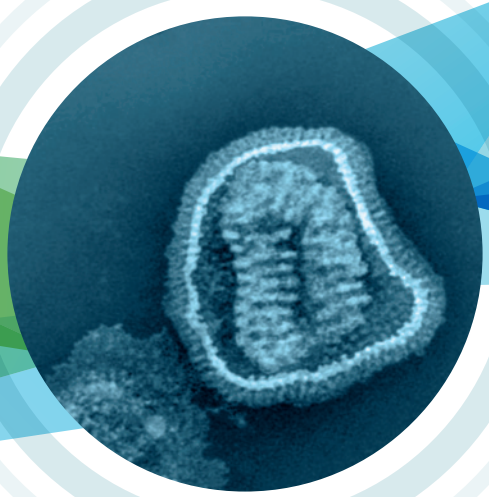
ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

World Health Organization
REGIONAL OFFICE FOR Europe



European external quality assessment programme for influenza virus

2018

ECDC/WHO REGIONAL OFFICE FOR EUROPE TECHNICAL REPORT

European external quality assessment programme for influenza virus

2018

This report was jointly commissioned by the European Centre for Disease Prevention and Control (ECDC) (framework contract 'Support of surveillance activities of human influenza in Europe 2017-2021- External quality assessment (EQA)' reference ID 7268; first specific contract 1 ECD.7737 implementing Framework Contract ECDC/20I7/002), coordinated by Cornelia Adlhoch (ECDC) and the World Health Organization (WHO) Regional Office for Europe (Agreement for Performance of Work 'External Quality Assurance (EQA) for influenza virus culture and antiviral susceptibility 2018' reference WCCPRD6918934 2018/804002), coordinated by Dmitriy Pereyaslov (WHO). It was produced by Dr Adam Meijer, National Institute for Public Health (RIVM), the Netherlands, on behalf of participants in the European Reference Laboratory Network for Human Influenza (ERLI-Net) in the European Union/European Economic Area (EU/EEA) and the network of National Influenza Centres (NICs) in the WHO European Region.

Authors

Gabriel Goderski, Ton Marzec, Mariam Bagheri, Pieter Overduin, Sharon van den Brink and Lisa Wijsman (RIVM) and Ruud van Beek and Prof Dr Guus Rimmelzwaan (Erasmus Medical Centre, Department of Viroscience, Rotterdam, the Netherlands) contributed to the design and planning of the exercise and production of the panel at the Respiratory Viruses Group, Division of Virology, Department of Respiratory and Enteric Viruses, Centre for Infectious Diseases Research, Diagnostics and Laboratory Surveillance, RIVM, Bilthoven, the Netherlands.

Pre-testing of the panel with specimens labelled in random order was performed at Erasmus Medical Centre, Department of Viroscience, Rotterdam, the Netherlands and Centre National de Référence Virus des Infections Respiratoire, Laboratoire de Virologie, Institut des Agents Infectieux, Lyon, France.

Panel distribution and data services were provided by Quality Control for Molecular Diagnostics (QCMD), Glasgow, UK.

Natalja Kuznetsova (NIC-Tallinn, Estonia) took care of Russian documentation translations and Russian-speaking laboratory support.

Colin Steel and Caterina Di Lorenzo, QCMD, and Dr Adam Meijer, RIVM, worked jointly to perform an initial analysis on the submitted data. Further analysis and report preparation were done by Dr Meijer.

Suggested citation: European Centre for Disease Prevention and Control and WHO Regional Office for Europe. European external quality assessment programme for influenza virus – 2018. Stockholm and Copenhagen: ECDC and WHO; 2019.

Stockholm, February 2019

ISBN 978-92-9498-305-3
DOI 10.2900/689354
Catalogue number TQ-04-19-132-EN-N

Contents

Figures

Tables

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
AV18	Influenza antiviral susceptibility determination specific specimens of the EEIQAP 2018 panel
CDC	Centers for Disease Control and Prevention
CPA	Clinical Pathology Accreditation
CPE	Cytopathic effect
Ct	Cycle threshold
EEIQAP	European External Influenza Quality Assessment Programme
EISN	European Influenza Surveillance Network
EISNINF_MD18	Programme in EEIQAP 2018 comprising molecular detection, typing, type A H- and N-subtyping and type B lineage determination
EISNINF_VI18	Programme in EEIQAP 2018 comprising virus isolation and antigenic and genetic characterisation
EISNINF_VS18	Programme in EEIQAP 2018 comprising antiviral (neuraminidase inhibitors) susceptibility determination
EISS	European Influenza Surveillance Scheme
ELISA	Enzyme linked immunosorbent assay
EQA	External quality assessment
EQAP	External quality assessment project
ErasmusMC	Erasmus Medical Centre
ERLI-Net	European Reference Laboratory Network for Human Influenza
GISAID	Global Initiative on Sharing All Influenza Data
GISRS	Global Influenza Surveillance and Response System
HA	Haemagglutinin
HI	Haemagglutination inhibition
HRI	Highly reduced inhibition
IC_{50}	50% inhibitory concentration
INF18	Influenza virus detection, typing, subtyping and lineage determination, virus isolation and genetic and antigenic characterisation and antiviral susceptibility determination specimens of EEIQAP 2018 panel
IQR	Interquartile range
ITEMS	Information Technology EQA Management System
MDCK	Madin-Darby Canine Kidney
MUNANA	20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid
NA	Neuraminidase
NAMRU	Naval Medical Research Unit
NAT	Nucleic acid amplification technologies
NGS	Next-generation sequencing
NI	Normal inhibition
NIC	National influenza centre
NIP	No interpretation possible due to partial NA segment information
nM	Nanomolar
NP	Not performed
PCR	Polymerase chain reaction
PHE	Public Health England
QCMD	Quality Control for Molecular Diagnostics
RBC	Red blood cells
RI	Reduced inhibition
RIVM	National Institute for Public Health and the Environment
RT-PCR	Reverse transcription polymerase chain reaction
SIAT	Human alpha-2,6-sialyltransferase
SKML	Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
$TCID_{50}$	50% tissue culture infectious dose
TESSy	The European Surveillance System
VN	Virus neutralisation
VTM	Virus transport medium
WHO CC	WHO Collaborating Centre for Reference and Research on Influenza
WHO CC London	WHO CC at the Francis Crick Institute Worldwide Influenza Centre

Executive summary

Influenza epidemics occur every winter with high impact on disease burden, hospitalisations and excess mortality in countries in the 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 of the WHO Global Influenza Surveillance and Response System (GISRS). External quality assessments (EQAs) are important instruments 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 ECDC-WHO Regional Office for Europe weekly influenza update (http://www.flunewseurope.org).

From March through July 2018, 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 characterisation and antiviral susceptibility testing. It was the fifth detection, isolation and strain characterisation panel and the fourth antiviral susceptibility testing panel that have all been organised with the support of the European Union (EU) and ECDC. EEIQAP 2018 was organised by the contractor RIVM for the European Reference Laboratory Network for Human Influenza (ERLI-Net; previously called Community Network of Reference Laboratories for Human Influenza in Europe; CNRL) with the support of ECDC. Participation in EEIQAP 2018 by laboratories in countries outside the EU/European Economic Area (EU/EEA) region was supported by the WHO Regional Office for Europe. For the first 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 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) influenza virus isolation and strain characterisation using antigenic and/or genetic techniques and antiviral susceptibility testing using genetic and/or phenotypic techniques, within a defined reporting timeframe.

This exercise aimed to provide participants with an independent assessment of their own laboratory's performance and a comparison with other reference laboratories for influenza in the region. Additionally, performance in EEIQAP 2018 provided a validity check of data reported to TESSy. All 63 reference laboratories for influenza in the region were actively approached to participate and 56 laboratories in 46 of the 50 countries with at least one reference laboratory for influenza participated in at least one of the three components of EEIQAP 2018.

The panel consisted of seven simulated clinical specimens containing variable amounts of live virus (one A(H1N1)pdm09 clade 6B.1, two A(H3N2) clade 3C.2a1, two B/Victoria clade 1A, two B/Yamagata clade 3), one negative simulated clinical specimen and two inactivated specimens, one containing A(H1N1)pdm09 N1-275YH mix showing highly reduced inhibition by oseltamivir and normal inhibition by zanamivir and one containing B/Victoria NA-E195K showing reduced inhibition by oseltamivir and highly reduced inhibition by zanamivir.

One of the 56 laboratories performed virus detection by virus isolation only. As with previous panels, the performance of 55 laboratories for rapid molecular detection, A/B typing and type A H-subtyping was of high accuracy, with 52 (95%) of these laboratories reporting correct results. Of the 21 laboratories that reported on the type A N-subtype, all reported correct results. Except for one laboratory that reported all results in the incorrect random order, 45/46 (98%) laboratories that reported on the type B lineage returned correct results. One laboratory reported a false positive result detecting type B influenza virus in the negative specimen and another a false negative result for an A(H3N2) influenza virus containing specimen. 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 reported to TESSy.

Of the 44 laboratories participating in the virus isolation component, 27 (61%) reported correct results. This low percentage was mainly caused by the failure of 17/44 (39%) laboratories to isolate type B influenza viruses from one or more of the four type B influenza virus containing specimens. In 2013, when the panel had also included two type B viruses of each lineage (but different from the 2018 panel), only 4/32 (13%) laboratories failed at influenza type B virus isolation. The percentage of laboratories capable of isolating the virus from the four type B influenza virus containing specimens in the EEIQAP 2018 ranged from 30/44 (68%) for the specimen with the lowest isolation rate through 40/44 (91%) for the specimen with the highest isolation rate. This low performance was possibly linked to a low sensitivity of the applied methods as the specimens with the lowest amount of virus posed greater difficulties. All participants except one succeeded in isolating A(H3N2) viruses. Additionally, three laboratories could not isolate the virus from the specimen with A(H1N1)pdm09 virus. The decreased number of isolated viruses limited the number of viruses that could be antigenically characterised and tested phenotypically for antiviral susceptibility.

Antigenic strain characterisation reported by 32 laboratories was fairly concordant across laboratories for the four type B viruses, ranging from 13/23 (57%) for the specimen with lowest concordance to 20/25 (80%) for the

specimen with highest concordance and the A(H1N1)pdm09 virus (23/30, 77%). The reporting of the antigenic group for A(H3N2) viruses caused some uncertainties as to which group the viruses belonged. Similar numbers of participants reported the A(H3N2) panel viruses as 'old' A/Hong Kong/4801/2014-like vaccine strain (TESSy reporting category for 2016/17 and 2017/18 seasons) or the 'new' A/Singapore/INFIMH-16-0019/2016-like vaccine strain (TESSy reporting category for 2017/18 season). The result for A(H3N2) viruses highly likely reflects the diversity in reagents used for haemagglutination inhibition (HI) assays (source of sera, source species of red blood cells (RBCs), use of oseltamivir to inhibit neuraminidase-associated haemagglutination that both viruses showed) as only two laboratories used virus neutralisation.

Genetic strain characterisation through sequencing of the haemagglutinin (HA) genome segment was more straightforward. Seventy-three percent (19/26) of reporting laboratories provided correct results. Five laboratories assigned A(H3N2) viruses to older clade 3C.2a1 or even clade 3C.2a representative strains, indicating some difficulty in interpreting phylogenetic and amino acid substitution data. Similarly, four laboratories incorrectly allocated B/Victoria viruses to the new deletion subgroups characterised by a 2-amino acid deletion at 162-163 or a 3-amino acid deletion at 162-164 in the HA as the test viruses did not have these deletions. One laboratory allocated a B/Yamagata virus to a B/Victoria category.

The results for antigenic and genetic characterisation indicate that weekly analysis and interpretation of these data during the season requires some caution. However, accession numbers for HA sequences are also 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 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. All 28 laboratories participating in the antiviral susceptibility component of EEIQAP 2018 correctly identified the A(H1N1)pdm09 N1-H275Y amino acid substitution, although only 17/28 (61%) identified the N1-275YH mixed nature of the specimen. Of the 25 laboratories testing the B/Victoria NA-E105K specimen, 23 (92%) identified the amino acid substitution. One laboratory erroneously reported the R292K HRI substitution as both wild-type A(H3N2) viruses and two laboratories erroneously reported the H275Y HRI substitution as wild-type A(H1N1)pdm09 virus. A major challenge was the correct interpretation of the amino acid composition analysis data in the context of the level of testing, e.g., single nucleotide polymorphism (SNP) test versus full-length neuraminidase (NA) genome segment sequencing. A definitive conclusion can only be drawn when detecting a substitution associated with (highly) reduced inhibition or sequencing full-length NA. Of the 28 laboratories, 16 (57%) reported one or more interpretation errors. These data need to be interpreted with some caution. Accession numbers for NA sequences are also reported to TESSy and offer an opportunity for additional analysis and validation of the reported amino acid composition data.

Phenotypic antiviral susceptibility results from the 23 laboratories that participated in this component of the EEIQAP 2018 were highly accurate for all specimens except one, including a (highly) reduced inhibited phenotype for the A(H1N1)pdm09 N1-275YH mixture-containing specimen. The exception was the B/Victoria NA-E105K-containing specimen tested for oseltamivir susceptibility, for which seven laboratories reported a too low IC_{50} and hence a 'normal inhibited' instead of 'reduced inhibited' phenotype. This result is partly due to the use of chemiluminescent neuraminidase inhibition assays; the four laboratories that used this type of assay reported this artefact. Three laboratories incorrectly identified one wild-type B/Victoria that had been isolated in the laboratory with a 'reduced inhibited' phenotype. However, one laboratory reported that this was caused by outgrowth of a minority variant with NA-T106P amino acid substitution present in the simulated clinical specimen. Virus isolation-induced reduced inhibition is a phenomenon laboratories should be aware of. Only one IC_{50} data interpretation error was made classifying the A(H1N1)pdm09 N1-275YH mix as 'reduced inhibited' instead of 'normal inhibited' by zanamivir.

The majority of laboratories (43/55, 78%) are ISO 15189-accredited or are preparing to apply for it and about 90% are required to participate in EQAs for detection and A/B typing, type A subtyping and B lineage determination to obtain and maintain this accreditation. However, only 44%-67% of laboratories require EQA participation for the other tests challenged in EEIQAP 2018. Adding all these tests to the scope of ISO 15189 accreditation could provide an additional instrument for systematically addressing issues identified by EQA.

The participating laboratories were provided the expected results with some background information as soon as the last laboratory had reported its results. After the analysis of the results, the final draft version of the current report was provided to the individual laboratories together with the participant code in order to assess their own results in the context of those achieved by other laboratories.

In conclusion, the overall performance of the participating laboratories was good. Certain laboratories are encouraged to enhance their testing performance by evaluating the sensitivity and specificity of the assays in place and apply necessary updates accordingly. Other issues (e.g. incorrect translation into TESSy categories) will be addressed jointly with ECDC and the WHO Regional Office for Europe through training or adapting validation and analysis of data captured in TESSy (e.g. making better use of reported HA and NA sequences and organising/providing training, especially focusing on sequence analysis and 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 may lead to antigenic shift if new H- and/or N-subtypes are introduced to which humans are vulnerable. This process is particularly important in influenza A virus because of its potential to generate new pandemic strains [7–8].

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 virologic surveillance of influenza in the WHO European Region is part of the WHO Global Influenza Surveillance and Response System (GISRS) [1]. The network consists of national influenza laboratories in 50 countries in 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 [2] and three WHO H5 reference laboratories in France, Russia and the United Kingdom [3]. National influenza laboratories in 43 countries in the WHO European Region are recognised by WHO as national influenza centres (NICs) [4] and laboratories in 30 countries of the EU/EEA participate in the European Reference Laboratory Network for Human Influenza (ERLI-Net) coordinated by ECDC [5]. The European Influenza Surveillance Network (EISN), which includes 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 (NAT) has led to the replacement of less sensitive rapid antigen detection assays by molecular methods that can rapidly detect influenza viruses with high sensitivity and specificity. These new techniques allow for simultaneous identification of the type of virus (A or B), the H- and N-subtype of influenza A viruses (H1N1, H3N2, H7N9) and the genetic lineage of influenza B viruses (B/Victoria/2/87-like: Victoria and B/Yamagata/16/88-like: Yamagata). As a result, these tests assume great practical relevance in diagnosing individual patients and surveillance.

Through 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 may escape (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 neuraminidase genome segment provides useful information on known markers for (highly) reduced inhibition by neuraminidase inhibitors (oseltamivir and zanamivir). However, the ability to accurately determine the antigenic susceptibility profile of an influenza virus still requires the ability to isolate the virus in cell culture or embryonated eggs and carry out serological tests (haemagglutination inhibition [HI] or virus neutralisation [VN] assays). Likewise, virus isolates are phenotypically tested (neuraminidase enzyme activity inhibition assays) to measure their level of susceptibility to neuraminidase inhibitors.

It is essential to assess such technologies through effective quality control to ensure the reliability and comparability of results reported to physicians and disease surveillance systems at the national and European levels [9]. An integral part of quality control is performed through external quality assessments (EQAs), which provide a means of independent and objective laboratory performance evaluation. Starting with the predecessors of EISN, the influenza laboratory network in Europe has performed EQA studies on all aspects of laboratory influenza surveillance as described above. The first was performed in 2000, with antiviral susceptibility testing added in 2010 [10–12]. The European influenza EQA programme was the first of its kind to include virus isolation, strain characterisation and antiviral susceptibility determination. Molecular EQA is covered bythe WHO external quality assessment programme (EQAP) for NICs since 2007 [13]. In the past, the European Influenza Surveillance Scheme (EISS) also participated in other international programmes that included human and zoonotic avian influenza virus subtypes [14]. Antiviral susceptibility determination has been added on an optional basis to the WHO EQAP since 2013, but only targeting A(H1N1)pdm09 NA-H275Y [13]. There is no international EQA programme available for virus isolation and antigenic and genetic strain identification, although the first virus isolation EQA was reported from the WHO South East Asia and Western Pacific Regions in 2017 [15]. However, that EQA did not include strain identification through antigenic or genetic characterisation. Hence, the European External Influenza Quality Assessment Programme (EEIQAP) still fills 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 (http://www.flunewseurope.org).

In 2018, a framework contract with ECDC was put in place for EEIQAP covering the period from 2017–2021 to ensure the reliability and comparability of results reported to TESSy and identify needs for improvement in laboratory influenza surveillance and diagnostic capability. An Agreement for Performance of Work with the WHO

Regional Office for Europe ensured the participation of NICs in countries outside the EU/EEA in EEIQAP 2018. For the first time, results are jointly presented for the WHO European Region as a whole.

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 [9]:

- 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); and
- preparedness (e.g., avian influenza viruses).

The operational public health objectives of ECDC-supported EQAs are [9]:

- assessment of the quality and comparability of surveillance data reported by EU/EEA Member States; and
- 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 EQAs, as also intended for the current EEIQAP 2018, include:

- assessment of 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; and
- identification of training needs.

This report presents the results of EEIQAP 2018 for influenza reference laboratories in the WHO European Region, designed and prepared by the contractor and funded by ECDC and the WHO Regional Office for Europe.

The major objective of EEIQAP 2018 was to assess the performance of individual influenza reference laboratories in the following areas:

- rapid detection by reverse transcription polymerase chain reaction (RT-PCR) or other NAT, 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 time frame of 36 calendar days; and
- determination of susceptibility to neuraminidase inhibitors oseltamivir and zanamivir by genotypic and/or phenotypic methods within a defined reporting time frame of 36 calendar days.

Although objective 1 is covered by WHO EQAP and other available EQA schemes and objective 3 is partly covered by WHO EQAP, objective 2 is not covered by any widely available EQA scheme and none integrate all three objectives in one comprehensive panel.

A secondary objective was to collect information on the (desired) accreditation status of laboratories and how EQA fits into this accreditation.

2 Study design

2.1 Organisation

The EEIQAP panel was designed by staff from the contractor, National Institute for Public Health and the Environment (RIVM), and the final composition was agreed upon with ECDC. The panel was prepared and tested by the Respiratory Viruses Group of the Department Emerging and Endemic Viruses, Division of Virology, Centre for Infectious Diseases Research, Diagnostics and Laboratory Surveillance, RIVM, Bilthoven, the Netherlands. Further pretesting was performed by subcontractors Erasmus Medical Centre (ErasmusMC), Department of Viroscience, Rotterdam, the Netherlands and Centre National de Référence Virus des Infections Respiratoire, Laboratoire de Virologie, Institut des Agents Infectieux, Lyon, France. The panel contents, frozen on dry ice, were distributed to participants from March to July 2018 by specialist courier organised by subcontractor QCMD. Participants submitted results to a web-based database, Information Technology EQA Management System (ITEMS), that is hosted by QCMD and was adapted to the needs of EEIQAP 2018.

2.2 Panel composition, preparation and validation

The EEIQAP panel consisted of eight simulated clinical specimens containing live influenza viruses of subtypes 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. In addition, two specimens of two vials each specifically designed for antiviral susceptibility testing were included in the panel that contained inactivated virus with amino acid substitutions causing HRI or RI by the neuraminidase inhibitors oseltamivir and/or zanamivir (Table 1).

Table 1. Panel composition, 2018 EEQIAP

Specimen code EISN_	Matrix[1]	Strain; antiviral amino acid substitution; GISAID isolate accession number[2]	Type and subtype/lineage	Ct value[3]	$TCID_{50}$/ml[4]	IC_{50} (nM)[5]	
						O	Z
INF18-01	A549 cells in VTM	A/Netherlands/751/2017; none; EPI_ISL_270970	A(H3N2)	15.92	2795	0.10	0.30
INF18-02	A549 cells in VTM	B/Netherlands/2424/2017; none; EPI_ISL_255661	B/Yamagata	18.04	2795	24	3.0
INF18-03	A549 cells in VTM	B/Netherlands/076/2014; none; EPI_ISL_166326	B/Victoria	17.15	559	22	3.1
INF18-04	A549 cells in VTM	A/Netherlands/502/2017; none; EPI_ISL_247427	A(H1N1)pdm09	16.82	1250	0.39	0.44
INF18-05	A549 cells in VTM	No influenza virus	n/a	n/a	n/a	n/a	n/a
INF18-06	A549 cells in VTM	B/Netherlands/2423/2017; none; EPI_ISL_255660	B/Victoria	19.09	167	15	3.2
INF18-07	A549 cells in VTM	A/Netherlands/757/2017; none; EPI_ISL_270971	A(H3N2)	25.79	4180	0.13	0.25
INF18-08	A549 cells in VTM	B/Netherlands/3066/2015; none; EPI_ISL_233380	B/Yamagata	16.57	374	14	1.4
AV18-01	VTM; Triton X-100-inactivated	Mixture of A/Netherlands/3165/2017 NA-275Y (EPI_ISL_312679) and A/Netherlands/502/2017 NA-275H A(H1N1)pdm09	A(H1N1)pdm09 (provided)	12.35	n/a	135	0.36
AV18-01	VTM; heat-inactivated	Mixture of A/Netherlands/3165/2017 NA-275Y (EPI_ISL_312679) and A/Netherlands/502/2017 N1-275H A(H1N1)pdm09	A(H1N1)pdm09 (provided)	13.15	n/a	n/a	n/a
AV18-02	VTM; formalin-inactivated	B/Netherlands/2518/2016; NA-E105K; EPI_ISL_239749	B/Victoria (provided)	15.94	n/a	375	338
AV18-02	VTM; heat-inactivated	B/Netherlands/2518/2016; NA-E105K; EPI_ISL_239749	B/Victoria (provided)	20.53	n/a	n/a	n/a

[1]VTM: virus transport medium.
[2]For all panel members, HA and NA segments are available in GISAID; for 5 full genomes and for 1 full genome except PB2.
[3]Matrix-gene based RT-PCR for type A influenza viruses and non-structural gene based RT-PCR for type B influenza viruses.
[4]50% tissue culture infectious dose ($TCID_{50}$) determined on Madin-Darby Canine Kidney (MDCK)-MIX cells, MDCK-I and MDCK cells stable expressing human alpha-2,6-sialyltransferase (MDCK-SIAT) in 96-well plate, static incubation and end-point determination of cytopathic effect (CPE) in each well; this produces a valuable estimate for relative comparison of the amount of live virus in the panel members used for virus isolation, but likely produces an underestimate of the true number of live viruses in the panel members as using routine rolling tube virus isolation in contractors' laboratory dilution of these panel members was positive in virus isolation well below the 0 $TCID_{50}$ dilution. n/a: not applicable.
[5]IC_{50}: 50% inhibitory concentration; O: oseltamivir; Z: zanamivir; n/a: not applicable.

All viruses were selected based on known antigenic (Annex Figure 1), genetic (Annex Figure 2) and antiviral susceptibility characteristics (Table 1) previously determined at RIVM and ErasmusMC. All viruses were grown in monolayers of 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. Initially, the specimens with viruses exhibiting reduced inhibition by antivirals were inactivated with 1% Triton X-100 for one hour at room temperature as this procedure preserves neuraminidase activity and RNA quality for direct sequencing [18]. However, validation of these panel specimens at RIVM showed that Triton X-100 inactivation of the specimen containing the B/Victoria NA-E105K mutant caused an atypical inhibition curve with neuraminidase inhibitors different from that obtained with native

virus, complicating IC_{50} determination (Annex Figure 3). Using 0.02% formalin inactivation for 18 hours at 37°C [18] of this virus, the inhibition curve was similar to that of the native live virus, although with a slightly higher IC_{50} (Annex Figure 3). Therefore, specimen EISN_AV18-02 containing this virus was prepared using formalin inactivation.

A mixture of wild-type and N1-H275Y A(H1N1)pdm09 virus for specimen EISN_AV18-01 was prepared using the wild-type specimen EISN_INF18-04 virus to be able to compare the IC_{50} results of specimen EISN_AV18-01 with that of the wild-type specimen. Triton X-100 inactivation shifted the IC_{50} of the mixture for oseltamivir slightly to a higher value, but still with an inhibition curve having a typical slope for a mixture (Annex Figure 3). The live virus specimens were diluted to a $TCID_{50}$ high enough for successful virus isolation in static virus isolation procedures in microtitre plates (Table 1) and the inactivated virus specimens were diluted to a neuraminidase enzyme activity high enough for direct use in phenotypic antiviral susceptibility testing. The live virus specimens were prepared in a virus transport medium (VTM) with a final concentration of $1x10^5$/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 sent frozen on dry ice to the two independent laboratories for pretesting. Pretesting by these laboratories also included pretesting of the online reporting system ITEMS at QCMD. During pretesting of the inactivated virus specimens and follow-up study at RIVM, it became clear that the inactivation procedure using Triton X-100 or formalin preserved the neuraminidase activity well, but that with longer storage times at -80°C, one-step full-length sequencing of the neuraminidase segment became cumbersome. Using heat inactivation for one hour at 60°C and longer storage time at -80°C worked well for one-step full-length neuraminidase segment sequencing. Because heat inactivation inactivates the neuraminidase [18], the specimens for antiviral susceptibility testing were inactivated by Triton X-100 or formalin for phenotypic testing and separately by heat for genotypic testing. The final panels were shipped frozen on dry ice to the laboratories from March to early July 2018. Expected results for all panel members are listed in Tables 2 and 3. For a final check on the viability of live viruses in the panel, one panel stored frozen at -80°C similar to the distributed panels was thawed at the end of September 2018 and the specimens on MDCK-MIX cells at the RIVM were cultured. All specimens with live virus became positive within seven calendar days, confirming viability after long storage time and freeze-thaw cycles similar to that in the participating laboratories.

2.3 Participation

Participation in EQAs is one of the key tasks of ECDC ERLI-Net laboratories [5] and plays a key role in strengthening the WHO GISRS' diagnostic capacity and preparedness to effectively respond to influenza outbreaks [1]. Participation in components of EEIQAP 2018 for which NICs/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 2018 exercise in advance on 19 January (EU/EEA by ECDC) and 22 January 2018 (non-EU/EEA by WHO) and asked to sign up by 10 February 2018 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 participants in EEIQAP 2018 can be found in Annex Table 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 components of EEIQAP 2018 using the tests routinely used in the laboratory, the weekly results of which are reported to TESSy. If usual laboratory procedures included virus isolation and strain characterisation and/or antiviral susceptibility testing and resulting data were reported to TESSy, it was strongly recommended to also complete the corresponding parts of EEIQAP 2018. For any tests used, the laboratories were asked to provide detailed information.

The laboratories were instructed to test for:

- EISNINF_MD18 programme – molecular detection, typing, type A H- and N-subtyping and type B lineage determination; specimens EISN_INF18-1 through -8) using RT-PCR or other NAT
- EISNINF_VI18 programme – virus isolation and antigenic and genetic characterisation; specimens EISN_INF18-1 through -8. Virus isolation was to be performed in cell culture and/or embryonated eggs. For antigenic characterisation, the viruses had to be isolated and propagated first, followed by HI assay or VN. For genetic characterisation, the simulated clinical specimen or virus isolate had to be sequenced by Sanger or next-generation sequencing (NGS) techniques.
- EISNINF_VS18 programme – antiviral (neuraminidase inhibitors) susceptibility determination; specimens EISN_AV18-1 and -2 and specimens EISN_INF18-1 through -8 by available phenotypic (neuraminidase enzyme activity inhibition assay) and/or genotypic (SNP RT-PCR, Sanger sequencing or NGS or

pyrosequencing) antiviral susceptibility determination methods. EISN_AV18 tubes labelled 'phenotypic' contained virus inactivated by validated methods that retain neuraminidase activity and could be used directly for phenotypic antiviral susceptibility testing. The EISN_AV18 tubes labelled 'sequencing' contained virus inactivated by heat-preserving RNA integrity and could be used directly for genotypic antiviral susceptibility testing, including one-step full segment Sanger or NGS. EISN_INF18-1 through -8 specimens first had to be isolated and propagated for phenotypic antiviral susceptibility testing.

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 and 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 adapted for collating EEIQAP 2018 data. The documentation for reporting to TESSy for the 2016/17 and 2017/18 seasons and guidance for strain-based reporting of antiviral susceptibility and antigenic and genetic characterisation data were used for adapting ITEMS [19–21]. The expected results for each programme are displayed in Tables 2–3.

For the molecular detection programme of EEIQAP 2018, 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 in predefined categories with the 'other' option.

For strain characterisation, participants were asked to report the result of antigenic and/or genetic characterisation using drop-down lists with categories reflecting the TESSy categories for the 2016/17 and 2017/18 seasons. In addition, details were asked on the methods used for antigenic and/or genetic characterisation.

For genotypic antiviral susceptibility testing, participants were asked to report the relevant amino acid positions screened as marker positions for a change in susceptibility to oseltamivir and zanamivir with the amino acid found in the format as requested for reporting to TESSy.

Table 2. Expected results panel, EEQIAP 2018 molecular detection, virus isolation and antigenic and genetic characterisation

Specimen code	Programme EISNINF_MD18	Programme EISNINF_VI18			
	Type and subtype/lineage	Virus isolation	Type and subtype/lineage	Antigenic category[1]	Genetic category[2]
EISN_INF18-01	A(H3N2)	Positive	A(H3N2)	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like (has neuraminidase induced HA)	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup
EISN_INF18-02	B/Yamagata	Positive	B/Yamagata	B(Yam) lineage not attributed to category (low reactor B/Phuket/3073/2013)	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013
EISN_INF18-03	B/Victoria	Positive	B/Victoria	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage)	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008
EISN_INF18-04	A(H1N1)pdm09	Positive	A(H1N1)pdm09	A(H1)pdm09 A/California/7/2009 (H1N1)-like	A(H1)pdm09 group 6B.1 representative A/Michigan/45/2015
EISN_INF18-05	n/a	Negative	n/a	n/a	n/a
EISN_INF18-06	B/Victoria	Positive	B/Victoria	B(Vic) lineage not attributed to category; low reactor B/Brisbane/60/2008	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008
EISN_INF18-07	A(H3N2)	Positive	A(H3N2)	A(H3) A/Hong Kong/4801/2014 (H3N2)-like (has neuraminidase induced HA)	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup
EISN_INF18-08	B/Yamagata	Positive	B/Yamagata	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage)	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013

[1]*Annex Figure 1*
[2]*Annex Figure 2.*

Table 3. Expected results panel, EEQIAP 2018 antiviral susceptibility

Specimen code	Programme EISNINF_VS18				
	Phenotypic[1]		Genotypic (expected result when full NA is sequenced)[2]		
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Accession number NA in GISAID
EISN_INF18-01	NI	NI	AANI	AANI	EPI1030650
EISN_INF18-02	NI	NI	AANI	AANI	EPI991483
EISN_INF18-03	NI	NI	AANI	AANI	EPI541780
EISN_INF18-04	NI	NI	AANI	AANI	EPI959344
EISN_INF18-05	n/a	n/a	n/a	n/a	n/a
EISN_INF18-06	NI	NI	AANI	AANI	EPI975564
EISN_INF18-07	NI	NI	AANI	AANI	EPI1030658
EISN_INF18-08	NI	NI	AANI	AANI	EPI832038
EISN_AV18-01	HRI	NI	AAHRI N1-H275Y(mixH/Y) [3]	AANI	EPI1244483 (H275Y)/EPI959344
EISN_AV18-02	RI [4]	HRI [4]	AANI for NA-E105K based on published phenotypic data[5] AARI for NA-E105K based on phenotypic data EISN_AV18-02 virus isolate[4]	AARI for NA-E105K based on published phenotypic data[5] AAHRI for NA-E105K based on phenotypic data EISN_AV18-02 virus isolate[4]	EPI868728

[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]AAHRI also correct when determined using partial NA segment sequencing, pyrosequencing or SNP RT-PCR.
[4]Fold-change compared to median IC_{50} of recent Dutch B/Victoria viruses for oseltamivir determined at 32 (RI) and for zanamivir at 115 (HRI) at the contractors' laboratory.
[5]According to WHO reference table:
http://www.who.int/influenza/gisrs_laboratory/antiviral_susceptibility/NAI_Reduced_Susceptibility_Marker_Table_WHO.pdf.

Participants were also asked to provide an interpretation of the generated results using drop-down lists with the categories used for reporting to TESSy, taking into account the level of testing, e.g. SNP test versus full length neuraminidase (NA) genome segment sequencing. A 'No interpretation for this drug possible from testing performed' category due to incomplete analysis of the neuraminidase segment was added. For phenotypic antiviral susceptibility testing, participants were asked to report IC_{50} values for oseltamivir and zanamivir and provide an interpretation of their results using drop-down lists with the categories used for reporting to TESSy. For both methodologies, details to put the reported results in the context of the used methodologies were asked. Additional data were collected on the accreditation status of laboratories, which EQA programmes they participate in for each aspect of laboratory surveillance of influenza and whether these are required for accreditation.

2.6 Data analysis

All challenges of EEIQAP 2018 were considered 'educational' and therefore no pass/fail criteria were defined. A scoring system was used in which a correct result for a specimen 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 (Figure 2 footnote). For each challenge, each laboratory received a cumulative performance score by summing up the individual specimen scores. For network performance, the percentages of laboratories with a specific cumulative performance score were calculated and plotted in overview bar graphs. The detailed scoring system used for each challenge is provided in the footnotes to these graphs and the individual laboratory result tables in the annexes. In addition, explanations of the judgements to conclude an error are provided for individual specimens in the footnotes of the individual laboratory result tables in the annexes. If a laboratory did not perform a particular test because it is not available in that laboratory, it was not counted as an error. Accordingly, 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 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 and virus isolation challenges, laboratories had already determined the type/subtype or lineage of the viruses. Since there was no clear proof of confirmation of the type/subtype or lineage of isolated viruses, although reported, these results are neither shown nor included in the scoring.

An 'Expected results' document for self-evaluation was shared with each participant through QCMD ITEMS through an email notification from the QCMD neutral office after the last participant had submitted its results in July 2018. The 'Expected results' document was made available in English and Russian. After the analysis of the results, the final draft version of the current report was provided to the individual laboratories in November 2018 through QCMD ITEMS. This was accompanied by email notification from the QCMD neutral office to each individual participant and included the participant code in order to submit their own results in the context of those achieved by other laboratories.

3 Results

3.1 Participating laboratories

A total of 56 laboratories in 46 of the 53 countries in the WHO European Region participated in EEIQAP 2018: 35 in 28 of the 31 EU/EEA countries and the other 21 in 18 non-EU/EEA countries. An aggregated breakdown of participating laboratories by challenge type is shown in Table 4 and participating laboratory in Annex Table 2. Only 16/56 (29%) laboratories participated in the full programme.

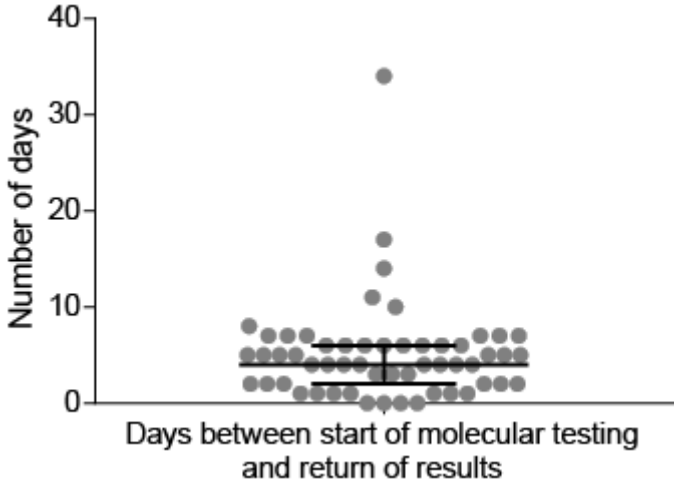
Table 4. Breakdown of number of participants by challenge type

Region	Number of participants for each challenge													
	Molecular detection		Virus isolation		Characterisation				Antiviral susceptibility testing				Full programme	
	Yes	No	Yes	No	Antigenic only	Genetic only	Both	None	Genetic only	Phenotypic only	Both	None	Yes	No
EU/EEA (n=35)	35	0	29	6	6	5	16	8	4	0	18	13	13	22
Total (n=56)	**55**	**1**	**44**	**12**	**12**	**6**	**20**	**18**	**6**	**1**	**22**	**27**	**16**	**40**

3.2 Molecular detection

A total of 55 of the 56 laboratories participated in the molecular detection, typing, type A H- and N-subtype and type B lineage determination challenges. The median delay between starting testing and reporting of results was four calendar days (range 0–34) with 50% (interquartile range) of the reports delayed by 2–6 calendar days, 75% of the reports by six calendar days and 89% available within the seven calendar day limit (Figure 1).

Figure 1. Calendar days between starting molecular testing and return of results for molecular detection, typing and subtyping/lineage determination



Start date of molecular testing taken as proxy for date of panel arrival in the laboratory. Large horizontal bar represents median, whiskers represent interquartile range.

An aggregated breakdown of the results is shown in Table 5 by specimen code, Table 6 by challenge type and Annex Table 3 by participating laboratory. The used methodologies are listed in aggregated form in Annex Figure 4 and by laboratory in Annex Table 3. An overview of the percentage of laboratories by cumulative performance score for detection, A/B typing, type A H-subtyping and the full challenge is shown in Figures 2 and 3 and by participating laboratory in Annex Table 3.

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

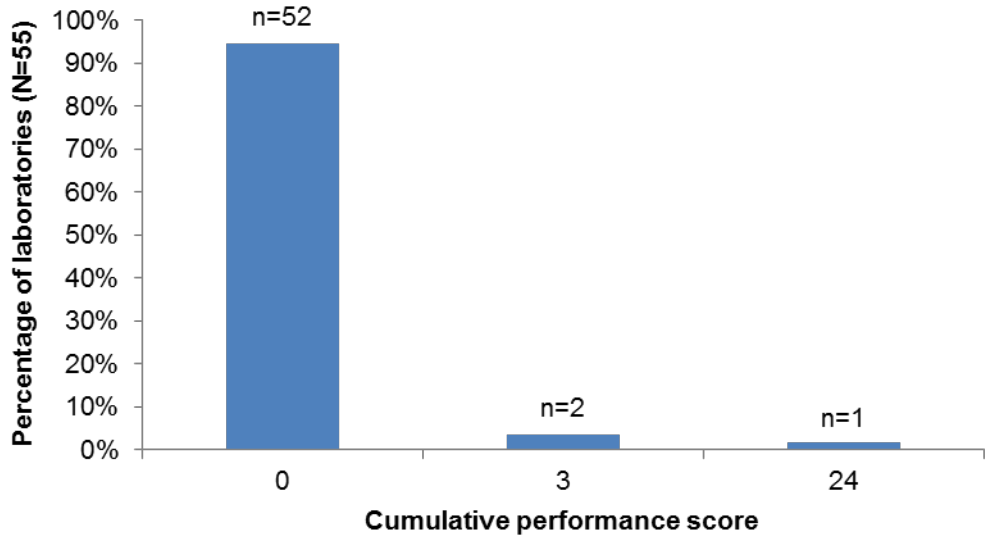
Specimen code (EISN_)	Expected results	Ct value by contractor laboratory	Molecular detection by type, A H- and N-subtyping and B lineage determination (n=55)[1]								
			Correct type & A H-subtype			Correct type and A H- and N-subtype/B-lineage			Incorrect result [2]		
			Result	n	%	Result	n	%	Result	n	%
INF18-01	A(H3N2)	15.9	A+H3	53	96.4	A(H3N2)	18	32.7	Negative	2	3.6
INF18-02	B/Yamagata	18.0	B	54	98.2	B/Yamagata	45	81.8	A(H1)pdm09	1	1.8
INF18-03	B/Victoria	17.2	B	54	98.2	B/Victoria	45	81.8	B/Yamagata	1	1.8
INF18-04	A(H1N1)pdm09	16.8	A+H1pdm09	54	98.2	A(H1N1)pdm09	19	34.5	B/Victoria	1	1.8
INF18-05	Negative	-	Negative	53	96.4	Negative	53	96.4	B; A(H3)	2	3.6
INF18-06	B/Victoria	19.1	B	54	98.2	B/Victoria	45	81.8	B/Yamagata	1	1.8
INF18-07	A(H3N2)	25.8	A+H3	54	98.2	A(H3N2)	19	34.5	B/Victoria	1	1.8
INF18-08	B/Yamagata	16.6	B	54	98.2	B/Yamagata	45	81.8	A(H3)	1	1.8

Ct: cycle threshold.
[1]Methodologies used are shown in Annex Figure 4.
[2]One laboratory reported results for all specimens in the incorrect random order and is therefore considered incorrect for all specimens.

Most laboratories (52/55, 95% with a cumulative performance score of 0) correctly identified influenza virus type and type A H-subtype in all eight panel specimens (Figure 2). One laboratory reported results for all eight specimens in the incorrect random order, one laboratory reported a false negative and another a false positive result (Tables 5–6). Only 17/55 (31%) of the participants 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 3). Many laboratories (34/55, 62%) did not report on type A N-subtyping and 9/55 (16%) did not report on type B lineage determination, likely because such assays were not available in the laboratory (Table 6, Annex Figure 4). Of the 19 laboratories that included type A N-subtyping, 18 (95%) identified the correct N subtype of the three type A influenza viruses included in the panel (Table 6). One laboratory did not detect influenza virus in one of the three type A influenza virus containing specimens and therefore did not perform N-subtyping of this specimen. Excluding the laboratory reporting all results in the incorrect random order, all 45 laboratories that included type B lineage determination correctly identified the lineage of the four influenza virus type B containing specimens (Table 6).

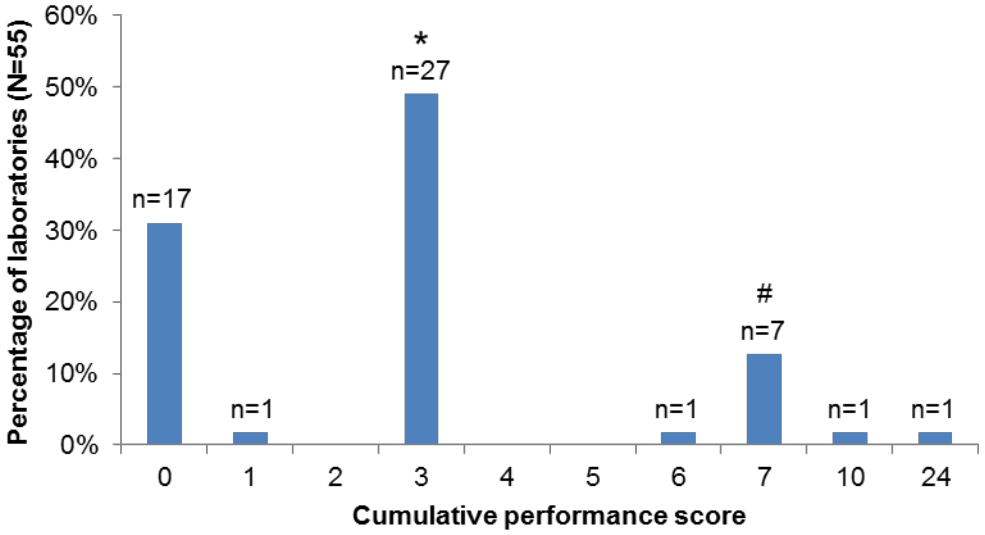
The majority of laboratories used in-house assays or primers and probes from the Centers for Disease Control and Prevention (CDC) for virus detection by type (A/B) (24/55, 44% and 20/55, 36% respectively), type A H-subtyping (22/55, 40% and 20/55, 36% respectively), type A N-subtyping (14/19, 74% and 1/19, 5% respectively) and type B lineage determination (27/46, 59% and 18/46, 39% respectively; Annex Figure 4). The high performance of laboratories using a wide variety of assays for the specific challenge types (Table 6) indicates that none of these assays had any issues (Annex Figure 4).

Figure 2. Overview of cumulative performance scores for molecular detection, typing (A/B) and type A H-subtyping.



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 3. Overview of cumulative performance scores for molecular detection, typing (A/B), type A H-and N-subtyping and type B lineage determination



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.*
#: missing N-subtype of type A influenza viruses and lineage of type B influenza viruses.

Table 6. Overview of molecular detection, typing, type A H- and N-subtype and type B lineage determination results by challenge type

Evaluation	Performance for all 55 participating laboratories					Performance for laboratories that used assays appropriate for indicated challenge type (column one)					
Challenge type (number of specimens with this challenge)	Labs with fully correct result	Labs with error or missing data	Specimens tested in challenge with correct result	Specimens with error or missing data	Type of error (number of specimens)	Labs included in evaluation[1]	Labs with fully correct result	Labs with error	Specimens tested in challenge with correct result	Specimens with error	Type of error (number of specimens)
positive/negative (8)	94.5%	3	97.7%	10	false positive (1); false negative (1); incorrect order (8)	54	96.3%	2	99.5%	2	false positive (1); false negative (1)
type A/B (7)	96.4%	2	97.9%	8	false negative (1); incorrect order (7)	54	98.1%	1	99.7%	1	false negative (1)
H1pdm09 subtype (1)	98.2%	1	98.2%	1	incorrect order (1)	54	100%	0	100%	0	
H3 subtype (2)	96.4%	2	97.3%	3	false negative (1); incorrect order (2)	54	98.1%	1	99.1%	1	false negative (1)
N1pdm09 subtype (1)	34.5%	36	34.5%	36	N-subtype missing (35); incorrect order (1)	19 [2]	100%	0	100%	0	
N2 subtype (2)	34.5%	36	33.6%	73	N-subtype missing (70); false negative (1); incorrect order (2)	19 [2]	98.1%	1	97.4%	1	false negative (1)
Victoria lineage (2)	81.8%	10	81.8%	20	B-lineage missing (18); incorrect order (2)	45 [3]	100%	0	100%	0	
Yamagata lineage (2)	81.8%	10	81.8%	20	B-lineage missing (18); incorrect order (2)	45 [3]	100%	0	100%	0	

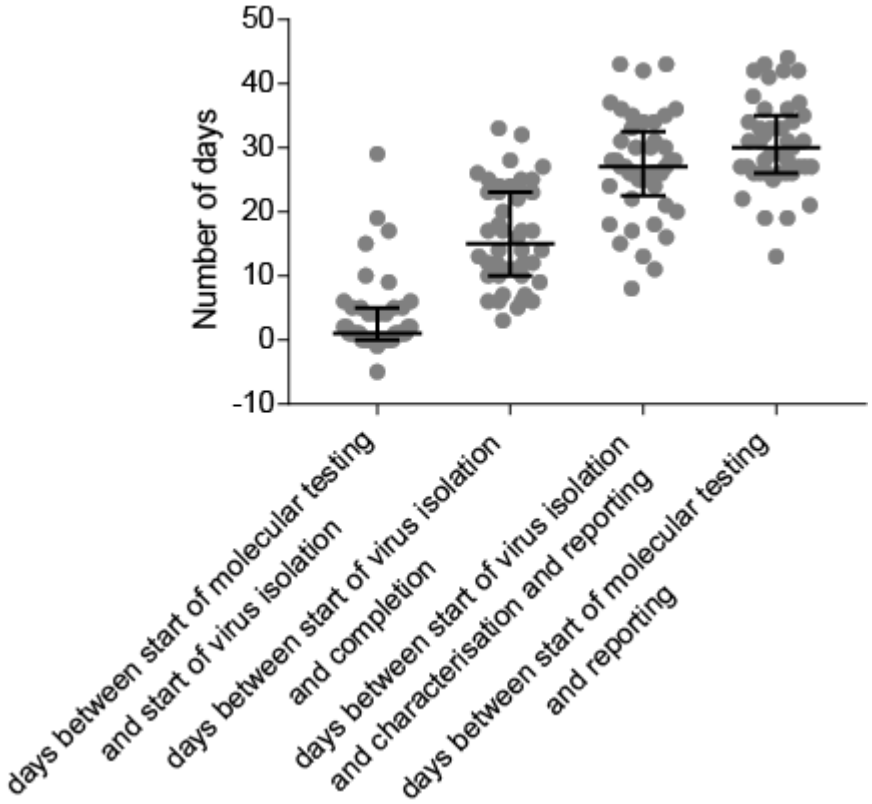
[1]Laboratory excluded that reported results of all specimens in the incorrect random order.
[2]Laboratories excluded that did not report on type A N-subtyping.
[3]Laboratories excluded that did not report on type B lineage determination.

3.3 Virus isolation and antigenic and genetic characterisation

A total of 44 of the 56 laboratories participated in the virus isolation and antigenic and genetic characterisation challenge. All 44 participated in the virus isolation, 20 participated in both the antigenic and genetic characterisation, 12 in antigenic characterisation only and six in genetic characterisation only. Six did not perform characterisations.

With a median of one day (IQR 0–5 days) after start of molecular testing, virus isolation was started (Figure 4). This indicates that virus isolation was started very shortly after confirming the presence of the virus and determining type and type A H-subtype in the specimens, likely to select the appropriate cell type for virus isolation. Virus isolation was completed after a median of 15 days (IQR 10–23 days; Figure 4). Results of this challenge were available 30 days (median; IQR 26–35 days) after starting molecular testing and for 81% of participating laboratories, within in time limit of 36 calendar days (Figure 4). The difference in days between start of virus isolation and reporting characterisations (12 days) indicates the number of days needed for antigenic characterisation.

Figure 4. Calendar days used for virus isolation and antigenic and genetic characterisation



Start date of molecular testing taken as proxy for panel arrival date in laboratory. Large horizontal bars represent median and whiskers interquartile range.

3.3.1 Virus isolation

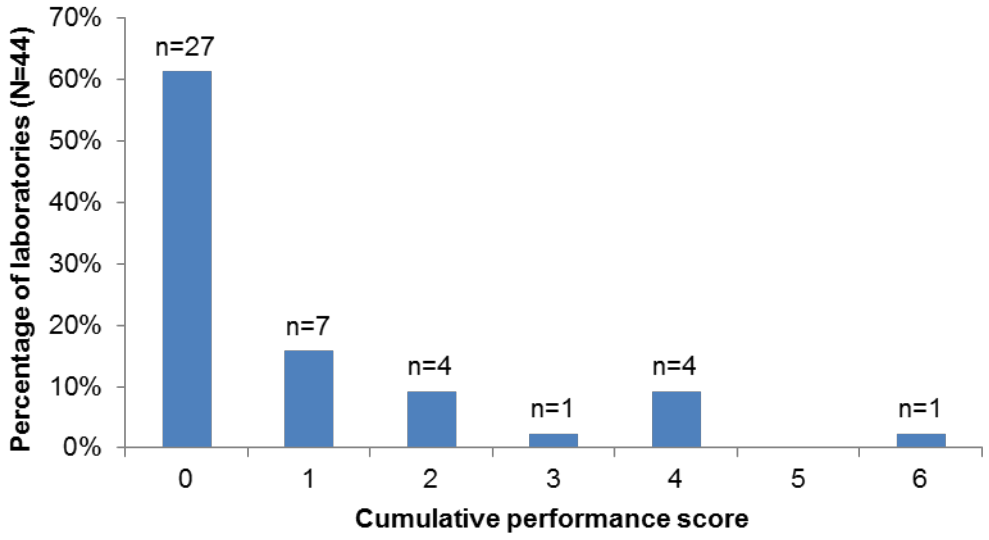
A total of 44 of the 56 laboratories participated in the virus isolation challenge (aggregated breakdown of results in Table 7; by participating laboratory and cumulative performance score in Annex Table 4). The methodologies used are listed in aggregated form in Annex Figure 5 and by laboratory in Annex Table 4. An overview of percentage of laboratories by cumulative performance score is shown in Figure 5.

Table 7. Overview of virus isolation results by specimen code

0.13	Virus	$TCID_{50}$/ml[1]; Ct value	Expected results	Viral isolation results (n=44)					
				Correct results			Incorrect results		
				Result	n	%	Result	n	%
EISN_INF18-01	A(H3N2)	2795; 15.9	Positive	Positive/Not attempted[2]	43	97.7	Negative	1	2.3
EISN_INF18-02	B/Yamagata	2795; 18.0	Positive	Positive	40	90.9	Negative	4	9.1
EISN_INF18-03	B/Victoria	559; 17.2	Positive	Positive	37	84.1	Negative	7	15.9
EISN_INF18-04	A(H1N1)pdm09	1250; 16.8	Positive	Positive	40	90.9	Negative	4	9.1
EISN_INF18-05	Negative	-; -	Negative	Negative/Not attempted[2]	44	100.0	-	0	0
EISN_INF18-06	B/Victoria	167; 19.1	Positive	Positive	30	68.2	Negative	14	31.8
EISN_INF18-07	A(H3N2)	4180; 25.8	Positive	Positive	43	97.7	Negative	1	2.3
EISN_INF18-08	B/Yamagata	374; 16.6	Positive	Positive	35	79.5	Negative	9	20.5

[1] $TCID_{50}$ determined on MDCK-MIX cells (mixed monolayer of MDCK-I and MDCK cells stable expressing human 2,6-sialtransferase [MDCK-SIAT]) in 96-well plate, static incubation and end-point determination of cytopathic effect in each well. This produces a valuable estimate for relative comparison of the amount of live virus in the panel members used for virus isolation, but likely produces an underestimate of the true number of live viruses in the panel members.
[2] Not attempted: laboratory did not attempt virus isolation because molecular testing was negative (Annex Tables 3–4) and the laboratories' algorithms likely include only virus-positive specimens in virus isolation. 'Not attempted' results are therefore considered correct.

Figure 5 Overview of cumulative performance scores for virus isolation



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.

Just over 60% of the laboratories (27/44; 61%) 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 5). Of all 44 laboratories, one failed to isolate the virus from both A(H3N2) containing specimens, four failed to isolate the virus from the A(H1N1)pdm09 containing specimen and 16 failed to isolate the virus from one or more of the four influenza B virus-containing specimens. Laboratories performed less well on the B/Victoria and B/Yamagata containing specimens with lower viral load compared to those with higher viral load (Table 7). No false positives were reported for virus isolation.

All laboratories used MDCK cells for virus isolation, although the type or types of MDCK cell lines used varied largely (Annex Figures 5A-C). MDCK-SIAT cells alone or in combination with other MDCK types were used more often for isolation of A(H3N2) influenza viruses (24/44, 55%) than for isolation of A(H1N1)pdm09 (13/44, 30%) and type B influenza viruses (15/44, 34%). The frequent failure to isolate type B influenza viruses was not clearly linked to the use of specific types of MDCK cells. However, 5/10 (50%) laboratories using MDCK-SIAT cells alone had one or more errors; four of the five failed to isolate B/Victoria virus from EISN_INF18-6. Of the five laboratories using another MDCK cell type in addition to MDCK-SIAT, three (60%) failed to isolate type B influenza viruses from at least one specimen and none of the three could isolate B/Yamagata from EISN_INF18-8. Among 29 laboratories not using MDCK-SIAT alone or in combination with other MDCK cell types, eight (28%) reported one or more failures to isolate type B influenza viruses and none of the eight could isolate B/Victoria virus from EISN_INF18-6.

The majority of laboratories used cytopathic effect (CPE; 35/44, 80%), mainly combined with haemagglutination (28/44, 64%) for detection and confirmation of virus growth (Annex Figure 5D). In addition to haemagglutination (37/44, 84%), RT-PCR (20/44; 46%) and neuraminidase activity assay (9/44, 21%) were most often used to detect and/or confirm growth of the virus. For haemagglutination, a wide variety of RBCs was used (Annex Figure 5E). Several laboratories used a combination of RBCs (Annex Table 4) or a specific type of RBC dependent on the influenza virus type and type A H-subtype (details not shown).

3.3.2 Antigenic characterisation

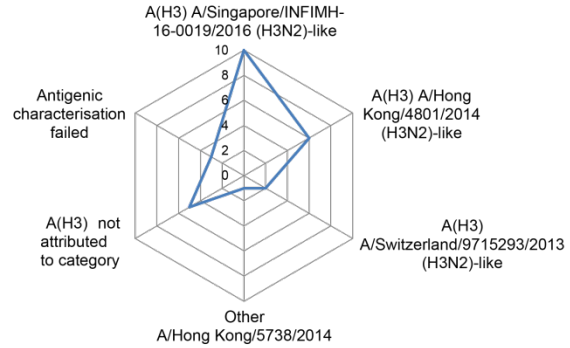
The evaluation of the reported antigenic characterisation results compared with the expected results was challenging. The returned antigenic characterisation category as preset in the TESSy reporting system largely depends on the specificity of the antisera used in HI and VN assays and the characteristics 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 neuraminidase-related haemagglutination (e.g. many of the current A(H3N2) influenza viruses). Individual specimen radar graphs to summarise the antigenic characterisation results were chosen to address these subjectivities and the wide range of reported categories per specimen (aggregated breakdown of the reported results in Figure 6; by participating laboratory in Annex Table 5). The used methodologies are listed by laboratory in Annex Table 5. Of the 32 laboratories reporting on antigenic characterisation, 31 (97%) used HI assay, one virus neutralisation and one HI assay or virus neutralisation on individual viruses. Of the 31 laboratories that used HI assay, one (3%) used

oseltamivir with all isolated viruses and 12 (39%) with the isolated A(H3N2) viruses only. In Annex Figure 6, an aggregated breakdown of type of sera used in HI assay and virus neutralisation 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 chicken hyperimmune sera.

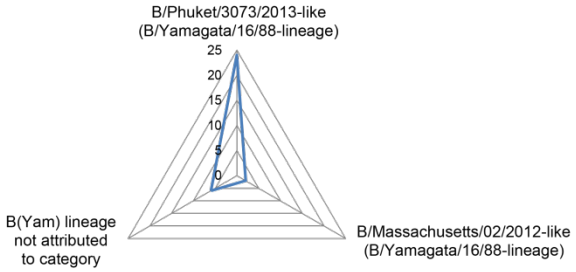
The one or two categories per specimen reported with the highest counts (Figure 6) can be considered correct. At the same time, this reflects the variety of results that can be reported to TESSy for the same virus depending on the laboratory that performed the analysis. The A(H1N1)pdm09 virus was mainly categorised as A/Michigan/45/2015-like (23/30, 77%; TESSy category for the 2016/17 and 2017/18 seasons) rather than the expected A/California/7/2009 (TESSy category for the 2016/17 season). This finding likely reflects the replacement of antisera against A/California/7/2009 with antisera against A/Michigan/45/2015, the A(H1N1)pdm09 vaccine component introduced in the 2017/2018 northern hemisphere vaccine. However, these two strains do not greatly differ antigenically (within fourfold HI titre difference, Annex Figure 1A). The two A(H3N2) viruses were mainly reported either as A/Singapore/INFIMH16-0019/2016-like (TESSy category for the 2017/18 season) or A/Hong Kong/4801/2014-like (TESSy category for the 2016/17 and 2017/18 seasons), possibly reflecting the difficulty to distinguish between these viruses or the limited use of multiple antisera against A(H3N2) viruses in HI assay or VN. Both A(H3N2) viruses showed neuraminidase-induced haemagglutination. However, there was no clear difference in reported categories between laboratories that used oseltamivir in the HI assay and those that did not (Annex Table 5). Both B/Victoria viruses were reported mainly as B/Brisbane/60/2008-like, although one of them (EISN_INF18-06) was expected to be a low reactor with antisera against B/Brisbane/60/2008-like vaccine virus (‘not attributed to category’ to be reported to TESSy). Similarly, one of the B/Yamagata viruses (EISN_INF18-02) was expected to be a low reactor with antisera against B/Phuket/3073/2013-like vaccine virus (‘not attributed to category’ to be reported to TESSy), whereas both B/Yamagata viruses were reported mainly as B/Phuket/3073/2013-like.

Figure 6. Overview summarising reported antigenic characterisation categories by specimen code

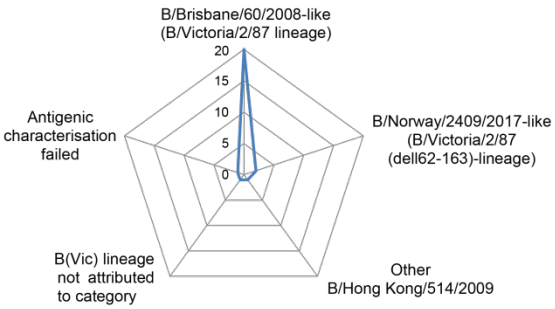
EISN_INF18-01 (N=27)



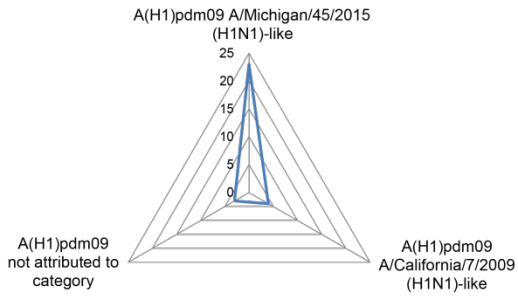
EISN_INF18-02 (N=32)



EISN_INF18-03 (N=25)



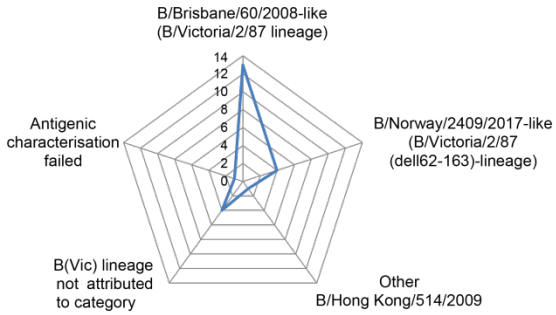
EISN_INF18-04 (N=30)



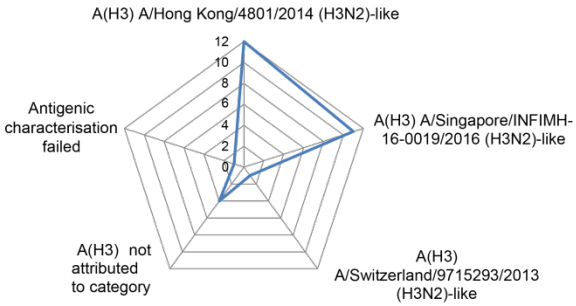
EISN_INF18-05 (N=32)

No isolates to characterise

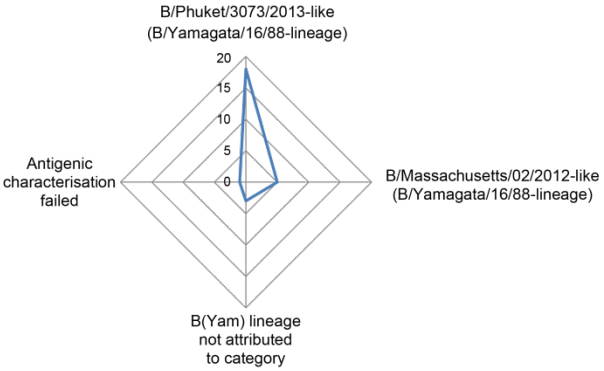
EISN_INF18-06 (N=23)



EISN_INF18-07 (N=29)



EISN_INF18-08 (N=27)

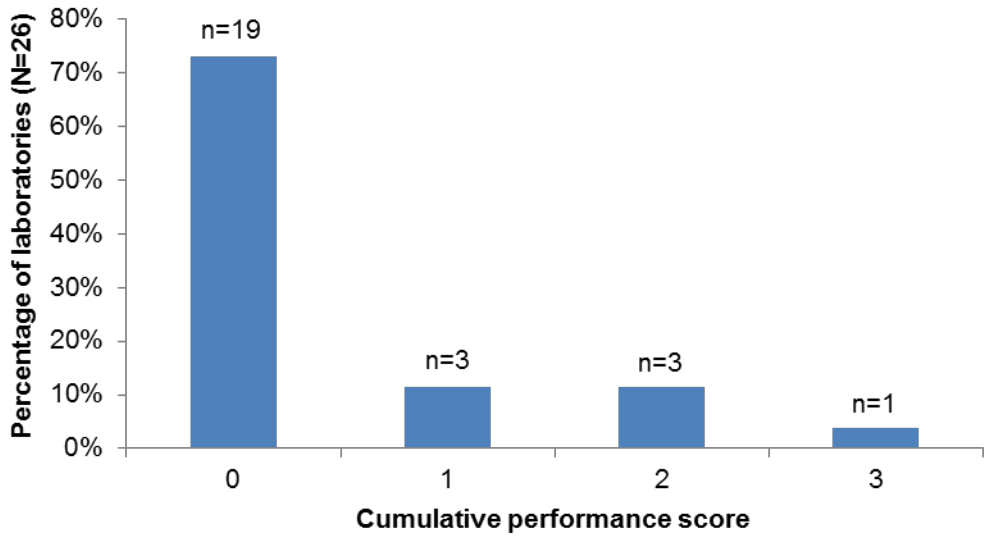


Total number of laboratories that obtained a virus isolate for characterisation indicated with each sample code. Expected result categories were: EISN_INF18-01: A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like; EISN_INF18-02: B(Yam) lineage not attributed to category; EISN_INF18-03 – B/Brisbane/60/2008-like (B/Victoria/2/87 lineage); EISN_INF18-04: A(H1)pdm09 A/California/7/2009 (H1N1)-like; EISN_INF18-05: N/A; EISN_INF18-06 – B(Vic) lineage not attributed to category; EISN_INF18-07 – A(H3) A/Hong Kong/4801/2014 (H3N2)-like; EISN_INF18-08: B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage). Both A(H3N2) influenza viruses had neuraminidase-induced haemagglutination. The B(Yam) and B(Vic) lineage viruses with expected result 'not attributed to category' were low reactors for B/Phuket/3073/2013 and B/Brisbane/60/2008 respectively.

3.3.3 Genetic characterisation

A total of 26 laboratories performed genetic characterisation (aggregated breakdown of reported results in Table 8; by participating laboratory and overall performance score in Annex Table 6). An overview of the percentage of laboratories by cumulative performance score is shown in Figure 7. The used methodologies are listed by laboratory in Annex Table 6. Of the 26 laboratories, 22 performed sequencing on the simulated clinical specimen, two on the simulated clinical specimen or the virus isolate, one on both and one on the virus isolate only. Nineteen of the laboratories used Sanger sequencing, six NGS and one Sanger sequencing and NGS.

Figure 7. Overview of cumulative performance scores for genetic characterisation



Scoring system used: correct: 0; incorrect: 1. Detailed arguments for allocating results as correct or incorrect inAnnex Table 6 footnote.

Just over 70% of the 26 laboratories (19/26, 73%) that performed genetic characterisation reported correct results for all virus-positive specimens (cumulative performance score of 0; (Figure 7, Table 8). The B/Victoria virus of specimen EISN_INF18-06 was assigned by some laboratories to genetic groups within clade 1A with deletion of amino acids 162-164 (representative B/Hong Kong/269/2017) or amino acids 162-163 (representative B/Norway/2409/2017) in HA1 (Table 8). However, this virus did not have these deletions and should have been assigned to the root 1A clade with B/Brisbane/60/2008 representative (Annex Figure 2C). Some laboratories assigned the A(H3N2) viruses to the older 3C.2a1 subgroup representative A/Bolzano/7/2016 subgroup or the root 3C.2a clade representative A/Hong Kong/4801/2014 subgroup (Table 8). However, both A(H3N2) panel viruses have the N121K amino acid substitution defining the 3C.2a1 subgroup represented by A/Singapore/INFIMH16-0019/2016 (Annex Figure 2B). EISN_INF18-01 virus has the N171K amino acid substitution further characterising this subgroup. The EISN_INF18-07 virus has a further amino acid substitution at this position, namely K171R, which is only discovered when a phylogenetic analysis in the context of the recommended reference viruses is performed (Annex Figure 2B).

Table 8. Overview summarising reported genetic characterisation categories by specimen code

Specimen code	Expected results Genetic category	Participants with results n	Genetic characterisation results													
			Correct							Incorrect[1]						
			Result	n	%	Result	n	%	Total	Result	n	%	Result	n	%	Total
EISN_INF18-01	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	26	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	24	92.3				92.3%	A(H3) clade 3C.2a1 representative A/Bolzano/7/2016 subgroup	2	7.7	-	-	-	7.7%
EISN_INF18-02	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	26	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	25	96.2				96.2%	B(Vic)-lineage clade 1A (del162-164 subgroup) representative B/Hong Kong/269/2017	1	3.8	-	-	-	3.8%
EISN_INF18-03	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	26	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	22	84.6	B/Johannesburg/3964/2012 (Clade 1A, B/Brisbane/60/2008) [2]	2	7.7	92.3%	B Victoria - clade 1A - not branched	1	3.8	B(Vic)-lineage clade 1B representative B/Hong Kong/514/2009	1	3.8	7.7%
EISN_INF18-04	A(H1)pdm09 group 6B.1 representative A/Michigan/45/2015	26	A(H1)pdm09 group 6B.1 representative A/Michigan/45/2015	25	96.2	A/Slovenia/2903/2015 (A(H1)pdm09 group 6B.1 A/Michigan/45/2015) [3]	1	3.8	100.0%	-	-	-	-	-	-	-
EISN_INF18-05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EISN_INF18-06	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	26	B(Vic)-lineage clade 1A representative B/Brisbane/60/2008	23	88.5				88.5%	B(Vic)-lineage clade 1A (del162-163 subgroup) representative B/Norway/2409/2017	2	7.7	B(Vic)-lineage clade 1A (del162-164 subgroup) representative B/Hong Kong/269/2017	1	3.8	11.5%
EISN_INF18-07	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	26	A(H3) clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup	22	84.6				84.6%	A(H3) clade 3C.2a representative A/Hong Kong/4801/2014 subgroup	1	3.8	A(H3) clade 3C.2a1 representative A/Bolzano/7/2016 subgroup	3	11.5	15.4%
EISN_INF18-08	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	25	B(Yam)-lineage clade 3 representative B/Phuket/3073/2013	25	100.0				100.0%	-	-	-	-	-	-	-

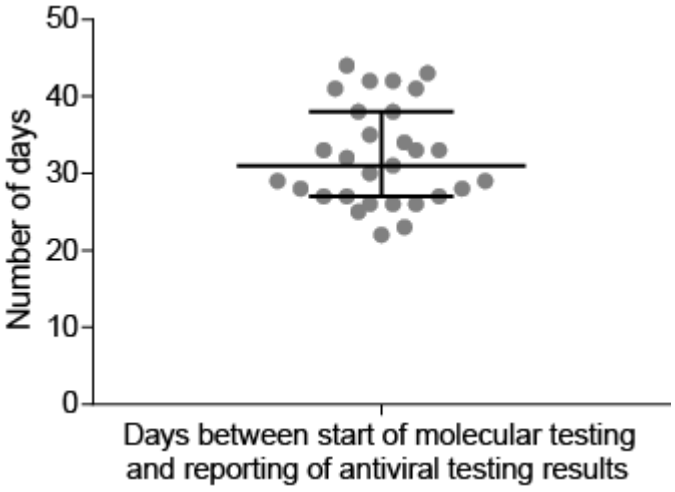
[1]Details on why the reported category is considered 'incorrect': in Annex Table 6 footnote.
[2]Although B/Johannesburg/3964/2012 is a more precise indication of the allocation in phylogenetic analysis (Annex Figure 2C), but still a B(Vic)-lineage clade 1A representative B/Brisbane/60/2008 and therefore scored 'correct', it is not a current TESSy reporting category.
[3]Although A/Slovenia/2903/2015 (Annex Figure 2A) has less in common in phylogenetic analysis with the current TESSy reporting category A(H1)pdm09 group 6B.1 representative A/Michigan/45/2015, it is a clade 6B.1 virus and is therefore considered a correct result.

3.4 Antiviral susceptibility

Twenty-nine laboratories participated in the antiviral susceptibility challenge; 22 performed genetic and phenotypic testing, six genetic testing only and one phenotypic testing only.

Antiviral testing results were reported with a median of 31 days (IQR 27–38 days) after the start of molecular testing , with 21/29 (72%) of laboratories reporting within the requested 36 calendar days (Figure 8).

Figure 8. Calendar days used for genetic and phenotypic antiviral susceptibility testing



Molecular testing start date taken as proxy for panel arrival date in laboratory. Large horizontal bar represents median and whiskers represent interquartile range.

3.4.1 Genetic testing

A total of 28 laboratories performed genetic testing. However, some tested only a subset of specimens depending on the type of tests available and deployed (e.g. laboratories with only RT-PCR for N1-H275Y tested only specimens containing A(H1N1)pdm09; Annex Table 7). Genetic testing results are shown in two tables: Table 9 with an aggregated breakdown of the identified amino acid substitutions and Table 10 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 Annex Table 7. The used methodologies are listed in aggregated form in Annex Figure 7 and by laboratory in Annex Table 7. An overview of the percentage of laboratories by cumulative performance score is shown in Figure 9.

All 28 laboratories identified the N1-H275Y amino acid substitution in specimen EISN_AV18-01 that contained a mixture of mutant N1-275Y and wild-type N1-275H. However, only 17/28 (61%) laboratories reported the mutant/wild-type mixed nature of the specimen. The specimen was prepared with SNP RT-PCR Ct values of 24 for 275H and 22 for 275Y and about 80% 275Y by sequencing. One laboratory reported Ct values of 22 for 275H and 20 for 275Y and eight laboratories reported a median percentage of 82% (range 70%–97%) for 275Y after sequencing, reflecting the intended nature of the mixture.

The NA-E105K amino acid substitution in the B/Victoria virus in specimen EISN_AV18-02 was reported by 23/25 (92%) laboratories that tested this specimen. One laboratory reported 150R instead. This was most likely a reporting error as the interpretation 'no amino acid substitutions associated with reduced inhibition' (AANI) for oseltamivir and zanamivir was given. The other laboratory reported 'no amino acid substitution' identified.

Two laboratories reported wild-type A(H1N1)pdm09 virus with N1-H275Y, likely a reporting error as both laboratories reported as interpretation either AANI for oseltamivir or 'no interpretation possible' (NIP). One of 24 laboratories reported the detection of N2-R292K amino acid substitution associated with (highly) reduced inhibition by neuraminidase inhibitors for both wild-type A(H3N2) viruses, likely a reporting error as the interpretation was reported as AANI for oseltamivir and zanamivir.

Table 9. Overview summarising the reported identified amino acid substitutions associated with reduced antiviral susceptibility by specimen code

Specimen code (EISN_)	Number tested[1]	Expected results[2]	Identification of amino acid substitutions[3]								
			Correct							Incorrect	
			Result	n	%	Result	n	%	Total	Result	n	%
AV18-01	28	275YHmix	257YHmix	17	60.7	257Y	11	39.3	100%	-	-	
AV18-02	25	E105K	E105K	23	92.0				92.0%	None, 150R	2	8.0
INF18-01	24	No substitutions	No substitutions	23	95.8				95.8%	292K	1	4.2
INF18-02	24	No substitutions	No substitutions	24	100				100%	-	-	
INF18-03	24	No substitutions	No substitutions	24	100				100%	-	-	
INF18-04	26	No substitutions	No substitutions	24	92.3				92.3%	275Y, 275YHmix	2	7.7
INF18-05	0	No virus	-	-						-	-	
INF18-06	24	No substitutions	No substitutions	24	100				100%	-	-	
INF18-07	24	No substitutions	No substitutions	23	95.8				95.8%	292K	1	4.2
INF18-08	24	No substitutions	No substitutions	24	100				100%	-	-	

[1]A number of laboratories did not perform genotypic antiviral susceptibility testing for all specimens depending on available tests.
[2]No substitutions: no amino acid substitutions associated with a reduction in neuraminidase inhibitor susceptibility following full NA segment sequencing.
[3]Reported result was translated from amino acid profiles reported and judged as correct or incorrect regardless of whether NA segment was fully or partially sequenced or whether only SNP detection assay was used; in the interpretation scoring these results, the level of testing was taken into account (Table 10; details: in Annex Table 7 footnote).

Table 10. Overview summarising reported interpretation of amino acid substitution identification associated with reduced antiviral susceptibility by specimen code

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	%
AV18-01	28	AAHRI	AAHRI	24	85.7	AARI, NIP	4	14.3	AANI	AANI	20	71.4	AANI, AARI, NIP	8	28.6
AV18-02	25	AANI or AARI	AANI or AARI	22	88.0	AANI, NIP	3	12.0	AARI or AAHRI	AARI or AAHRI	21	84.0	AANI, NIP	4	16.0
INF18-01	24	AANI	AANI	19	79.2	AANI, NIP	5	20.8	AANI	AANI	17	70.8	AANI, AARI, NIP	7	29.2
INF18-02	24	AANI	AANI	20	83.3	AANI, NIP	4	16.7	AANI	AANI	20	83.3	AANI, NIP	4	16.7
INF18-03	24	AANI	AANI	21	87.5	AANI, NIP	3	12.5	AANI	AANI	21	87.5	AANI, NIP	3	12.5
INF18-04	26	AANI	AANI	21	80.8	AANI, NIP	5	19.2	AANI	AANI	21	80.8	AANI, NIP	5	19.2
INF18-05	0	No virus	-	-	-	-	-		No virus	-	-	-	-	-	
INF18-06	24	AANI	AANI	21	87.5	AANI, NIP	3	12.5	AANI	AANI	21	87.5	AANI, NIP	3	12.5
INF18-07	24	AANI	AANI	20	83.3	AANI, NIP	4	16.7	AANI	AANI	20	83.3	AANI, NIP	4	16.7
INF18-08	24	AANI	AANI	21	87.5	AANI, NIP	3	12.5	AANI	AANI	21	87.5	AANI, NIP	3	12.5

[1]A number of laboratories did not perform genotypic antiviral susceptibility testing for all specimens depending on available tests.

[2]Expected result when 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 (SNP RT-PCR, partial or pyrosequencing).
[3]Judged as correct or incorrect after comparison with expected result taking into account the level of testing. For viruses without amino acid substitution, AANI is correct if full NA segment has been sequenced and no amino acid substitution previously associated with reduced antiviral susceptibility has been identified, otherwise NIP should have been reported. If full NA segment has been sequenced, NIP is incorrect as the presence or absence of an amino acid substitution previously associated with reduced antiviral susceptibility could have been identified (details in Annex Table 7).

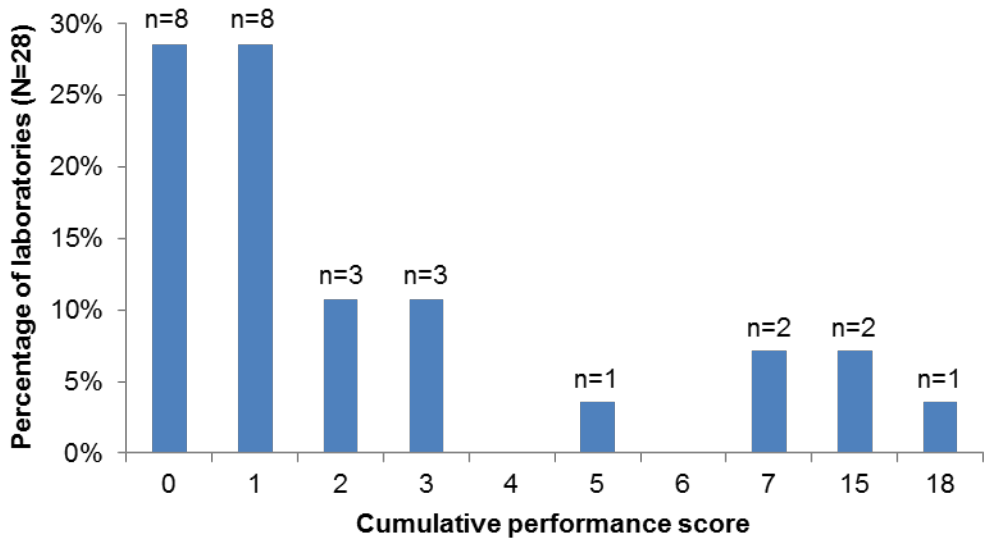
Thirteen laboratories had errors in the interpretation of their amino acid substitution analysis, often with the same underlying problem for all specimens (Table 10, Annex Table 7).

Common errors made were over the interpretation of results from partial sequencing or SNP detection by pyrosequencing or RT-PCR only. Five laboratories that used this approach reported wild-type viruses as AANI that should have been reported as NIP because the full-length NA was not analysed. On the other hand, two laboratories that had applied full-length NA sequencing under-interpreted their results and reported all or a number of wild-type viruses as NIP, whereas the more specific interpretation AANI for wild-type viruses should have been used. These errors due to not taking the level of genetic testing into account in the interpretation were reported by six laboratories for the EISN_AV18-01 and EISN_AV18-02 specimens. Examples are reporting detection of N1-H275Y with SNP assay or partial sequencing as AANI (over-interpretation, as should be NIP) or with full NA segment sequencing as NIP (under-interpretation, as should be AANI) for zanamivir.

Four laboratories detected N1-H275Y, but reported 'amino acid substitution associated with reduced inhibition' (AARI; n=2) or NIP (n=2) for oseltamivir instead of what should have been 'amino acid substitution associated with highly reduced inhibition' (AAHRI). Similarly, two laboratories detected B/Victoria NA-E105K, but reported NIP for zanamivir instead of what should have been AARI or AAHRI.

Figure 9 shows the cumulative performance score for the amino acid substitution analysis and interpretation. Only 8/28 (29%) laboratories had fully correct results (cumulative performance score of 0; specimens not tested were not scored). Given the high percentage of correct results for the amino acid substitution analysis by specimen (Table 9, Annex Table 7), this relatively low percentage of laboratories with fully correct results is obviously caused by failing to report the 275YH mixed nature for EISN_AV18-01 (11/28; 39%) and interpretation errors (13/28; 46%; Table 10, Annex Table 7).

Figure 9. Overview of cumulative performance scores for genetic antiviral susceptibility determination, amino acid substitution analysis and interpretation of this analysis



Scoring system used (only those specimens scored for which a result was reported):
EISN_AV18-01 substitutions – 275HY mix found, 0; 275Y found, 1; none found, 2; not tested, not scored.
EISN_AV18-01 interpretation oseltamivir – 275Y and any test and 'amino acid substitution associated with highly reduced inhibition' (AAHRI), 0; rest, 1; not tested, not scored.
EISN_AV18-01 interpretation zanamivir – full NA sequenced AND 'no amino acid substitution associated with (highly) reduced inhibition' (AANI), 0; SNP OR partial sequenced and 'no interpretation possible' (NIP), 0; any other, 1; not tested, not scored.
EISN_AV18-02 substitutions: E105K found, 0; none found, 1; not tested, not scored.
EISN_AV18-02 interpretation oseltamivir – E105K and any test and AANI or 'amino acid substitution associated with reduced inhibition' (AARI), 0; rest, 1; not tested, not scored.

EISN_AV18-02 interpretation zanamivir – E105K and any test and AARI or AAHRI, 0; rest, 1; not tested, not scored.
EISN_INF18-01 – 08 (except 05) substitution – none found, 0, any other, 1; not tested, not scored.
EISN_INF18-01 – 08 (except 05) interpretation oseltamivir and zanamivir – full NA sequenced and AANI, 0; SNP or partial sequenced and NIP, 0; any other, 1; not tested, not scored.

3.4.2 Phenotypic testing

A total of 23 participants performed phenotypic testing. However, not all specimens were tested by all laboratories and not all were tested for both oseltamivir and zanamivir (Table 11). Although the virus first had to be isolated for specimen EISN_INF01-08, at least one laboratory used one or more of the specimens without preceding virus isolation. Figure 10 shows an aggregated overview of the IC_{50} values reported and Figure 11 an aggregated overview of IC_{50} fold changes of EISN_AV18-01 and 02 calculated using IC_{50} values reported for wild-type EISN_INF18-04 and wild-type EISN_INF18-03,06 respectively (by participating laboratory in Annex Figures 8–9). Table 11 shows an aggregated breakdown of the interpretations of the reported IC_{50} values and by participating laboratory with cumulative performance score in Annex Table 8. An overview of the percentage of laboratories by cumulative performance score is shown in Figure 12. One laboratory included in Table 11 could not be included in Figures 10–11 because this laboratory indicated that it reported IC_{50} fold change values instead of the requested IC_{50} values. The methodologies used are listed in aggregated form in Table 12 and by laboratory for the used type of neuraminidase inhibition assay only in Annex Table 8.

Table 11. Overview of phenotypic antiviral susceptibility testing results by specimen code

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	%
AV18-01	23	HRI or RI	HRI or RI	23	100	-	-		22	NI	NI	21	95.5	RI[4]	1	4.5
AV18-02	23	HRI or RI	HRI or RI	16	69.6	NI[3]	7	30.4	22	HRI or RI	HRI or RI	22	100	-	-	
INF18-01	21	NI	NI	21	100	-	-		20	NI	NI	20	100	-	-	
INF18-02	20	NI	NI	20	100	-	-		19	NI	NI	19	100	-	-	
INF18-03	16	NI	NI	16	100	-	-		15	NI	NI	15	100	-	-	
INF18-04	19	NI	NI	19	100	-	-		18	NI	NI	18	100	-	-	
INF18-05	0	No virus	-	-	-	-	-		0	No virus	-	-	-	-	-	
INF18-06	15	NI	NI	15	100	-	-		14	NI	NI	11	78.6	RI[5]	3	21.4
INF18-07	21	NI	NI	21	100	-	-		20	NI	NI	20	100	-	-	
INF18-08	18	NI	NI	18	100	-	-		17	NI	NI	17	100	-	-	

[1]A number of laboratories that participated in the antiviral susceptibility challenge did not perform any phenotypic testing; some laboratories tested only a limited number of specimens and not always for both oseltamivir and zanamivir; not testing is mainly due to not having isolated a virus.
[2]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.
[3]In five cases for which it could be confirmed, IC_{50} fold change compared to EISN_INF18-3,6 was <5 and NI interpretation of own result is correct, but it was incorrect compared to expected result; in one case, the IC_{50} fold change compared to EISN_INF18-3,6 was >5; in one case, no EISN_INF18-3,6 data was available for confirmation of the IC_{50} fold change.
[4]IC_{50} fold change compared to EISN_INF18-4 <10.
[5]Likely due to culture selected NA-T106P amino acid substitution confirmed to be present as minority variant in simulated clinical specimen and in one of three isolates. For two laboratories, the fold change relative to wild-type EISN_INF18-3 could be calculated >5 and < 50, indicating interpretation was correct, but incorrect compared to expected result.

The overall performance of the laboratories in phenotypic testing was good; 12/23 (52%) laboratories had all specimens correct and the other 11/23 (48%) reported an incorrect result only for one specimen and one antiviral (Figure 12, Annex Table 8). A variety of techniques and approaches for IC_{50} measurement and calculations were used (Table 12), although the majority of laboratories used an in-house MUNANA substrate-based assay with pre-titration of NA activity and an 'HPA Excel template' for IC_{50} calculation, reflecting training activities performed in the past at Public Health England.

The EISN_AV18-01 specimen with a mixture of A(H1N1)pdm09 N1-275YH was correctly determined as HRI (n=18) or RI (n=5) for oseltamivir by all 23 laboratories (Table 11, Figures 10– 11). However, when comparing reported IC_{50} values with those available for wild-type EISN_INF18-04, one laboratory reporting HRI had an RI fold change (79-fold) and two laboratories reporting RI had an HRI fold change (139-fold and 691-fold; Figure 11).

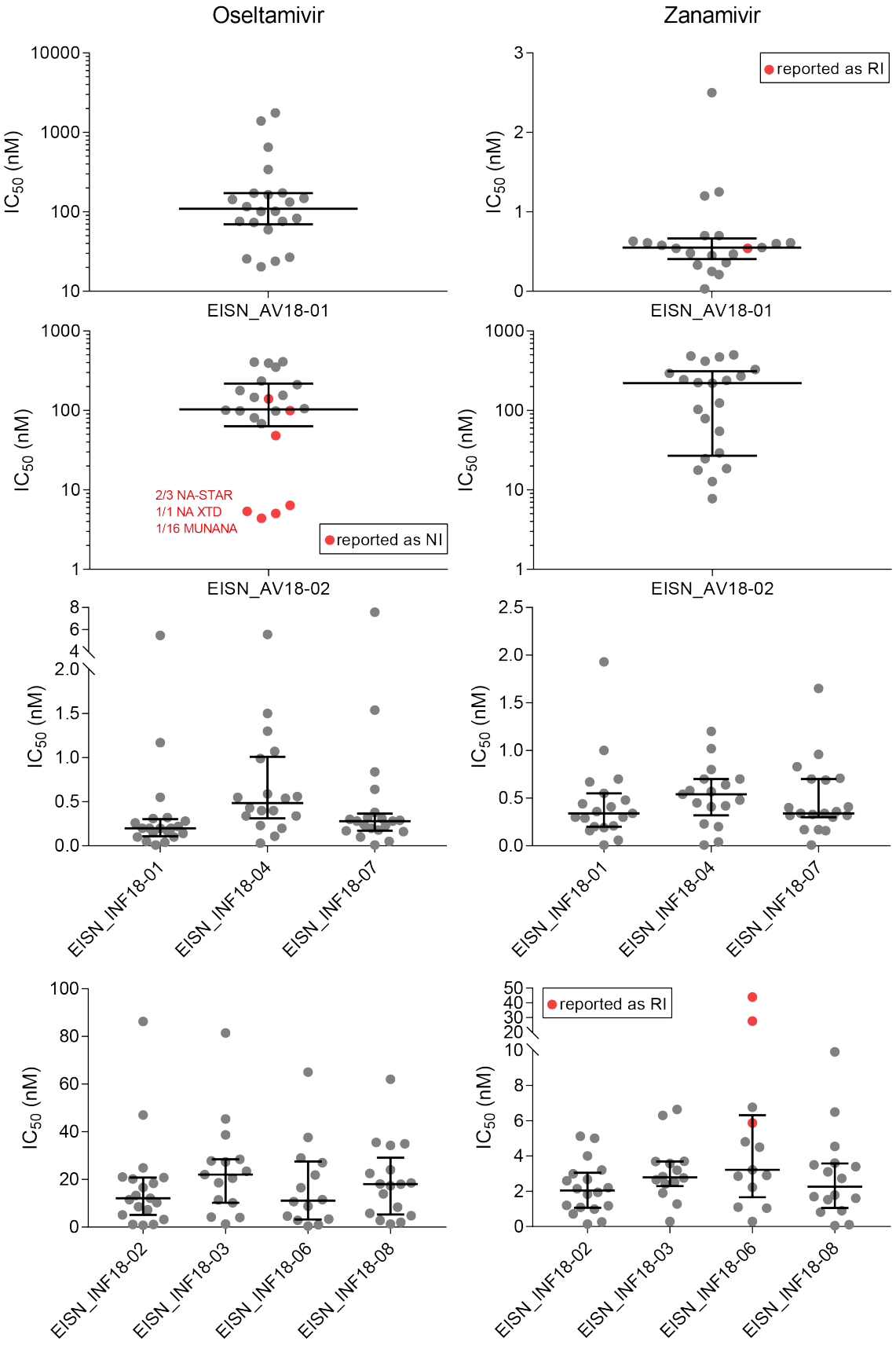
Table 12. Methodologies used by laboratories to determine and evaluate IC_{50} values

Method	Number of laboratories	
Assay type[1]		
MUNANA in-house	16	
NA-Fluor Kit	3	
NA-STAR Kit	3	
NA XTD Kit	1	
Drug range (nM) tested	Lowest concentration	Highest concentration
Median	0.010	4 000
Mean	0.028	16 759
Minimum	0	1 000
Maximum	0.32	200 000
Pre-titration NA activity		
Yes	21	
No	2	
Measurements		
Duplicate	21	
Single	2	
Control viruses[2]		
In-house	8	
ISIRV	8	
CDC	2	
ISIRV; in-house	2	
CDC; WHO London	1	
Reference strain	1	
Vaccine reference strains	1	
Software to calculate IC_{50}[3]		
HPA Excel template	14	
GraphPad Prism	6	
Excel template	1	
SigmaPlot	1	
MyAssay (http://www.myassays.com)	1	
Evaluation of IC_{50} against[4]		
wild-type virus	7	
median previous	5	
mean current	2	
mean previous; wild-type virus	2	
mean previous	1	
median current	1	
median current; mean current	1	
median previous; mean previous	1	
median previous; median current	1	
median previous; median current; wild-type virus	1	
wild-type virus; known resistant virus	1	

[1]MUNANA: 20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid substrate producing a fluorescent product after neuraminidase cleavage; NA: neuraminidase
[2]ISIRV: International Society for Influenza and other Respiratory Virus Diseases antiviral working group (ISIRV-AVG stopped providing reference viruses in 2018); CDC: Centers for Disease Control and Prevention, International Reagent Resource; WHO London: WHO Collaborating Centre, London, UK.
[3]HPA Excel template: Excel template file provided by Health Protection Agency, currently Public Health England.
[4]previous: previous season; current: current season.

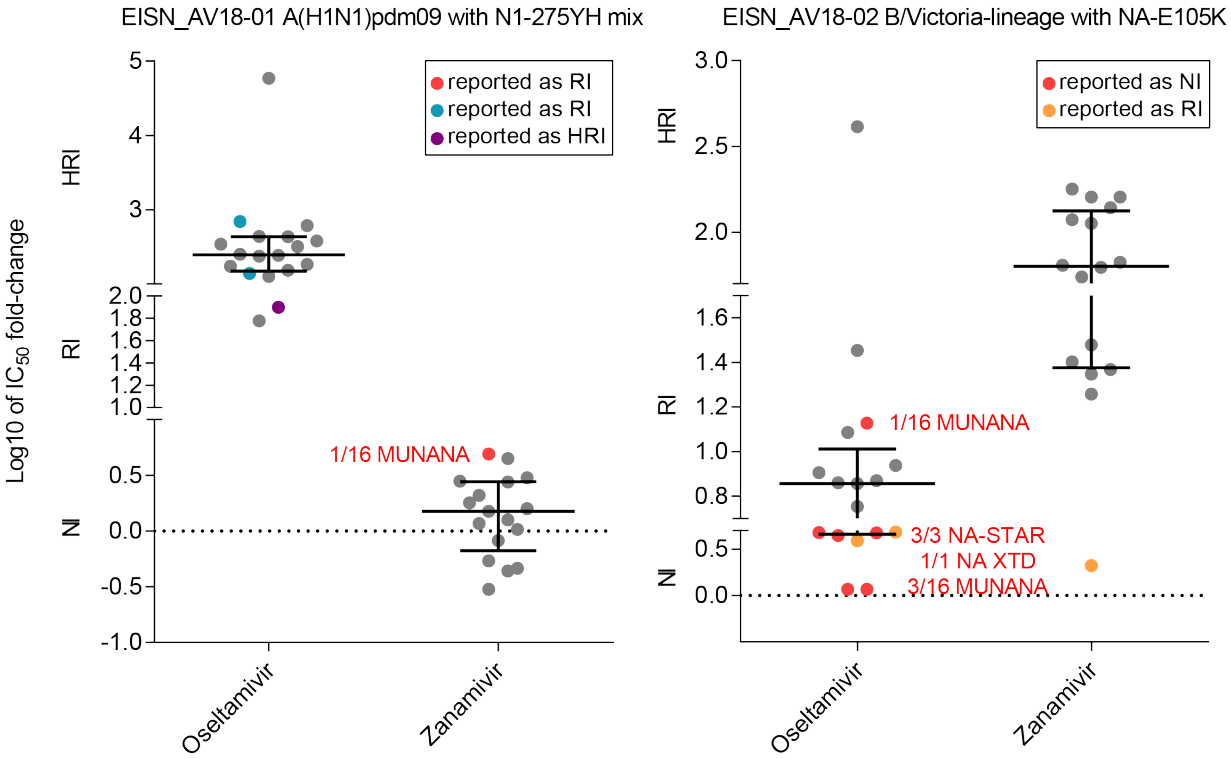
Among the laboratory-specific fold changes that could be calculated for 19 laboratories reporting IC_{50} values for both EISN_AV18-01 and EISN_INF18-04, 17 resulted in an HRI fold change (15 laboratories reported HRI and 2 RI) and two in an RI fold change (one laboratory reported HRI and one RI; Figure 11). The differences in interpretation likely depend on what is used as a reference for the calculation of the fold changes (Table 12). For zanamivir and specimen EISN_AV18-01, one laboratory erroneously reported RI, although the laboratory-specific fold change compared to EISN_INF18-04 was <10, indicating an NI phenotype for zanamivir (Figure 11). Overall, 22/23 (96%) laboratories had a correct result for this specimen (Figure 12).

Figure 10. Overview of reported IC_{50} values by specimen code



Red dots indicate specimens with incorrect result: IC_{50} level not as expected and/or interpretation not as expected (details by laboratory in Annex Table 8, Annex Figure 8). Large horizontal bars represent median and whiskers represent interquartile range.

Figure 11. Overview of calculated IC_{50} fold-change values for EISN_AV18-01 and 02 specimens

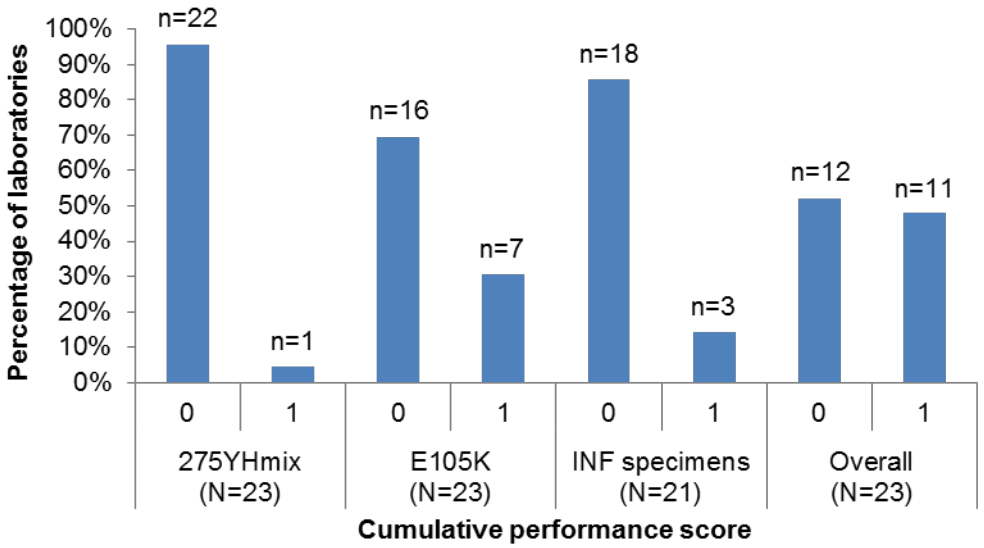


Laboratory-specific fold change values calculated for laboratories that also reported IC_{50} values for wild-type viruses of same subtype or lineage included in EISN_INF18 specimens, EISN_INF18-04 and mean EISN_INF18-03, 06 (3 RI outliers excluded) respectively. Large horizontal bars represent median and whiskers represent 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 dots: specimens with incorrect result; IC_{50} level not as expected and/or interpretation not as expected (details by laboratory in Annex Table 8, Annex Figure 9). Orange dots: laboratory correctly reported RI compared to expected result, although fold-change calculated with mean of EISN_AV18-03,06 indicated fold change below RI threshold. Blue dots: laboratory reported RI, while fold change calculated with EISN_INF18-04 indicated HRI. Purple dot: laboratory reported HRI, while fold change calculated with EISN_INF18-04 indicated RI.

All 22 laboratories that tested the EISN_AV18-02 specimen (B/Victoria NA-E105K) reported correct results with HRI (n=12) or RI (n=10) for zanamivir (Table 11, Figures 10–11). However, when laboratory-specific IC_{50} fold changes relative to the average of the EISN_INF18-03,06 specimens were calculated, the RI result of one laboratory returned an NI fold change (Figure 11). IC_{50} measurement and interpretation for the EISN_AV18-02 specimen and oseltamivir was challenging, as seven laboratories reported an NI interpretation of the IC_{50} obtained (Table 11). For six of them, the laboratory-specific fold change relative to the average of the EISN_INF18-03,06 specimens could be calculated and five turned out as NI (Figure 11). Of these five, three were obtained with the NA-STAR or NA XTD assay and two with the MUNANA in-house assay. Furthermore, an additional NA-STAR assay and MUNANA in-house assay result reported as RI returned an NI result after a laboratory-specific fold change calculation (Figure 11). These results indicate that chemiluminescent assays experience difficulties in generating an RI result for this specimen that is mildly RI for oseltamivir when using fluorescent-based MUNANA or NA-Fluor assay types (Figure 11, Annex Table 8). Overall, 16/23 (70%) laboratories reported a correct result for this specimen (Figure 12).

Among the seven EISN_INF18 specimens with wild-type viruses tested by 21 laboratories, three laboratories reported an incorrect result for the same single specimen (EISN_INF18-06; B/Victoria): RI instead of the expected NI for zanamivir (Table 11, Figure 11). However, for two of the three laboratories, it was confirmed by comparing the IC_{50} values with those of the EISN_INF18-3 specimen that the laboratories had correctly reported RI (fold changes of 7.7 and 17.6; Figure 10). As reported by one of the laboratories, the most likely explanation is that cell culture-induced amino acid substitution NA-T106P caused this shift in IC_{50} for zanamivir, similar to what is observed for the NA-E105K amino substitution in specimen EISN_AV18-02. Deep sequencing of the original simulated clinical specimen showed the presence of NA-T106P as a minority variant. Overall, 18/21 (86%) laboratories had a correct result for this specimen (Figure 12).

Figure 12. Overview of cumulative performance scores for phenotypic antiviral susceptibility determination



Scoring system used (only those specimens scored for which a result was reported):
EISN_AV18-01: oseltamivir: HRI or RI, 0; other, 1; zanamivir: NI, 0; other, 1; not tested or no isolate, not scored.
EISN_AV18-02: oseltamivir: RI or HRI, 0; other, 1; zanamivir: HRI or RI, 0; other, 1; not tested or no isolate, not scored.
EISN_INF18-01 - 08 (except 05): oseltamivir and zanamivir: NI, 0; other 1; not tested or no isolate, not scored.

3.5 Accreditation requirements

Participation in EQA programmes is an important element in laboratory accreditation. Table 13 outlines th accreditation status of the laboratories, their participation in other EQA programmes and the requirement for the laboratory to participate in such EQAs for accreditation purposes.

Table 13. Summary of survey on laboratory accreditation and participation in external quality assessment programmes

Question and response[1]	Further specification and numbers of laboratories		
Is your laboratory accredited?	n		
Yes			
ISO 15189	16		
ISO 17025	6		
WHO	4		
ISO 9001	2		
CPA	1		
ISO 15189; ISO 17025	1		
ISO 15189; WHO	1		
Ministry of Health Slovenia	1		
National Academy of Medical Science of Ukraine	1		
Total	33		
No, in the process of obtaining accreditation			
ISO 15189	9		
ISO 15189; ISO 17025	1		
Total	10		
No, and not in the process of obtaining accreditation	12		
Question and response	**Further specification and numbers of laboratories**		
Which EQA panel(s) do you use for detection, typing, subtyping and lineage determination?	Detection and typing (n)	Type A subtyping (n)	Type B lineage determination (n)
WHO	26	26	22
WHO; ERLI-NET	4	5	5
WHO; QCMD	4	4	2
CDC	2	2	3
ERLI-NET	2	2	3
QCMD	2	2	2
CDC; INSTAND	1	1	1
EQA Finland; ERLI-NET	1	1	1
INSTAND	1	1	1

Question and response	Further specification and numbers of laboratories		
WHO; ERLI-NET; INSTAND	1	1	1
WHO; ERLI-NET; EQUALIS; NEQAS	-	-	1
WHO; ERLI-NET; VSR, local NIC; EQUALIS; NEQAS	1	1	-
WHO; INSTAND	1	1	1
WHO; PHE	1	1	1
WHO; PNEQA	1	-	
WHO; QCMD, local NIC	1	1	1
WHO; QCMD; NEQAS	1	1	1
WHO; QCMD; NEQAS; CAP	1	1	-
WHO; QCMD; SKML	1	1	1
None	-	-	2
NA	-	-	2
Total	**52**	**52**	**51**
Required for accreditation? (number/number with response)	19/21	18/20	16/18
Which EQA panel(s) do you use for virus isolation?	In cells (n)	In eggs (n)	
ERLI-NET	18	4	
WHO	4	1	
ERLI-NET; NEQAS	2	-	
CAP	1	-	
ERLI-NET; WHO	1	-	
INFHT, QCMD	1	-	
Local NIC	1	1	
SKML	1	-	
None	7	18	
N/A	3	7	
Total	**39**	**31**	
Required for accreditation? (number/number with response)	7/11	2/4	
Which EQA panel(s) do you use for virus characterisation?	Antigenic (n)	Genetic (n)	
ERLI-NET	14	8	
ERLI-NET; WHO	1	4	
WHO	4	4	
ERLI-NET; WHO; local NIC	-	1	
CAP	1	-	
FluPEP-SEQ, CDC	-	1	
INFHT, QCMD	1	1	
Local NIC	1	1	
PHE	-	1	
None	9	14	
None (performed at other NIC location)	1	-	
N/A	2	2	
Total	**34**	**37**	
Required for accreditation? (number/number with response)	4/9	6/9	

Question and response	Further specification and numbers of laboratories
Which EQA panel(s) do you use for antiviral susceptibility testing?	n
ERLI-NET; WHO	11
WHO	8
ERLI-NET	6
PHE	1
WHO; PHE	1
None	11
N/A	2
Total	**40**
Required for accreditation (number/number with response)	7/11

[1]CDC: Centers for Disease Control and Prevention; CPA: Clinical Pathology Accreditation, UK based; EQUALIS: Equalis AB; ERLI-NET: European Reference Laboratory Network for Human Influenza; FluPEP-SEQ, CDC: Influenza Molecular Diagnostic Performance Evaluation Panel, CDC; INFHT: influenza virus hemagglutinin typing panel QCMD; INSTAND: Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V.; N/A: not applicable; NEQAS: UK NEQAS; NIC: national influenza centre; PHE: Public Health England; PNEQA: unknown EQA programme abbreviation; QCMD: Quality Control for Molecular Diagnostics; SKML: Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek; VSR: unknown EQA programme abbreviation; WHO: World Health Organization; although not always explicitly indicated, assumed to refer to EQAP.

Data on accreditation were available for 55 laboratories. Of the 55 laboratories, 33 (60%) were accredited, of which 18 (55%) by ISO 15189 (medical laboratories). A further 10/55 (18%) laboratories were in the process of obtaining ISO 15189 accreditation. The laboratories reported to have participated in a wide variety of EQA panels (Table 13). Although not reported by all laboratories in the survey, all have used the current EEIQAP 2018 and many EU/EEA laboratories (31 in 2008, 38 in 2015 for molecular rapid testing) also participated in previous ERLI-Net-provided panels for external quality assessment [22]. A high percentage reported the use of the WHO

EQAP panel for detection and typing (43/52, 83%), subtyping influenza A viruses (43/52, 83%), lineage determination of type B viruses (36/51, 71%) and antiviral susceptibility testing (20/40, 50%). Participation in WHO EQAP panels for detection and typing and type A H-subtyping is a requirement for WHO-recognised NICs. Type A N-subtyping, type B lineage determination and antiviral susceptibility testing are so far optional in that project. The ERLI-Net panel is the most often used panel for virus isolation (21/29, 72%), antigenic characterisation (15/34, 44%) and genetic characterisation (13/37, 35%). A relatively high number of laboratories reported not using EQA panels for virus isolation, antigenic and genetic characterisation and antiviral susceptibility testing. This corresponded with a low percentage of laboratories reporting EQA for these techniques as a requirement for accreditation. However, EEIQAP 2018 shows a high percentage participation in molecular detection, virus isolation and antigenic and/or genetic characterisation and a lower percentage participation in antiviral susceptibility testing (Table 4).

4 Discussion

There was a high participation in EEIQAP 2018 among the 53 WHO European Region Member State countries. Forty-six of the 50 countries with at least 1 NIC/national influenza reference laboratory participated. Of the 63 laboratories in the WHO European Region actively approached to sign up, 56 laboratories participated. As such, EEIQAP 2018 provides a good overview of the capabilities and performance of laboratories in the WHO European Region Member States and WHO GISRS and ECDC EISN ERLI-Net influenza reference laboratories for different aspects of influenza surveillance in the region.

Panel distribution started in March 2018 and the last panel was delivered in early July 2018 due to delayed distribution to non-EU/EEA countries and the required paperwork for passing local customs. The return result was relatively good, with 49/55 (89%) laboratories reporting within the expected time limit of seven calendar days for molecular detection and 35/43 (81%) and 21/29 (72%) laboratories reporting within the expected time limit of 36 calendar days for virus isolation with strain characterisation and antiviral susceptibility testing respectively. The time required for sequencing was not separately recorded. Nevertheless, many laboratories used genetic strain characterisation (26/56, 46%) and sequencing for antiviral susceptibility marker identification (25/56, 45%). This indicated that these laboratories have the capability for a relatively rapid (within one week) genetic strain characterisation and antiviral susceptibility marker analysis using sequencing directly on clinical specimens when an emerging situation requires it. Virus isolation is the limiting factor for antigenic characterisation and phenotypic testing for antiviral susceptibility, taking a median of 15 days until completion.

Fifty-five laboratories performed very well in applying molecular testing to influenza virus detection, A/B typing and type A H-subtyping of the current seasonal circulating A(H1N1)pdm09, A(H3N2), B/Victoria and B/Yamagata influenza viruses. Overall, 52/55 (95%) laboratories reported correct results for all specimens, reconfirming the correct and reliable reporting of surveillance detection data by the network laboratories to TESSy. The percentage of laboratories with correct results was higher than that reported by 38 ERLI-Net laboratories in EU/EEA countries in the 2015 EQA study (90%) and 46 NICs in the WHO European Region in the 2017 WHO EQAP study (89%), which also included avian type A subtypes [22,23]. EEIQAP 2018 challenged participating laboratories to determine the Nsubtype and B-lineage for the first time. Fewer laboratories performed N-subtyping of type A influenza viruses (21/55, 38%) than lineage determination of type B viruses (46/55, 84%), both included in the dataset for reporting to TESSy. Nevertheless, all laboratories that reported the N-subtype and/or B-lineage did so correctly when the incorrect results of one laboratory due to incorrect random order reporting were excluded. 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 [24]. 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 [25]. In 2018, one H1N2 reassortant virus was rapidly identified in routine surveillance because N-subtyping was included [26]. B-lineage is important to know for the (re)emergence and distribution of lineages [25], the lineage match with the strain included in trivalent vaccine and the differential impact of both lineages, e.g. lineage-specific vaccine effectiveness [27].

After the proportion of laboratories with correct results increased from 21/30 (70%) in the 2010 ERLI-Net EQA to 26/32 (81%) in the 2015 ERLI-Net EQA [22], it dropped to 27/44 (61%) in EEIQAP 2018. This is explained by the high number of isolation failures for influenza virus type B, especially for specimens with the lowest concentration of virus and was also observed in the first virus isolation EQA in the Asia-Pacific region [15]. Type B isolation failures in EEIQAP 2018 were randomly distributed among laboratories. However, if a laboratory failed to isolate B/Victoria from the EISN_INF-06 specimen, the laboratory often also failed to isolate type B viruses from one or more of the other type B specimens. There was no obvious correlation with the wide variety of MDCK cells used and known to be suitable for influenza virus isolation [28]. However, of laboratories using MDCK-SIAT cells alone, 50% faced influenza type B virus isolation failures compared to 28% of those that did not use MDCK-SIAT cells alone or in combination with other MDCK cell types. MDCK-SIAT has been developed specifically to support cell-based assays for measuring neuraminidase 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 [29]. 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 [30]. Therefore, the observed high number of failures to isolate influenza type B viruses cannot be solely explained by the use of MDCK-SIAT cells. Similar to the observations for the Asia–Pacific region [15], there is clearly an issue with the sensitivity of influenza type B virus isolation procedures. As virus isolation is required for antigenic characterisation and phenotypic antiviral susceptibility testing, failure to isolate the virus had an immediate effect on the number of EEIQAP 2018 specimens that could be included in these analyses. In a broader perspective, failure to isolate the influenza virus dependent on the type of virus or viral load in the clinical specimen reduces the number of characterisations that can be reported to TESSy and the selection of viruses with specific characteristics to be forwarded to WHO CC London.

Comparable antigenic characterisation of influenza viruses across laboratories remains a challenging task, even among WHO CCs. Assays are difficult to standardise and changes in the viruses may prevent them from agglutinating RBCs or cause neuraminidase-induced haemagglutination [31,32]. This is also reflected in the struggle of NICs to generate accurate antigenic characterisation data as shown in previous EISN EQAs [22] and the current EEIQAP 2018. Low reactors of the B/Victoria and B/Yamagata viruses included in EEIQAP 2018 were not readily identified in HI assay with ferret sera against B/Brisbane/60/2008 and B/Phuket/3073/2013 respectively. Both A(H3N2) viruses were attributed almost equally to the A/Singapore/INFIMH-16-0019/2016 and the older A/Hong Kong/4801/2014 reference TESSy reporting categories. Despite the high variability of the assays used, there was good concordance between laboratories in the reported antigenic characterisation categories for all virus-containing panel specimens. However, the exercise also demonstrates that antigenic characterisation data reported to TESSy should be interpreted with caution.

Genetic characterisation of the haemagglutinin segment of the panel viruses after sequencing was more straightforward, with 19/26 (73%) laboratories reporting correct results for all panel specimens, slightly less than in 2015 (15/20, 75%) [22]. However, four laboratories did not genetically characterise all panel specimens in 2015. The main error made with A(H3N2) viruses in EEIQAP 2018 was allocating them to an older category of clade 3C.2a viruses, 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 [20]. A specific challenge with EISN_INF18-07 was to interpret the K171R amino acid substitution correctly as a further evolution of the clade-characterising N171K amino acid substitution. A particular error made with both B/Victoria viruses was allocating them to the subclades of clade 1A that harbour the deletion in HA at positions 162-163 or 162-164, although the viruses included in the panel did not have these amino acid deletions. Similar errors in data reported to TESSy might lead to flawed estimates when analysing the emergence and spread of these deletion 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 an impact on vaccine match like deletion variants.

Reporting of amino acid substitution as required for TESSy reporting [21] was cumbersome, as the relevant amino acid positions were often not reported accurately. Nevertheless, determination of the A(H1N1)pdm09 N1-275Y/H mixed virus and B/Victoria NA-E105K virus was very good, with all 28 (100%) laboratories detecting 275Y and 23/25 (92%) detecting NA-E105K. This is consistent with the high accuracy in detecting N1-275Y in the 2017 WHO EQAP and 2015 ERLI-Net EQA [23,33], a sporadic amino acid substitution in influenza B/Victoria, NA-I221L in the 2015 ERLI-Net EQA [33,34] and NA-E105K in the current EEIQAP 2018 [35]. Identification of the A(H1N1)pdm09 N1-275Y/H mix, in which N1-275H was present at approximately 20%, was achieved by 17/28 (61%) laboratories, a decline from 17/23 (74%) laboratories in the 2015 ERLI-Net EQA [33]. This observation is possibly explained by the percentage of 20% N1-275H in the mixture being close to the detection limit for Sanger sequencing or the inclusion of a higher number of laboratories with less extensive training on this aspect of antiviral susceptibility monitoring in EEIQAP 2018. Still, the capability to accurately detect a mixture is important as specimens with a (highly) reduced inhibited virus quasispecies are a common scenario for laboratories that receive clinical specimens as part of a surveillance programme or treatment evaluation. Several laboratories reported amino acid substitutions associated with (highly) reduced inhibition for wild-type specimens, likely a reporting error as in all cases, the interpretation reported was different throughout. The major problem in genetic antiviral susceptibility testing was similar to that in the 2015 ERLI-Net EQA [33] related to the interpretation of the amino acid substitution analysis relative to the level of testing performed, e.g. SNP RT-PCR versus full-length NA sequencing. Frequently, data were either over- or under-interpreted. These errors were often made by the same laboratories for all or a large number of specimens. In the past, webinars specifically addressed this issue and they are still available on the EISN extranet. Phenotypic testing for antiviral susceptibility and interpretation of IC_{50} values were highly accurate for all specimens except the B/Victoria NA-E105K specimen and oseltamivir. Although the provided interpretations 'normal inhibited' for this specimen for the determined IC_{50}s for oseltamivir by seven laboratories was correct, the determined IC_{50}s were too low, resulting in fold changes compared to wild-type B/Victoria viruses included in the panel just below the 'reduced inhibited' threshold. All four laboratories that used chemiluminescent-based assays had this issue, compared to three of 16 laboratories that used fluorescent-based assays. Chemiluminescent assays were also associated with reduced sensitivity to generate a 'reduced inhibited' result with an A(H1N1)pdm09 N1-275Y/H mixture in the 2015 ERLI-Net EQA [33]. In EEIQAP 2018, this issue was not observed, likely because of the relatively high percentage of approximately 80% of 275Y in the specimen. A particular issue previously described in surveillance of antiviral susceptibility of influenza viruses is virus isolation-induced amino acid substitution in the neuraminidase resulting in (highly) reduced inhibition [36]. This can only be addressed by sequencing the neuraminidase gene segment of both the clinical specimen and virus isolate and must be done especially if an unknown amino acid substitution is found in a virus isolate with a (highly) reduced inhibited phenotype.

A relatively high number of laboratories in the network have obtained ISO 15189 accreditation for medical laboratories or is working towards obtaining this standard. This is a good development, although many laboratories reported that EQAs on several aspects of laboratory influenza surveillance challenged in EEIQAP 2018 and other EQAs are not required for accreditation. As ISO 15189-accredited tests require recorded corrective action when failing in EQAs, adding tests to the scope of the accreditation could be helpful in addressing errors and further improving the performance of laboratory testing and surveillance of the network as a whole.

5 Conclusions

Overall, the performance of the network in molecular detection, simultaneous typing and type A H-subtyping of seasonal influenza viruses can be rated as very good. Laboratories determining the N-subtype of type A influenza viruses and lineage of type B viruses demonstrated excellent performance. However, an increase in the number of laboratories with these latter capabilities would be desirable. Surveillance data derived from molecular testing can be considered accurate.

EEIQAP 2018 has clearly identified an issue in the performance of the network in virus isolation, especially for type B influenza viruses. This is possibly linked to a lack of sensitivity of the procedures used and probably at least partly due to inappropriate use of only MDCK-SIAT cells. Not being able to isolate the virus has immediate repercussions on the number of isolates available for antigenic characterisation and phenotypic antiviral susceptibility testing in laboratories and reporting the results to TESSy. It also limits the provision of viruses to WHO CC London for further analysis in an even more international context aimed to inform the WHO vaccine composition recommendation meetings.

Although there is good concordance among laboratories in the reported antigenic characterisation categories for the EEIQAP panel specimens, the results also indicate that subtle antigenic differences between viruses are not picked up accurately and results reported to TESSy in the predefined categories should therefore be interpreted with caution.

Genetic characterisation of the HA of seasonal influenza viruses by the network is highly accurate. However, certain laboratories had difficulties with allocating certain viruses to the correct predefined categories. Therefore, genetic categories reported to TESSy should also be analysed and interpreted with caution.

Genetic and phenotypic testing of antiviral susceptibility by the network is of high accuracy. However, amino acid substitution data and their interpretation in the TESSy format and categories are inaccurate for a relatively high number of laboratories. This complicates the analysis of antiviral susceptibility data reported to TESSy.

A high number of laboratories have obtained ISO 15189 accreditation or are in the process of applying for it, suggesting that the performance of tests for laboratory surveillance of influenza should be of high standard if included in the scope. For molecular detection and typing, A-subtyping and B-lineage determination, this holds as shown in EEIQAP 2018. However, for other tests challenged in EEIQAP 2018, many laboratories indicated that EQAs were not required for ISO 15189 accreditation.

6 Recommendations

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

One participant only taking part in the molecular detection component of the panel reported the correct number of viruses by type, type A H-subtype, type B-lineage and a negative specimen, but in the incorrect random order. This indicates an issue in the reporting of data rather than a testing protocol issue. A review of standard operating procedures (SOPs) for the reporting of test data should resolve this issue.

One participant reported a false positive in the molecular detection component of the panel. This indicates an issue with specimen contamination or switching and therefore a specimen handling error rather than a testing protocol issue. A review of SOPs for specimen reception and sample handling should resolve this issue.

Another participant reported a false negative for an A(H3N2)-containing specimen and therefore did not perform further analyses on this specimen. This is likely an error in the generic influenza virus type A detection assay. Repeating this specimen and a review of used primers and probes should resolve this issue.

Laboratories currently not performing type A N-subtyping and/or type B-lineage determination should consider adding this capability to their assay repertoire.

Seasonal reviews 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 high number of laboratories failed to isolate influenza type B viruses. Furthermore, some laboratories failed to isolate the A(H1N1)pdm09 virus and one failed to isolate all except one B/Yamagata virus despite multiple attempts. A review of all relevant SOPs is recommended as these errors are usually due to reagent, cell or sensitivity issues. Laboratories using MDCK-SIAT cells alone may want to consider using an additional MDCK cell type. This lack of sensitivity must be further addressed in discussion with the laboratories concerned.

Continued support of and 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.

Strain characterisation

The number of laboratories performing antigenic and/or genetic strain characterisation is considerable (38/56; 68%). However, given that 27 of these laboratories are based in the EU/EEA, it suggests somewhat unequal distribution across the WHO European Region. Increasing this capability in a number of additional laboratories would improve timely surveillance of circulating variants across the whole region.

Antigenic characterisation

Antigenic characterisation of influenza viruses is considered of good quality based on concordance in the reported antigenic TESSy categories. However, it appeared difficult to allocate A(H3N2) viruses to one category, possibly reflecting differences in used ferret sera and other assay characteristics, such as species source of RBCs andthe use of oseltamivir in HI assay to inhibit neuraminidase-induced haemagglutination of current A(H3N2) viruses. Standardisation of assays and the reagents used and testing with an increased number of ferret sera covering strains seen in multiple seasons may be an approach to increasing accuracy. However, distribution of high volumes and 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. In addition, for accurate reporting in TESSy, a mechanism should be in place for timely updating of the antigenic categories in response to the emergence of new antigenic variants. This should be accompanied by making the appropriate ferret reference sera and influenza reference strains for antigenic characterisation available.

Genetic characterisation

A couple of laboratories had difficulty allocating certain viruses to the correct TESSy categories. Errors made were possibly due to incorrect interpretation of obtained results after phylogenetic and amino acid substitution analysis. For laboratories with errors, training in these types of analysis and appropriate use of online tools like FluSurver [37] is recommended.

One laboratory correctly identified a B/Yamagata-containing specimen, but incorrectly reported it as a B/Victoria lineage B/Hong Kong/269/2017 virus with deletion 162-164 in its HA. This could be the result of sample switching or a reporting error. Review of the original sequencing results against the reported category should resolve this issue. 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 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.

Reporting of appropriate genetic categories to TESSy requires updating these categories to reflect emerging clades during the season. In the absence of such updates, laboratories that wish to correctly report the outcomes of phylogenetic analysis are forced to allocate viruses to an inappropriate category or report it in a free text comment, which complicates weekly analysis for Flu News Europe. A mechanism to update genetic categories more frequently would be desirable.

Antiviral susceptibility testing

Relatively few (29/56; 52%) laboratories are performing some level of antiviral susceptibility testing and they are unequally distributed across the region (22 in the EU/EEA). Increasing this capability in a number of additional laboratories would benefit the timely estimation of antiviral susceptibility across the Region and reporting to TESSy.

Genetic antiviral susceptibility testing

Two laboratories reported the detection of N1-H275Y in the wild-type A(H1N1)pdm09 virus specimens and one laboratory (ID 200) reported R292K in both wild-type A(H3N2) specimens. These laboratories did not report the HRI interpretation for the amino acid substitutions. Errors may have been made in the reporting of the amino acid positions screened and results obtained. Several laboratories reported the amino acid substitutions obtained from FluSurver [37] by comparison with the nearest vaccine strain instead of using the amino acid positions associated with reduced antiviral susceptibility that can be found one layer deeper in FluSurver [37] or in the list provided by the WHO working group on surveillance of influenza antiviral susceptibility [38]. In addition, a considerable number of laboratories made over- or under-interpretation errors based on the level of testing (e.g. SNP RT-PCR versus full-length NA segment sequencing). These observations suggest that laboratories are not fully familiar with data interpretation and reporting to TESSy.

Similar to genetic strain characterisation, reporting correct data is the responsibility of the submitting laboratory. However, laboratories are also asked to report GISAID accession numbers for NA sequences to TESSy. Therefore, interpretation of genetic antiviral susceptibility data reported to TESSy is backed up by the possibility of performing additional sequence analysis for validation of suspicious results.

A number of laboratories did not report the mixed nature of the A(H1N1)pdm09 N1-275YH mixed specimen. Careful review of SNP detection or sequencing results should identify whether the wild-type was not detected or overlooked after detecting 275Y. Alternatively, these laboratories may not have been familiar with how to report the mixed nature of this specimen.

Laboratories would benefit from training on interpretation and reporting of antiviral susceptibility data. Recordings of previous webinars and instruction documents are available on the EISN extranet.

Phenotypic antiviral susceptibility testing

A number of laboratories incorrectly identified the B/Victoria NA-E105K containing virus with an IC_{50} value considered 'normal inhibited'. This was not a testing error in itself, but mainly the result of using a chemiluminescent assay that is known to be less sensitive in detecting mixtures of wild-type and (highly) reduced inhibited virus, the A(H3N2) NA-E119V variant and other variants like the B/Victoria NA-E105K variant with mildly reduced inhibition. Laboratories using a chemiluminescent assay should consider switching to in-house MUNANA or commercial fluorescent-based neuraminidase inhibition assay for routine use.

Accreditation

ISO 15189 recommends external 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 accreditation. Laboratories would also benefit from inclusion of tests used in routine laboratory surveillance of influenza in the scope of ISO 15189 accreditation.

References

1. World Health Organization (WHO). Global Influenza Surveillance and Response System (GISRS) [Internet]. Geneva: WHO; 2019. Available from: http://www.who.int/influenza/gisrs_laboratory.
2. World Health Organization (WHO). WHO Collaborating Centres for influenza and their terms of reference [Internet]. Geneva: WHO; 2018. Available from: http://www.who.int/influenza/gisrs_laboratory/collaborating_centres/list.
3. World Health Organization (WHO). WHO reference laboratories for diagnosis of influenza A/H5 infection [Internet]. Geneva: WHO; 2017. Available from: http://www.who.int/influenza/gisrs_laboratory/h5_reflabs/list.
4. World Health Organization (WHO). National Influenza Centres – European Region of WHO [Internet]. Geneva: WHO; 2018. Available from: http://www.who.int/influenza/gisrs_laboratory/national_influenza_centres/list/en/index3.html.
5. European Centre for Disease Prevention and Control. European Reference Laboratory Network for Human Influenza (ERLI-Net) [Internet]. Stockholm: ECDC; 2017. Available from: http://ecdc.europa.eu/en/healthtopics/influenza/laboratory_network/Pages/laboratory_network.aspx.
6. European Parliament and European Council. Regulation (EC) No 851/2004 of the European Parliament and the Council of 21 April 2004 establishing a European centre of disease prevention and control. Strasbourg: European Parliament and European Council; 2004. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32004R0851.
7. Wu A, Su C, Wang D, Peng Y, Liu M, Hua S, et al. Sequential Reassortments Underlie Diverse Influenza H7N9 Genotypes in China. Cell Host Microbe. 2013 Oct 16;14(4):446-52.
8. Westgeest KB, Russell CA, Lin X, Spronken MI, Bestebroer TM, Bahl J, et al. Genomewide Analysis of Reassortment and Evolution of Human Influenza A(H3N2) Viruses Circulating between 1968 and 2011. J Virol. 2014 Mar;88(5):2844-57.
9. European Centre for Disease Prevention and Control. Strategy for the external quality assessment of public health microbiology laboratories. Stockholm: ECDC; 2018. Available from: http://ecdc.europa.eu/publications-data/strategy-external-quality-assessment-public-health-microbiology-laboratories.
10. Valette M, Aymard M. Quality control assessment of influenza and RSV testing in Europe: 2000-01 season. Euro Surveill. 2002 Nov;7:161-5. Available from: http://www.eurosurveillance.org/content/10.2807/esm.07.11.00375-en.
11. Meijer A, Valette M, Manuguerra JC, Pérez-Breña P, Paget J, Brown C, et al. Implementation of the community network of reference laboratories for human influenza in Europe. J Clin Virol. 2005 Oct;34(2):87-96.
12. Thompson CI, Lackenby A, Daniels RS, McCauley JW, Pereyaslov D, Broberg EK, et al. Evaluation of influenza virus antiviral susceptibility testing in Europe: results from the first external quality assessment exercise. J Clin Virol. 2013 Mar;56(3):212-8.
13. World Health Organization. WHO External Quality Assessment Project for the detection of influenza viruses by PCR. Geneva: WHO; 2012 Available from: http://www.who.int/influenza/gisrs_laboratory/external_quality_assessment_project.
14. Mackay WG, van Loon AM, Niedrig M, Meijer A, Lina B, Niesters HG. Molecular detection and typing of influenza viruses: are we ready for an influenza pandemic? J Clin Virol. 2008 Jun;42(2):194-7.
15. Reading PC, Leung VK, Buettner I, Gillespie L, Deng YM, Shaw R, et al. The first external quality assessment of isolation and identification of influenza viruses in cell culture in the Asia Pacific region, 2016. J Clin Virol. 2017 Dec;97:54-58.
16. Snacken R, Brown C. New developments of influenza surveillance in Europe. Euro Surveill. 2015 Jan 29;20(4). Available from: http://www.eurosurveillance.org/content/10.2807/ese.20.04.21020-en.
17. World Health Organization. Laboratory quality management system: handbook. Geneva: WHO; 2011. Available from: http://www.who.int/ihr/publications/lqms.
18. Jonges M, Liu WM, van der Vries E, Jacobi R, Pronk I, Boog C, et al. Influenza Virus Inactivation for Studies of Antigenicity and Phenotypic Neuraminidase Inhibitor Resistance Profiling. J Clin Microbiol. 2010 Mar;48(3):928-40.

19. European Centre for Disease Prevention and Control. Influenza virus characterisation guidelines for the northern hemisphere influenza season 2016-2017. Stockholm: ECDC; October 2016.

20. European Centre for Disease Prevention and Control. Influenza virus characterisation guidelines for the northern hemisphere influenza season 2017-2018. Stockholm: ECDC; October 2017.

21. European Centre for Disease Prevention and Control. Reporting and interpretation of antiviral susceptibility data. Stockholm: ECDC; November 2014.

22. European Centre for Disease Prevention and Control. External quality assessment scheme for detection, isolation and characterisation of influenza viruses for the European Reference Laboratory Network for Human Influenza – 2015. Stockholm: ECDC; 2016. Available from: http://ecdc.europa.eu/publications-data/external-quality-assessment-scheme-detection-isolation-and-characterisation.

23. World Health Organization. Detection of influenza viruses by reverse transcription polymerase chain reaction: WHO external quality assessment programme summary analysis, 2017. Wkly Epidemiol Rec. 2018 Jan 12;93(2):9-16.

24. Gregory V, Bennett M, Orkhan MH, Al Hajjar S, Varsano N, Mendelson E, et al.. Emergence of Influenza A H1N2 Reassortant Viruses in the Human Population during 2001. Virology. 2002 Aug 15;300(1):1-7.

25. Paget WJ, Meerhoff TJ, Goddard NL; EISS. Mild to moderate influenza activity in Europe and the detection of novel A(H1N2) and B viruses during the winter of 2001-02. Euro Surveill. 2002 Nov;7(11):147-57. Available from: http://www.eurosurveillance.org/content/10.2807/esm.07.11.00373-en.

26. Meijer A, Swaan CM, Voerknecht M, Jusic E, van den Brink S, Wijsman LA, et al. Case of seasonal reassortant A(H1N2) influenza virus infection, the Netherlands, March 2018. Euro Surveill. 2018 Apr;23(15). Available from: http://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2018.23.15.18-00160.

27. Kissling E, Valenciano M, Pozo F, Vilcu AM, Reuss A, Rizzo C, et al. 2015/16 I-MOVE/I-MOVE+ multicentre case-control study in Europe: Moderate vaccine effectiveness estimates against influenza A(H1N1)pdm09 and low estimates against lineage-mismatched influenza B among children. Influenza Other Respir Viruses. 2018 Jul;12(4):423-437.

28. Dukes JD, Whitley P, Chalmers AD. The MDCK variety pack: choosing the right strain. BMC Cell Biol. 2011 Oct 7;12:43.

29. Matrosovich M, Matrosovich T, Carr J, Roberts NA, Klenk HD. Overexpression of the α-2,6-Sialyltransferase in MDCK Cells Increases Influenza Virus Sensitivity to Neuraminidase Inhibitors. J Virol. 2003 Aug;77(15):8418-25.

30. Hatakeyama S, Sakai-Tagawa Y, Kiso M, Goto H, Kawakami C, Mitamura K, et al. Enhanced Expression of an α2,6-Linked Sialic Acid on MDCK Cells Improves Isolation of Human Influenza Viruses and Evaluation of Their Sensitivity to a Neuraminidase Inhibitor. J Clin Microbiol. 2005 Aug;43(8):4139-46.

31. Ampofo WK, Azziz-Baumgartner E, Bashir U, Cox NJ, Fasce R, Giovanni M, et al. Strengthening the influenza vaccine virus selection and development process: Report of the 3rd WHO Informal Consultation for Improving Influenza Vaccine Virus Selection held at WHO headquarters, Geneva, Switzerland, 1-3 April 2014. Vaccine. 2015 Aug 26;33(36):4368-82.

32. Hampson A, Barr I, Cox N, Donis RO, Siddhivinayak H, Jernigan D, et al. Improving the selection and development of influenza vaccine viruses - Report of a WHO informal consultation on improving influenza vaccine virus selection, Hong Kong SAR, China, 18-20 November 2015. Vaccine. 2017 Feb 22;35(8):1104-1109.

33. European Centre for Disease Prevention and Control. External quality assessment scheme for influenza antiviral susceptibility for the European Reference Laboratory Network for Human Influenza – 2015. Stockholm: ECDC; 2016. Available from: http://ecdc.europa.eu/publications-data/external-quality-assessment-scheme-influenza-antiviral-susceptibility-european.

34. Escuret V, Collins PJ, Casalegno JS, Vachieri SG, Cattle N, Ferraris O, et al. A Novel I221L Substitution in Neuraminidase Confers High-Level Resistance to Oseltamivir in Influenza B Viruses. J Infect Dis. 2014 Oct 15;210(8):1260-9.

35. Fujisaki S, Takashita E, Yokoyama M, Taniwaki T, Xu H, Kishida N, et al. A single E105K mutation far from the active site of influenza B virus neuraminidase contributes to reduced susceptibility to multiple neuraminidase-inhibitor drugs. Biochem Biophys Res Commun. 2012 Dec 7;429(1-2):51-6.

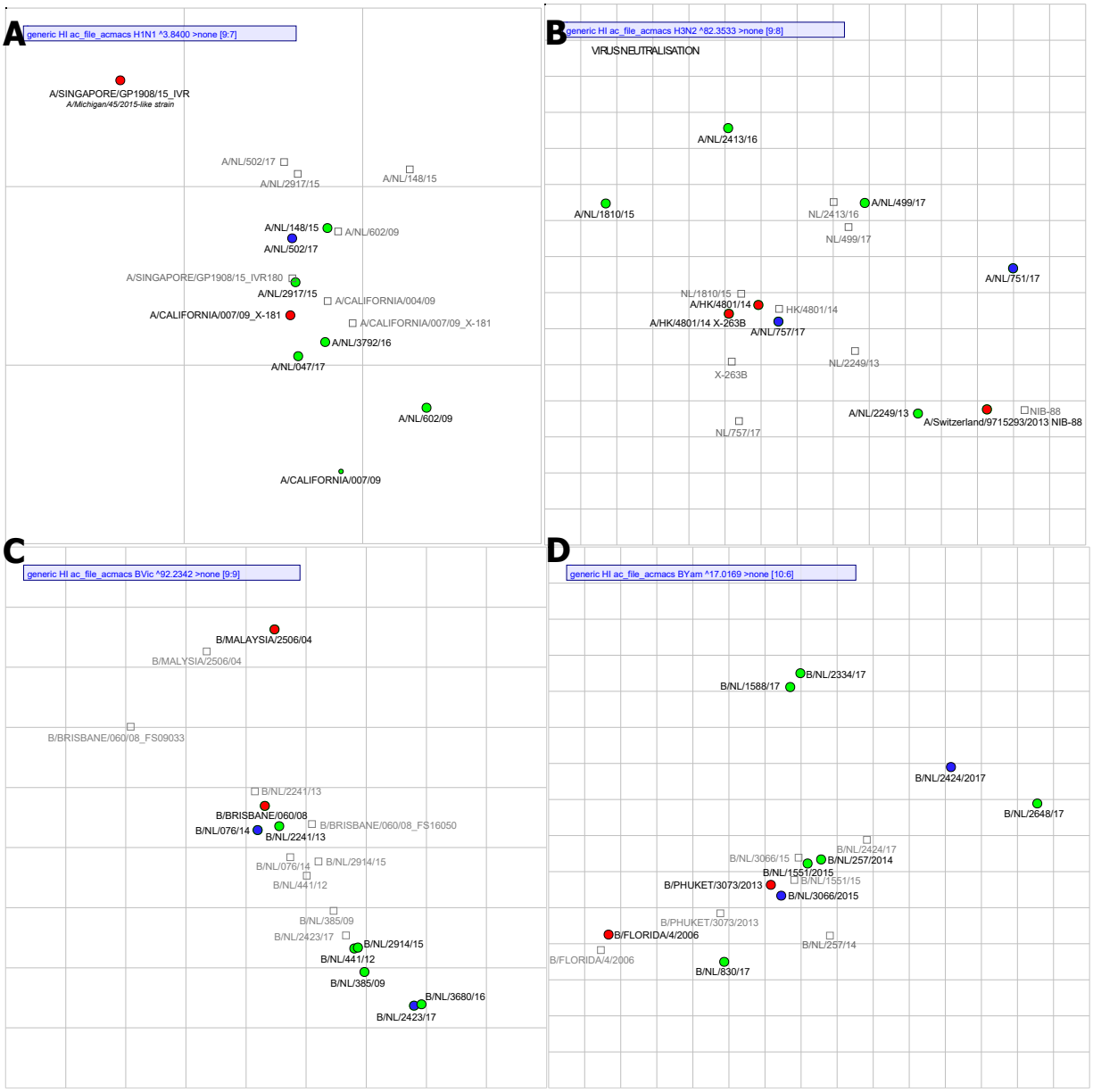
36. Lackenby A, Besselaar TG, Daniels RS, Fry A, Gregory V, Gubareva LV, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors and status of novel antivirals, 2016-2017. Antiviral Res. 2018 Sep;157:38-46.

37. A*STAR Bioinformatics Institute. FluSurver Influenza surveillance – Prepared for the next wave. Singapore: A*STAR; 2019. Available from: http://flusurver.bii.a-star.edu.sg.

38. World Health Organization. Summary of neuraminidase amino acid substitutions associated with reduced inhibition by neuraminidase inhibitors. Geneva: WHO; 2018. Available from: http://www.who.int/influenza/gisrs_laboratory/antiviral_susceptibility/NAI_Reduced_Susceptibility_Marker_Table_WHO.pdf.

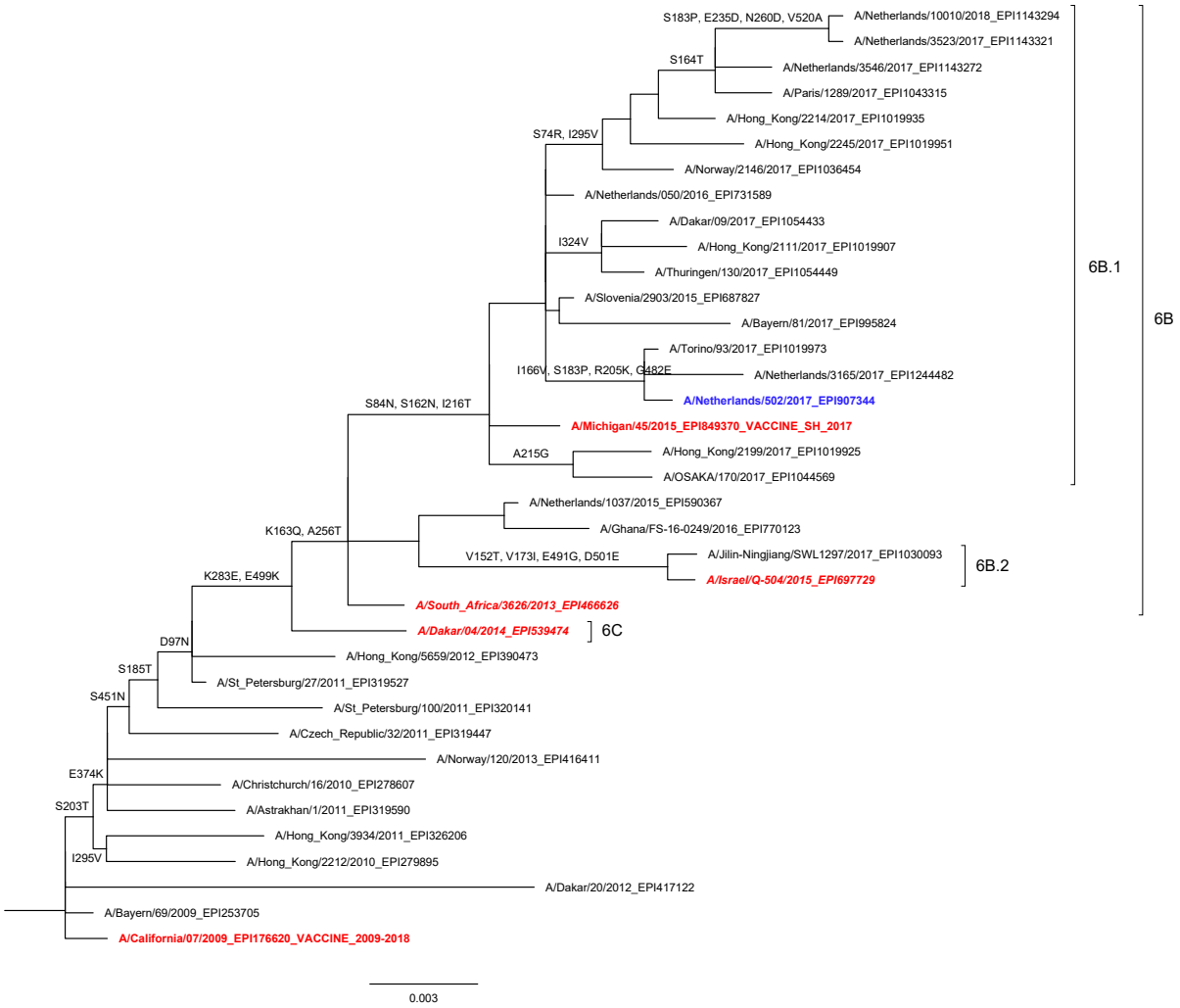
Annexes: extra tables and figures

Annex Figure 1. Antigenic cartography maps created based on HI-assay data for A(H1N1)pdm09 (A), B/Victoria (C) and B/Yamagata influenza viruses (D) and based on virus neutralisation data for A(H3N2) influenza virus (B)



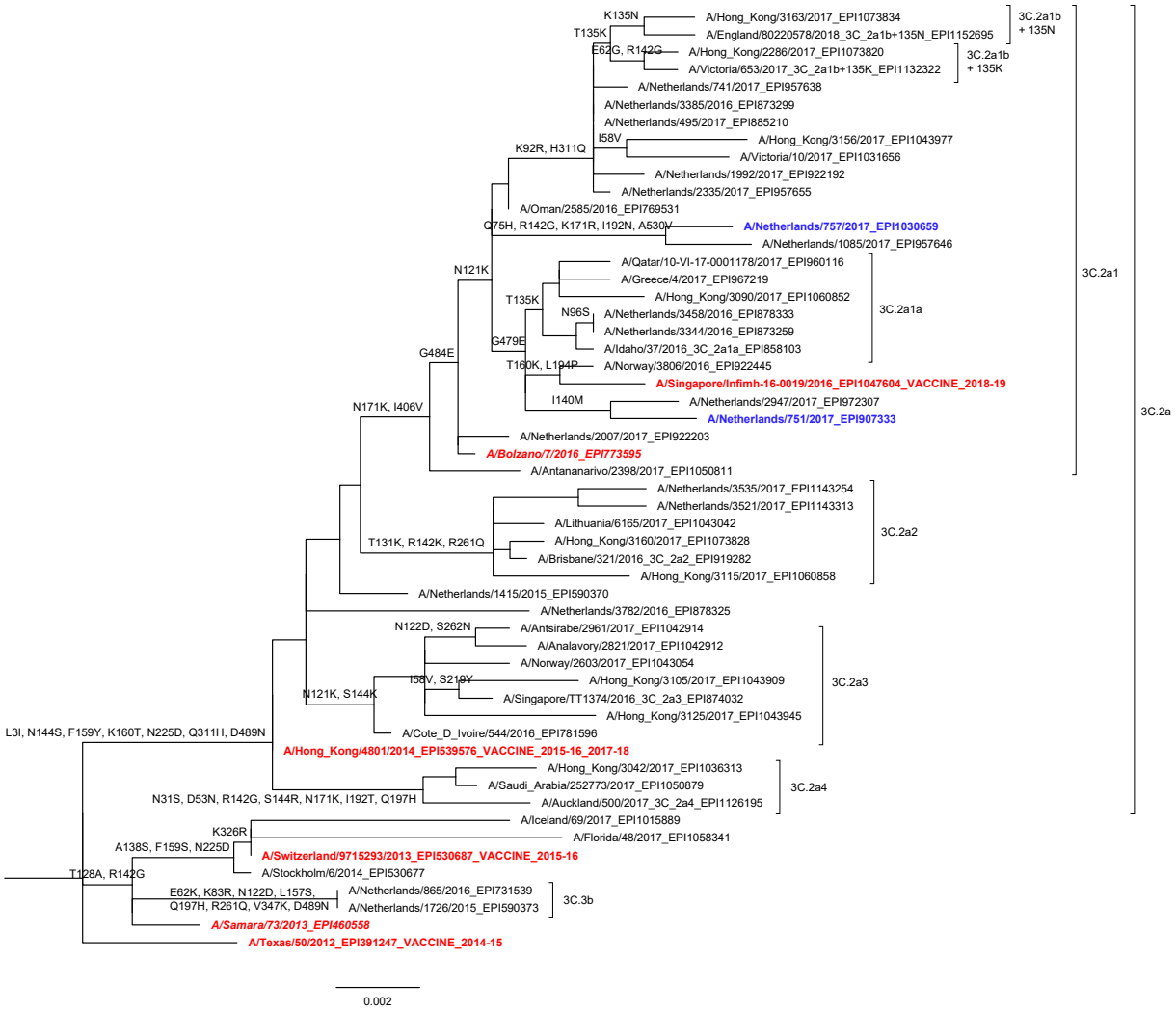
Generated at the Dutch National Influenza Centre, ErasmusMC, Rotterdam, the Netherlands. Vaccine viruses indicated with red dot, viruses included in the panel indicated with blue dot and other recent viruses indicated with green dot. Spacing between grid lines is one unit of antigenic distance, corresponding to a twofold dilution of antiserum in the HI or virus neutralisation assay.

Annex Figure 2A. Phylogenetic tree of full HA of A(H1N1)pdm09 influenza viruses with common amino acid changes for viruses at indicated branch



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 indicated in bold red font and other reference viruses for reporting TESSy categories indicated in bold red italic font. Viruses included in panel indicated in bold blue font.

Annex Figure 2B. Phylogenetic tree of full HA of A(H3N2) influenza viruses with common amino acid changes for viruses at indicated branch

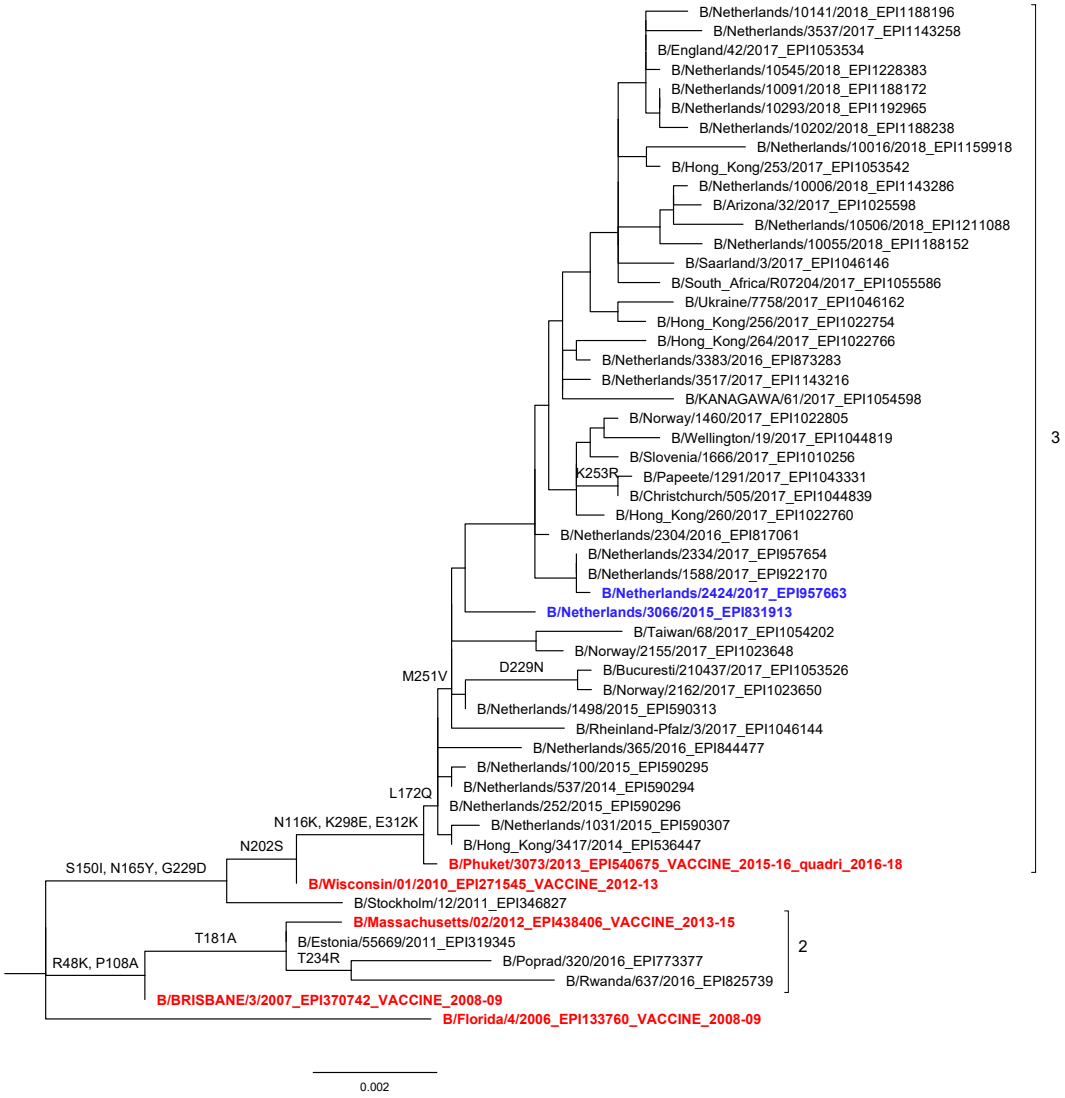


Reference virus set recommended by ECDC for analysis of viruses to be reported to TESSy supplemented with relevant viruses from the Netherlands have been used to infer the phylogenetic tree. Vaccine viruses indicated in bold red font and other reference viruses for reporting TESSy categories indicated in bold red italic font. Viruses included in the panel indicated in bold blue font.

Annex Figure 2C. Phylogenetic tree of full HA of B/Victoria influenza viruses with common amino acid changes for viruses at indicated branch

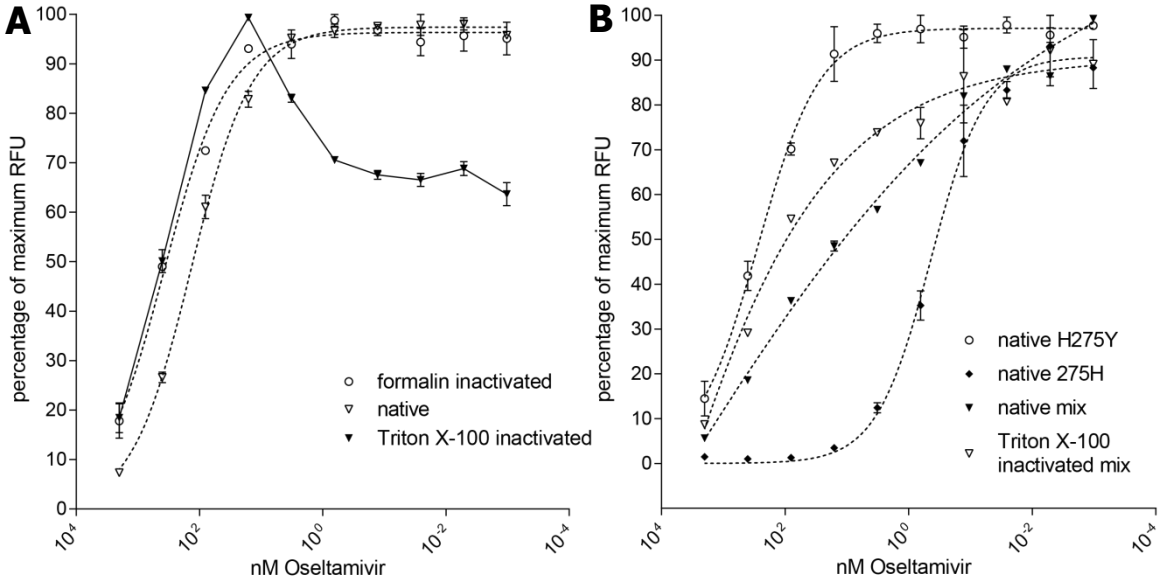
Reference virus set recommended by ECDC for analysis of viruses to be reported to TESSy supplemented with relevant viruses from the Netherlands have been used to infer the phylogenetic tree. Vaccine viruses indicated in bold red font and other reference viruses for reporting TESSy categories indicated in bold red italic font. Viruses included in the panel indicated in bold blue font.

Annex Figure 2D. Phylogenetic tree of full HA of B/Yamagata influenza viruses with common amino acid changes for viruses at indicated branch



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 indicated in bold red font for reporting TESSy categories. Viruses included in the panel indicated in bold blue font.

Annex Figure 3. Neuraminidase inhibition curves for determination of IC50 for oseltamivir of B/Victoria NA- E105K mutant (A) and A(H1N1)pdm09 N1-H275Y(mixH/Y) mutant (B) using native virus and inactivated by Triton X-100 or formalin



Annex Table 1. List of participants

Country	City	Organisation
Albania	Tirana	Albanian National Institute of Public Health National Virology Laboratory, National Influenza laboratory
Armenia	Yerevan	National Centre for Disease Control and Prevention (NCDCP)
Austria	Vienna	Center of Virology, Medical University Vienna
Azerbaijan	Baku	Republican Anti-Plague Station – virological laboratory
Belarus	Minsk	Republik Research-Practical Centre of Epidemiology & Microbiology
Belgium	Brussels	National Influenza Centre, Scientific Institute of Public Health
Bosnia and Herzegovina	Sarajevo	OU Clinical Microbiology, Clinical Center University of Sarajevo
Bosnia and Herzegovina, Republic of Srpska	Banja Luka	Public Health Institute of the Republic of Srpska
Bulgaria	Sofia	Department of Virology, National Laboratory 'Influenza and ARD'
Cyprus	Nicosia	Microbiology Department, Nicosia General Hospital
Czech Republic	Prague	National Institute of Public Health, Centre for Epidemiology and Microbiology, Reference Laboratory for Influenza
Denmark	Copenhagen	Statens Serum Institut
Estonia	Tallinn	Central Laboratory of Communicable Diseases
Finland	Helsinki	National Institute for Health and Welfare (THL), Expert Microbiology Unit
Former Yugoslav Republic of Macedonia	Skopje	Institute of Public Health of Republic of Macedonia, Laboratory for Virology and Molecular Diagnostics
France	Lyon	CNR Virus des infections respiratoires, Laboratoire de Virologie, Centre de Biologie & Pathologie Nord – IAI
France	Paris	Institut Pasteur, Centre National de Reference Virus des infections respiratoires (dont la grippe)
Georgia	Tbilisi	National Center for Disease Control & Public Health
Germany	Berlin	Robert Koch-Institut, NRZ Influenza
Greece	Athens	Hellenic Pasteur Institute, National Influenza Reference Laboratory for Southern Greece
Greece	Thessaloniki	National Influenza Centre for Northern Greece, Microbiology Department, Medical School, Aristotle University of Thessaloniki
Hungary	Budapest	National Public Health Institute, Directorate of Clinical and Public Health Microbiology , Department Respiratory Viruses
Iceland	Reykjavik	Virus laboratories, Landspital –- National University Hospital
Ireland	Dublin	UCD National Virus Reference Laboratory, University College Dublin
Israel	Ramat Gan	Central Virology Laboratory, Sheba Medical Centre
Italy	Rome	National Influenza Centre, Department of Infectious Diseases, Istituto Superiore di Sanità
Kazakhstan	Almaty	National Reference Laboratory for Control of Viral Infections, Research-Practical Center of Sanitary-Epidemiological Expertise and Monitoring
Kyrgyzstan	Bishkek	Department of the State Sanitary and Epidemiological Surveillance, Ministry of Health, Centre of Molecular-Genetic and Microbiological Investigations
Latvia	Riga	Riga East University Hospital, Latvian Centre of Infectious Diseases, National Microbiology Reference Laboratory
Lithuania	Vilnius	National Public Health Surveillance Laboratory
Luxembourg	Luxembourg	Laboratoire National de Santé, Service Virologie-Serologie
Malta	Msida	Molecular Diagnostics, Pathology Department, Mater Dei Hospital
Moldova	Chisinau	National Center for Public Health, Laboratory of Viral Infections
Montenegro	Podgorica	Institute of Public Health of Montenegro
The 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)
The Netherlands	Rotterdam	Erasmus Medical Centre, Department of Viroscience
Norway	Oslo	Norwegian Institute of Public Health, Department of Influenza

Country	City	Organisation
Portugal	Lisboa	Instituto Nacional de Saúde Dr. Ricardo Jorge, Laboratório Nacional de Referência Vírus Gripe
Romania	Bucharest	Institutul Național de Cercetare-Dezvoltare Medico-Militară (INCDMM) 'Cantacuzino', Laboratory for Viral Respiratory Infections, National Influenza Center Romania
Russia	Moscow	Influenza Etiology and Epidemiology Laboratory, D.I. Ivanovsky Institute of Virology 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), Department of Zoonotic Infections and Influenza
Russia	Saint Petersburg	Research Institute of Influenza, WHO-recognised National Influenza Centre of the Russian Federation
Serbia	Belgrade	Institute of Virology, Vaccine and Sera 'Torlak', Respiratory Department
Slovakia	Bratislava	National Influenza Center, Public Health Authority of the Slovak Republic
Slovenia	Ljubjana	Laboratory for Public Health Virology, National Laboratory for Health, Environment and Food
Spain	Barcelona	Laboratory of Microbiology, Hospital Clinic
Spain	Madrid	Instituto de Salud Carlos III, Influenza and Respiratory Virus Laboratory, National Center for Influenza Madrid
Spain	Valladolid	Centro Nacional de Gripe de Valladolid, Servicio de Microbiología e Inmunología, Hospital Clínico Universitario de Valladolid
Sweden	Solna	Public Health Agency of Sweden, Unit for Laboratory Surveillance of Viral Pathogens and Vaccine Preventable Diseases
Switzerland	Geneva	National Center of Influenza, University of Geneva Hospitals, Laboratory of Virology
Turkey	Ankara	Public Health Institution of Turkey, Virology Reference and Research Laboratory, National Influenza Centre
Ukraine	Kyiv	L.V. Gromashevsky Institute of Epedemiology and Infectious Diseases, National Academy of Medical Science of Ukraine
United Kingdom, England	London	Public Health England, Respiratory Virus Unit, Virus Reference Department, National Infection Service, Colindale
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
Uzbekistan	Tashkent	Republican Centre of State Sanitary Epidemiological Surveillance of the Ministry of Health of the Republic of Uzbekistan

Annex Table 2. Overview in which challenge type each participating laboratory participated

Participant ID[1]	Challenge type				Full programme
	Molecular detection	Virus isolation	Characterisation (antigenic, genetic)	Antiviral susceptibility testing (genetic, phenotypic)	
50	Yes	Yes	Antigenic only	None	No
95	Yes	Yes	Both	Both	Yes
112	Yes	No	None	None	No
117	Yes	No	None	None	No
200	Yes	Yes	Both	Both	Yes
1159	Yes	Yes	Both	Both	Yes
1299	Yes	Yes	Both	Both	Yes
1643	Yes	Yes	Genetic only	Both	No
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
2270	Yes	No	None	None	No
2271	Yes	Yes	Both	Both	Yes
2272	Yes	No	None	None	No
2274	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	None	No
3442	Yes	Yes	Both	Both	Yes
10007	Yes	Yes	Both	Genetic only	No
10014	Yes	Yes	Both	Genetic only	No
10023	Yes	Yes	Genetic only	Both	No
10078	Yes	Yes	None	None	No
10080	Yes	Yes	Genetic only	Both	No
10104	Yes	Yes	Genetic only	Both	No
10115	Yes	Yes	Antigenic only	Both	No
10144	Yes	Yes	Both	Both	Yes
10205	Yes	Yes	None	Genetic only	No
10461	Yes	Yes	Antigenic only	Genetic only	No
10462	Yes	Yes	Genetic only	None	No
10464	Yes	Yes	Both	Both	Yes
10465	Yes	Yes	Both	Both	Yes
10466	Yes	Yes	Both	Both	Yes
1600	Yes	Yes	Antigenic only	None	No
1991	Yes	Yes	None	None	No
2295	Yes	Yes	None	None	No
2812	Yes	No	None	None	No
2813	Yes	No	None	None	No
2814	No	Yes	Antigenic only	None	No
2815	Yes	Yes	None	None	No
2817	Yes	Yes	Both	Both	Yes
2826	Yes	Yes	Antigenic only	Genetic only	No
3558	Yes	Yes	Both	Both	Yes
4344	Yes	Yes	Both	Both	Yes
10053	Yes	Yes	Both	Genetic only	No
10142	Yes	No	None	None	No
10206	Yes	Yes	Antigenic only	None	No
10248	Yes	No	None	None	No
10261	Yes	Yes	Antigenic only	Phenotypic only	No
10492	Yes	Yes	None	None	No
10493	Yes	No	None	None	No
10494	Yes	No	None	None	No
10498	Yes	Yes	Genetic only	Both	No
10507	Yes	Yes	Antigenic only	None	No

[1]*Cell with orange shading: laboratory located in EU/EEA member country.*

Annex Table 3. 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 used methodology

Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Type and A H-subtype score [4]	Overall score [5]	Assay type			
Expected result	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A (H3N2)	B/Yam			Type A/B	A H-subtype	A N-subtype	B-lineage
Participant ID[1]														
50	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
95	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house[6]	In-house	In-house	In-house
112	A(H3)	B	B	A(H1N1)pdm09	Negative	B	A(H3)	B	0	6	In-house, Luminex NxTag RPP kit	In-house, Luminex NxTag RPP kit	Not performed	Not performed
117	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
200	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house	In-house	In-house
1159	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house	In-house	In-house
1299	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house	In-house	In-house
1643	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
2125	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	sequencing	CDC
2126	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
2253	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	CDC	CDC	CDC	CDC
2258	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
2270	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	Sacace Influenza A/B kit	In-house	In-house	In-house
2271	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	CDC	In-house	CDC
2272	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
2274	A(H3)	B	B	A(H1pdm09)	Negative	B	A(H3)	B	0	7	Fast Track Diagnostics Respiratory Screen 33	Fast Track Diagnostics A-Subtyping	Not performed	Not performed
2276	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
2277	A(H3)	B	B	A(H1pdm09)	Negative	B	A(H3)	B	0	7	EliGene Influenza A/B/Pandemic LC kit	EliGene Influenza A/B/Pandemic LC kit	Not performed	Not performed
2278	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
2820	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
3442	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3) [3]	B/Yam	0	3	In-house	Prodesse ProFast+ kit	Not performed	In-house
10007	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	sequencing	In-house
10014	A(H3)	B	B	A(H1pdm09)	Negative	B	A(H3)	B	0	7	Luminex NxTag RPP	Luminex NxTag RPP	Not performed	Not performed
10023	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
10078	A(H3)	B	B	A(H1pdm09)	Negative	B	A(H3)	B	0	7	CDC	CDC	Not performed	Not performed
10080	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house	In-house	In-house
10104	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house	In-house	In-house
10115	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house, CDC	In-house, CDC	In-house	In-house
10144	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	In-house, CDC	Not performed	In-house
10205	A(H3)	B	B	A(H1pdm09)	Negative	B	A(H3)	B	0	7	In-house	In-house	Not performed	Not performed
10461	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	Luminex NxTag RPP	CDC	Not performed	CDC
10462	A(H3)	B	B	A(H1pdm09)	B	B	A(H3)	B	3	10	Seegene Allplex Respiratory Panel 1	Seegene Allplex Respiratory Panel 1	Not performed	Not performed
10464	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	CDC	CDC	Sequencing	CDC
10465	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	CDC	In-house, CDC	In-house	In-house
10466	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house	In-house	In-house
1600	A(H3)	B	B	A(H1pdm09)	Negative	B	A(H3)	B	0	7	CDC	CDC	Not performed	Not performed
1991	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
2295	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
2812	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC

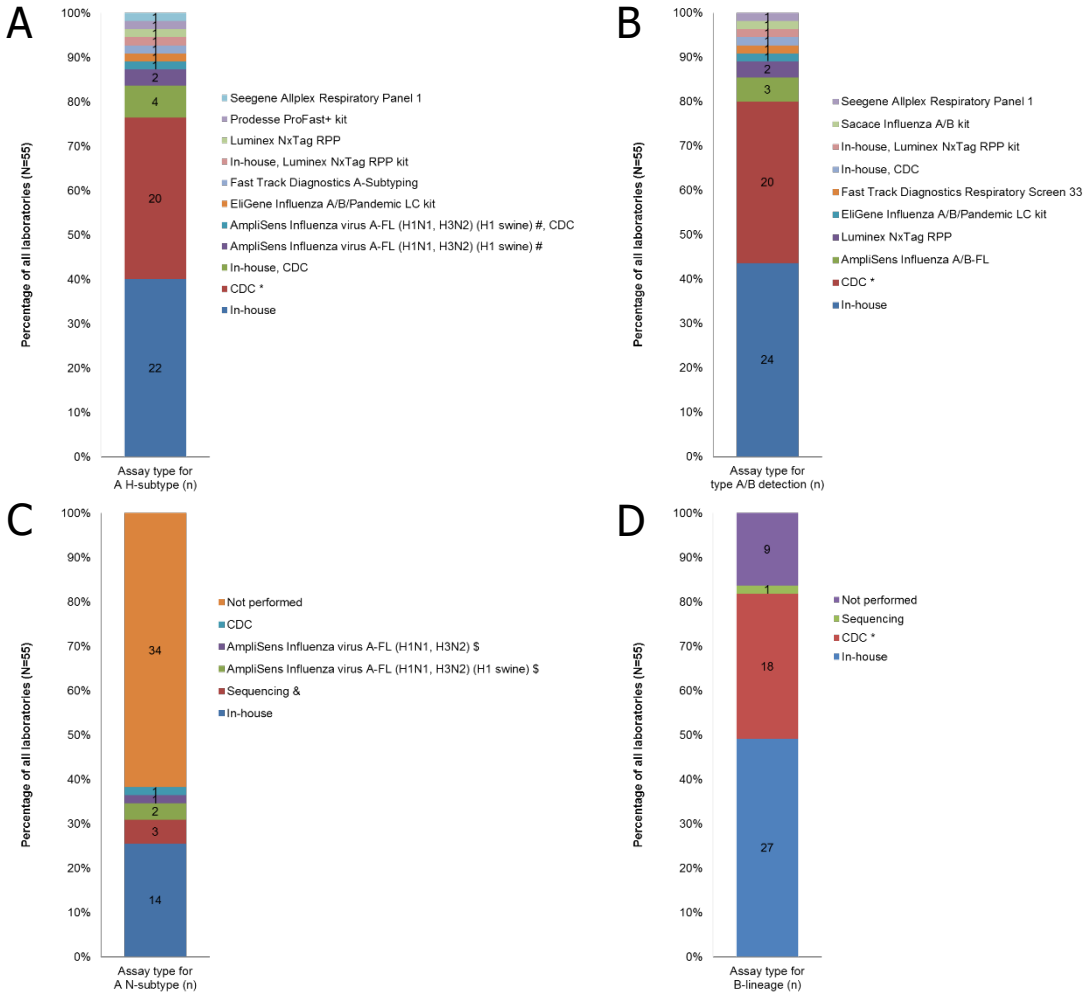
Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Type and A H-subtype score [4]	Overall score [5]	Assay type			
Expected result	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A (H3N2)	B/Yam			Type A/B	A H-subtype	A N-subtype	B-lineage
Participant ID[1]														
2813[2]	Negative	A(H1pdm09)	B/Yam	B/Vic	A(H3)	B/Yam	B/Vic	A(H3)	24	24	In-house	In-house	Not performed	In-house
2815	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
2817	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	AmpliSens Influenza A/B-FL	AmpliSens Influenza virus A-FL (H1N1, H3N2) (H1 swine)	AmpliSens Influenza virus A-FL (H1N1, H3N2) (H1 swine) [5]	Sequencing
2826	Negative	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	3	3	In-house	In-house	In-house	In-house
3558	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house, CDC	In-house	In-house
4344	A(H3N2)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3N2)	B/Yam	0	1	AmpliSens Influenza A/B-FL	AmpliSens Influenza virus A-FL (H1N1, H3N2) (H1 swine), CDC	AmpliSens Influenza virus A-FL (H1N1, H3N2)	CDC
10053	A(H3)	B	B	A(H1pdm09)	Negative	B	A(H3)	B	0	7	CDC	CDC	Not performed	Not performed
10142	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
10206	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
10248	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
10261	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	AmpliSens Influenza A/B-FL	AmpliSens Influenza virus A-FL (H1N1, H3N2) (H1 swine)	AmpliSens Influenza virus A-FL (H1N1, H3N2) (H1 swine) [8]	In-house
10492	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC [7]	CDC [7]	Not performed	CDC [7]
10493	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	In-house	In-house	Not performed	In-house
10494	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam	0	0	In-house	In-house	In-house	In-house
10498	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC
10507	A(H3)	B/Yam	B/Vic	A(H1pdm09)	Negative	B/Vic	A(H3)	B/Yam	0	3	CDC	CDC	Not performed	CDC

[1]Cell with orange shading = laboratory located in EU/EEA member country.
[2]Laboratory returned correct number of virus detections of specific type and subtype/lineage, but in incorrect random order and therefore all results are considered incorrect; hence scores of 24 and 24 respectively in the scoring columns.
[3]Laboratory returned 'Negative' for N-subtype.
[4]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 without H-subtype (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; and*
- *Negative specimen – negative (green shading), 0; all other results (red shading), 3.*

[5]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 without H- and N-subtype (shading not applicable), 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.
[6]In-house: in own laboratory developed or implemented or modified from published or personally obtained from elsewhere primers and probes or if not further specified; use of CDC primers and probes or kit indicated separately.
[7]Laboratory actually reported WHO; this presumably refers to CDC primers and probes used widely in eastern part of region.
[8]Kit insert of H1 swine test shows that assay is unsuitable for N-subtyping.

Annex Figure 4. Molecular methodologies used by 55 laboratories in 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)



In-house: in own laboratory developed or implemented or modified from primers and probes published or personally obtained from elsewhere or if methodology not further specified; use of CDC primers and probes or kit indicated separately as CDC.
**One laboratory actually reported WHO; this presumably refers to CDC primers and probes used widely in region.*
[#]Kit insert of (H1N1, H3N2) test states that assay should not be used for H1pdm09-subtyping and the (H1 swine) test used instead.
[$]Kit inserts of (H1N1, H3N2) and (H1 swine) tests state that assays are unsuitable for N1pdm09-subtyping.
[&]Two of three laboratories reporting using sequencing for N-subtyping did not report N-subtype in molecular diagnostics.
Numbers in bars indicate number of laboratories.

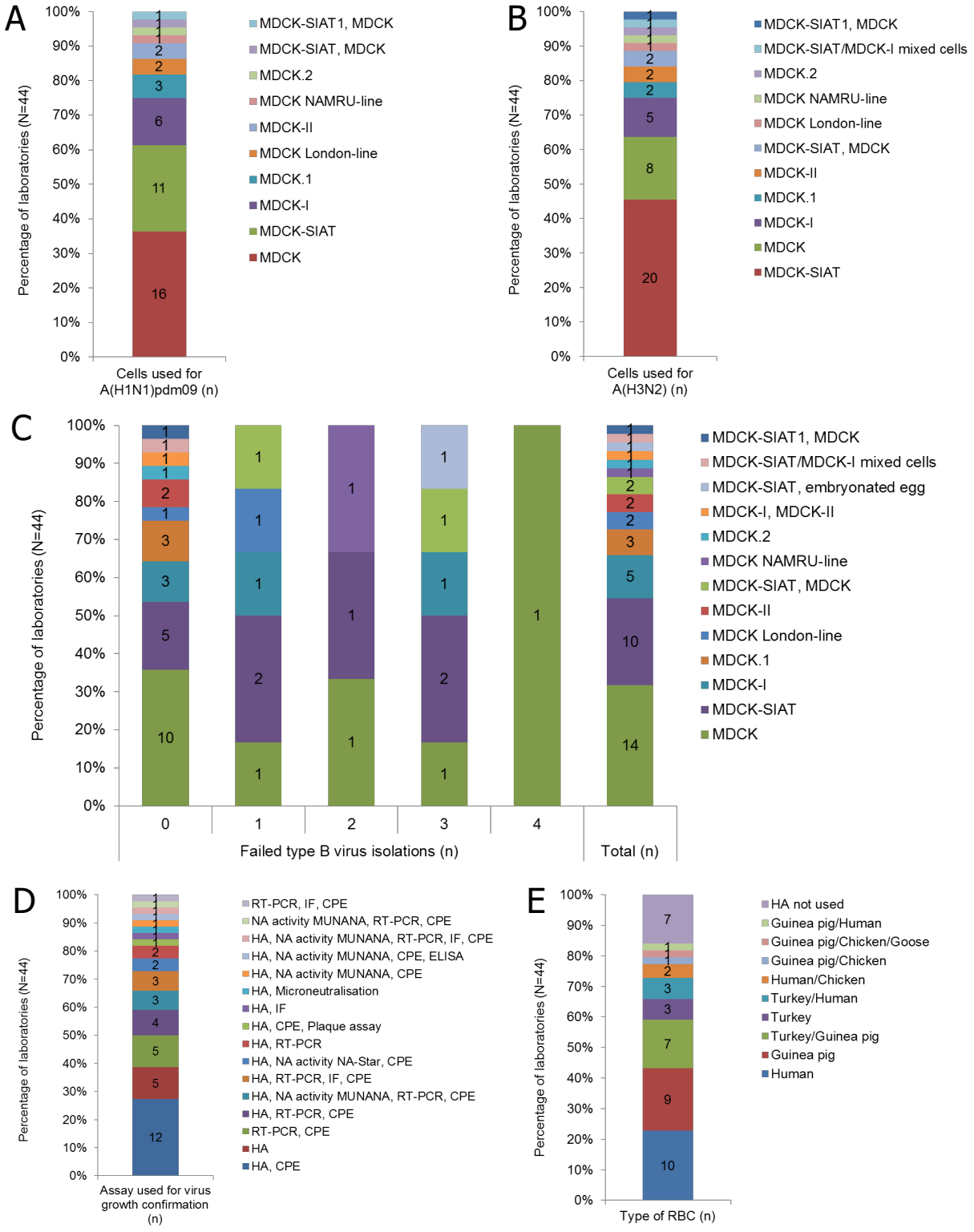
Annex Table 4. Overview of virus isolation results with performance score and used methodology

Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Overall score[5]	Isolation method (n specimens)[7]	Confirmation method virus isolation (n specimens)[7]						
Subtype/lineage	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam			HA-assay	NA activity		RT-PCR	IF	CPE	Other
Expected result	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive			RBC species	MUNANA	NA-STAR				
Participant ID[1]																	
50	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Positive	1	MDCK-I (8)	Human (8)	0	0	0	0	8	0
95	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Negative	2	MDCK-SIAT (8)	Turkey (8)	0	7	0	0	8	0
200	Positive	Positive	Positive	Positive*	Not attempted	Positive	Positive	Negative*	1	MDCK-SIAT (6) MDCK (2*)	Guinea pig (1)	0	0	1	1	7	0
1159	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive	2	MDCK-SIAT (8)	HA not used	0	0	8	0	8	0
1299	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK (8)	Guinea pig (8)	0	0	0	0	8	0
1643	Positive	Negative	Positive*	Negative	Negative	Negative	Positive	Negative	4	MDCK-SIAT (7) Embryonated egg (1*)	Turkey (8) Guinea pig (8)	6	0	1	7	7	0
2125	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK (8)	Turkey (1) Guinea pig (8)	0	7	0	0	8	0
2126	Positive	Positive*	Positive*	Positive	Negative	Positive	Positive	Positive*	0	MDCK.1 (5) MDCK-SIAT (3*)[6]	Turkey (5) Guinea pig (2)	0	0	0	0	8	0
2253	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-II (8)	Human (8)	0	0	8	0	7	0
2271	Positive*	Positive	Positive	Positive	Not attempted	Positive	Positive*	Positive	0	MDCK-SIAT (2*) MDCK London line (5)	Guinea pig (7)	0	0	0	0	0	0
2276	Positive*	Positive	Positive	Positive	Not attempted	Positive	Positive*	Positive	0	MDCK-SIAT (2*) MDCK (5)	Turkey (7) Guinea pig (7)	7	0	0	0	7	0
2277	Positive	Positive	Positive	Positive	Negative*	Positive	Positive	Positive	0	MDCK-I (7) MDCK-II (1*)	Human (8)	0	0	0	0	0	0
2278	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK (8) MDCK-SIAT1 (8)	Human (8) Chicken (2)	0	0	8	8	8	0
2820	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-SIAT (8)	Guinea pig (8)	0	0	0	0	8	0
3442	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Negative	4	MDCK-SIAT (8)	Guinea pig (8)	0	0	0	0	0	0
10007	Positive*	Positive	Positive	Positive	Not attempted	Positive	Positive*	Positive	0	MDCK-SIAT (2*) MDCK (5)	Turkey (5) Guinea pig (2)	0	0	0	0	0	0
10014	Positive*	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-SIAT (1*) MDCK (7)	Guinea pig (8)	0	0	0	0	0	0
10023[2]	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative	4	MDCK (8)	HA not used	0	0	3	0	8	0
10078	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK (8)	HA not used	0	0	4	8	8	0
10080	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-SIAT and MDCK-I mixed cells (8)	HA not used	8	0	7	0	8	0
10104	Positive*	Positive	Positive	Positive*	Not attempted	Positive	Positive*	Positive	0	MDCK-SIAT (3*) MDCK.1 (4)	Guinea pig (5)	0	0	0	0	7	7 [8]
10115	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-I (8)	Guinea pig (4) Chicken (6)	7	0	0	0	8	8 [9]
10144	Positive*	Positive	Positive	Positive	Negative	Negative	Positive*	Positive	1	MDCK-SIAT (2*) MDCK (6)	Turkey (5) Human (1)	7	0	8	0	8	0
10205	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-SIAT (8)	HA not used	0	0	8	0	8	0
10461	Positive*	Positive	Positive	Positive	Negative*	Positive	Positive*	Positive	0	MDCK-SIAT (3*) MDCK.1 (5)	Human (3) Chicken (6)	0	0	8	8	8	0
10462	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK.1 (8)	Human (8)	0	0	0	0	8	0

Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Overall score[5]	Isolation method (n specimens)[7]	Confirmation method virus isolation (n specimens)[7]						
Subtype/lineage	A(H3N2)	B/Yam	B/Vic	A(H1N1)pdm09	Negative	B/Vic	A(H3N2)	B/Yam			HA-assay	NA activity		RT-PCR	IF	CPE	Other
Expected result	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive			RBC species	MUNANA	NA-STAR				
Participant ID[1]																	
10464	Positive*	Positive	Positive	Positive	Negative	Positive	Positive*	Positive	0	MDCK-SIAT (2*) MDCK (6)	Turkey (8) Guinea pig (2)	7	0	8	0	8	0
10465	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK.2 (8)	Guinea pig (4)	0	0	0	0	0	3[10]
10466	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-SIAT (8)	Turkey (6) Guinea pig (7)	0	0	0	0	8	0
1600	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK (8)	Turkey (8)	0	0	0	0	8	0
1991	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Positive	1	MDCK London line (8)	Human (8)	0	0	0	2	0	0
2295	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Negative	2	MDCK NAMRU line (8)	Turkey (5)	0	0	0	0	8	0
2814	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-II (8)	Human (8)	0	0	8	0	7	0
2815	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	0	MDCK-I (8)	Human (8)	0	0	8	0	7	0
2817	Positive	Positive	Negative	Positive	Negative	Negative	Positive	Positive	2	MDCK (8)	Guinea pig (8) Chicken(5) Goose(1)	8	0	8	0	8	0
2826	Not attempted [4]	Positive	Positive	Positive	Not attempted	Positive	Positive	Positive	0	MDCK (6)	Human (6)	0	0	6	0	6	0
3558	Positive	Positive	Positive	Positive	Not attempted	Positive	Positive	Positive	0	MDCK-SIAT (7)	Guinea pig (7)	0	0	0	0	7	0
4344	Positive	Positive	Negative	Positive	Not attempted	Negative	Positive	Negative	3	MDCK (7)	Turkey (4) Human (8)	0	0	0	0	7	0
10053	Positive*	Positive#	Positive	Positive#	Not attempted	Positive	Positive*	Positive	0	MDCK-SIAT (2*) MDCK-I (2#) MDCK-II (3)	HA not used	0	0	7	0	7	0
10206	Positive	Positive	Positive	Positive	Not attempted	Negative	Positive	Positive	1	MDCK-SIAT (7)	Guinea pig (2) Human (4)	0	0	0	0	7	0
10261	Positive*	Positive	Negative	Positive	Negative	Positive	Positive*	Positive	1	MDCK-SIAT (2*) MDCK (6)	Human (8)	0	0	0	0	8	0
10492	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	4	MDCK-I (8)	Human (8)	0	0	6	0	0	0
10498	Positive	Negative	Positive	Positive	Not attempted	Positive	Positive	Positive	1	MDCK-SIAT (7)	HA not used	0	0	7	0	6	0
10507[3]	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	6	MDCK (8) MDCK-SIAT (8)	Turkey (8) Human (8)	0	0	8	0	0	0

[1]Cell with orange shading: laboratory located in EU/EEA member country.
[2]Laboratory reported: We currently experience general problems with isolation of influenza B.
[3]Laboratory reported: To study the samples, we used 5 passages, twice.
[4]Not attempted: considered correct following widely used algorithm to take into virus isolation only those specimens that are positive in molecular detection.
[5]Scoring: Positive specimens – ositive (green shading) and Not attempted (yellow shading because of negative result in molecular detection), 0; Negative and all other (red shading), 1.
Negative specimens: Negative (green shading) and Not attempted (yellow shading because of negative result in molecular detection), 0; all other (red shading), 1.
[6]Laboratory reported: Isolation attempted in MDCK and MDCK-SIAT cells. Cell line with highest haemagglutination titer given.
[7]MDCK: Madin Darby Canin Kidney; SIAT: human alpha 2,6-sialyltransferase; NAMRU line: obtained from Naval Medical Research Unit; London line: obtained from WHO CC London, UK; 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.
[8]Plaque assay.
[9]Enzyme linked immunosorbent assay (ELISA).
[10]Microneutralisation assay.
** and # indicate isolation method used with same label in Isolation method column; no indication mark refers to isolation method without mark in Isolation method column.*

Annex Figure 5. Methodologies used by 44 laboratories in virus isolation; type of cells or eggs 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)



Abbreviations: Table 4 footnote. Numbers in bars: number of laboratories.

Annex Table 5. Overview of virus antigenic characterisation results with used methodology

Individual antigenic characterisation results										Antigenic characterisation method				
Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Number of virus isolates subjected to characteri-sation	RBC used in HA assay[6]	HI assay			VN
Expected result	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like[2]	B(Yam) lineage not attributed to category[4]	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage)	A(H1)pdm09 A/California/7/2009 (H1N1)-like	N/A	B(Vic) lineage not attributed to category[5]	A(H3) A/Hong Kong/4801/2014 (H3N2)-like [2]	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage)		Species (n viruses)	Oseltamivir used (n viruses)		Sera (source and species)	n viruses
Participant ID[1]											Yes	No		
50	A(H3) not attributed to category	B(Yam) lineage not attributed to category	B/Norway/2409/2017-like (B/Victoria/2/87 (dell62-163)-lineage)	A(H1)pdm09 not attributed to category	-	Not isolated	A(H3) not attributed to category	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	6	Human (8)	0	6	WHO CC Atlanta ferret WHO CC London ferret	0
95	Isolate available, characterisation not done	B(Yam) lineage not attributed to category	Isolate available, characterisation not done	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Not isolated	Isolate available, characterisation not done	Not isolated	2	Turkey (8)	0	2	In-house ferret	0
200	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/California/7/2009 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	Not isolated	6	Guinea pig (1)	0	6	WHO CC London ferret	0
1159	Antigenic characterisation failed	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	Not isolated	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Not isolated	Antigenic characterisation failed	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	5	HA not used	2	3	WHO CC London ferret	0
1299	A(H3) A/Switzerland/9715293/2013 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	Other B/Hong Kong/514/2009	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Other B/Hong Kong/514/2009	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Guinea pig (8)	2	5	WHO CC London ferret	0
1643	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	Turkey (8) Guinea pig (8)	NA	NA	NA	NA
2125	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Massachusetts/02/2012-like (B/Yamagata/16/88-lineage	7	Turkey (1) Guinea pig (8)	1	7	WHO CC London ferret	0
2126	A(H3) not attributed to category	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Turkey (5) Guinea pig (2)	0	7	In-house ferret	0
2253	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Massachusetts/02/2012-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Norway/2409/2017-like (B/Victoria/2/87 (dell62-163)-lineage)	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Human (8)	0	7	WHO CC London ferret	0

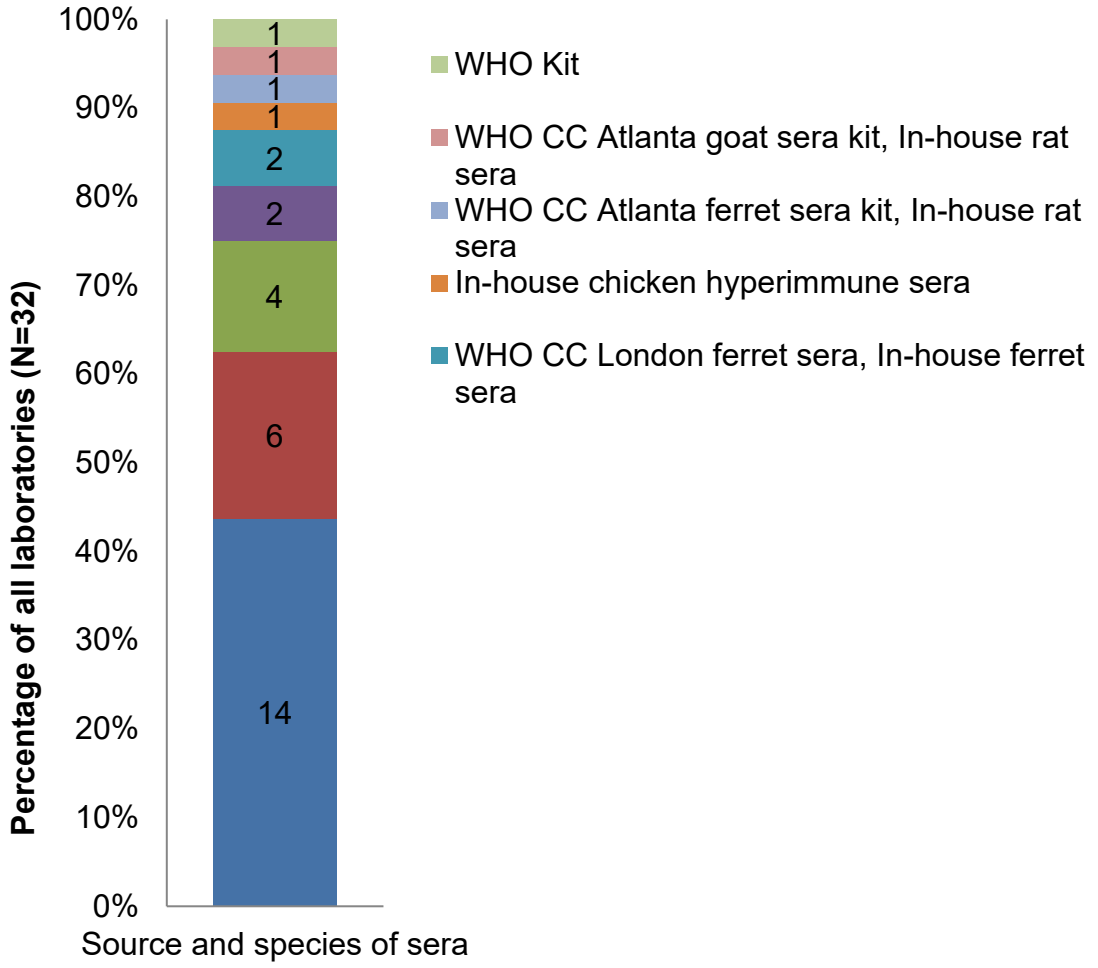
Individual antigenic characterisation results										Antigenic characterisation method				
Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Number of virus isolates subjected to characteri-sation	RBC used in HA assay[6]	HI assay			VN
Expected result / Participant ID[1]	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like[2]	B(Yam) lineage not attributed to category[4]	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage)	A(H1)pdm09 A/California/7/2009 (H1N1)-like	N/A	B(Vic) lineage not attributed to category[5]	A(H3) A/Hong Kong/4801/2014 (H3N2)-like [2]	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage)		Species (n viruses)	Oseltamivir used (n viruses)		Sera (source and species)	n viruses
											Yes	No		
2271	Other A/Hong Kong/5738/2014	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Guinea pig (7)	0	7	WHO CC Atlanta ferret WHO CC London ferret	0
2276	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B(Vic) lineage not attributed to category	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Turkey (7) Guinea pig (7)	1	6	WHO CC London ferret	0
2277	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Human (8)	0	7	WHO CC London ferret	0
2278	Antigenic characterisation failed	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	Antigenic characterisation failed	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Antigenic characterisation failed	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	Antigenic characterisation failed	7	Human (8) Chicken (2)	2	7	WHO CC London ferret	0
2820	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/California/7/2009 (H1N1)-like	-	B/Norway/2409/2017-like (B/Victoria/2/87 (dell62-163)-lineage)	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Massachusetts/02/2012-like (B/Yamagata/16/88-lineage	7	Guinea pig (8)	0	7	WHO CC Atlanta ferret WHO CC London ferret	0
3442	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	Not isolated	Not isolated	-	Not isolated	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	Not isolated	3	Guinea pig (8)	0	3	WHO CC London ferret	0
10007	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Norway/2409/2017-like (B/Victoria/2/87 (dell62-163)-lineage)	A(H3) A/Switzerland/9715293/2013 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Turkey (5) Guinea pig (2)	2	5	WHO CC London ferret	0
10014	A(H3) not attributed to category	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Guinea pig (8)	0	7	WHO CC Atlanta ferret	0
10023	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	HA not used	NA	NA	NA	NA
10078	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	HA not used	NA	NA	NA	NA
10080	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	HA not used	NA	NA	NA	NA
10104	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	Guinea pig (5)	NA	NA	NA	NA

Individual antigenic characterisation results										Antigenic characterisation method				
Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Number of virus isolates subjected to characteri-sation	RBC used in HA assay[6]	HI assay			VN
Expected result / Participant ID[1]	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like[2]	B(Yam) lineage not attributed to category[4]	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage)	A(H1)pdm09 A/California/7/2009 (H1N1)-like	N/A	B(Vic) lineage not attributed to category[5]	A(H3) A/Hong Kong/4801/2014 (H3N2)-like [2]	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage)		Species (n viruses)	Oseltamivir used (n viruses) Yes	Oseltamivir used (n viruses) No	Sera (source and species)	n viruses
10115	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Massachusetts/02/2012-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Massachusetts/02/2012-like (B/Yamagata/16/88-lineage	7	Guinea pig (4) Chicken (6)	0	7	WHO CC London ferret In-house ferret	0
10144	Antigenic characterisation failed	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Not isolated	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Massachusetts/02/2012-like (B/Yamagata/16/88-lineage	6	Turkey (5) Human (1)	2	4	WHO CC London ferret	0
10205	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	HA not used	NA	NA	NA [8]	NA
10461	A(H3) not attributed to category	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 not attributed to category	-	B(Vic) lineage not attributed to category	A(H3) not attributed to category	B(Yam) lineage not attributed to category	7	Human (3) Chicken (6)	0	7	WHO CC Atlanta ferret WHO CC London ferret	0
10462	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	Human (8)	NA	NA	NA [8]	NA
10464	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Turkey (8) Guinea pig (2)	2	5	WHO CC London ferret	0
10465	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B(Vic) lineage not attributed to category	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Guinea pig (4)	1	3	In-house chicken hyperimmune	3
10466	Isolate available, characterisation not done	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Norway/2409/2017-like (B/Victoria/2/87 (dell62-163)-lineage)	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Norway/2409/2017-like (B/Victoria/2/87 (dell62-163)-lineage)	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	6	Turkey (6) Guinea pig (7)	6	0	WHO CC London ferret In-house ferret	0
1600	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B(Yam) lineage not attributed to category	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/California/7/2009 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Massachusetts/02/2012-like (B/Yamagata/16/88-lineage	7	Turkey (8)	0	0	WHO CC Atlanta ferret	7
1991	Other[3]	Other[3]	Other[3]	Other[3]	-	Other[3]	Other[3]	Othe [3]	-	Human (8)	NA	NA	NA	NA
2295	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	Turkey (5)	NA	NA	NA	NA
2814	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/California/7/2009 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Human (8)	0	7	WHO CC Atlanta ferret	0
2815	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	Human (8)	NA	NA	NA [8]	NA
2817	A(H3) not attributed to category	B(Yam) lineage not attributed to category	Not isolated	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Not isolated	A(H3) not attributed to category	B(Yam) lineage not attributed to category	5	Guinea pig (8) Chicken(5) Goose(1)	2	3	WHO CC Atlanta ferret	0

Individual antigenic characterisation results										Antigenic characterisation method				
Specimen (EISN_INF18)	01	02	03	04	05	06	07	08	Number of virus isolates subjected to characteri-sation	RBC used in HA assay[6]	HI assay			VN
Expected result	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like[2]	B(Yam) lineage not attributed to category[4]	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage)	A(H1)pdm09 A/California/7/2009 (H1N1)-like	N/A	B(Vic) lineage not attributed to category[5]	A(H3) A/Hong Kong/4801/2014 (H3N2)-like [2]	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage)		Species (n viruses)	Oseltamivir used (n viruses)		Sera (source and species)	n viruses
Participant ID[1]											Yes	No		
2826	Not isolated	B(Yam) lineage not attributed to category	B(Vic) lineage not attributed to category	A(H1)pdm09 not attributed to category	-	B(Vic) lineage not attributed to category	A(H3) not attributed to category	B(Yam) lineage not attributed to category	6	Human (6)	0	6	WHO CC Atlanta ferret	0
3558	A(H3) A/Switzerland/9715293/2013 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	7	Guinea pig (7)	0	7	WHO CC London ferret	0
4344	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	Not isolated	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Not isolated	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	Not isolated	4	Turkey (4) Human (8)	2	2	WHO CC Atlanta ferret In-house rat	0
10053	Isolate available, characterisation not done	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	Isolate available, characterisation not done	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	5	HA not used	0	5	WHO kit	0
10206	A(H3) A/Singapore/INFIMH-16-0019/2016 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	Not isolated	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	6	Guinea pig (2) Human (4)	0	6	WHO CC London ferret	0
10261	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	Not isolated	A(H1)pdm09 A/Michigan/45/2015 (H1N1)-like	-	B/Brisbane/60/2008-like (B/Victoria/2/87 lineage	A(H3) A/Hong Kong/4801/2014 (H3N2)-like	B/Phuket/3073/2013-like (B/Yamagata/16/88-lineage	6	Human (8) [7]	2	4	WHO CC Atlanta goat In-house rat	0
10492	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	Human (8)	NA	NA	NA	NA
10498	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	-	HA not used	NA	NA	NA	NA
10507	Not isolated	B(Yam) lineage not attributed to category	Not isolated	Not isolated	-	Not isolated	Not isolated	Not isolated	1	Turkey (8) Human (8)	0	1	WHO CC Atlanta ferret	0

[1]Cell with orange shading: laboratory located in EU/EEA member country. Cells with grey shading: results excluded from analysis.
[2]Has neuraminidase induced haemagglutination (HA).
[3]Laboratory reported: Only type and subtype/lineage identified using the WHO identification kit.
[4]Low reactor B/Pucket/3073/2013.
[5]Low reactor B/Brisbane/60/2008.
[6]Because it was not explicitly asked, it was assumed that laboratories used the same type of RBCs for antigenic characterisation if not otherwise indicated.
[7]Laboratory reported: Rat sera were used in haemagglutination inhibition (HI) assay.
[8]Laboratories reported using sera for antigenic characterisation, but no antigenic characterisations were reported. Two of these laboratories reported using HA assay and one not using HA assay for virus growth confirmation.

Annex Figure 6. Source and species of sera used for antigenic characterisation in HI assay and virus neutralisation



Annex Table 6. Overview of genetic characterisation results with performance score and used methodology

Individual genetic characterisation results[1]									Overall score[13]	Genetic characterisation					
Sample (EISN_INF18):	01	02	03	04	05	06	07	08		Number of specimens genetically character-ised	On specimen type			Technique	
Expected tesult:	A(H3) clade 3C.2a1 A/Singapore/ INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	N/A	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/ INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013			Simulated clinical specimen	Virus isolate	Both	Sanger	NGS
Participant ID[2]															
50	Not performed[3]	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA[14]	0	-	-	-	-	-
95	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	0	7	7	0	0	0	7
200	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	0	7	7	0	0	7	7
1159	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	0	7	2	5	0	7	0
1299	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a A/Hong Kong/4801/2014 subgroup [11]	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	1	7	7	0	0	0	7
1643	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	0	7	7	0	0	7	0
2125	A(H3) clade 3C.2a1 A/Bolzano/7/2016 subgroup[4]	B(Vic)-lineage clade 1A (del162-164 subgroup) B/Hong Kong/269/2017[5]	Other Brisbane like - B/Johannesburg/ 3964/2012 - like[6]	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Bolzano/7/2016 subgroup [4]	Not done[12]	3	6	3	0	3	6	0
2126	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	0	7	7	0	0	0	7
2253	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
2271	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Bolzano/7/2016 subgroup [4]	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	1	7	7	0	0	7	0
2276	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	Other B/Johannesburg/ 3964/2012 (Clade 1A, B/Brisbane/60/ 2008)[6]	Other A/Slovenia/2903/ 2015 (A(H1)pdm09 group 6B.1 A/Michigan/ 45/2015) [9]	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	0	7	7	0	0	7	0
2277	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
2278	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H1)pdm09 group 6B.1 A/Michigan/45/ 2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/ 2008	A(H3) clade 3C.2a1 A/Bolzano/7/2016 subgroup [4]	B(Yam)-lineage clade 3 B/Phuket/3073/ 2013	1	7	0	0	7	7	0
2820	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-

Individual genetic characterisation results[1]									Overall score[13]	Genetic characterisation					
Sample (EISN_INF18):	01	02	03	04	05	06	07	08		Number of specimens genetically character-ised	On specimen type			Technique	
Expected result:	A(H3) clade 3C.2a1 A/Singapore/ INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	N/A	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/ INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013			Simulated clinical specimen	Virus isolate	Both	Sanger	NGS
Participant ID[2]															
3442	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	0	7
10007	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10014	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10023	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	0	7
10078	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
10080	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10104	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10115	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
10144	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10205	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
10461	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
10462	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	Other B Victoria - clade 1A - not branched[7]	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A (del162-163 subgroup) B/Norway/2409/2017[10]	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	2	7	7	0	0	0	7
10464	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10465	A(H3) clade 3C.2a1 A/Bolzano/7/2016 subgroup [4]	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A (del162-164 subgroup) B/Hong Kong/269/2017[10]	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	2	7	7	0	0	7	0
10466	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	0	7	0	7	0

Individual genetic characterisation results[1]									Overall score[13]	Genetic characterisation					
Sample (EISN_INF18):	01	02	03	04	05	06	07	08		Number of specimens genetically character-ised	On specimen type			Technique	
Expected tesult: / Participant ID[2]	A(H3) clade 3C.2a1 A/Singapore/ INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	N/A	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/ INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013			Simulated clinical specimen	Virus isolate	Both	Sanger	NGS
1600	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
1991	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
2295	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
2814	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
2815	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
2817	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
2826	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
3558	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
4344	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10053	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A B/Brisbane/60/2008	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	0	7	7	0	0	7	0
10206	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
10261	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
10492	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-
10498	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	B(Vic)-lineage clade 1B B/Hong Kong/514/2009[8]	A(H1)pdm09 group 6B.1 A/Michigan/45/2015	-	B(Vic)-lineage clade 1A (del162-163 subgroup) B/Norway/2409/2017[10]	A(H3) clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup	B(Yam)-lineage clade 3 B/Phuket/3073/2013	2	7	4	3	0	7	0
10507	Not performed	Not performed	Not performed	Not performed	-	Not performed	Not performed	Not performed	NA	0	-	-	-	-	-

[1]All strain indications refer to representative strain as indicated in the guidance for TESSy categories.
[2]Cell with orange shading: laboratory located in EU/EEA member country.
[3]Not performed: laboratory did not perform genetic characterisation.
[4]Virus has N121K, which is the indication to allocate virus in clade 3C.2a1 A/Singapore/INFIMH-16-0019/2016 subgroup, as is also confirmed by phylogenetic analysis (Annex Figure 2B).
[5]Incorrect B lineage reported.
[6]Although B/Johannesburg/3964/2012 is a more precise indication of the allocation in phylogenetic analysis (Annex Figure 2C) (but still B(Vic)-lineage clade 1A representative B/Brisbane/60/2008) and therefore is scored 'correct', it is not a current TESSy reporting category.
[7]In phylogenetic analysis, virus is clearly branched and can be allocated to B(Vic)-lineage clade 1A representative B/Brisbane/60/2008 (Annex Figure 2C).
[8]Incorrect clade reported.
[9]AlthoughA/Slovenia/2903/2015 has less in common in phylogenetic analysis with current TESSy reporting category A(H1)pdm09 group 6B.1 representative A/Michigan/45/2015, it is scored 'correct' (Annex Figure 2A).
[10]Although virus allocates to clade 1A in phylogenetic analysis, it definitely does not have a 2- or 3-amino acid deletion in HA1 (Annex Figure 2C).

[11]Although virus also allocates in phylogenetic analysis to clade 3C.2a, it has typical amino acid changes to allocate it to clade 3C.2a1 representative A/Singapore/INFIMH-16-0019/2016 subgroup (Annex Figure 2C).
[12]Laboratory has capacity, but unclear why not done for this specimen.
[13]Scoring: category as expected (green shading) or close (yellow shading) – 0; category not as expected (red shading) – 1. Result cells with grey shading: results excluded from analysis.
[14]NA: not applicable as none of the specimens have been tested.

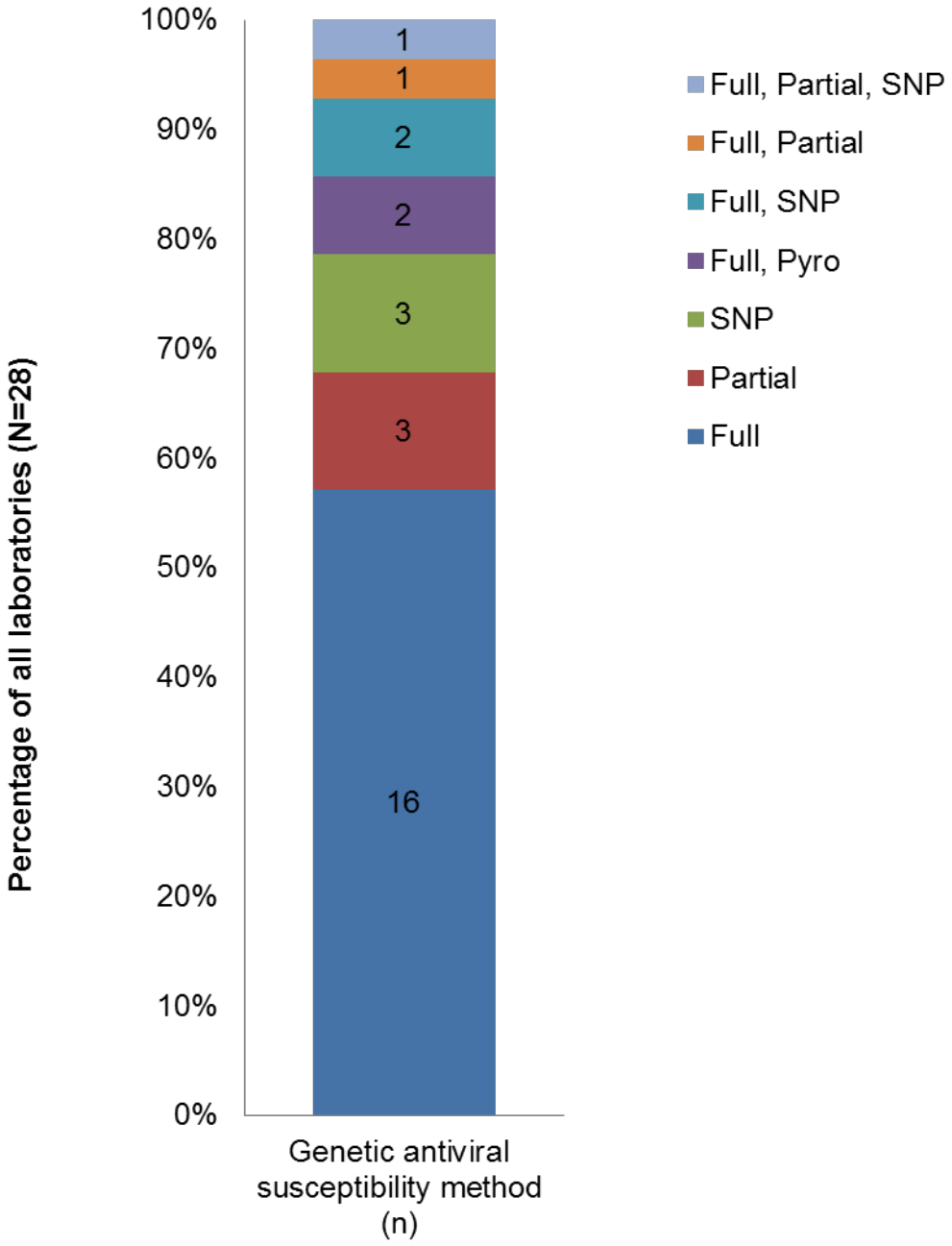
Annex Table 7. Overview of genetic antiviral susceptibility testing results with performance score and used methodology

Specimen	EISN_AV18-01			EISN_AV18-02			EISN_INF18-01			EISN_INF18-02			EISN_INF18-03			EISN_INF18-04		
Subtype	A(H1N1)pdm09			B/Victoria			A(H3N2			B/Yamagata			B/Victoria			A(H1N1)pdm09		
Result type	Genotype	Interpretation[2]		Genotype	Interpretation		Genotype	Interpretation		Genotype	Interpretation		Genotype	Interpretation		Genotype	Interpretation	
		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir
Expected result	275YHmix	AAHRI	AANI	E105K	AANI or AARI	AARI or AAHRI	None[11]	AANI	AANI	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI
Participant ID[1]																		
95	275YHmix	AAHRI	AANI	150R	AANI	AANI[9]	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
200	275Y	AAHRI	AANI	105K	AANI	AARI	292K	AANI[12]	AANI[12]	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
1159	275YHmix	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
1299	275YHmix	AAHRI	AANI	105K	AANI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
1643	275YHmix	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	275YH	AANI[12]	AANI[12]
2125	275Y	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
2126	275YHmix	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
2271	275YHmix	NIP[3]	NIP[4]	105K	NIP[7]	NIP[10]	none	NIP[4]	NIP[4]	none	NIP[4]	NIP[4]	none	NIP[4]	NIP[4]	none	NIP[4]	NIP[4]
2276	275YHmix	AAHRI	AANI	105K	AANI	AAHRI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
3442	275YHmix	AAHRI	AANI	105K	AANI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10007	275YHmix	AAHRI	AARI[5]	105K	AANI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10014	275YHmix	AAHRI *	AANI[6*]	105K	AANI	AARI	none	AANI [6*]	AANI [6*]	none	AANI	AANI	none	AANI	AANI	none	AANI[6*]	AANI[6*]
10023	275YHmix	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10080	275YHmix	AAHRI	AANI	105K	AANI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10104	275YHmix	AAHRI	AARI[5]	105K	NIP[7]	NIP[10]	none	NIP[4]	NIP[4]	none	NIP[4]	NIP[4]	none	AANI	AANI	none	AANI	AANI
10115	275YHmix	AAHRI	AANI[6*]	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	none	AANI[5]	AANI[5]
10144	275YHmix	AAHRI	AANI	105K	AARI	AARI	none	AANI	AARI[13]	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10205	275Y	AAHRI*	AANI[6*]	none	AANI[8*]	AANI *	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10461	275Y	AAHRI	NIP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10464	275Y	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10465	275Y	AARI[3]	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10466	275YHmix	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
2817	275Y	AAHRI	AANI	105K	AANI	AARI	none	AANI	AARI[13]	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
2826	275Y	NIP[3]	NIP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	H275Y	NIP [12]	NIP [12]
3558	275Y	AAHRI*	AANI[6*]	105K	AANI	AARI	none	AANI[6*]	AANI[6*]	none	AANI[6*]	AANI[6*]	none	AANI[6*]	AANI[6*]	none	AANI[6*]	AANI[6*]
4344	275Y	AAHRI	AANI	105K	AARI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI
10053	275YHmix	AARI[3*]	AANI[6*]	105K	AANI	AARI	none	AANI[6*]	AANI[6*]	none	AANI[6*]	AANI[6*]	none	AANI[6*]	AANI[6*]	none	AANI[6*]	AANI[6*]
10261	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10498	275Y	AAHRI	AANI	105K	AANI	AARI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI	none	AANI	AANI

Sample	EISN_INF18-05			EISN_INF18-06			EISN_INF18-07			EISN_INF18-08			Overall score[15]	Methods used (n specimens)			
Subtype	Negative			B/Victoria			A(H3N2)			B/Yamagata				SNP detection		Sequencing NA	
Result type	Genotype	Interpretation[2]		Genotype	Interpretation		Genotype	Interpretation		Genotype	Interpretation			SNP RT-PCR	Pyro-sequencing	Partial	Full
		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir		Oseltamivir	Zanamivir					
Expected result	NA[14]	NA	NA	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI					
Participant ID[1]																	
95	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	2	0	0	0	9
200	-	-	-	None	AANI	AANI	292K	AANI[12]	AANI[12]	None	AANI	AANI	3	0	0	0	9
1159	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	0	0	0	9
1299	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	0	0	0	9
1643	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	1	0	0	0	9
2125	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	1	0	0	0	9
2126	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	0	9[20]	0	9
2271	-	-	-	None	NIP[4]	NIP[4]	None	NIP[4]	NIP[4]	None	NIP[4]	NIP[4]	18	0	0	0	9
2276	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	2[18]	0	1	9
3442	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	0	0	0	9
10007	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	1	0	0	0	9
10014	-	-	-	None	AANI	AANI	None	AANI[6*]	AANI[6*]	None	AANI	AANI	7	0	0	4*	5
10023	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	0	0	0	9
10080	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	1[18]	0	0	9
10104	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	7	0	0	0	9
10115	-	-	-	NP	NP	NP	NP	NP	NP	NP	NP	NP	3[16]	2[18]	0	0	0
10144	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	1	0	2[21]	0	9
10205	-	-	-	NP	NP	NP	NP	NP	NP	NP	NP	NP	5 [16]	0	0	2*	0
10461	-	-	-	NP	NP	NP	NP	NP	NP	NP	NP	NP	1[16]	1[19]	0	0	0
10464	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	1	0	0	0	9
10465	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	2	1[18]	0	0	9
10466	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	0	0	0	0	9
2817	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	2	0	0	0	9
2826	-	-	-	NP	NP	NP	NP	NP	NP	NP	NP	NP	3[16]	2[18]	0	0	0
3558	-	-	-	None	AANI[6*]	AANI[6*]	None	AANI[6*]	AANI[6*]	None	AANI[6*]	AANI[6*]	15	0	0	9*	0
4344	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	1	0	0	0	9
10053	-	-	-	None	AANI[6*]	AANI[6*]	None	AANI[6*]	AANI[6*]	None	AANI[6*]	AANI[6*]	15	0	0	9*	0
10261	-	-	-	NP	NP	NP	NP	NP	NP	NP	NP	NP	NA[17]	0	0	0	0
10498	-	-	-	None	AANI	AANI	None	AANI	AANI	None	AANI	AANI	1	0	0	0	9

[1]Cell with orange shading: laboratory located in EU/EEA member country.
*[2]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); NP: not performed; * indicate specimens that have only be partial sequenced.*
[3]Because H275Y was detected, interpretation should have been AAHRI.
[4]Because full segment was sequenced, interpretation should have been other than NIP.
[5]H275Y does not cause RI for zanamivir.
[6]Because only partial NA segment information available (SNP PCR, partial- or pyrosequencing), interpretation should have been NIP.
[7]Because E105K detected and full segment sequenced, interpretation should have been AANI or AARI.
[8]Indicated that 105 position was screened and did not detect E105K.
[9]E105K not detected.
[10]Because E105K was detected, interpretation should have been AARI or AAHRI.
[11]None: no amino acid substitutions associated with RI or HRI detected after full segment sequencing. Reported result translated from substitutions reported and allocated to correct or incorrect independent from whether the full NA segment was sequenced or only partial or only SNP detection assay was used; in scoring of interpretation of these results, level of testing taken into account (see note 15).
[12]Considered correct because correct compared to expected result, but actually incorrect compared to reported amino acid substitution.
[13]No relevant amino acid substitution reported associated with AARI.
[14]NA: not applicable as no virus in specimen.
[15]Scoring systems used:
EISN_AV18-1 substitutions – 275HY mix found (green shading), 0; 275Y found without indication of mix with 275H (yellow shading), 1; none found (red shading), 2; not tested (grey shading), not scored.
EISN_AV18-1 interpretation oseltamivir – 275Y AND any test AND AAHRI (green shading), 0; rest (red shading), 1; not tested (grey shading), not scored.
EISN_AV18-1 interpretation zanamivir – full NA sequenced 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_AV18-2 substitutions – E105K found (green shading), 0; none found (red shading), 1; not tested (grey shading), not scored.
EISN_AV18-2 interpretation oseltamivir – E105K AND any test AND AANI OR AARI (green shading), 0; rest (red shading), 1; not tested (grey shading), not scored.
EISN_AV18-2 interpretation zanamivir – E105K AND any test AND AARI OR AAHRI (green shading), 0, rest (red shading), 1; not tested (grey shading), not scored.
EISN_INF18-01 – 08 (except 05) substitution – none found (green shading), 0; any other (red shading), 1; not tested (grey shading), not scored.
EISN_INF18-01 – 08 (except 05) interpretation oseltamivir and zanamivir: full NA sequenced 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.
[16]Score for tested specimens only.
[17]NA: not applicable as this laboratory did not perform genotypic antiviral susceptibility testing; performed only phenotypic antiviral susceptibility testing.
[18]In-house RT-PCR for detection H275Y amino acid substitution.
[19]Liveriver TM H274Y kit.
[20]Pyrosequencing for: A(H1N1)pdm09: 275; A(H3N2): 119,245-9,292-4; B: 150,197,221.
[21]Pyrosequencing for 275 position in A(H1N1)pdm09 virus.
**indicates specimens that have only been partial sequenced.*

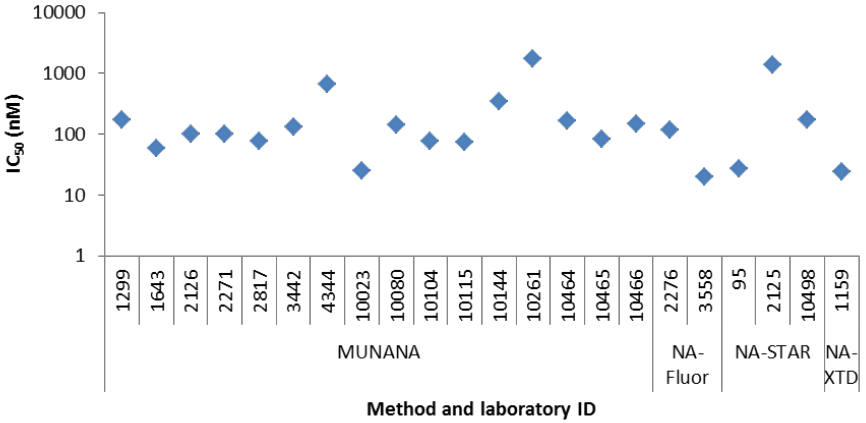
Annex Figure 7. Methods used for genetic antiviral susceptibility determination



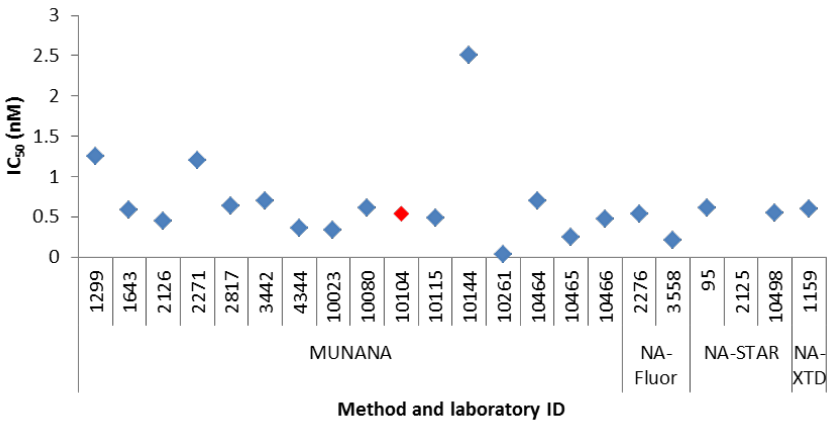
Full: full length neuraminidase gene sequencing
Partial: partial neuraminidase gene sequencing
Pyro: pyrosequencing (1 for N1-H275Y only, 1 for N1-H275Y, N2-119,245-9,292-4, B-150,197,221)
SNP: single nucleotide polymorphism for N1-H275Y (5 In-house developed, 1 Liveriver™ H274Y kit).

Annex Figure 8. Overview of reported IC50 values by method and participant ID of laboratories participating in the phenotypic antiviral susceptibility determination challenge

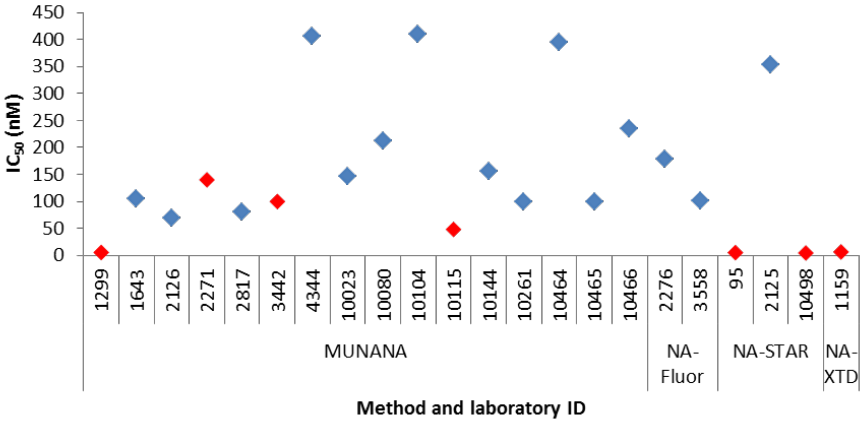
EISN_AV18-01 Oseltamivir A(H1N1)pdm09 NA-275YH mix



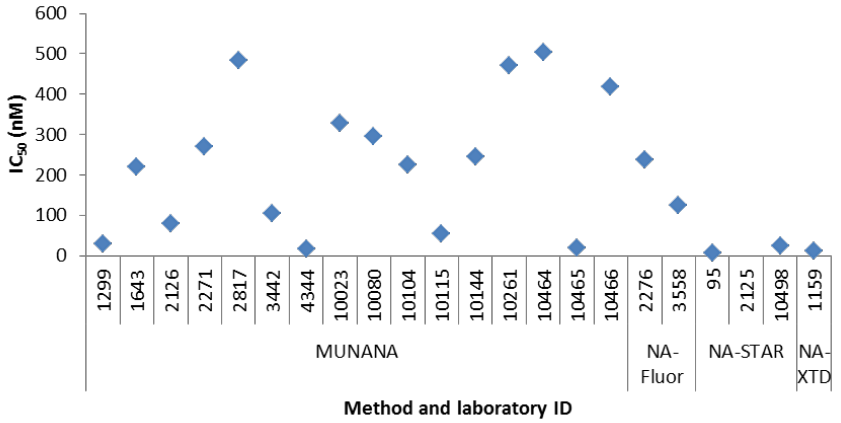
EISN_AV18-01 Zanamivir A(H1N1)pdm09 NA-275YH mix



EISN_AV18-02 Oseltamivir B/Victoria lineage NA-E105K

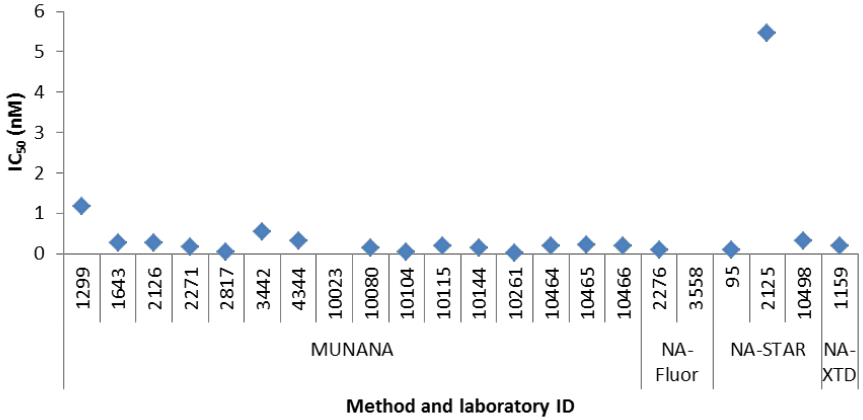


EISN_AV18-02 Zanamivir B/Victoria lineage NA-E105K

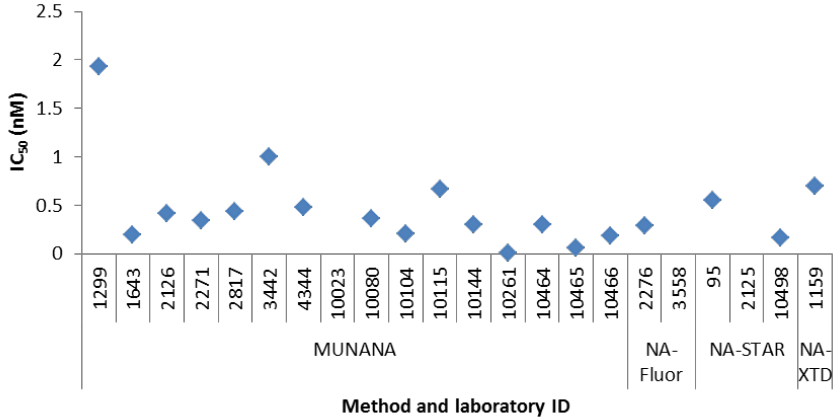


Legend: see below.

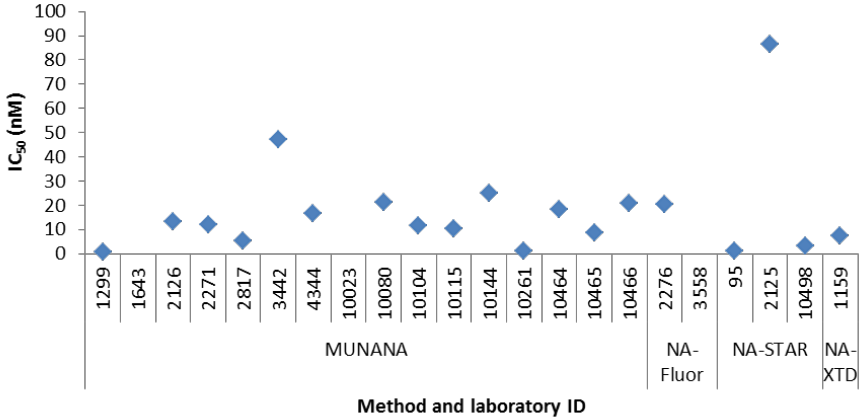
EISN_INF18-01 Oseltamivir A(H3N2) NA-wild type



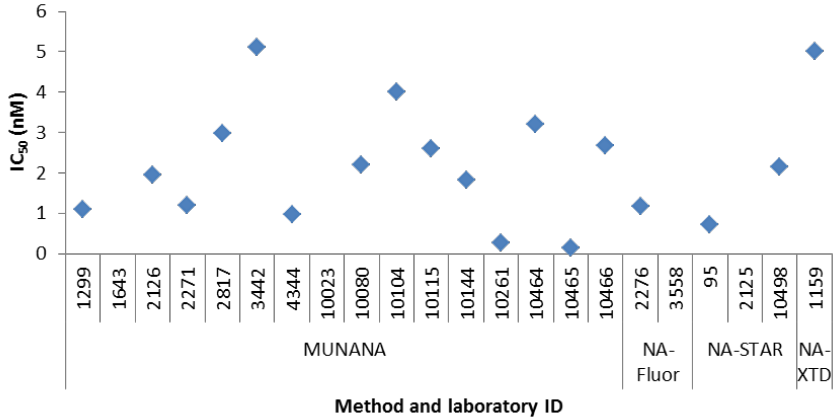
EISN_INF18_01 Zanamivir A(H3N2) NA-wild type



EISN_INF18-02 Oseltamivir B/Yamagata NA-wild type

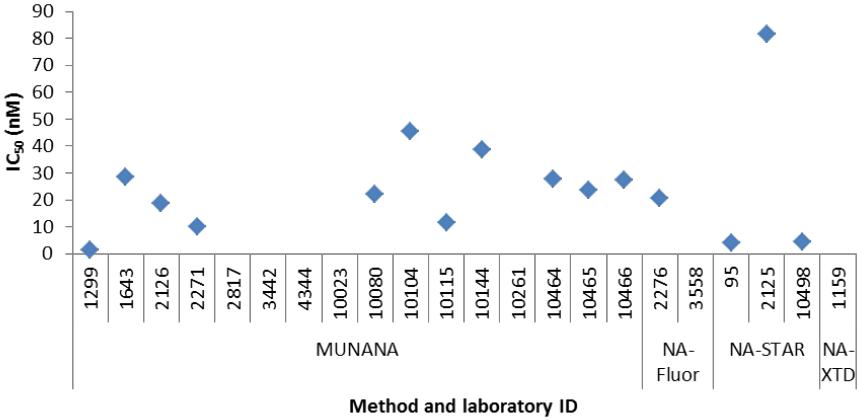


EISN_INF18-02 Zanamivir B/Yamagata NA-wild type

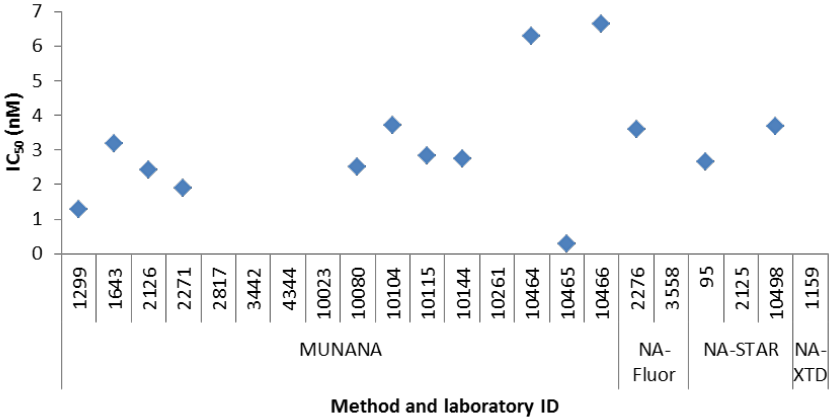


Legend: see below.

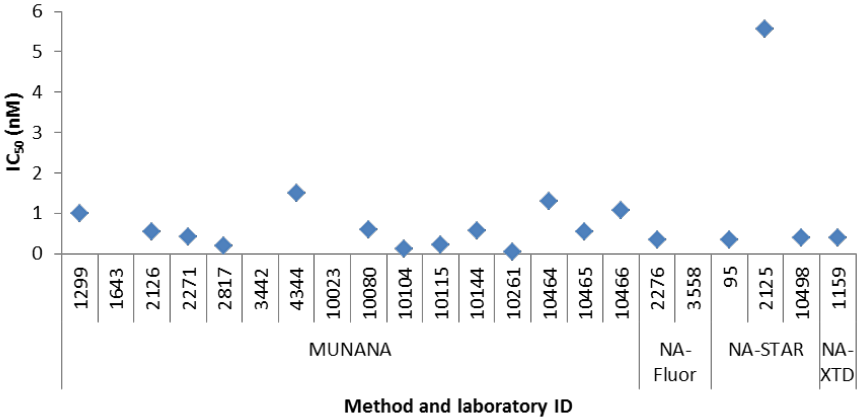
EISN_INF18-03 Oseltamivir B/Victoria NA-wild type



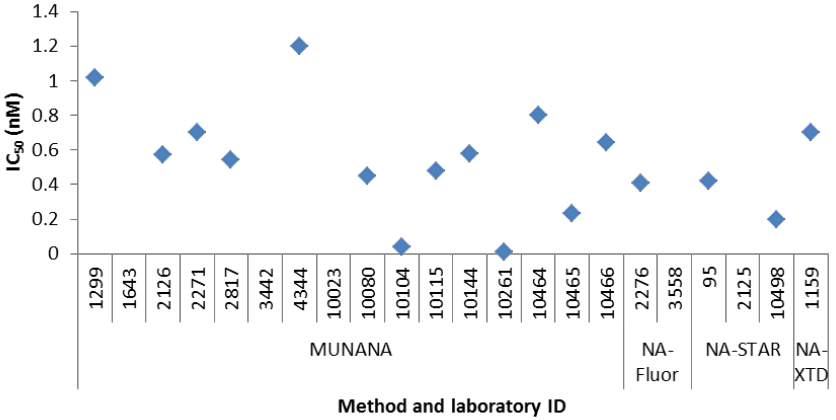
EISN_INF18-03 Zanamivir B/Victoria NA-wild type



EISN_INF18-04 Oseltamivir A(H1N1)pdm09 NA-wild type

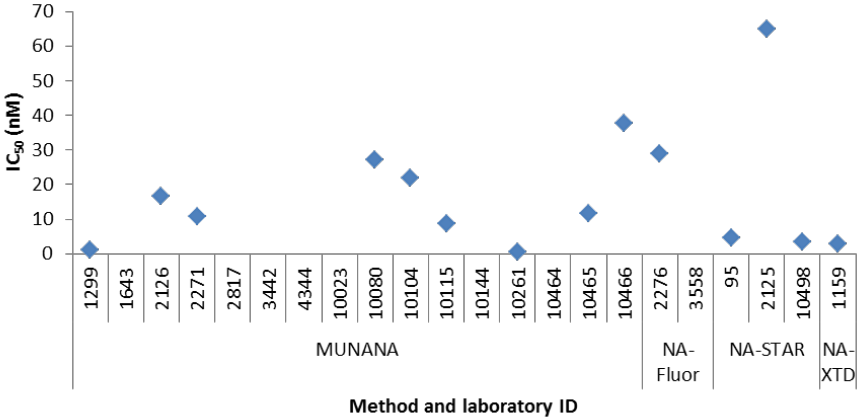


EISN_INF18-04 Zanamivir A(H1N1)pdm09 NA-wild type

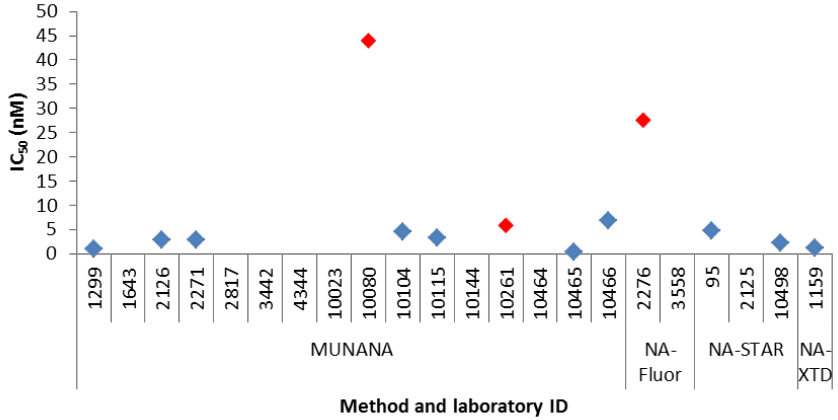


Legend: see below.

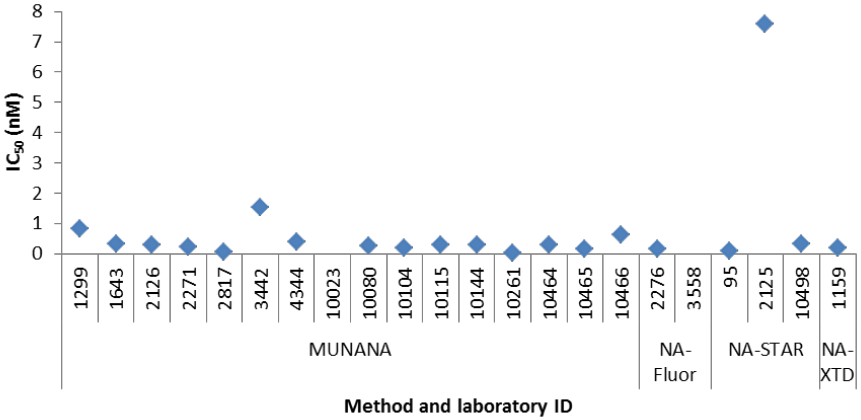
EISN_INF18-06 Oseltamivir B/Victoria NA-wild type



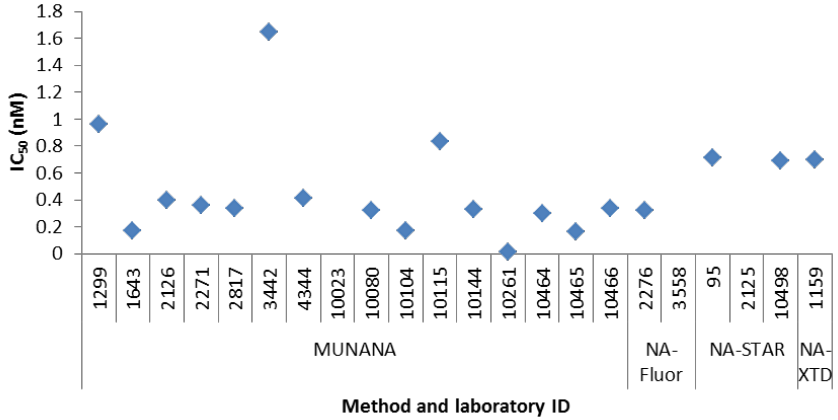
EISN_INF18-06 Zanamivir B/Victoria NA-wild type



EISN_INF18-07 Oseltamivir A(H3N2) NA-wild type

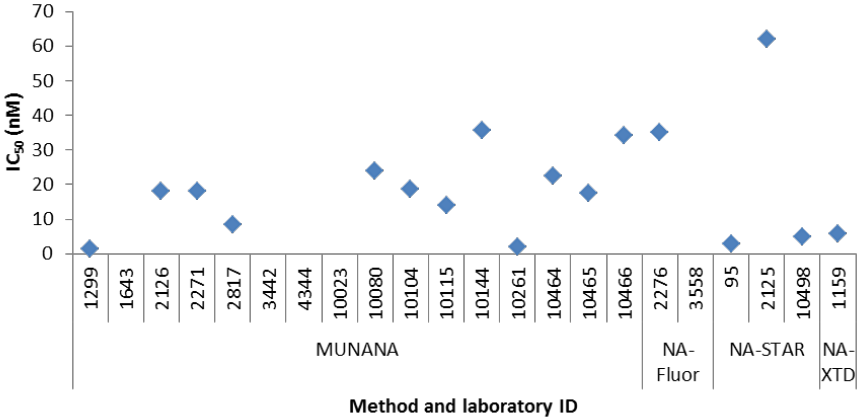


EISN_IF18-07 Zanamivir A(H3N2) NA-wild type

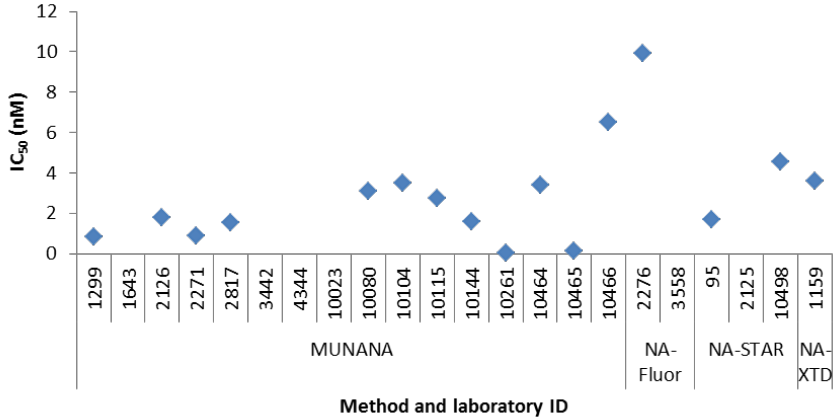


Legend: see below.

EISN_INF18-08 Oseltamivir B/Yamagata NA-wild type



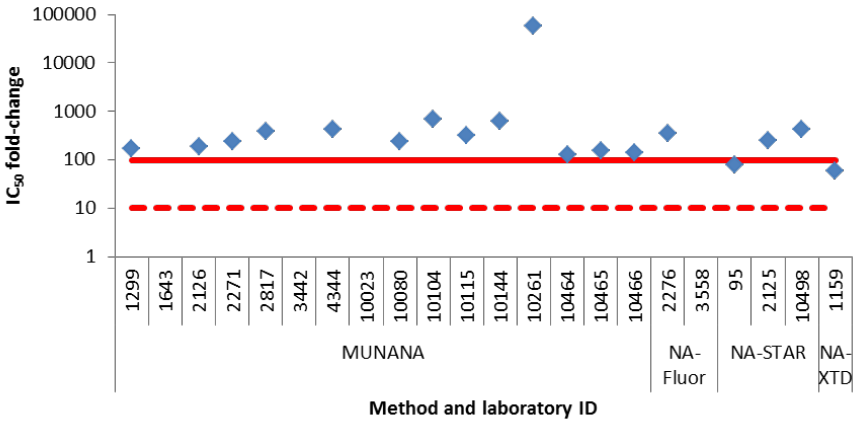
EISN_INF18-08 Zanamivir B/Yamagata NA-wild type



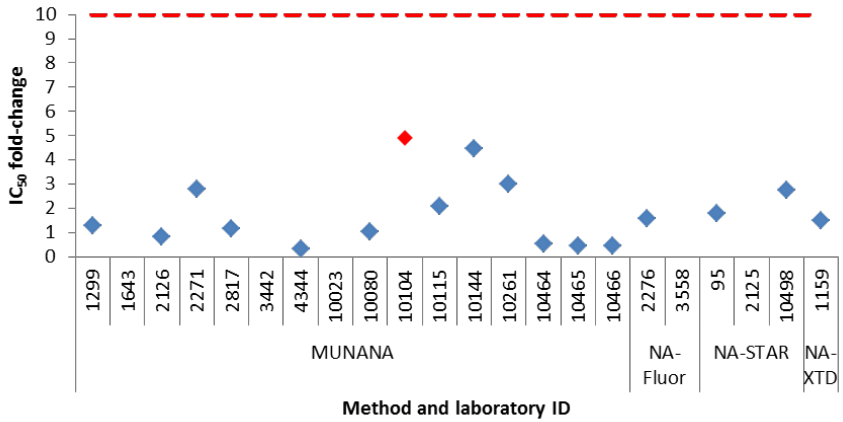
Red dots indicate specimens with incorrect result: IC_{50} level not as expected and/or interpretation not as expected (Annex Table 8). If IC_{50} value for a particular participant ID is not shown, this is either because laboratory did not isolate the virus or did not determine IC_{50} for all specimens or both oseltamivir and zanamivir (Annex Table 8). For participant ID 200, data not shown as laboratory reported that fold changes were reported instead of IC_{50} values.

Annex Figure 9. Overview of calculated IC50 fold-change values by method and participant ID of EISN_AV18-01 and 02 specimens

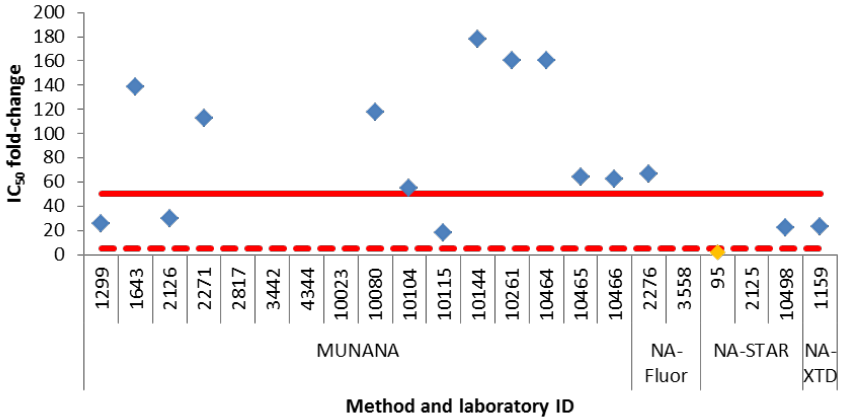
EISN_AV18-01 Oseltamivir A(H1N1)pdm09 NA-275YH mix



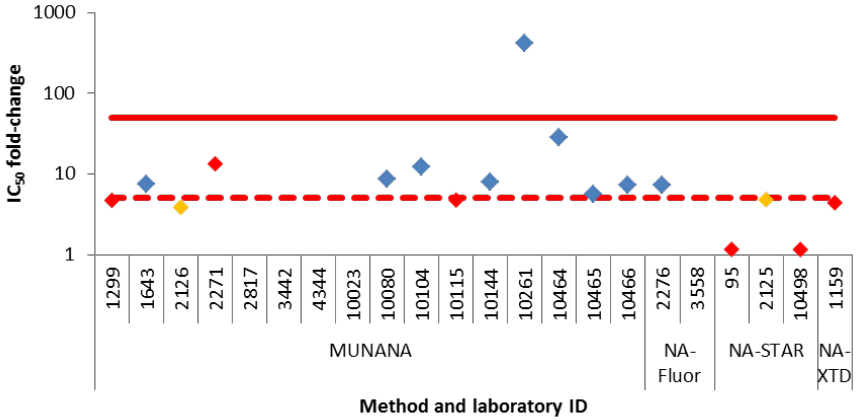
EISN_AV18-01 Zanamivir A(H1N1)pdm09 NA-275YH mix



EISN_AV18-02 Oseltamivir B/Victoria NA-E105K



EISN_AV18-02 Zanamivir B/Victoria NA-E105K



Results plotted for laboratories that reported also IC_{50} values for wild-type viruses of the same subtype or lineage included in the EISN_INF18 specimens, EISN_INF18-04 and mean EISN_INF18-03, 06 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, for type B viruses: NI – IC_{50} fold change < 5; RI – IC_{50} fold change ≥ 5 – ≤ 50; HRI – IC_{50} fold change > 50. 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 (Annex Table 8). Orange dots: laboratory correctly reported RI compared to expected result, although fold-change calculated with mean of EISN_AV18-03,06 indicated fold change below RI threshold.

Annex Table 8. Overview of phenotypic antiviral susceptibility testing results with performance score and used methodology (assay type only)

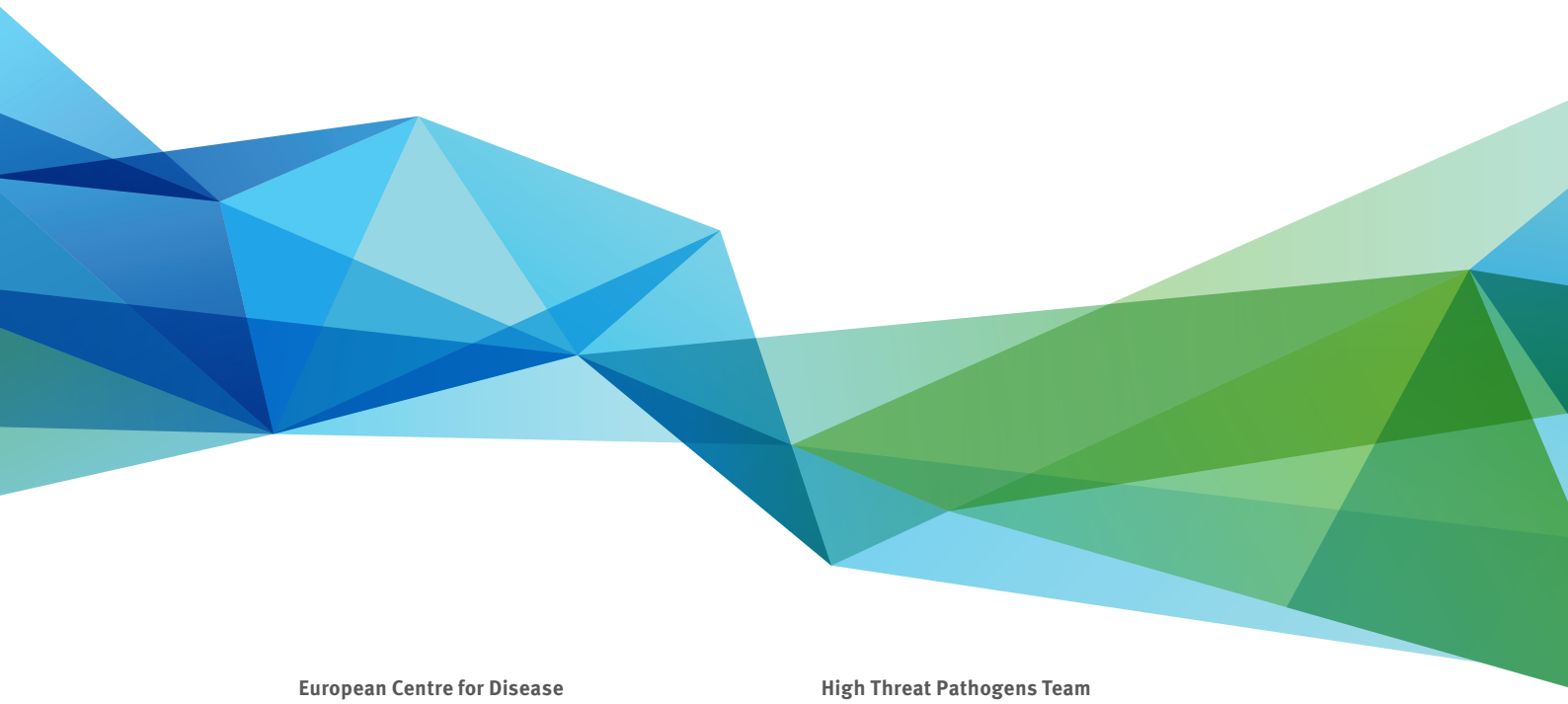
Specimen	EISN_AV18-01		EISN_AV18-02		EISN_INF18-01		EISN_INF18-02		EISN_INF18-03		EISN_INF18-04	
Subtype	A(H1N1)pdm09		B/Victoria		A(H3N2)		B/Yamagata		B/Victoria		A(H1N1)pdm09	
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir
Expected result:	HRI or RI[2]	NI[2]	RI or HRI	HRI or RI	NI	NI	NI	NI	NI	NI	NI	NI
Participant ID[1]												
95	HRI	NI	NI[5]	RI	NI	NI	NI	NI	NI	NI	NI	NI
200	HRI	NI	RI	HRI	NI	NI	NI	NI	NI	NI	NI	NI
1159	RI	NI	NI[5]	RI	NI	NI	NI	NI	No isolate	No isolate	NI	NI
1299	HRI	NI	NI[5]	RI	NI	NI	NI	NI	NI	NI	NI	NI
1643	RI	NI	RI	HRI	NI	NI	No isolate[8]	No isolate	NI	NI	No isolate	No isolate
2125	HRI	Not tested [3]	RI	Not tested	NI	Not tested	NI	Not tested	NI	Not tested	NI	Not tested
2126	HRI	NI	RI	HRI	NI	NI	NI	NI	NI	NI	NI	NI
2271	HRI	NI	NI[6]	RI	NI	NI	NI	NI	NI	NI	NI	NI
2276	HRI	NI	RI	HRI	NI	NI	NI	NI	NI	NI	NI	NI
3442	HRI	NI	NI[7]	RI	NI	NI	NI	NI	No isolate	No isolate	No isolate	No isolate
10007	NP 2	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10014	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10023	RI	NI	RI	HRI	Not tested	Not tested	No isolate	No isolate	No isolate	No isolate	Not tested	Not tested
10080	HRI	NI	RI	HRI	NI	NI	NI	NI	NI	NI	NI	NI
10104	RI	RI[4]	HRI	HRI	NI	NI	NI	NI	NI	NI	NI	NI
10115	HRI	NI	NI[5]	RI	NI	NI	NI	NI	NI	NI	NI	NI
10144	HRI	NI	RI	HRI	NI	NI	NI	NI	NI	NI	NI	NI
10205	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10461	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10464	HRI	NI	RI	HRI	NI	NI	NI	NI	NI	NI	NI	NI
10465	HRI	NI	RI	RI	NI	NI	NI	NI	NI	NI	NI	NI
10466	RI	NI	RI	RI	NI	NI	NI	NI	NI	NI	NI	NI
2817	HRI	NI	RI	HRI	NI	NI	NI	NI	No isolate	No isolate	NI	NI
2826	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
3558	HRI	NI	RI	HRI	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
4344	HRI	NI	HRI	RI	NI	NI	NI	NI	No isolate	No isolate	NI	NI
10053	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
10261	HRI	NI	HRI	HRI	NI	NI	NI	NI	No isolate	No isolate	NI	NI
10498	HRI	NI	NI[5]	RI	NI	NI	NI	NI	NI	NI	NI	NI

Specimen	EISN_INF18-05	EISN_INF18-06	EISN_INF18-07	EISN_INF18-08	Overall score[12]	Method used

Subtype	Negative		B/Victoria		A(H3N2)		B/Yamagata			
	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir	Oseltamivir	Zanamivir		
Expected result	NA[9]	NA[9]	NI	NI	NI	NI	NI	NI		
Participant ID[1]										
95	-	-	NI	NI	NI	NI	NI	NI	1	NA-STAR Kit
200	-	-	NI	NI	NI	NI	NI	NI	0	NA Fluor Kit
1159	-	-	NI	NI	NI	NI	NI	NI	1[13]	NA XTD Kit
1299	-	-	NI	NI	NI	NI	NI	NI	1	MUNANA in-house
1643	-	-	No isolate	No isolate	NI	I	No isolate	No isolate	0[13]	MUNANA in-house
2125	-	-	NI	Not tested	NI	Not tested	NI	Not tested	0[13]	NA-STAR Kit
2126	-	-	NI	NI	NI	NI	NI	NI	0	MUNANA in-house
2271	-	-	NI	NI	NI	NI	NI	NI	1	MUNANA in-house
2276	-	-	NI	R [11]	NI	NI	NI	NI	1	NA Fluor Kit
3442	-	-	No isolate	No isolate	NI	NI	No isolate	No isolate	1[13]	MUNANA in-house
10007	-	-	NP	NP	NP	NP	NP	NP	NA[14]	-
10014	-	-	NP	NP	NP	NP	NP	NP	NA[14]	-
10023	-	-	No isolate	No isolate	Not tested	Not tested	No isolate	No isolate	0[13]	MUNANA in-house
10080	-	-	NI	RI[11]	NI	NI	NI	NI	1	MUNANA in-house
10104	-	-	NI	NI	NI	NI	NI	NI	1	MUNANA in-house
10115	-	-	NI	NI	NI	NI	NI	NI	1	MUNANA in-house
10144	-	-	No isolate	No isolate	NI	NI	NI	NI	0 [13]	MUNANA in-house
10205	-	-	NP	NP	NP	NP	NP	NP	NA[14]	-
10461	-	-	NP	NP	NP	NP	NP	NP	NA[14]	-
10464	-	-	Not tested[10]	Not tested[10]	NI	NI	NI	NI	0[13]	MUNANA in-house
10465	-	-	NI	NI	NI	NI	NI	NI	0	MUNANA in-house
10466	-	-	NI	NI	NI	NI	NI	NI	0	MUNANA in-house
2817	-	-	No isolate	No isolate	NI	NI	NI	NI	0[13]	MUNANA in-house
2826	-	-	NP	NP	NP	NP	NP	NP	NA[14]	-
3558	-	-	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	0[13]	NA Fluor Kit
4344	-	-	No isolate	No isolate	NI	NI	No isolate	No isolate	0[13]	MUNANA in-house
10053	-	-	NP	NP	NP	NP	NP	NP	NA[14]	-
10261	-	-	NI	RI[11]	NI	NI	NI	NI	1[13]	MUNANA in-house
10498	-	-	NI	NI	NI	NI	NI	NI	1	NA-STAR Kit[15]

[1]Cell with orange shading: laboratory located in EU/EEA member country.
[2]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; NP – not performed, meaning laboratory does not have test available.
[3]Not tested: laboratory has test available, but has not performed test on indicated specimen or does not have antiviral agent available.
[4] In addition, IC_{50} fold change compared to EISN_INF18-04 < 10 (Annex Figure 2).
[5]Incorrect to compared to expected result, although IC_{50} fold change compared to EISN_INF18-03,06 < 5 (Annex Figure 2).
[6]Incorrect compared to expected result, in addition IC_{50} fold change compared to EISN_INF18-03,06 > 5.
[7]Incorrect compared to expected result, no results for EISN_INF18-03,06 available for IC_{50} fold-change confirmation.
[8]No isolate: virus isolation not successful and therefore could not be performed.
[9]NA: not applicable as the specimen did not contain virus.
[10]Laboratory reported: NA activity of EISN_INF18-06 was not sufficient for NAI susceptibility assay; although laboratory isolated virus (Annex Table 3), it is unclear whether test was done on isolated virus or simulated clinical specimen.
[11]RI likely due to culture selected T106P amino acid substitution confirmed to be present as minority variant in simulated clinical specimen and 1 of 3 isolates.
[12]Scoring systems used:
EISN_AV18-01 oseltamivir – HRI or RI (green shading), 0; other (red shading), 1; zanamivir: NI (green shading), 0; other (red shading), 1; not tested, no isolate (grey shading): not scored.
EISN_AV18-02 oseltamivir – RI or HRI (green shading), 0; other (red shading), 1; zanamivir: HRI or RI (green shading), 0; other (red shading), 1; not tested, no isolate (grey shading): not scored.
EISN_INF18-01–08 (except 05) oseltamivir and zanamivir – NI (green shading), 0; other (red shading), 1; not tested, no isolate (grey shading): not scored.
[13]Score for specimens tested only.
[14]NA: not applicable as this laboratory did not perform phenotypic antiviral susceptibility testing.
[15]Laboratory likely performed at least for specimens from which no virus could be isolated phenotypic test directly on simulated clinical specimen (for virus isolation see Annex Table 3).

TQ-04-19-132-EN-N



European Centre for Disease Prevention and Control (ECDC)

Gustav III:s boulevard 40, SE-16973 Solna, Sweden

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

Contact us
publications@ecdc.europa.eu

Follow us on Twitter
@ECDC_EU

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

High Threat Pathogens Team
WHO Health Emergencies Program
WHO Regional Office for Europe

UN City, Marmorvej 51, 2100 Copenhagen Ø, Denmark

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

Contact us
euinfluenza@who.int

Follow us on Twitter
@WHO_EUROPE

Like our Facebook page
www.facebook.com/WHOEurope

ISBN 978-92-9498-305-3