(Vic) lineage not attributed to category	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Washington/02/2019-like (B/Victoria/2/87 (del162-164)-lineage)	6	Human (8)	0	8	WHO CC London ferret sera
2820	A(H3) A/Kansas/14/2017 (H3N2)-like	Isolate available, not able to characterize	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	Isolate available, not characterised	B(Vic) lineage not attributed to category	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	No isolate obtained	5	Guinea pig (7)	0	7	WHO CC London ferret sera
10007	Antigenic characterisation failed	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	7	Turkey (4)/Guinea pig (3)	3	4	WHO CC London ferret sera
10115	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/South Australia/34/2019 (H3N2)-like	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	7	Turkey (4)/Guinea pig (5)	0	9	WHO CC London ferret sera
10464	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/South Australia/34/2019 (H3N2)-like	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	7	Turkey (4)/Guinea pig (3)	3	4	WHO CC London ferret sera
10466	A(H3) A/South Australia/34/2019 (H3N2)-like	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/South Australia/34/2019 (H3N2)-like	Antigenic characterisation failed	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	7	Turkey (7)/Guinea pig (7)	14		In-house generated ferret sera
2817	No isolate obtained	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B(Vic) lineage not attributed to category	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	6	Turkey (6)/Guinea pig (2)	3	5	WHO CC Atlanta ferret sera kit/ In-house generated ferret sera

Individual antigenic characterisation results										Antigenic characterisation method			
Specimen (EISN_INF20):	1	2	3	4	5	6	7	8	Total number of virus isolates subjected to characterisation	RBC used in HI-assay	HI-assay		
Expected Result:	A(H3) A/Hong Kong/4801/2014 (H3N2)-like (has neuraminidase-induced hemagglutination)	No virus	A(H1)pdm09 A/Brisbane/02/2018 -like but also very similar to A/Michigan/45/2015	A(H3) A/South Australia/34/2019-like (did not agglutinate RBC at WHO CC)	B/Vic lineage not attributed to category; low reactor B/Brisbane/60/2008 -like	Bit further away from the most recent vaccine strain B/Phuket/3073/2013, but still considered B/Yam B/Phuket/3073/2013-like	A(H3) A/Kansas/14/2017-like; similar to A/England/538/2018, which is a representative of recent 3C.3a viruses similar to A/Kansas/14/2017 (WHO CC data)	B/Vic B/Colorado/06/2017-like		Species (n viruses)	Oseltamivir used (n viruses)		Sera (Source and species)[2]
											Yes	No	
Participant ID[1]													
3558	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	-	A(H1)pdm09 A/California/7/2009 (H1N1)-like	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	7	Guinea pig (7)	0	8	WHO CC London ferret sera
10261	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	-	A(H1)pdm09 A/Brisbane/02/2018 (H1N1)-like	A(H3) A/South Australia/34/2019 (H3N2)-like	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	No isolate obtained	A(H3) A/Kansas/14/2017 (H3N2)-like	B/Colorado/06/2017-like (B/Victoria/2/87 (del162-163)-lineage)	6	Human (8)	3	5	In-house generated rat sera

[1] Cells with orange shading = laboratory located in an EU/EEA country. Result cells with grey shading indicate results for which no antigenic characterisation was reported with explanation.
[2] A summary of the sera/viruses by reference strain is shown in Figure A7.

Figure A6. Summary overview of source and species of sera (A) and source of RBC (B) used for antigenic characterisation in HI-assays, European External Influenza Quality Assessment Programme, WHO European Region, 2020

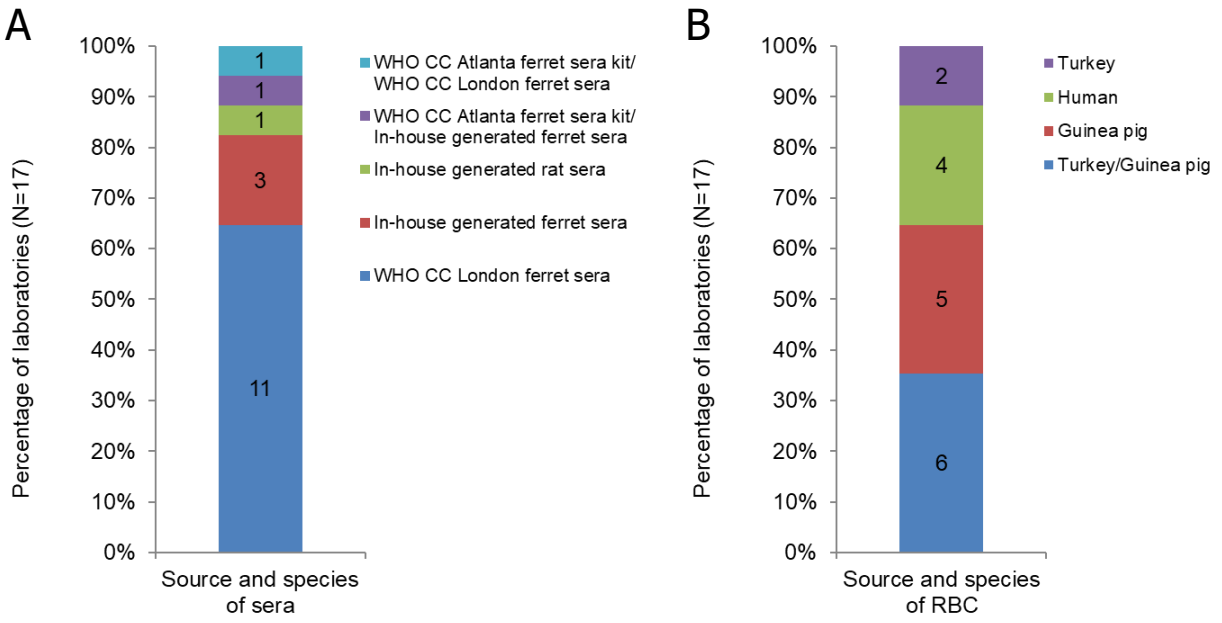
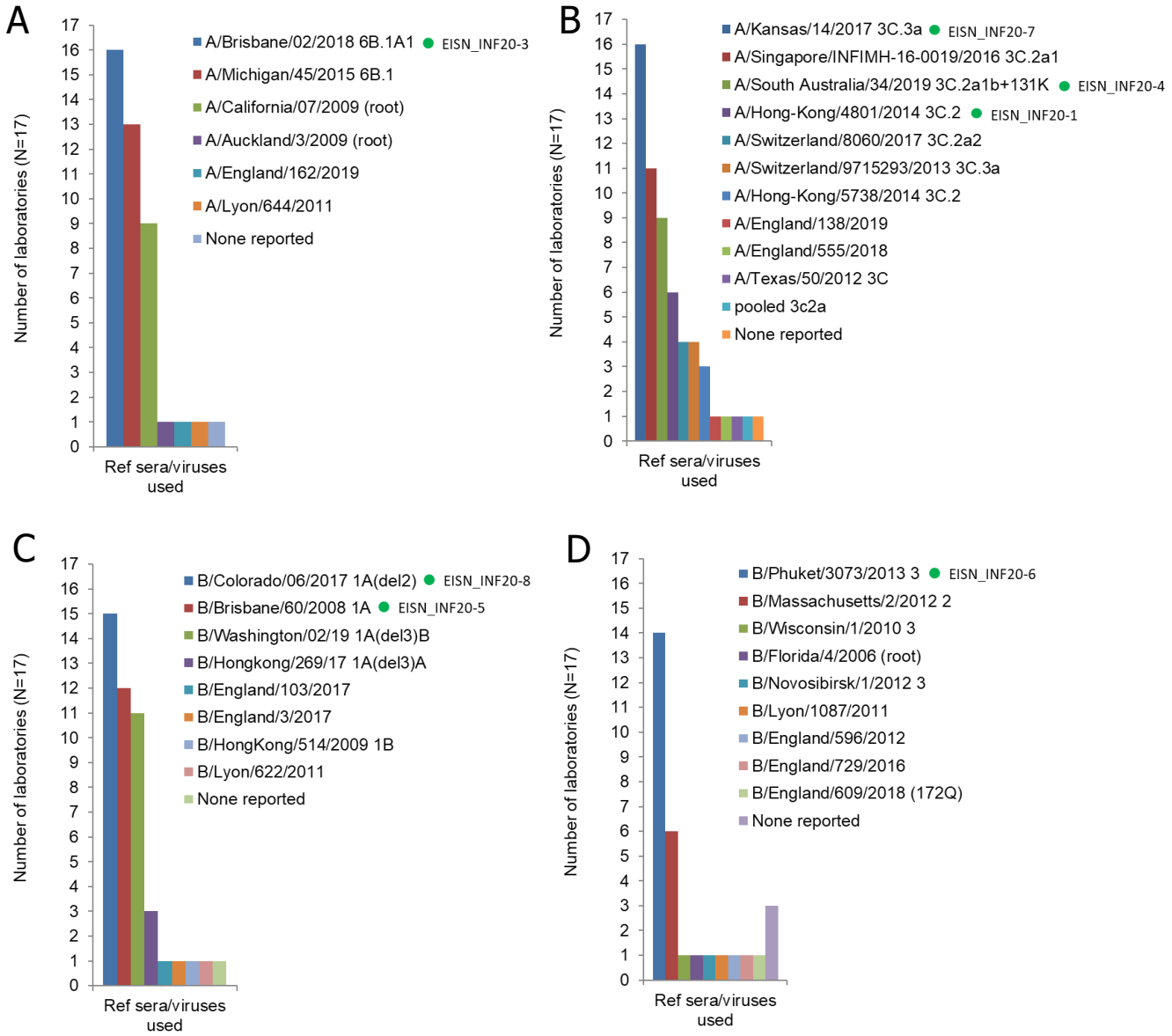
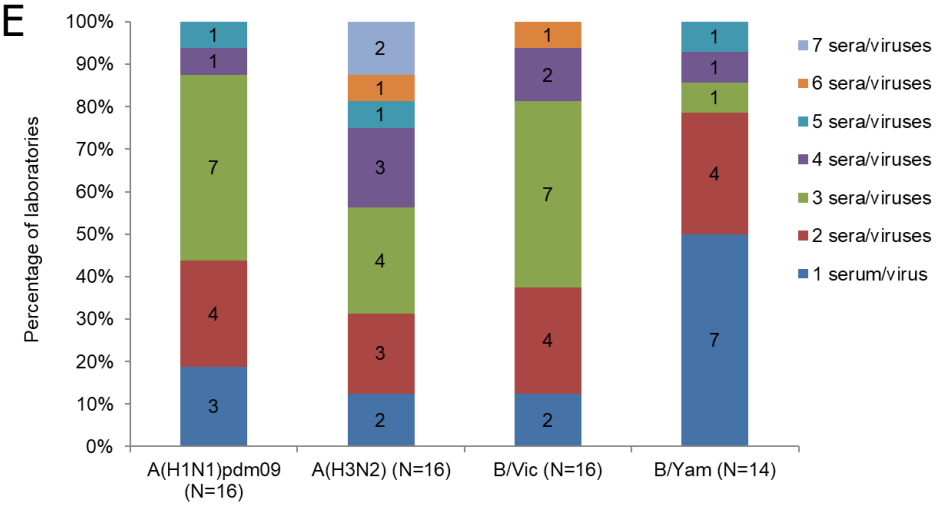


Figure A7. Summary overview of specification of the sera/viruses used for antigenic characterisation, by virus strain (A(H1N1)pdm09 virus (EISN_INF20-3) (A), A(H3N2) viruses (EISN_INF20-1, 4, 7) (B), B/Victoria viruses (EISN_INF20-5 and 8) (C) and B/Yamagata virus (EISN_INF-6) (D)) and the number of laboratories using a specified number of sera/viruses per test virus subtype or lineage (E), European External Influenza Quality Assessment Programme, WHO European Region, 2020



E



None reported means that the laboratory did not obtain a virus isolate or the virus was not antigenically characterised for another reason (See Table A5). Green dots indicate the 'like'-viruses included in the EEIQAP panel. In panel E, the number of laboratories using a specified number of sera/viruses per test virus subtype or lineage The N indicates the number of laboratories that performed characterisation; there are less than 17 because for each virus subtype or lineage there was one or more laboratory that did not obtain an isolate or did not characterise it (those are 'None reported' in the panels A to D).

Table A6. Overview of genetic characterisation results with performance score and methodology used, European External Influenza Quality Assessment Programme, WHO European Region, 2020

Individual genetic characterisation results[2]									Overall score[3]	Genetic characterisation				
Sample (EISN_INF20):	1	2	3	4	5	6	7	8		Total number of specimens genetically characterised	On specimen type	Technique		Sequence uploaded[4]
Expected Result:	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	No virus	A(H1)pdm09 clade 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017				Sanger	NGS	
Participant ID[1]														
95	A(H3) clade 3C.2a1a representative A/Greece/4/2017 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	1	7	Simulated specimen	0	7	7
200	A(H3) clade 3C.2a1a representative A/Greece/4/2017 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	1	7	Simulated specimen	0	7	0
1159	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	7[5]
2125	A(H3) clade 3C.2a1a representative A/Greece/4/2017 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A (del162-164A subgroup) representative B/Hong Kong/269/2017	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	2	7	Simulated specimen	7	0	7[6]
2126	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	0	7	7
2271	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	0
2276	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A1 representative A/Brisbane/02/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1B (del162-164B subgroup) representative B/Washington/02/2019	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	2	7	Virus isolate	0	7	6[7]

Individual genetic characterisation results[2]									Overall score[3]	Genetic characterisation				
Sample (EISN_INF20):	1	2	3	4	5	6	7	8		Total number of specimens genetically characterised	On specimen type	Technique		Sequence uploaded[4]
Expected Result:	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	No virus	A(H1)pdm09 clade 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017				Sanger	NGS	
Participant ID[1]														
2278	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Virus isolate	7	0	7[8]
10007	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	7
10023	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	0	7	7
10080	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	7
10115	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	0	7	7
10144	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	7[9]
10205	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B/Ireland/3154/2016(Vic)-like 1A	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	7

Individual genetic characterisation results[2]									Overall score[3]	Genetic characterisation				
Sample (EISN_INF20):	1	2	3	4	5	6	7	8		Total number of specimens genetically characterised	On specimen type	Technique		Sequence uploaded[4]
Expected Result:	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	No virus	A(H1)pdm09 clade 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017				Sanger	NGS	
Participant ID[1]														
10464	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	7[10]
10465	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	1	7	Virus isolate	7	0	7[11]
10466	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Both	0	7	7
2817	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen (1)/Virus isolate (6)	0	7	7
3558	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b + 131K A/South Australia/34/2019 subgroup	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	0	7	Simulated specimen	7	0	7[12]
10053	A(H3) clade 3C.2a1b representative A/Alsace/1746/2018 subgroup	–	A(H1)pdm09 group 6B.1A5A representative A/Norway/3433/2018	A(H3) clade 3C.2a1b representative A/Alsace/1746/2018 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	3	7	Both	7	0	0
10261	A(H3) clade 3C.2a3 representative A/Cote d'Ivoire/544/2016 subgroup	–	A(H1)pdm09 group 6B.1A1 representative A/Brisbane/02/2018	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	B(Vic)-lineage clade 1A (del162-164A subgroup) representative B/Hong Kong/269/2017	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	A(H3) clade 3C.3a representative A/Kansas/14/2017 subgroup	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Colorado/06/2017	4	7	Simulated specimen (1)/Virus isolate (6)	0	7	7[13]

[1] Cells with orange shading = laboratory located in an EU/EEA country.
[2] All strain indications refer to representative strain, as indicated in the guidance for TESSy categories.
[3] Scoring: category as expected (green shading) or close (yellow shading): 0; category not as expected (red shading): 1.

[4] Most reported sequences were full length or nearly full-length HA segment unless otherwise indicated.
[5] All sequences HA1.
[6] Sequences of B/Vic and B/Yam HA2.
[7] EISN_INF20-6 was not uploaded.
[8] EISN_INF20-5 had many nucleotide mutations compared to the original sequence.
[9] All sequences HA1.
[10] Sequence of B/Yam HA1.
[11] EISN_INF20-1 identical to EISN_INF20-4; EISN_INF20-3 had several nucleotide mutations.
[12] All sequences HA1.
[13] EISN_INF20-1 was very similar to EISN_INF20-4, although there were a considerable number of dissimilar nucleotides; EISN_INF20-5 sequence has a three-amino acids deletion, but the original virus is not a deletion variant.

Table A7. Overview of genetic antiviral susceptibility testing results with performance score and methodology used, European External Influenza Quality Assessment Programme, WHO European Region, 2020

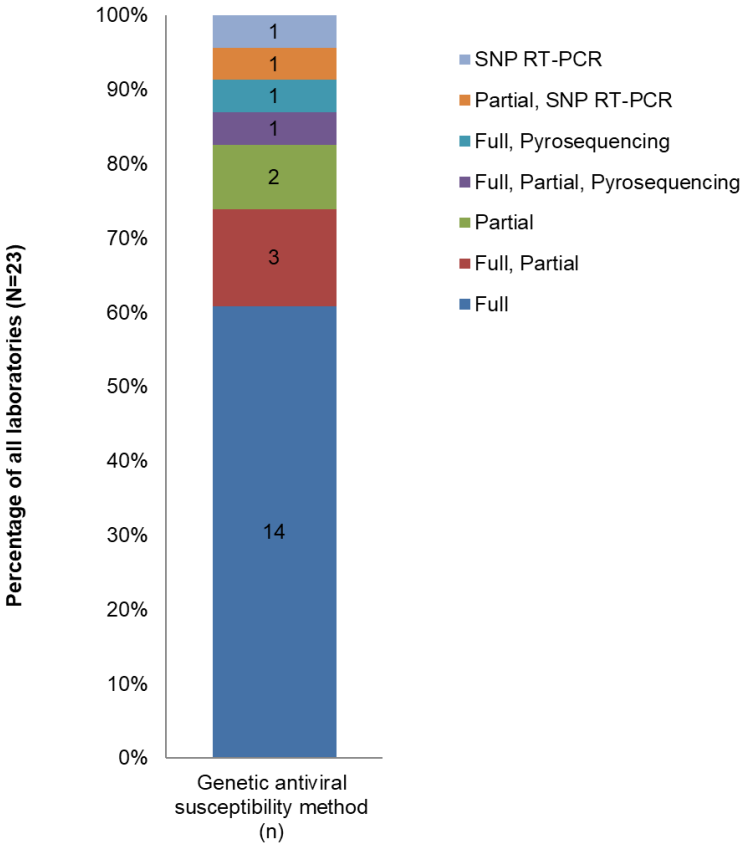
Sample:	EISN_AV20-1				EISN_AV20-2				EISN_INF20-1				EISN_INF20-2		
Subtype	A(H1N1)pdm09				A(H3N2)				A(H3N2)				No virus		
Result type:	Genotype[2]		Interpretation[3]		Genotype[2]		Interpretation[3]		Genotype[2]		Interpretation[3]		Genotype[2]	Interpretation[3]	
Expected Result:	D199E		Oseltamivir AARI	Zanamivir AANI	E119V; 245-248del		Oseltamivir AAHRI	Zanamivir AAHRI	None		Oseltamivir AANI	Zanamivir AANI	N/A	Oseltamivir N/A	Zanamivir N/A
Participant ID[1]	Sequence	Report			Sequence	Report			Sequence	Report					
95	Not tested	Not tested	Not tested	Not tested	Not uploaded	E119V; 245-248del	AARI	AARI	None	None	AANI	AANI	_	–	–
112	D199E	None	AANI	AANI	E119V; 245-248del	E119V	AAHRI	AANI	None	None	AANI	AANI	–	–	–
200	Not uploaded	D199E	AANI	AANI	Not uploaded	E119V; 244-247del	AAHRI	AANI	Not uploaded	None	AANI	AANI	–	–	–
1159	D199E	None	AANI	AANI	E119V; 245-248del	E119V	AAHRI	AARI	None	None	AANI	AANI	–	–	–
2125	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 246-248del	AAHRI	NIP	None	None	AANI	AANI	–	–	–
2126	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 244-247del	AAHRI	AARI	None	None	AANI	AANI	–	–	–
2271	Not uploaded	D199E	AARI	AANI	Not uploaded	E119V	AAHRI	AANI	Not uploaded	None	AANI	AANI	–	–	–
2276	D199E	D199E	AANI	AANI	E119V; at 245-248 SASG amino acids	E119V	AAHRI	AAHRI	None	None	AANI	AANI	–	–	–
2820	Only SNP H275Y assay	None	AANI	NIP	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	–
3442	Not uploaded	D199E	AARI	AANI	Not uploaded	E119V; 245-248del	AAHRI	AAHRI	Not tested	Not tested	Not tested	Not tested	–	–	–
10007	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 245-248del	AAHRI	AARI	None	None	AANI	AANI	–	–	–
10023	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 245-248del	AAHRI	AARI	None	None	AANI	AANI	–	–	–
10080	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 245-248del	AAHRI	AAHRI	None	None	AANI	AANI	–	–	–
10104[5]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	–
10115	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 245-248del	AAHRI	AARI	None	None	AANI	AANI	–	–	–
10144	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 244-247del	AAHRI	AARI	None	None	AANI	AANI	–	–	–
10205	D199E	None	AANI	AANI	E119V; 245-248del	E119V; 244-247del	AAHRI	AANI	None	None	AANI	AANI	–	–	–
10464	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 245-248del	AAHRI	AAHRI	None	None	AANI	AANI	–	–	–
10465	Not uploaded	None	AANI	AANI	Sequence to short for E119V; 245-248del	None	AANI	AANI	Is identical to EISN_INF20-4	None	AANI	AANI	–	–	–
10466	D199E	D199E	AARI	AANI	E119V; 245-248del	E119V; 245-248del	AAHRI	AARI	None	D161N	AANI	AANI	–	–	–
2817	D199E	None	AANI	AANI	E119V; 245-248del	E119V; 247-250del	AAHRI	AARI	None	None	AANI	AANI	–	–	–
3558	D199E	None	AANI	AANI	E119V; 245-248del	E119V; 245-248del	AAHRI	AARI	None	None	AANI	AANI	–	–	–
4344[5]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	–
10053	Not uploaded	None	AANI	AANI	Not uploaded	E119V; 245-248del	AAHRI	AANI	Not uploaded	None	AANI	AANI	–	–	–
10261	Not uploaded	D199E	AARI	AANI	Not uploaded	E119V; 244-247del	AAHRI	AARI	None	None	AANI	AANI	–	–	–

Sample:	EISN_INF20-3				EISN_INF20-4				EISN_INF20-5				EISN_INF20-6			
Subtype	A(H1N1)pdm09				A(H3N2)				B/Victoria				B/Yamagata			
Result type:	Genotype[2]		Interpretation[3]		Genotype[2]		Interpretation[3]		Genotype[2]		Interpretation[3]		Genotype[2]		Interpretation[3]	
Expected Result:	None		Oseltamivir AANI	Zanamivir AANI	None		Oseltamivir AANI	Zanamivir AANI	None		Oseltamivir AANI	Zanamivir AANI	None		Oseltamivir AANI	Zanamivir AANI
Participant ID[1]	Sequence	Report			Sequence	Report			Sequence	Report			Sequence	Report		
95	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
112	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
200	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI
1159	None; short	None	AANI	AANI	None; short	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
2125	Not tested	Not tested	Not tested	Not tested	None	None	AANI	AANI	None; short	None	AANI	AANI	None; short	K360R	AARI	AANI
2126	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
2271	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI
2276	None	None	AANI	AANI	None	None	AANI	AANI	None; short	None	AANI	AANI	None	None	AANI	AANI
2820	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
3442	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
10007	None	None	AANI	AANI	None	N329S	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10023	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10080	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10104[5]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
10115	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10144	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10205	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10464	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10465	None; short	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
10466	None	None	AANI	AANI	None	D161N	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
2817	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
3558	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI
4344[5]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
10053	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI
10261	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI	None	None	AANI	AANI

Sample:	EISN_INF20-7				EISN_INF20-8				Overall score[4]	Methods used (n specimens)				
Subtype	A(H3N2)				B/Victoria					SNP detection		Sequencing neuraminidase gene		
Result type:	Genotype[2]		Interpretation[3]		Genotype[2]		Interpretation[3]							
Expected Result:	None		Oseltamivir AANI	Zanamivir AANI	None		Oseltamivir AANI	Zanamivir AANI		SNP RT-PCR	Pyrosequencing	Partial	Full length	Sequence uploaded
Participant ID[1]	Sequence	Report			Sequence	Report								
95	None	None	AANI	AANI	None	None	AANI	AANI	2	0	0	0	8	7
112	None	None	AANI	AANI	None	None	AANI	AANI	3	0	0	0	9	9
200	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	3	0	0	0	9	0
1159	None	None	AANI	AANI	None	None	AANI	AANI	5	0	0	9	0	9
2125	None	None	AANI	AANI	None; short	None	AANI	AANI	4	0	0	2	6	8
2126	None	None	AANI	AANI	None	None	AANI	AANI	1	0	9	0	9	9
2271	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	1	0	0	0	9	0
2276	Not uploaded	None	AANI	AANI	None	None	AANI	AANI	4	0	0	0	9	8
2820	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	2	1	0	0	0	0
3442	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	0	0	0	0	2	0
10007	None	None	AANI	AANI	None	None	AANI	AANI	2	0	0	0	9	9
10023	None	None	AANI	AANI	None	None	AANI	AANI	1	0	0	0	9	9
10080	None	None	AANI	AANI	None	None	AANI	AANI	0	0	0	0	9	9
10104[5]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	0	0	0	0	0
10115	None	None	AANI	AANI	None	None	AANI	AANI	1	0	0	0	9	9
10144	None	None	AANI	AANI	None	None	AANI	AANI	1	0	2	4	5	9
10205	None	None	AANI	AANI	None	None	AANI	AANI	4	0	0	5	4	9
10464	None	None	AANI	AANI	None; short	None	AANI	AANI	0	0	0	1	8	9
10465	None	None	AANI	AANI	None	None	AANI	AANI	8	1	0	8	0	8
10466	None	None	AANI	AANI	None	None	AANI	AANI	3	0	0	0	9	9
2817	None	None	AANI	AANI	None	None	AANI	AANI	3	0	0	0	9	9
3558	None	None	AANI	AANI	None	None	AANI	AANI	3	0	0	0	9	9
4344[5]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	0	0	0	0	0
10053	Not uploaded	None	AANI	AANI	Not uploaded	None	AANI	AANI	4	0	0	9	0	0
10261	None	None	AANI	AANI	None	None	AANI	AANI	1	0	0	0	9	7

[1] Cells with orange shading = laboratory located in an EU/EEA country.
[2] None = no amino acid substitution previously associated with (highly) reduced inhibition identified. 'Sequence' column based on the assessment at RIVM using the reported sequence and 'Report' column contains the reported result by the laboratory based on their own assessment using the obtained sequence from the panel specimen. N/A = not applicable.
[3] 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; NIP = no interpretation possible due to partial NA segment information (SNP PCR, partial- or pyrosequencing). N/A = not applicable.
[4] Scoring system used:
EISN_AV20-1 substitutions – D199E found (green shading), 0; any other (red shading), 1; not tested (grey shading), not scored
EISN_AV20-1 interpretation oseltamivir – D199E AND any test AND AARI (green shading), 0; any other (red shading), 1; not tested (grey shading), not scored.
EISN_AV20-1 interpretation zanamivir – NA sequenced covering known RI/HRI amino acid substitutions AND AANI (green shading), 0; SNP OR partial sequenced AND no interpretation possible (NIP) (green shading), 0; any other (red shading), 1; not tested (grey shading), not scored.
EISN_AV20-2 substitutions – E119V and 245-248 del (or del at approximate location but with different numbering) found (green shading), 0; only E119V found (yellow shading), 1; none found (red shading), 2; not tested (grey shading), not scored.
EISN_AV20-2 interpretation oseltamivir – E119V AND 245-248 del AND any test AND AAHRI (green shading), 0; E119V AND 245-248 del AND any test AND AARI (yellow shading), 1; E119V only AND any test AND AAHRI or AARI (green shading), 0; any other (red shading), 2; not tested (grey shading), not scored.
EISN_AV20-2 interpretation zanamivir – E119V AND 245-248 del AND any test AND AAHRI (green shading), 0; E119V AND 245-248 del AND any test AND AARI (yellow shading), 1; E119V only AND any test AND AANI (green shading), 0; any other (red shading), 2; not tested (grey shading), not scored.
EISN_INF20-01 – 08 (except 02) substitution – none found (green shading), 0; any other (red shading), 1; not tested (grey shading), not scored.
EISN_INF20-01 – 08 (except 02) interpretation oseltamivir and zanamivir: NA sequenced covering known RI/HRI amino acid substitutions AND AANI (green shading), 0; SNP OR partial sequenced AND no interpretation possible (NIP) (green shading), 0; any other (red shading), 1; not tested (grey shading), not scored.
[5] Laboratory performed only phenotypic testing for antiviral susceptibility and for comparison retained in this table (See Table A8).

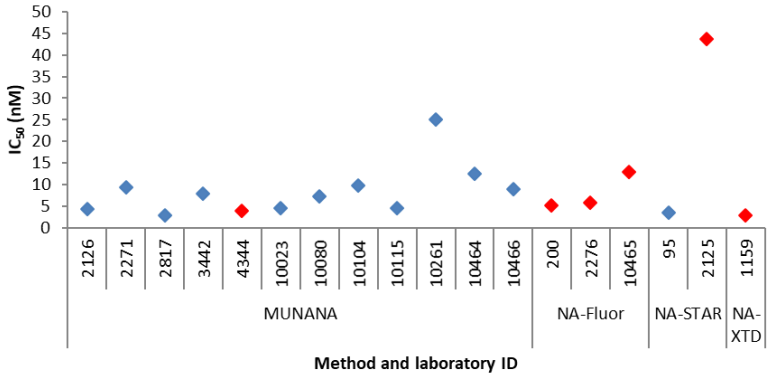
Figure A8. Methods used for genetic antiviral susceptibility determination, European External Influenza Quality Assessment Programme, WHO European Region, 2020



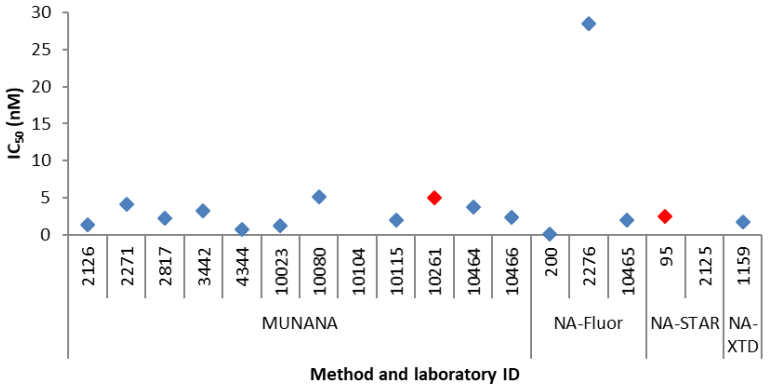
Full = full length neuraminidase gene sequencing; partial = partial neuraminidase gene sequencing; SNP RT-PCR = single nucleotide polymorphism for N1-H275Y (n = 2).
Pyrosequencing: N1-H275Y (n = 1); N1 223;275, N2-119;243-250;292-294 and B-150,197,221 (n = 1).

Figure A9. Overview of reported IC_{50} values by method and participant ID of laboratories participating in the phenotypic antiviral susceptibility determination challenge, European External Influenza Quality Assessment Programme, WHO European Region, 2020

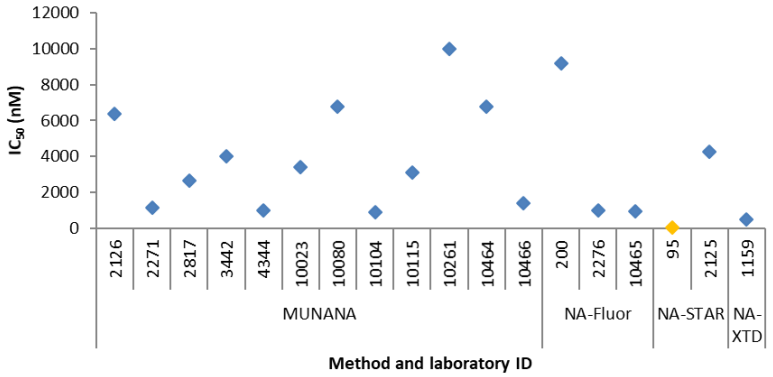
EISN_AV20-1 Oseltamivir; A(H1N1)pdm09 D199E



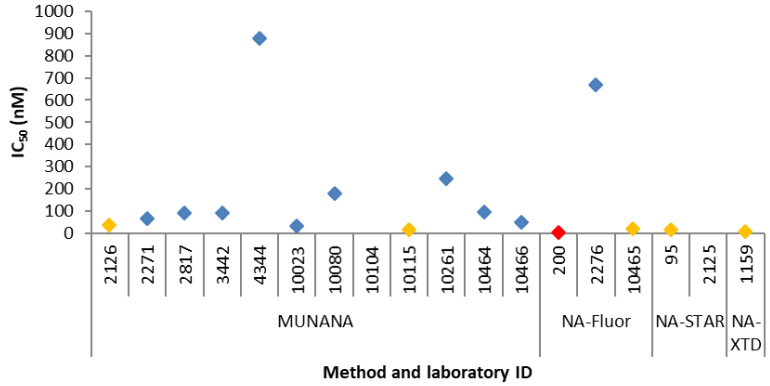
EISN_AV20-1 Zanamivir; A(H1N1)pdm09 D199E



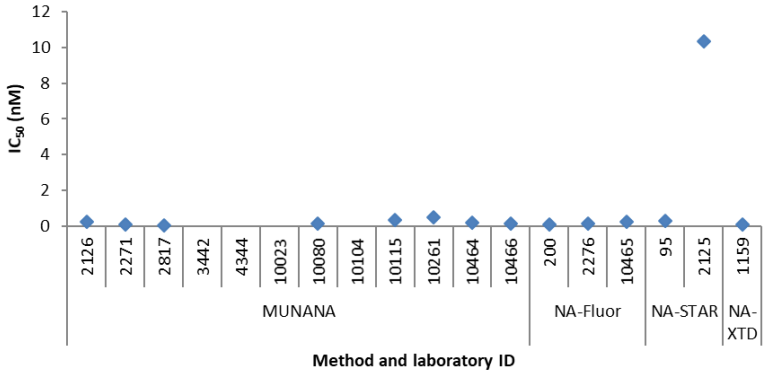
EISN_AV20-2 Oseltamivir; A(H3N2) E119V, 245-248 del



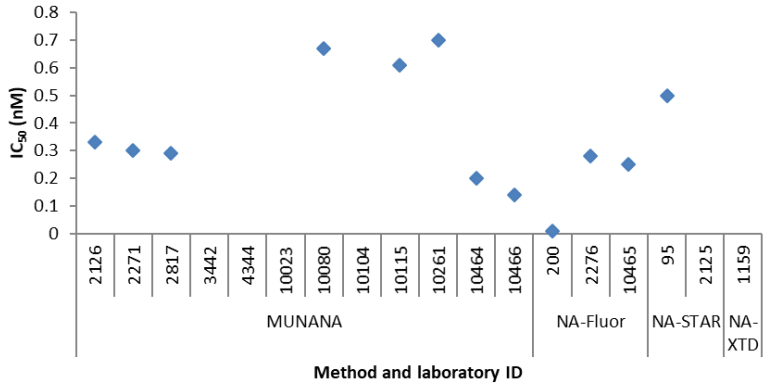
EISN_AV20-2 Zanamivir; A(H3N2) E119V, 245-248 del



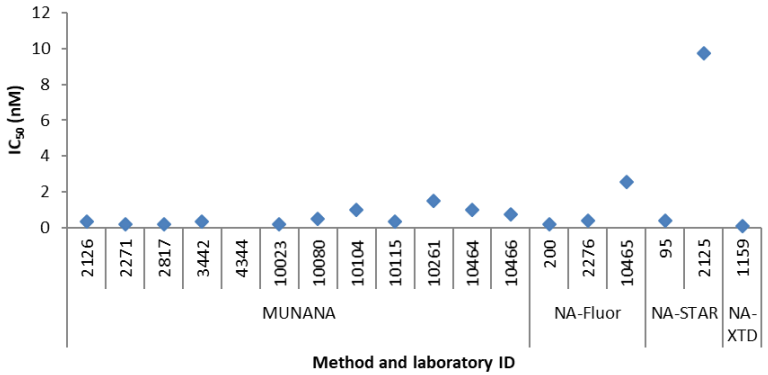
EISN_INF20-1 Oseltamivir; A(H3N2) NA-wild type



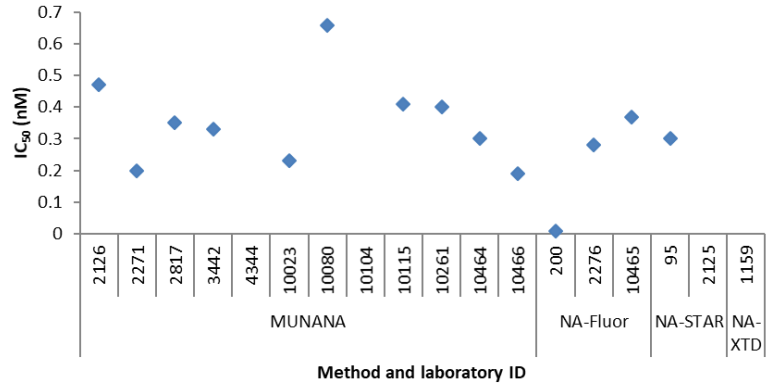
EISN_INF20-1 Zanamivir; A(H3N2) NA-wild type



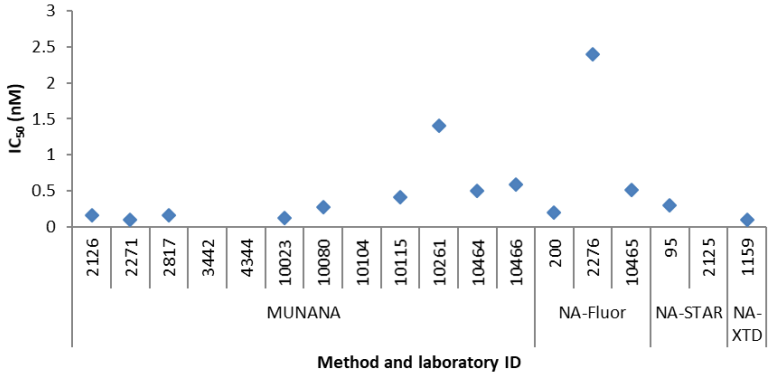
EISN_INF20-3 Oseltamivir; A(H1N1)pdm09 NA-wild type



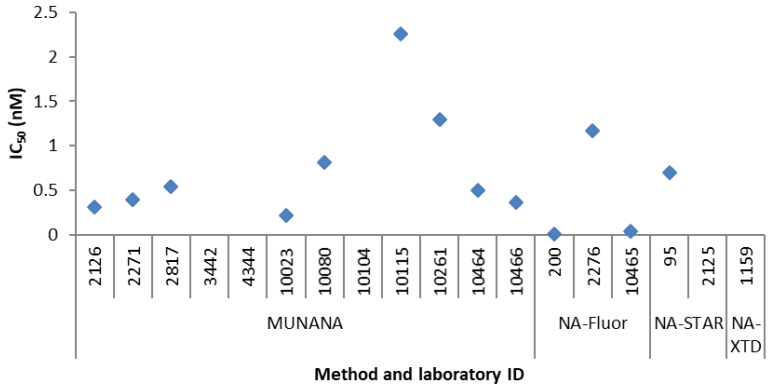
EISN_INF20-3 Zanamivir; AH1N1)pdm09 NA-wild type



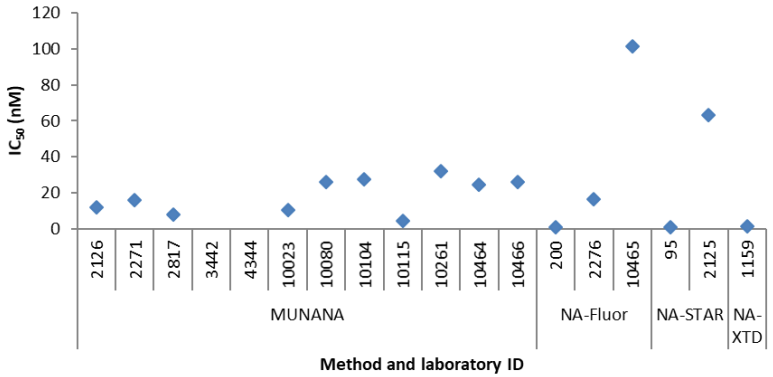
EISN_INF20-4 Oseltamivir; A(H3N2) NA-wild type



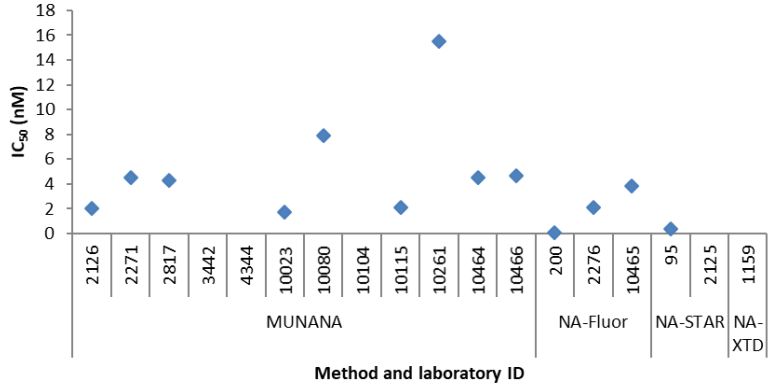
EISN_INF20-4 Zanamivir; A(H3N2) NA-wild type



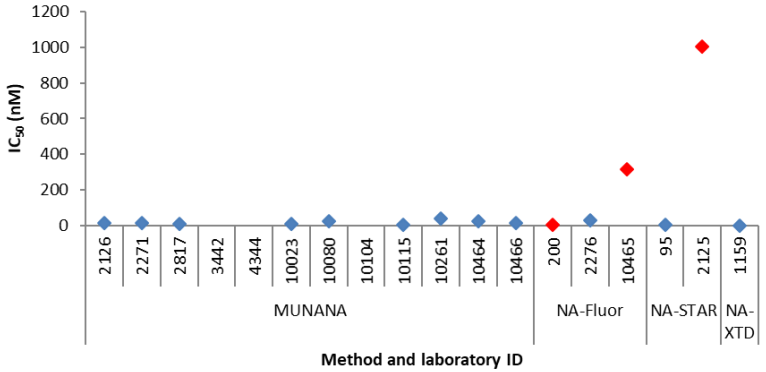
EISN_INF20-5 Oseltamivir; B/Victoria NA-wild type



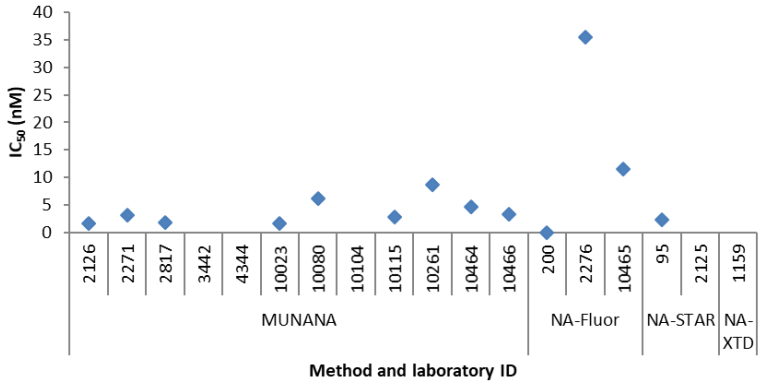
EISN_INF20-5 Zanamivir; B/Victoria NA-wild type



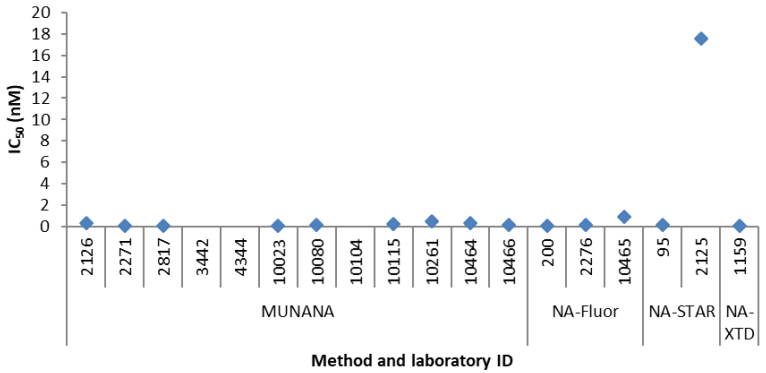
EISN_INF20-6 Oseltamivir; B/Yamagata NA-wild type



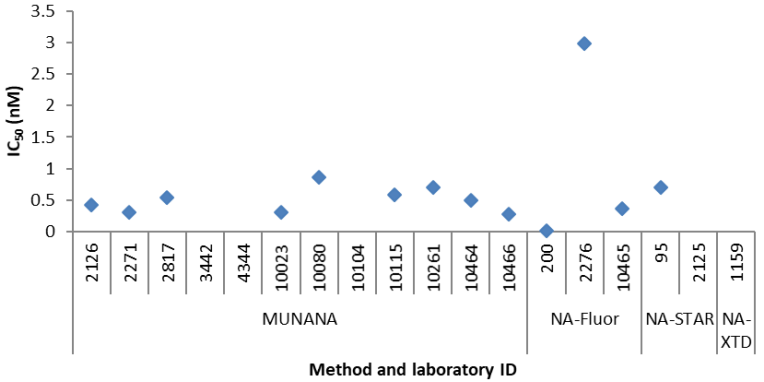
EISN_INF20-6 Zanamivir; B/Yamagata NA-wild type



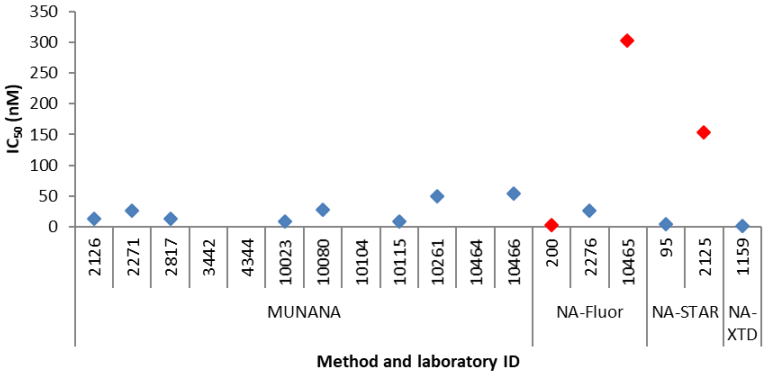
EISN_INF20-7 Oseltamivir; A(H3N2) NA-wild type



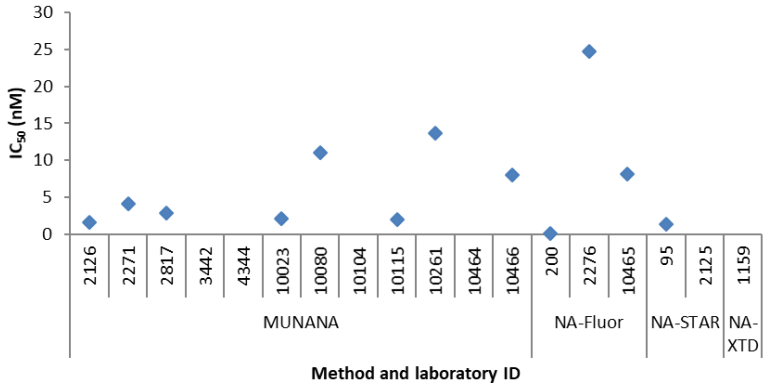
EISN_INF20-7 Zanamivir; A(H3N2) NA-wild type



EISN_INF20-8 Oseltamivir; B/Victoria NA-wild type



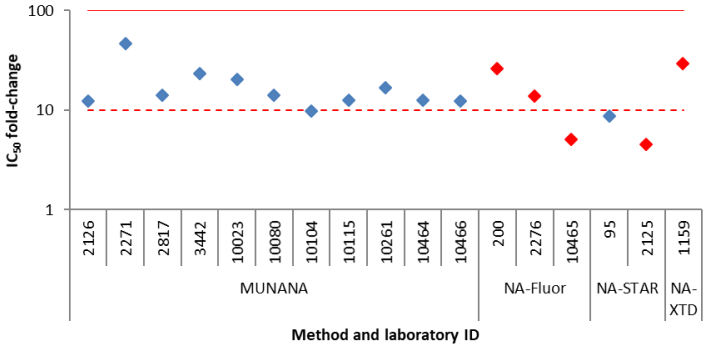
EISN_INF20-8 Zanamivir; B/Victoria NA-wild type



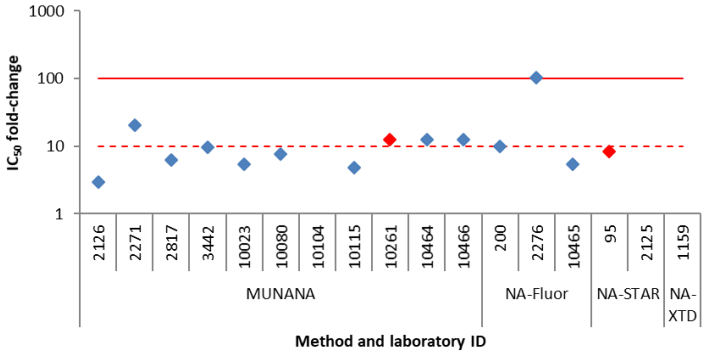
Red dots indicate specimens with incorrect result: IC_{50} level not as expected and/or interpretation not as expected (for details see Table A8). Orange dots indicate specimen with RI result reported, whereas the expected results is HRI; RI result not considered incorrect according to calculated fold-change in Figure 10 and Figure A10. If an IC_{50} value for a particular participant ID is not shown, this is either because the laboratory did not isolate the virus or did not determine the IC_{50} for all specimens or both oseltamivir and zanamivir (for details see Table A8).

Figure A10. Overview of calculated IC_{50} fold-change values by method and participant ID of the EISN_AV20-1 and -2 specimens, European External Influenza Quality Assessment Programme, WHO European Region, 2020

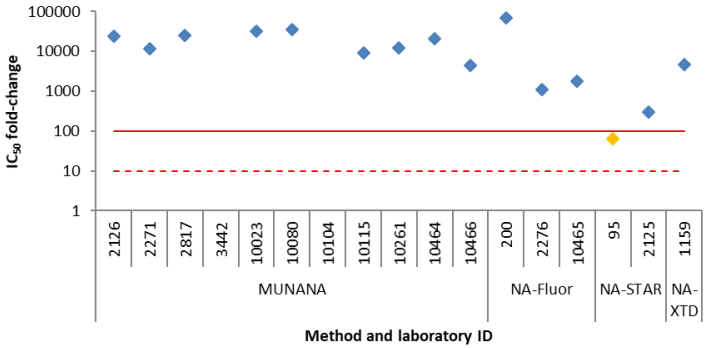
EISN_AV20-1 Oseltamivir A(H1N1)pdm09 D199E



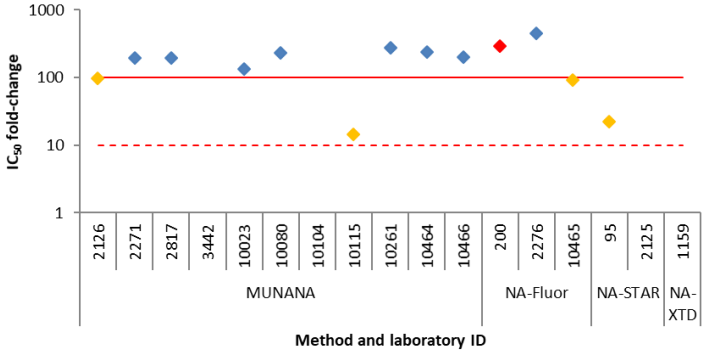
EISN_AV20-1 Zanamivir; A(H1N1)pdm09 D199E



EISN_AV20-2 Oseltamvir; A(H3N2) E119V, 245-248 del



EISN_AV20-2 Zanamivir; A(H3N2) E119V, 245-248 del



Results are plotted for the laboratories that also reported IC_{50} values for the wild type viruses of the same subtype in the EISN_INF20 specimens, EISN_INF20-3 and mean EISN_INF20-1, -4 and -7 respectively. 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. Dashed red line: RI threshold; continuous red line: HRI threshold. Red dots: specimens with incorrect result (IC_{50} level not as expected and/or interpretation not as expected). Orange dots: specimens with RI result reported, whereas the expected result is HRI; RI result not considered incorrect because fold-change calculated at RIVM indicates indeed RI for the reporting laboratories. For details see Table A8.

Table A8.A Overview of phenotypic antiviral susceptibility testing results with performance score and methodology used (assay type only), European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen:	EISN_AV20-1		EISN_AV20-2		EISN_INF20-1		EISN_INF20-2		EISN_INF20-3		EISN_INF20-4	
Subtype	A(H1N1)pdm09		A(H3N2)		A(H3N2)		No Virus		A(H1N1)pdm09		A(H3N2)	
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir
Expected Result:[1]	RI	NI	HRI	HRI	NI	NI	N/A	N/A	NI	NI	NI	NI
Participant ID[2]												
95	RI	RI	RI	RI	NI	NI	–	–	NI	NI	NI	NI
112[4]	Not tested[5]	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
200	NI	NI	HRI	NI	NI	NI	–	–	NI	NI	NI	NI
1159	NI	NI	HRI	RI	NI	Not tested	–	–	NI	Not tested	NI	Not tested
2125	NI	Not tested	HRI	Not tested	NI	Not tested	–	–	NI	Not tested	Not tested	Not tested
2126	RI	NI	HRI	RI	NI	NI	–	–	NI	NI	NI	NI
2271	RI	NI	HRI	HRI	NI	NI	–	–	NI	NI	NI	NI
2276	NI	NI	HRI	HRI	NI	NI	–	–	NI	NI	NI	NI
2820[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
3442	RI	NI	HRI	HRI	Not tested	Not tested	–	–	NI	NI	Not tested	Not tested
10007[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
10023	RI	NI	HRI	HRI	Not tested	Not tested	–	–	NI	NI	NI	NI
10080	RI	NI	HRI	HRI	NI	NI	–	–	NI	NI	NI	NI
10104	RI	Not tested	HRI	Not tested	No isolate	No isolate	–	–	NI	Not tested	No isolate	No isolate
10115	RI	NI	HRI	RI	NI	NI	–	–	NI	NI	NI	NI
10144[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
10205[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
10464	RI	NI	HRI	HRI	NI	NI	–	–	NI	NI	NI	NI
10465	NI	NI	HRI	RI	NI	NI	–	–	NI	NI	NI	NI
10466	RI	NI	HRI	HRI	NI	NI	–	–	NI	NI	NI	NI
2817	RI	NI	HRI	HRI	NI	NI	–	–	NI	NI	NI	NI
3558[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
4344	NI	NI	HRI	HRI	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
10053[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	–	–	Not tested	Not tested	Not tested	Not tested
10261	RI	RI	HRI	HRI	NI	NI	–	–	NI	NI	NI	NI

[1] For type A viruses: NI = IC_{50} fold-change < 10; RI = IC_{50} fold-change ≥ 10 – ≤ 100; HRI = IC_{50} fold change > 100. For type B viruses: NI = IC_{50} fold-change < 5; RI = IC_{50} fold-change ≥ 5 – ≤ 50; HRI = IC_{50} fold change > 50. Not tested means that the laboratory does not have the test available or has the test available, but did not test the virus for the neuraminidase inhibitor indicated. N/A = not applicable.

[2] Cells with orange shading = laboratory located in an EU/EEA country.

[3] Scoring system used:

EISN_AV20-1: oseltamivir: RI (green shading), 0; other (red shading), 2; zanamivir: NI (green shading), 0; other (red shading), 2; Not tested (grey shading): not scored.

EISN_AV20-2: oseltamivir: HRI (green shading), 0; RI (yellow shading), 1; other (red shading), 2; zanamivir: HRI (green shading), 0; RI (yellow shading), other (red shading), 2; Not tested (grey shading): not scored.

EISN_INF20-1 - 8 (except 05): oseltamivir and zanamivir: NI (green shading), 0; other (red shading), 2; Not tested (grey shading): not scored. N/A = not applicable.

[4] Laboratory performed only genotypic testing for antiviral susceptibility and for comparison retained in this table (see Table A7).

[5] Not tested = the laboratory has the test available, but has not performed the test on the indicated specimen; does not have the antiviral agent available; does not have the test available; or was not able to perform the test due to COVID-19-related workload.

Table A8.B Overview of phenotypic antiviral susceptibility testing results with performance score and methodology used (assay type only), European External Influenza Quality Assessment Programme, WHO European Region, 2020

Specimen:	EISN_INF20-5		EISN_INF20-6		EISN_INF20-7		EISN_INF20-8		Overall score[3]	Method used
Subtype	B/Victoria		B/Yamagata		A(H3N2)		B/Victoria			
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir		
Expected Result:[1]	NI	NI	NI	NI	NI	NI	NI	NI		
Participant ID[2]										
95	NI	NI	NI	NI	NI	NI	NI	NI	4	NA-STAR
112[4]	Not tested[5]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	N/A
200	NI	NI	RI	NI	NI	NI	RI	NI	8	NA-Fluor
1159	NI	Not tested	NI	Not tested	NI	Not tested	NI	Not tested	3	NA-XTD
2125	NI	Not tested	HRI	Not tested	NI	Not tested	RI	Not tested	6	NA-STAR
2126	NI	NI	NI	NI	NI	NI	NI	NI	1	MUNANA
2271	NI	NI	NI	NI	NI	NI	NI	NI	0	MUNANA
2276	NI	NI	NI	NI	NI	NI	NI	NI	2	NA-Fluor
2820[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	N/A
3442	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	0	MUNANA
10007[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	N/A
10023	NI	NI	NI	NI	NI	NI	NI	NI	0	MUNANA
10080	NI	NI	NI	NI	NI	NI	NI	NI	0	MUNANA
10104	NI	Not tested	Not tested	Not tested	No isolate	No isolate	No isolate	No Isolate	0	MUNANA
10115	NI	NI	NI	NI	NI	NI	NI	NI	1	MUNANA
10144[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	N/A
10205[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	N/A
10464	NI	NI	NI	NI	NI	NI	Not tested	Not tested	0	MUNANA
10465	NI	NI	RI	NI	NI	NI	RI	NI	7	NA-Fluor
10466	NI	NI	NI	NI	NI	NI	NI	NI	0	MUNANA
2817	NI	NI	NI	NI	NI	NI	NI	NI	0	MUNANA
3558[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	N/A
4344	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	2	MUNANA
10053[4]	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	N/A	N/A
10261	NI	NI	NI	NI	NI	NI	NI	NI	2	MUNANA

[1] For type A viruses: NI = IC_{50} fold-change < 10; RI = IC_{50} fold-change ≥ 10 – ≤ 100; HRI = IC_{50} fold change > 100. For type B viruses: NI = IC_{50} fold-change < 5; RI = IC_{50} fold-change ≥ 5 – ≤ 50; HRI = IC_{50} fold change > 50. Not tested means that the laboratory does not have the test available or has the test available, but did not test the virus for the neuraminidase inhibitor indicated. N/A = not applicable.

[2] Cells with orange shading = laboratory located in an EU/EEA country.

[3] Scoring system used:
EISN_AV20-1: oseltamivir: RI (green shading), 0; other (red shading), 2; zanamivir: NI (green shading), 0; other (red shading), 2; Not tested (grey shading): not scored.
EISN_AV20-2: oseltamivir: HRI (green shading), 0; RI (yellow shading), 1; other (red shading), 2; zanamivir: HRI (green shading), 0; RI (yellow shading), other (red shading), 2; Not tested (grey shading): not scored.
EISN_INF20-1 - 8 (except 05): oseltamivir and zanamivir: NI (green shading), 0; other (red shading), 2; Not tested (grey shading): not scored. N/A = not applicable.

[4] Laboratory performed only genotypic testing for antiviral susceptibility and for comparison retained in this table (see Table A7).

[5] Not tested = the laboratory has the test available, but has not performed the test on the indicated specimen; does not have the antiviral agent available; does not have the test available; or was not able to perform the test due to COVID-19-related workload.

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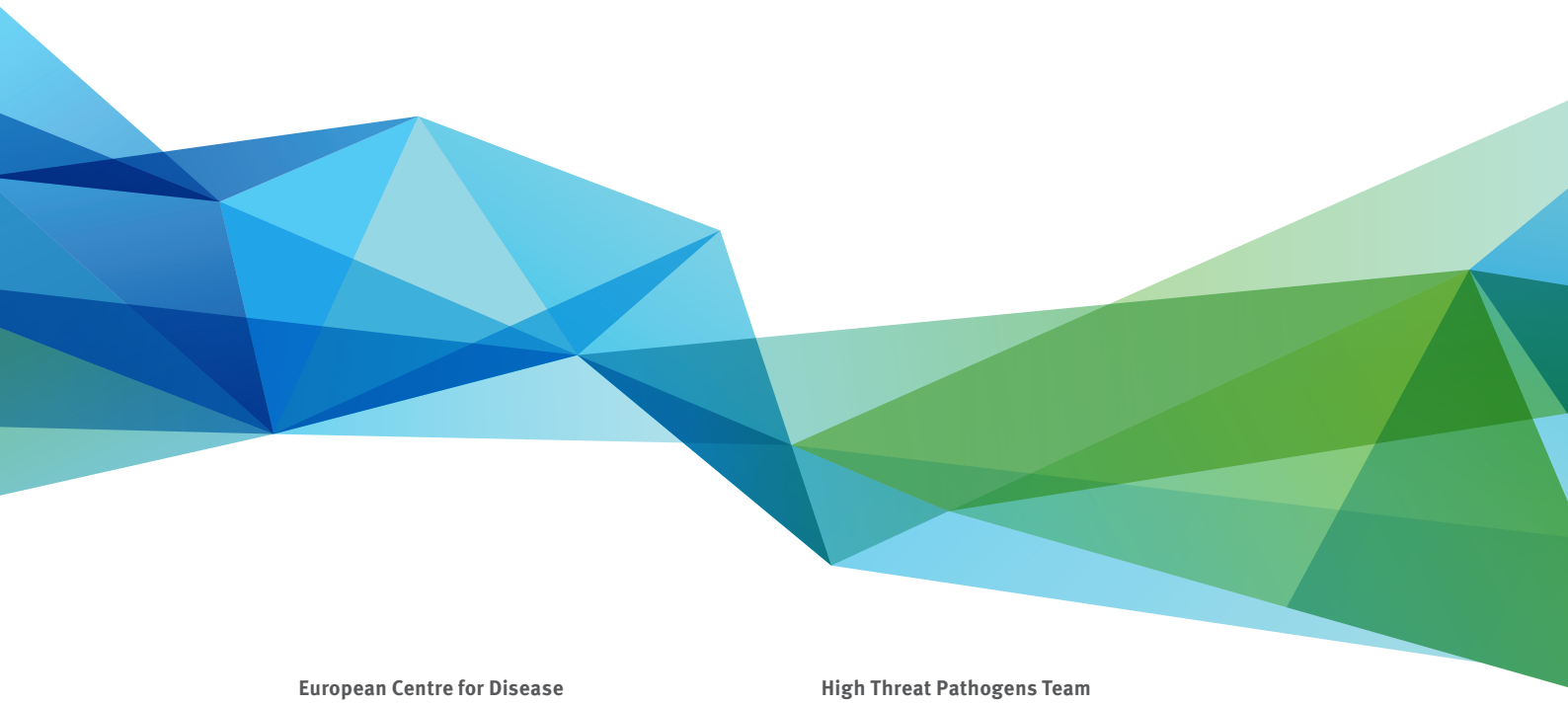

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