

Annexes - European Influenza virus EQA programme 2023

List of abbreviations

ATG	Adenine-Thymine Guanine start codon
BXM	baloxavir marboxil
CO ₂	carbon dioxide
CPE	cytopathic effect
ECDC	European Centrer for Disease Prevention and Control
EEIQAP	European External (Influenza) Quality Assessment Programme for Influenza
EPI	EPI prefix, used for tracking viral sequences (specific to the GISAID database)
EU/EEA	European Union/European Economic Area
GISAID	Global Initiative on Sharing All Influenza Data
GTR	general time-reversible (e.g. GTR GAMMA model)
HA	hemagglutinin
HRI	highly reduced inhibited
IF	immunofluorescence
IRR	International Reagent Resource
MDCK	Madin Derby Canin Kidney
MRE	majority rule extended
MUNANA	20-(4-methylumbelliveryl)-a-D-N-acetylneuraminic acid
NA	neuraminidase
ND	not done
NI	normal inhibited
N/A	Not applicable
NO	no interpretation possible due to partial neuraminidase segment information
PA	Polymerase acidic
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
RI	Reduced inhibited
RIVM	National Institute for Public Health and the Environment, the Netherlands
RS	reduced susceptibility
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SIAT	sialyltransferase
SNP	single nucleotide polymorphisms
TC	tissue culture-treated
TESSy	The European Surveillance System
WHO	World Health Organization
WHO CC	World Health Organization Collaborating Centre

Annex 1. Summary of virus isolation survey following the European External (Influenza) Quality Assessment Programme for Influenza (EEIQAP) 2023

Participating laboratories

During the European External Influenza Quality Assessment Programme (EEIQAP) organised in 2023, laboratories were able to participate in a virus isolation challenge. Following up on a signal related to culturing issues with the EEIQAP 2023 panel, an additional voluntary survey was initiated on influenza virus isolation procedures. A total of 13 laboratories filled in the survey: seven of these originally participated in the EEIQAP 2023 virus isolation challenge and six were European laboratories that had not participated. The corresponding participant ID is shown in column 'EEIQAP ID', along with colour coding according to the score of their performance in the virus isolation, as defined in Table A2.

Table A1. Region of the responding laboratories, as reported in the survey

EEIQAP ID	Region
2820	EU/EEA member country
10080	EU/EEA member country
11111	EU/EEA member country
10104	EU/EEA member country
3442	EU/EEA member country
2271	EU/EEA member country
3558	non-EU/EEA member country
N/A 1	Unspecified
N/A 2	Unspecified
N/A 3	World Health Organization (WHO) European Region not within the remit of the European Centre for Disease Prevention and Control (ECDC)
N/A 4	EU/EEA member country
N/A 5	EU/EEA member country
N/A 6	EU/EEA member country

Performance of laboratories participating in the virus isolation challenge of EEIQAP 2023

The performance for each lab that filled in the survey and participated in the EEIQAP 2023 virus isolation challenge is shown below by specimen – i.e. whether they were successful in isolating the virus (or determining that there was no virus in the case of EISN_INF23-05). For each sample, successful isolation of a specimen is shown in light green, unsuccessful in light red and no attempt in white. Laboratories were scored with one point per unsuccessful isolation, meaning that 0 is the best possible score and 8 the worst. The 'EEIQAP ID' is colour-coded according to this score in all tables throughout Annex 1. Scores between 0 and 4 are coloured in a shade of green (the best result is in dark green), and scores between 5 and 8 are coloured in a shade of red (the worst result is in dark red).

Table A2. Virus isolation results obtained by each laboratory responding to the survey and overall performance score

EEIQAP ID	EISN_INF23-01 A (H1N1) pdm09	EISN_INF23-02 A (H3N2)	EISN_INF23-03 B/Vic	EISN_INF23-04 A (H3N2)	EISN_INF23-05 Negative	EISN_INF23-06 A (H1N1) pdm09	EISN_INF23-07 B/Vic	EISN_INF23-08 A (H3N2)	Score
2820									0
10080									0
11111									2
10104									3
3442									6
2271									7
3558									7

Pretreatment of clinical samples

The type of pretreatment that each laboratory executes routinely and during the EEIQAP 2023 is summarised below. Each type of pretreatment (filtration, centrifugation, vortexing) is shown in a different shade of blue. Most of the laboratories that participated in the survey did not carry out any pretreatment. It should be noted that two laboratories which routinely execute pretreatment, opted not to do any for the EEIQAP 2023 isolation challenge. Moreover, both laboratories that routinely carry out filtration reported using 0 µm openings. As these are not a valid measurement, they were placed in italics.

Table A3. Clinical sample pretreatment, as reported by each responding laboratory

EEIQAP ID	Routine pretreatment	EEIQAP 2023
2820	Filtration (<i>0 µm openings</i>)	No
10080	Centrifugation (3773 x g)	Centrifugation (3773 x g)
11111	No	No
10104	Vortexing +1/10 dilution	Vortexing +1/10 dilution
3442	No	No
2271	Centrifugation (400 x g)	No
3558	No	No
N/A 1	No	No
N/A 2	No	No
N/A 3	Centrifugation (1000 x g)	Centrifugation (1000 x g)
N/A 4	No	No
N/A 5	Filtration (<i>0 µm openings</i>)	No
N/A 6	No	No

Virus isolation system

The types of virus isolation system are shown below, coloured in a shade of blue for matching systems and in white for the two laboratories using a different system to the others. In terms of temperature, laboratory 'N/A 2' uses a distinctly lower temperature than the other laboratories that reported temperatures ranging between 35 and 37. Just over half of the laboratories supply carbon dioxide (CO₂) to the system, a few of these also increased the humidity (shown in orange). There were no laboratories that increased humidity without supplying CO₂.

Table A4. Cell type and physical parameters used for virus isolation, as reported by each responding laboratory

EEIQAP ID	Virus isolation system	Temperature	CO ₂	Increased humidity
2820	Monolayer in 24-well TC-treated plate	35		
10080	Monolayer in rolling TC-treated tube	35		
11111	Monolayer in 24-well TC-treated plate	37		
10104	Monolayer in 24-well TC-treated plate	35		
3442	Cell culture tubs, sterile, 7ml, PS, NUCLON (ref ^a 734-2037)	35		
2271	Monolayer in T25 TC-treated flask	36		
3558	Monolayer in 24-well TC-treated plate	37		
N/A 1	Monolayer in T25 TC-treated flask	37		
N/A 2	Monolayer in rolling TC-treated tube	33		
N/A 3	Culture flasks Nunc 3 ml	35		
N/A 4	Monolayer in rolling TC-treated tube	35		
N/A 5	Monolayer in 24-well TC-treated plate	36		
N/A 6	Monolayer in rolling TC-treated tube	35		

Media used

The specifics of the media used for maintaining cells and for the virus isolation are summarised below, including type of medium, producer of the medium and any additions to the medium. When used by the laboratory, the additions to the medium are shown in orange cells with details listed. Orange cells with no text indicate that participants did not provide information about the addition, even though they indicated having used one.

There was a great deal of variation in how specifically the participants completed the survey for this question. The resulting tables are more akin to an interpretation/summary of the data. The full answers sometimes contain more information.

Media used for maintaining cells

There was considerable variation between the laboratories in the media and additions they use for maintaining cells. There was no clear difference that would explain the discrepancy between ill- and well-performing laboratories.

Table A5. Media and additives used for maintaining cells in culture, as reported by each responding laboratory

EEIQAP ID	Medium	Brand	Additions to the medium					
			Antibiotics	Anti-fungals	Amino acids	Extra buffer	Serum replacement	Other
2820	DMEM		Penicillin, Streptomycin, Geneticin (SIAT-MDCK)			HEPES	FBS	
10080	DMEM	various	Penicillin, Streptomycin	Nystatin	Gln		FBS	
11111	RPMI-1640	Capricorn			Gln, Gly, L-Ala, L-Asn, L-Asp, L-Pro, L-Ser	HEPES	FBS	
10104		ThermoFisher	Penicillin, Streptomycin				FBS	
3442	DMEM	Gibco	Penicillin, Streptomycin, Neomycin		Gln, MEM NEAA			
2271	DMEM	VWR	Penicillin, Streptomycin		L-Gln	HEPES	FBS	
3558	MEM (MDCK), DMEM (SIAT)	Gibco	Penicillin, Streptomycin, Geneticin (SIAT-MDCK)		Gln	HEPES	FBS	
N/A 1	DMEM	Gibco	Penicillin, Streptomycin		Gln	HEPES	FBS	
N/A 2	MEM	Sigma & Gibco			Gln, Gly, L-Ala, L-Asn, L-Asp, L-Glu, L-Pro, L-Ser	HEPES	FBS	NaHCO ₃
N/A 3	Alpha MEM	Biolot					FBS	
N/A 4	DMEM	Biowest	Geneticin, Gentamicin		Gln, MEM NEAA	HEPES	FBS	
N/A 5	MEM	ThermoFisher	Penicillin, Streptomycin		GlutaMAX	HEPES	FBS	
N/A 6	DMEM		Gentamicin				FBS	

DMEM = Dulbecco's Modified Eagle Medium

MDCK = Madin Derby Canin Kidney

MEM = Minimal Essential Medium

RPMI 1640 = Roswell Park Memorial Institute (RPMI) 1640 Medium

SIAT = human alpha 2,6-sialyltransferase.

Media used for virus isolation

For virus isolation, most laboratories used the same media as for maintaining cells, but with different additions. While all laboratories added FBS (fetal bovine serum) to their cell-maintaining medium, only one added FBS to the virus isolation medium. Furthermore, several laboratories chose to add anti-fungals to their medium.

Table A6. Media and additives used for virus isolation in cell culture, as reported by each responding laboratory

EEIQAP ID	Medium	Brand	Additions to the medium					
			Antibiotics	Anti-fungals	Amino acids	Extra buffer	Serum replacement	Other
2820	DMEM-virus growth medium		Penicillin, Streptomycin			HEPES		
10080	DMEM	various	Penicillin, Streptomycin	Nystatin, Amphotericin B	Gln	HEPES		
11111	RPMI-1640	Capricorn	Penicillin, Streptomycin	Nystatin	Gln, Gly, L-Ala, L-Asn, L-Asp, L-Pro, L-Ser	HEPES		
10104	MEM (for MDCK and SIAT)	ThermoFisher	Penicillin, Streptomycin					
3442	DMEM	Gibco	Penicillin, Streptomycin, Neomycin	Amphoterecin B		HEPES		
2271	DMEM	VWR	Penicillin, Streptomycin		L-Gln	HEPES		
3558	MEM (MDCK), DMEM (SIAT)	Gibco	Penicillin, Streptomycin, Geneticin (SIAT)	Fungizone	Gln	HEPES		
N/A 1	DMEM	Gibco	Penicillin, Streptomycin		Gln	HEPES	FBS	DMSO
N/A 2	Medium 199	Sigma & Gibco	Vancomycin, Amikacin	Nystatin	Gln			NaHCO ₃
N/A 3	Alpha MEM	Biolot	?			?	?	
N/A 4	DMEM	Biowest	Geneticin		Gln			Trypsin-PBS
N/A 5	MEM	ThermoFisher	Penicillin, Streptomycin		GlutaMAX	HEPES		
N/A 6	DMEM		Penicillin, Streptomycin			HEPES		TPCK trypsin

DMEM = Dulbecco's Modified Eagle Medium

MDCK = Madin Derby Canin Kidney

MEM = Minimal Essential Medium

RPMI 1640 = Roswell Park Memorial Institute (RPMI) 1640 Medium

SIAT = human alpha 2,6-sialyltransferase.

Inoculation

Two laboratories reported a trypsin concentration 1000x higher than the others, which was possibly an error in recording the units. Moreover, those laboratories that performed poorly appeared to use a larger volume of clinical specimen for inoculation. There was also some variation in what laboratories assess as being successful virus isolation. Intermediate assessments are shown in light orange, and the final check in dark orange. Most laboratories used comparable volumes for inoculation and second passage. From the limited data points available, it appears that those laboratories which performed poorly used larger volumes.

Table A7. Infection conditions for virus isolation, as reported by each responding laboratory

EIQAP ID	Trypsin brand	Trypsin concentration (units/ml)	Volume of clinical specimen used for inoculation (µl)	Removed the inoculum before adding medium	Incubation time (h)	Final volume for virus isolation (ml)	Freeze-thaw cycles	Assessment of virus isolation success of first passage (dpi)												Blind passage for second round	Second passage if first negative or not convincing	Volume (µl) from first passage used in second
								1	2	3	4	5	6	7	8	9	10	11	12			
2820		5000	200	No	1	1	1													Yes	Yes	200
10080	Sigma-Aldrich	1	250	No	0	2	1													No	Yes	250
11111	Sigma-Aldrich	28	100	No	1	1	1													Yes	Yes	100
10104	Sigma-Aldrich	2	200	No	1	2	1													Yes	Yes	200
3442	Sigma-Aldrich	3	250	No	1	1	1													No	Yes	200
2271	Sigma-Aldrich	20	800	No	1	5	2													No	Yes	800
3558	Sigma-Aldrich	1	400	No	1	1	1													No	Yes	400
N/A 1	Sigma-Aldrich	0	300	No	1	3	2													No	No	
N/A 2	Sigma-Aldrich	3	100	No	0	1	1													No	Yes	100
N/A 3	Sigma-Aldrich	2	200	No	1	2	1													Yes	Yes	200
N/A 4	Biowest	1	200	No	1	2	3													Yes	Yes	200
N/A 5	Sigma-Aldrich	10000	100	Yes	2	100	2													No	Yes	100
N/A 6	Sigma-Aldrich	2	200	No	1	4	1													Yes	Yes	200

ml = millilitre

µl = microlitre

dpi = days post infection

Conclusion

Due to the limited amount of data on laboratories participating in both the survey and the influenza isolation part of EEIQAP 2023, it is not possible to make direct connections between answers in the survey and performance in the isolation.

Annex 2

Antigenic cartography maps (Figures A1, A2 and A3) were created based on hemagglutinin inhibition (HI)-assay data generated at the Dutch National Influenza Centre, location Erasmus Medical Centre (MC), Rotterdam, The Netherlands. For one virus information from the World Health Organization (WHO) Collaborating Centre (London) has been used (Table A1). Vaccine viruses are indicated by abbreviations and a grey halo, viruses included in the panel are indicated with red panel number. The spacing between grid lines is one unit of antigenic distance, corresponding to a two-fold dilution of antiserum in the HI assay.

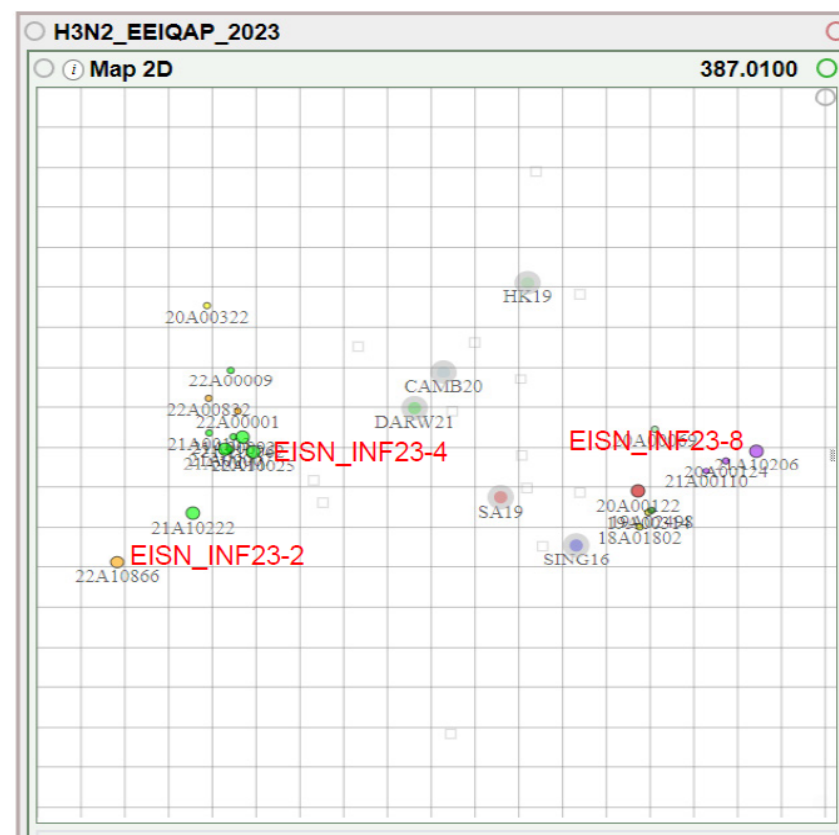
Figure A1. Antigenic cartography of the A(H1N1)pdm09 panel members

(number in red). Vaccine viruses (with grey halo) are CINC-1909GD19 (green), IVR-180SING15 (dark purple), MICH15 (light purple), IVR-190Brisb18 (yellow), IVR-215VICT19 (red). See Table A1. Small dots are other, Dutch, reference viruses with associated sera included in the HI-assay. Large dots are the test viruses among which the viruses included in the panel.



Figure A2. Antigenic cartography of the A(H3N2) panel members

(number in red). Vaccine viruses (with grey halo) are DARW21 (darker green), CAMB20 (light blue), HK19 (light green), SA19 (red), SING16 (purple). Small dots are other, Dutch, reference viruses with associated sera included in the HI-assay. Large dots are the test viruses among which the viruses included in the panel.



The selected virus A/Netherlands/11742/2022 A(H1N1)pdm09 not included in Figure A1 replaced the 6B.1A.5a.2 virus initially selected and indicated in Figure A1 that was similar in amino acid composition to A/Netherlands/11742/2022, but did not grow well to high titer for the panel. The A/Netherlands/11742/2022 virus is antigenically similar to the vaccine strain IVR-215 (A/Victoria/2570/2019) and cell and egg grown A/Sydney/5/2021 vaccine strains, as shown in Table A8 below (courtesy of WHO Collaborating Centre, London).

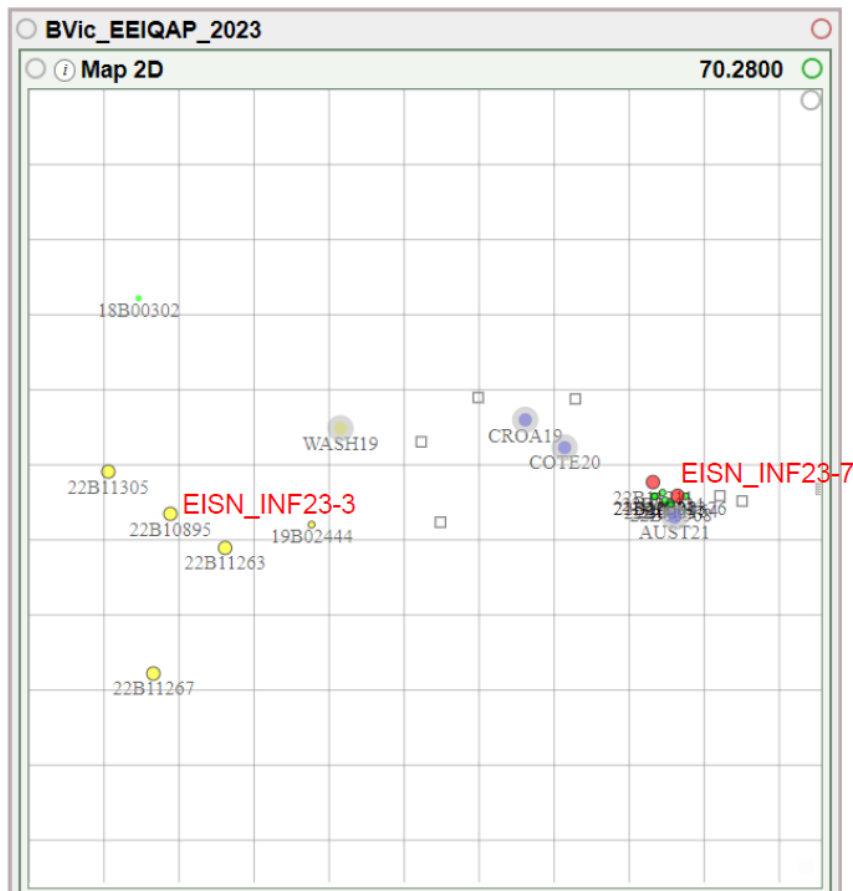
Table A8. Antigenic characterisation of EISN_INF23-1, WHO CC, London, data

Viruses	Other information	Passage history	Collection date	Passage history	Haemagglutination inhibition titre							
					Post-infection fereret antisera							
					A/G-M SWL1536/19 MDCK	A/G-M SWL1536/19 Egg	A/Ghana 1894/21 Egg	A/Lyon 820/21 Egg	A/Denmark 3280/19 MDCK	A/Nor 25089/22 MDCK	IVR-215 A/Vic/2570/19 Egg	A/Sydney 5/21 Egg
					F09/20	F12/20	F02/22	F06/22	F28/20	F38/22	F37/21	F04/22
					Genetic group	6B.1A.5a.1	6B.1A.5a.1	6B.1A.5a.1	6B.1A.5a.1	6B.1A.5a.2	6B.1A.5a.2	6B.1A.5a.2
REFERENCE VIRUSES												
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	C2/MDCK1	1280	2560	1280	320	40	40	80	40
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	E3/E2	1280	1280	640	320	40	<40	80	40
A/Ghana/1894/2021		6B.1A.5a.1	2021-07-21	E2/E1	2560	2560	2560	320	80	<40	160	80
A/Lyon/820/2021		6B.1A.5a.1	2021-11-16	E1/E2	320	320	160	640	40	40	40	40
A/Denmark/3280/2019		6B.1A.5a.2	2019-11-10	MDCK4/MDCK5	80	80	<40	80	2560	1280	2560	1280
A/Norway/25089/2022		6B.1A.5a.2	2022-06-15	MDCK2	<40	40	<40	<40	1280	2560	1280	1280
IVR-215 (A/Victoria/2570/2019)		6B.1A.5a.2	2018-11-22	E4/D7/E2	160	80	40	80	1280	1280	2560	1280
A/Sydney/5/2021		6B.1A.5a.2	2021-10-16	MDCK3/MDCK1	<40	<40	<40	<40	640	320	640	640
A/Sydney/5/2021		6B.1A.5a.2	2022-10-31	E3/E2	80	40	<40	40	640	640	1280	1280
TEST VIRUSES												
A/Netherlands/11714/2022	P137S, K142R	6B.1A.5a.2	2022-08-03	MDCK-MIX1/MDCK1	<40	<40	<40	<40	640	1280	1280	640
A/Netherlands/11699/2022	P137S, K142R	6B.1A.5a.2	2022-08-11	MDCK-MIX1/MDCK1	<40	<40	<40	<40	640	1280	1280	1280
A/Netherlands/11711/2022	P137S, K142R	6B.1A.5a.2	2022-08-17	MDCK-MIX1/MDCK1	<40	40	<40	<40	1280	1280	1280	1280
A/Netherlands/11733/2022	P137S, K142R	6B.1A.5a.2	2022-09-06	MDCK-MIX1/MDCK1	<40	40	<40	<40	1280	1280	1280	1280
A/Netherlands/11742/2022	P137S, K142R	6B.1A.5a.2	2022-09-20	MDCK-MIX1/MDCK1	<40	<40	<40	<40	640	1280	1280	640
< relates to the lowest dilution of antiserum used						Vaccine			Vaccine			Vaccine
ND = Not Done						NH 2020-21			SH 2021			SH 2023
									NH 2021-22			
									SH 2022			
									NH 2022-23			

Results obtained for ferret antisera corresponding to seasonal vaccine strains are highlighted in yellow.

Figure A3. Antigenic cartography of the B/Victoria lineage panel

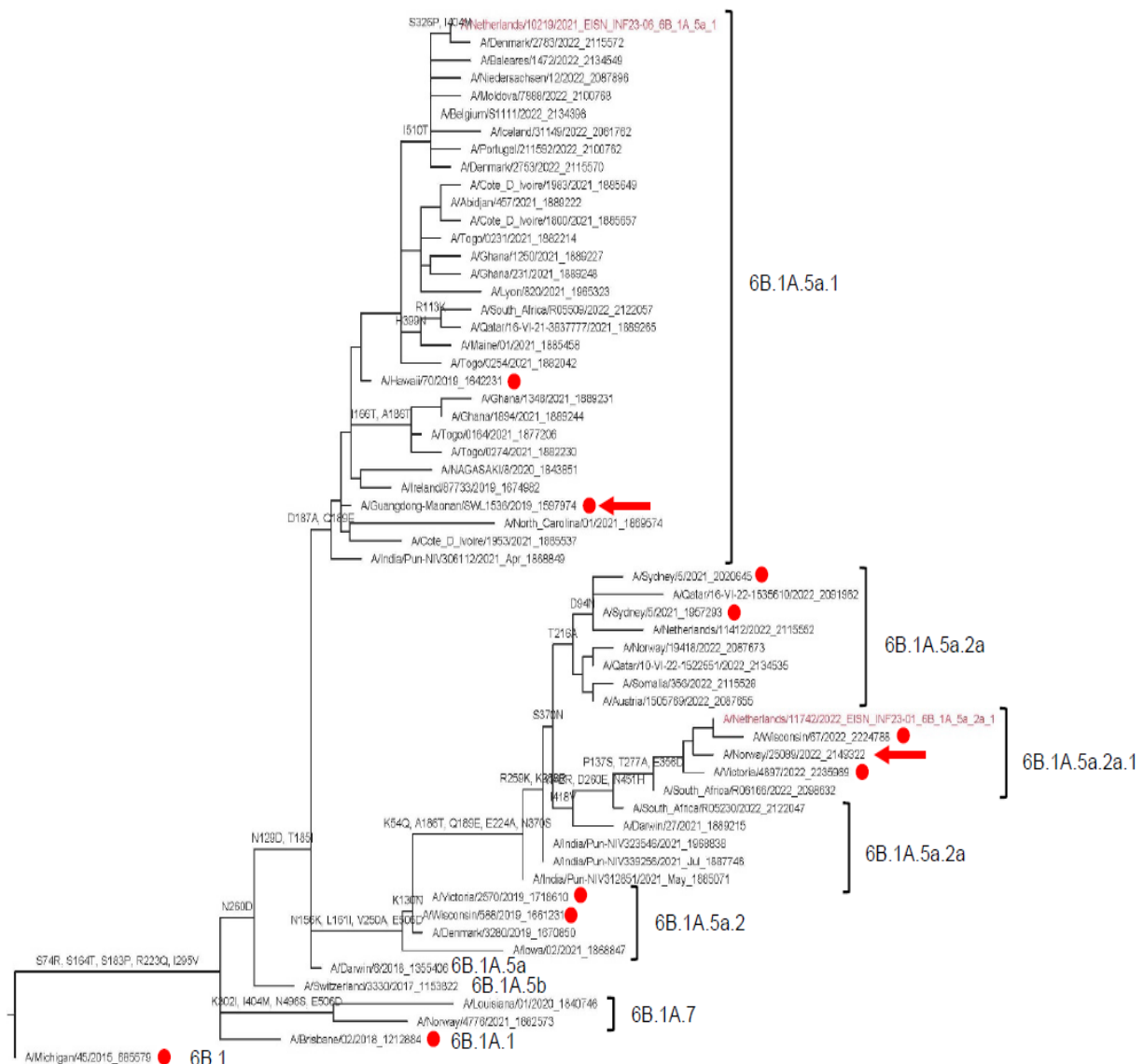
members (numbers in red). Vaccine viruses (with grey halo) are WASH19 (yellow) and AUST21 (blue), and further reference viruses (with grey halo) are CROA19 (purple) and COTE20 (purple). Small dots are other, Dutch, reference viruses with associated sera included in the HI-assay. Large dots are the test viruses among which the viruses included in the panel.



Phylogenetic trees are presented below with common amino acid changes for viruses at branches after the indicated branch (Figure A4, A5 and A6). The non-panel viruses included in the trees are derived from the reference virus sets provided in the ECDC/WHO seasonal guidance for seasons 2021/2022 and 2022/2023, and vaccine strains selected in February 2023 have been added. Phylogenetic trees annotated with amino acid substitutions have been inferred from the generated influenza virus hemagglutinin sequences using the protein coding parts, devoid of signal peptide and stop codon. First, the sequences were aligned using BioEdit software version 7.2.5 with ClustalW algorithm and manually refined, taking into account the right reading frame for translation. They were then trimmed to remove signal peptide and stop codon. The annotated phylogenetic analysis was executed by making use of the treesub package (<https://github.com/tamuri/treesub>) which combines Maximum Likelihood methodology of RAxML, and the annotated tree inference and branch length estimation of the baseml function in PAML. A maximum-likelihood analysis was performed by fitting to the GTRGAMMA model. Bootstrapping was then done using the auto majority-rule extended (MRE) function of RAxML which performs bootstraps until convergence. The optimal tree, as indicated by RAxML, was provided for the respective amino acid substitutions by baseml, as called by treesub. FigTree 1.4.3 6 was used to visualise the trees, pre-prepare for publication and export in pdf format.

Figure A4. Phylogenetic tree full hemagglutinin (HA) A(H1N1)pdm09 viruses

The Global Initiative on Sharing All Influenza Data (GISAID) hemagglutinin (HA) sequence accession number without EPI follows the strain name. Panel viruses have the strain name in red and the expected clade indicated. Red dots represent vaccine strains. A red arrow indicates the expected WHO/ECD/CDC European Surveillance System (TESSy) genetic strain characterisation reporting category, as mentioned in Table A8.



The GISAID HA sequence accession number without EPI follows the strain name. Panel viruses have the strain name in red and the expected clade indicated. Red dots represent vaccine strains. A red arrow indicates the expected WHO/ECDC TESSy genetic strain characterisation reporting category, as mentioned in Table A8.

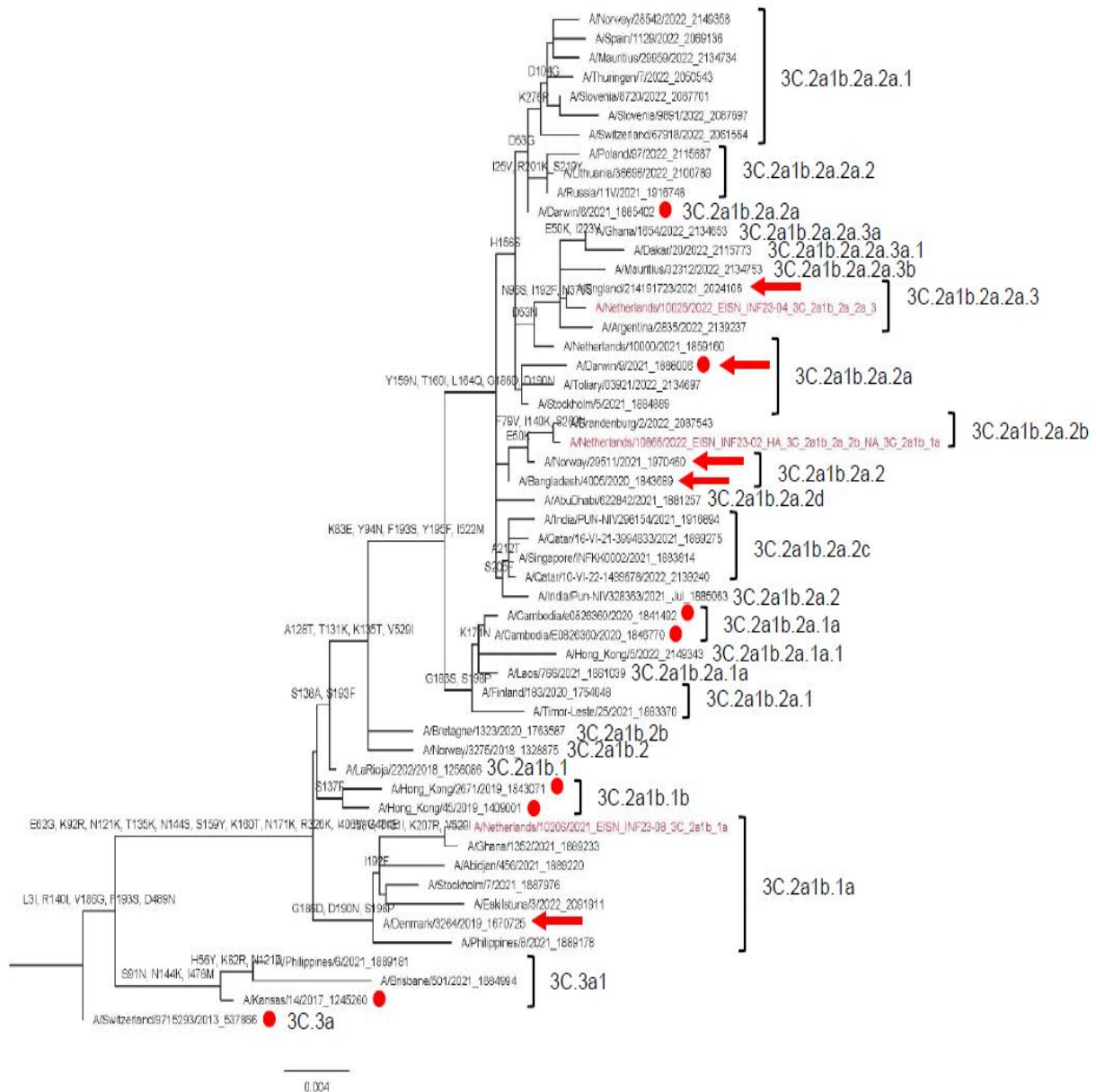


Figure A6. Phylogenetic tree full HA B/Victoria viruses

The GISAID HA sequence accession number without EPI follows the strain name. Panel viruses have the strain name in red and the expected clade indicated. Red dots represent vaccine strains. A red arrow indicates the expected WHO/ECDC TESSy genetic strain characterisation reporting category, as mentioned in Table A8.



Table A9. List of participants, European External Quality Assessment Programme, Influenza, 2023

Country	City	Institute
Albania	Tirana	Institute of Public Health "Hulo Hajderi"
Armenia	Yerevan	National Center for Disease Control and Prevention, Ministry of Health of the Republic of Armenia
Austria	Vienna	Medical University Vienna
Azerbaijan	Baku	Azerbaijan Republic Ministry of Health "Special Dangerous Infections Control Center" Public Legal entity
Belgium	Brussels	Institute of Public Health
Bosnia and Herzegovina	Sarajevo	University Clinical Center
Bulgaria	Sofia	Center of Infectious & Parasitic Disease
Croatia	Zagreb	National Institute of Public Health
Cyprus	Nicosia	Nicosia General Hospital
Czechia	Prague	National Institute of Public Health
Denmark	Copenhagen	Statens Serum Institute
Estonia	Tallinn	Health Protection Inspectorate
Finland	Helsinki	National Institute for Health & Welfare
France	Lyon Cedex 4	Centre de Biologie Nord
France	Paris CEDEX 15	CNR de la Grippe - Institute Pasteur
Germany	Berlin	Robert Koch-Institut
Greece	Athens	National Influenza Center for South Greece
Greece	Thessaloniki	National Influenza Center for North Greece
Hungary	Budapest	National Public Health Center
Iceland	Reykjavik	Landspítali-University Hospital
Ireland	Dublin	University College Dublin
Israel	Tel Hashomer	Chaim Sheba Medical Center
Italy	Rome	Istituto Superiore di Sanità
Latvia	Riga	Latvian Centre of Infectious Diseases of Riga East University Hospital, NIC of Latvia
Liechtenstein	Vaduz	Dr Risch Medical Laboratory
Lithuania	Vilnius	National Public Health Surveillance Laboratory
Luxembourg	Dudelange	Laboratoire National de Santé
Malta	Msida	Mater Dei Hospital
Moldova	Chisinau	National Agency for Public Health
Netherlands	Rotterdam	Erasmus MC
Netherlands	Bilthoven	National Institute for Public Health and the Environment (RIVM)
North Macedonia	Skopje	Republic Institute for Health
Norway	Oslo	Norwegian Institute of Public Health
Poland	Warsaw	National Institute of Hygiene
Portugal	Lisbon	INSA Doutor Ricardo Jorge
Republic of Montenegro	Montenegro	Institute of Public Health
Republic of Serbia	Belgrade	Inst. of Virology, Vaccine & Sera
Romania	Bucharest	Cantacuzino National Military Medical Institute for Research and Development
Romania	Bucharest	National Institute for Public Health Romania
Russian Federation	St Petersburg	Research Institute of Influenza - RAMS
Russian Federation	Moscow	D.I.Ivanovsky research Institute of Virology MoH&SD
Slovakia	Bratislava	Public Health Authority of the Slovak Republic
Slovenia	Ljubljana	National Institute of Public Health
Spain	Majadahonda, Madrid	Instituto de Salud Carlos III
Spain	Barcelona	Hospital Clinic i Provincial
Spain	Valladolid	Hospital Clínico Universitario de Valladolid
Sweden	Solna	Public Health Agency of Sweden
Switzerland	Geneva 14	Hopital Universitaire de Geneve
Türkiye	Ankara	Public Health Institutions of Türkiye
Ukraine	Kiev	Institute of Epidemiology & Infectious Diseases
United Kingdom	Belfast	Royal Victoria Hospital
United Kingdom	Cardiff	Public Health Wales NHS Trust
United Kingdom	Glasgow	Glasgow Royal Infirmary
United Kingdom	London	UK Health Security Agency

Participating laboratories are listed here in alphabetical order.

Table A10. Overview of challenge types in which each laboratory participated

Participant ID ¹	Molecular detection	Virus isolation	Characterisation (antigenic, genetic)	Antiviral susceptibility testing (genetic, phenotypic)	Full programme
95	Yes	Yes	Both	No	No
112	Yes	No	No	Genotypic	No
117	Yes	No	No	No	No
200	Yes	Yes	Both	Both	Yes
1159	Yes	Yes	Genetic	Both	Yes
1299	Yes	Yes	Both	Both	Yes
1600	Yes	Yes	No	No	No
1643	Yes	Yes	Genetic	Both	Yes
1991	Yes	No	No	No	No
2125	Yes	No	No	No	No
2126	Yes	Yes	Both	Genotypic	Yes
2253	Yes	No	No	No	No
2258	Yes	Yes	No	No	No
2270	Yes	No	No	No	No
2271	Yes	Yes	Genetic	Phenotypic	Yes
2272	Yes	No	No	No	No
2274	Yes	No	No	No	No
2275	Yes	Yes	No	Phenotypic	Yes
2277	Yes	Yes	Antigenic	No	No
2278	Yes	Yes	Both	No	No
2812	Yes	No	No	No	No
2816	Yes	No	No	Both	No
2819	Yes	No	No	No	No
2820	Yes	Yes	No	No	No
3442	Yes	Yes	Both	Both	Yes
3558	Yes	Yes	Genetic	Both	Yes
4209	Yes	No	No	No	No
10007	Yes	Yes	Both	Genotypic	Yes
10014	Yes	No	No	No	No
10023	Yes	Yes	Genetic	Both	Yes
10040	Yes	No	No	No	No
10053	Yes	Yes	Genetic	Genotypic	Yes
10078	Yes	Yes	No	No	No
10080	Yes	Yes	Genetic	Both	Yes
10104	Yes	Yes	Genetic	No	No
10115	Yes	Yes	Both	Both	Yes
10142	Yes	No	No	Genotypic	No
10144	Yes	Yes	Genetic	Genotypic	Yes
10205	Yes	No	No	Genotypic	No
10206	Yes	No	No	No	No
10248	Yes	No	No	No	No
10261	No ²	Yes	No	Phenotypic	No
10461	Yes	Yes	Genetic	Genotypic	Yes
10462	Yes	Yes	Genetic	Genotypic	Yes
10464	Yes	Yes	Both	Both	Yes
10466	Yes	Yes	Both	Phenotypic	Yes
10492	Yes	No	No	No	No
10498	Yes	Yes	Genetic	Genotypic	Yes
10507	Yes	Yes	No	No	No
11111	Yes	Yes	Antigenic	Both	Yes
11119	Yes	Yes	Both	Both	Yes
13261	Yes	No	No	No	No
13262	Yes	No	No	No	No
13271	Yes	No	No	No	No

¹ The participant ID assigned for EEIQAP 2023 is a registration number at QCMD, not linked to alphabetical order.

Cell with orange shading = laboratory located in an EU/EEA country. Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by World Health Organization's Regional Office for Europe.

² The laboratory did not receive the panel (in time) to participate in the molecular detection challenge.

Table A11. 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_INF23): Expected result:	01	02	03	04	05	06	07	08	Overall score ²	Assay type			
	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)		type A/B	A H-subtype	A N-subtype	B-lineage
Participant ID ¹													
95	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	In-house, Oxford Nanopore technologies			
112	A (H1N1) pdm09	A/H3	B	A/H3	Negative	A (H1N1) pdm09	B	A/H3	0	Luminex NxTAG RPP+SARS-CoV2, In-house ABI 7500 Fast H1/H3/H5/H7			
117	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	In-house, J Clin Virol. 2005 Aug; 33(4):341-4., HPA H1 ASSAY EISS H3 ASSAY, Supplied by Yves Thomas Jan 2006, Real-time RT-PCR for influenza A H5 viruses, Method supplied by Joanna Ellis PHE			Yamagata and Victoria by real-time PCR
200	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	In-house	In-house	In-house (N1), In-house (N2)	In-house
1159	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	TibMolBiol CatNo 53-0101-96 and 58-0102-96,	adapted from WHO NIC Hong Kong SAR	adapted from WHO NIC Hong Kong SAR	adapted from Department of Virology, Norway
1299	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A/H3, N Negative	1	In-house MA, MB	In house H3 specific, H1pdm09 specific	In-house N2 specific, N1pdm09 specific	In-house Bvic specific
1600	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC (Influenza A/B Typing panel), CDC Influenza Virus Real Time RT-PCR Panel	CDC Influenza A (H3/H1pdm09) subtyping panel		CDC Influenza B Lineage (Genotyping panel)
1643	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	RT-PCR_Influenza ABC	Influenza HA Subtyping		HB Lineage
1991	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza Virus Real-Time PCR panel			CDC Influenza B Lineage genotyping panel VER 1.1
2125	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza Virus Real-time RT-PCR Influenza A/B Typing Panel	CDC Influenza A Subtyping Kit (VER 3), Influenza A/H5 Subtyping Kit (VER 4), Influenza A/H7 (Eurasian Lineage) Assay (RUO), H9 Veterinary		CDC Influenza B Lineage Genotyping Panel (VER1.1)
2126	A/H1pdm09	A/H3	B	A/H3	Negative	A/H1pdm09	B	A/H3	0	In-house UKHSA-RVU (H1,H3 unpublished; A M, B NP WHO February 2021)			
2253	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Realtime RT-PCR (rRTPCR) protocol for detection influenza, Altona diagnostics RealStar® Influenza Screen&Type RT-PCR	RT-PCR of INF viruses (WHO, 7th revision: February 21		RT-PCR of INF viruses (WHO, 7th revision: February 2021
2258	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza A/B Typing Panel	CDC Influenza A (H3/H1pdm09) Subtyping Panel, Influenza A(H5) (Asian Lineage) Subtyping Panel, Influenza A(H7) (Eurasian Lineage) Assay		CDC Influenza B Lineage Genotyping Panel

Specimen (EISN_INF23): Expected result: Participant ID ¹	01	02	03	04	05	06	07	08	Overall score ²	Assay type			
	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)		type A/B	A H-subtype	A N-subtype	B-lineage
2270	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	Cepheid GeneXpert	Conventional PCR		
2271	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza virus Real-time RT-PCR A/B typing panel	CDC Influenza A(H3/H1pdm09) subtyping panel		CDC Influenza B lineage genotyping Panel
2272	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	In house IRR - CAT.NO. FluVD03-9,	In house IRR - CAT.NO. FluRUO-15		In house IRR - CAT.NO. FluRUO-11
2274	A (H1N1) pdm09	A/H3, N Negative	B	A (H3N2)	Negative	A (H1N1) pdm09	B	A/H3, N Negative	2	FTD Respiratory Pathogen 33, Sacace Biotechnologies Influenza A H1N1 & H3N2 RT-PCR, Sacace Biotechnologies Influenza A H5 H7 H9 Typing FRT			
2275	A/H1pdm09	A/H3	Negative	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	3	Seegene Allplex Respiratory Panel RV1			Primers and Probes obtained from WHO
2277	A/H1pdm09	A/H3	B	A/H3	Negative	A/H1pdm09	B	A/H3	0	Multiplex Respira Screen 4 Diff	In-house		
2278	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	In-house AusDiagnostics Respiratory Viruses (16-well), Ref.: 20602, ChenY.2011.JClinMicrobiol.49:4:1653-1656,	CDC Influenza A (H3/H1pdm09) Subtyping (Ver 3)		In-house JClinMicrobiol 38(4)1552-1558; JClinMicrobiol 42(11)5189-5198)
2812	A/H1pdm09	A/H Negative	B	A/H3	Negative	A/H1pdm09	B	A/H3	1	CDC Real-Time PCR Typing and Subtyping Influenza virus			
2816	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC primers and probes	CDC primers and probes		CDC primers and probes
2819	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC influenza in-house protocol, 1copy™ COVID-19/FluA/FluB/RSV qPCR Kit, 1drop Inc	CDC influenza in-house protocol		
2820	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC IRR Primers and probes received from CDC Atlanta, USA	CDC IRR Primers and probes received from CDC Atlanta, USA		CDC IRR Primers and probes received from CDC, Atlanta, USA
3442	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	SARS-CoV-2/FluA/FluB/RSV Assay, HPA VSOP086C (January 2013)	WHO inf for the MD of Flu viruses Inst Pasteur Feb 2021		Norwegian Institute of Public Health, Norway
3558	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	CDC A/B	Subtyping Quadruplex (H1, N1, H3, N2)		Duplex BVIC/BYam (HA)
4209	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC influenza detection panel			
10007	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A/H3, N Negative	1	Influenza A/influenza B/RSV A/RSV B	H1pdm09/H3/N1pdm09/N2	H1pdm09/H3/N1pdm09/N2	BVICT/Yama
10014	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0		CDC Influenza Virus Real-Time RT-PCR Influenza A (H3/H1pdm09)		CDC Influenza B Lineage Genotyping Panel
10023	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	In-house realtime-PCR	In-house RT-PCR		In-house RT-PCR
10040	A (H1N1) pdm09	A (H3N2)	B	A (H3N2)	Negative	A (H1N1) pdm09	B	A (H3N2)	0	In-house Bosphore Influenza A and B assay			
10053	A	A	B	A	Negative	A	B	A	0	Allplex™ RV Essential Assay			
10078	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC WHOCC Atlanta (FluRUO-01)			
10080	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	In-house SOP-F351	In-house SOP-F351	In-house SOP-F351	In-house SOP-F351

Specimen (EISN_INF23): Expected result:	01	02	03	04	05	06	07	08		Assay type			
Participant ID ¹	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	Overall score ²	type A/B	A H-subtype	A N-subtype	B-lineage
10104	A/H1pdm09, N negative	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	1	SOP13/3/08 triplex A/B/RP (CDC/Van Elden/Hummel),	SOP13/3/106 duplex H1-N2, SOP 13/3/29 simplex H1 or H3 (CDC protocol/Overduin, 2009)	SOP13/3/30 simplex N1 or N2 (Overduin, 2009/Pasteur), SOP13/3/106 duplex H1-N2 (CDC protocol 2009/ Overduin 2009)	SOP13/3/31 duplex YAM-VIC (Hungnes, NIC Norway, 2009)
10115	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A/H3, N Negative	1	In-house	In-house	In-house	In-house
10142	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza SARS-CoV-2 Multiplex Assay	CDC Influenza A(H3/H1pdm09) Subtyping Panel		CDC Influenza B Lineage Genotyping Panel
10144	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay	CDC H3, H1pdm09 (RKI, Schulze et al 2010)		In-house, WHO method
10205	A/H1pdm09	A/H3	B	A/H3	Negative	A/H1pdm09	B	A/H3	0	Modulab LightMixes, Roche			
10206	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Centers for Diseases Control and Prevention kit			
10248	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC rtRT-PCR InfA/H1pdm09 Subtyping Panel v.3, CDC rtRT-PCR InfA/H5 Asian Lineage Subtyping Panel v.4, CDC rtRT-PCR InfA/H7 Eurasian Lineage Assay, CDC rtRT-PCR InfB Lineage Genotyping Panel v.1.1,			
10461	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza Virus RT-PCR Panel (RUO) A, H3, H1pdm09, CDC Influenza Virus RT-PCR Panel (RUO) B, Yamagata, Victoria,			
10462	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	Allplex RV Essential Assay (SEEGENE), Allplex respiratory Panel 1A (SEEGENE),	HA NGS sequencing (Illumina)	NA NGS Sequencing (Illumina)	
10464	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	CDC Influenza A/B Typing Panel (VER 2) - Atlanta, CDC Influenza A/B SARS-CoV-2 Multiplex assay (FluSC2-RUO),	CDC Influenza A (H3/H1 pdm09) Subtyping Panel (VER 3) - Atlanta	In-house PCR with own-designed primers	CDC Influenza B Lineage Genotyping Panel (VER 1.1)-Atlanta
10466	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)	0	In-house, AM	In-house, H1pdm09, H3	In-house, N1pdm09, N2	In-house, B-YamVic
10492	A/H1pdm09	A/H3	B/Vic	A/H3	Not Tested	A/H1pdm09	B/Vic	A/H3	0	AgPath-IDTM One-Step RT-PCR Reagents			
10498	A (H1N1) pdm09	A/H3	B/Negative	A/H3	Negative	A (H1N1) pdm09	B/Negative	A/H3	4	CDC Real-time PCR Kits			
10507	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza Virus Real-Time RT -PCR Panel			
11111	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC Influenza SC2 Multiplex kit.	CDC Influenza A Subtyping Kit		CDC Influenza B Lineage Genotyping Panel

Specimen (EISN_INF23): Expected result: Participant ID ¹	01	02	03	04	05	06	07	08	Overall score ²	Assay type			
	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)		type A/B	A H-subtype	A N-subtype	B-lineage
11119	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A/H1pdm09, N negative	B/Vic	A (H3N2)	1	In-house Ward et al. L.Clin.Virol 29:179-188, 204,	WHO manual, RIVM protocol	In-house J. Virological Methods 173 (2011) 259-265, J of Molecular Diagnostics, Vol.15, No.3, May 2013	In-house Barbara Biere et al. J.Clin.Microbiol Apr.2010
13261	A/H1pdm09	A/H3	B	A/H3	Negative	A/H1pdm09	B	A/H3	0	Allplex Respiratory Panel 1, BioFire Respiratory Panel 2.1 plus			
13262	A	A	B	A	Negative	A	B	A	0	Xpert Xpress CoV-2/Flu/RSV plus			
13271	A/H1pdm09	A/H3	B/Vic	A/H3	Negative	A/H1pdm09	B/Vic	A/H3	0	CDC IRR Influenza SARS- CoV-2 Multiplex Assays FR1766, Influenza Real-time RT-PCR Influenza A(H3/H1) FLURUO-15, Influenza Real- time RT-PCR B Lineage Real- time RT-PCR B Lineage FLURUO-11, Influenza virus A/H5(Asian Lineage) FluRUO*13, Real-Time TR- PCR Influenza A/H7 FLURUO-07			

IRR – International Reagent Resource

CDC – US Centres for Disease Prevention and Control, Atlanta, USA.

PCR – Polymerase chain reaction

RT-PCR – Reverse Transcription Polymerase Chain Reaction.

Fully correct (type, A H- and N-subtype or B-lineage) reported results by participants are shaded in dark green. Reported results with correct type and A H-subtype are shaded in light green. Incorrect results are shaded in red.

¹ Cell with orange shading = laboratory located in an EU/EEA country. Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by WHO Regional Office for Europe.

² Scoring for molecular detection:

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

In-house = primers and probes developed or implemented in own laboratory, or modified from published, or personally obtained from elsewhere, if not further specified; use of CDC primers and probes or kit indicated separately.

Table A12. Overview of virus isolation results with performance score and used methodology

Specimen (EISN_INF23):	01	02	03	04	05	06	07	08	Overall score ²	Isolation method (n specimens) ³	Confirmation method virus isolation (n specimens) ⁴						
Subtype/lineage:	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)			HA-assay	NA activity		RT-PCR	IF	CPE	Other
Expected result isolated:	YES	YES	YES	YES	NO	YES	YES	YES			RBC species	MUNANA	NA-STAR				
Participant ID ¹																	
95	YES	YES	YES	YES	YES	YES	YES	YES	1	MDCK-hCK (8)	Turkey (8)	0	0	0	0	0	0
200	NO	YES	NO	YES	NOT_ATTEMPTED	NO	YES	YES	3	MDCK-SIAT (5), MDCK and SIAT (2)	Guinea Pig (7)	0	0	0	0	7	0
1159	YES	YES	NO	NO	NO	NO	NO	YES	4	MDCK-SIAT (8)		0	0	8	0	8	0
1299	YES	YES	NO	YES	NOT_ATTEMPTED	YES	YES	YES	1	MDCK-SIAT (7)	Guinea Pig (7)	0	0	7	0	7	0
1600	YES	YES	NO	YES	NO	YES	YES	NO	2	MDCK-ATL (8)	Turkey (8)	0	0	0	0	5	0
1643	NO	NO	NO	NO	NO	NO	NO	NO	7	MDCK-SIAT (8)	Turkey (8), Guinea Pig (8)	4	0	0	8	8	0
2126	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK-I (2), MDCK-SIAT (2)	Turkey (5), Guinea Pig (4)	0	0	0	0	8	1
2258	YES	YES	YES	YES	NO	YES	YES	YES	0	MDSC (4), MDCK-SIAT (4)	N/A	0	0	0	0	8	0
2271	NO	NO	NO	NO	NOT_ATTEMPTED	NO	NO	NO	7	MDCK-SIAT (7)	Guinea Pig (7)	0	0	7	0	7	0
2275	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK-I (8)	Turkey (1)	0	0	7	0	0	0
2277	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK-I (8)	Human (8)	0	0	0	0	8	0
2278	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK (1), MDCK London line (7)	Human (8)	0	0	8	8	8	0
2820	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK-1 (2), MDCK-2(2), MDCK-SIAT (4)	Human (8)	0	0	0	0	8	0
3442	NO	NO	NO	NO	NO	NO	YES	NO	6	MDCK (2), MDCK-SIAT (6)	Guinea Pig (8)	0	0	0	1	8	0
3558	NO	NO	NO	NO	NOT_ATTEMPTED	NO	NO	NO	7	MDCK_2 (7)	Guinea Pig (7)	0	0	1	0	6	0
10007	NO	YES	NO	NO	NOT_ATTEMPTED	NO	YES	NO	5	MDCK (4), MDCK-SIAT (3)	Turkey (4), Guinea Pig (4)	0	1	0	1	0	0
10023	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK-SIAT (8)	N/A	0	0	8	0	8	0
10053	NO	NO	NO	NO	NO	NO	NO	NO	7	MDCK-SIAT (8)	N/A	0	0	7	0	0	0
10078	YES	NO	NO	NO	NO	YES	YES	NO	4	MDCK (8)	N/A	0	0	8	8	8	0

Specimen (EISN_INF23):	01	02	03	04	05	06	07	08	Overall score ²	Isolation method (n specimens) ³	Confirmation method virus isolation (n specimens) ⁴						
Subtype/lineage:	A (H1N1) pdm09	A (H3N2)	B/Vic	A (H3N2)	Negative	A (H1N1) pdm09	B/Vic	A (H3N2)			HA-assay	NA activity		RT-PCR	IF	CPE	Other
Expected result isolated:	YES	YES	YES	YES	NO	YES	YES	YES			RBC species	MUNANA	NA-STAR				
Participant ID ¹																	
10080	YES	YES	YES	YES	NO	YES	YES	YES	0	Mixed MDCK-I and MDCK-SIAT (8)	N/A	8	0	0	0	8	0
10104	NO	YES	NO	YES	NOT_ATTEMPTED	NO	YES	YES	3	MDCK and SIAT (3), MDCK (1), SIAT (3)	Chicken (7)	0	0	0	0	6	0
10115	YES	YES	NO	YES	NO	YES	YES	YES	1	MDCK (8)	Turkey (4), Guinea Pig (5)	0	0	1	2	8	0
10144	YES	YES	NO	YES	NOT_ATTEMPTED	NO	YES	YES	2	MDCK (4), MDCK-SIAT (3)	N/A	0	0	7	0	7	0
10261	NO	NO	NO	NO	NO	NO	NO	NO	7	MDCK (8)	Human (8)	0	0	0	0	8	0
10461	YES	YES	YES	YES	NO	NO	NO	YES	2	MDCK (5), MDCK-SIAT (3)	N/A	0	0	0	8	0	0
10462	NOT_ATTEMPTED	NOT_ATTEMPTED	NOT_ATTEMPTED	NOT_ATTEMPTED	NOT_ATTEMPTED	NOT_ATTEMPTED	NOT_ATTEMPTED	NOT_ATTEMPTED									
10464	NO	YES	NO	YES	NOT_ATTEMPTED	YES	YES	YES	2	MDCK (4), MDCK-SIAT (3)	Turkey (4), Guinea Pig (3)	5	0	7	0	7	0
10466	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK-SIAT (8)	Turkey (7)	0	0	0	0	8	1
10498	YES	YES	YES	YES	NO	YES	YES	YES	0	MDCK-SIAT (8)	N/A	0	0	8	0	0	0
10507	NO	YES	NO	YES	NO	NO	YES	NO	4	MDCK (8)	Guinea Pig (8)	0	0	8	0	3	0
11111	YES	NO	NO	YES	NO	YES	YES	YES	2	MDCK-II (7), embryonated (1)	Guinea Pig (8), Rooster (8)	0	0	8	0	5	0
11119	NO	YES	YES	YES	NO	YES	YES	YES	1	MDCK-SIAT (8)	Turkey (8), Guinea Pig (8)	7	0	8	0	8	0

RBC = red blood cells, N/A – not applicable

¹ Cell with orange shading = laboratory located in an EU/EEA country. Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by WHO Regional Office for Europe.

² Scoring

- Positive specimens: Positive and Not Attempted (because of negative result in molecular detection) (green shading), 0; Negative and all other (pink shading), 1.
- Negative specimens: Negative and Not Attempted (because of negative result in molecular detection) (green shading), 0; all other (pink shading), 1.

³ MDCK = Madin Darby Canine Kidney; SIAT = human alpha 2,6-sialyltransferase; London line = Obtained from WHO CC London, UK; hCK = humanised Canine Kidney cell line (a Madin-Darby canine kidney cell line that expresses high levels of human influenza virus receptors and low levels of avian virus receptors).

⁴ HA = haemagglutinin; RBC = Red Blood Cells; NA = neuraminidase; MUNANA = 20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid; RT-PCR = reverse transcription polymerase chain reaction; IF = immunofluorescence; CPE = cytopathic effect observed microscopically.

Table A13. Overview of virus antigenic characterisation results with methodology used

Individual antigenic characterisation results										Antigenic characterisation method ²			
Specimen EISN_INF23_	01	02	03	04	05	06	07	08	Total number of virus isolates subjected to characteri- sation	RBC used in HA-assay ²	HI-assay		
Expected Result:	A(H1)pdm09 A/Victoria/2 570/2019 (H1N1)-like	A(H3) not attributed to category	B(Vic) lineage not attributed to category	A(H3) A/Darwin/9/2 021 -like	neg	A(H1)pdm09 A/Guangdong- Maonan/SWL153 6/2019 (H1N1)- like	B/Austria/135941 7/2021-like	A(H3) not attributed to category		Species (n viruses)	Oseltamivir used (n viruses)		Sera (source and species)
											Yes	No	
Participant ID ¹													
95	A(H1) A/Victoria/257 0/2019 (H1N1)-like	A(H3) A/Darwin/9/2021- like	B/Netherlands/1 1267/2022 (B/Victoria/2/87 (V1A.3)-lineage)	A(H3) A/Darwin/9/2021 -like		A(H1) A/Guangdong- Maonan/SWL1536/ 2019 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	FAILED	7	Turkey (7)	0	7	In-house generated ferret sera
200		OTHER: A/Stockholm/5/202 1 and A/Darwin/9/2021		A(H3) A/Darwin/9/2021 -like			B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	A(H3) A/Darwin/9/2021- like	4	Guinea pig (7)	0	4	WHO CC London ferret sera
1299	A(H1) A/Victoria/257 0/2019 (H1N1)-like	A(H3) A/Darwin/9/2021- like		A(H3) A/Darwin/9/2021 -like		A(H1) A/Guangdong- Maonan/SWL1536/ 2019 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	FAILED	6	Guinea pig (7)	3	3	WHO CC London ferret sera
2126	A(H1) A/Victoria/257 0/2019 (H1N1)-like	A(H3) A/Darwin/9/2021- like	B(Vic) lineage not attributed to category	A(H3) A/Darwin/9/2021 -like		A(H1) A/Guangdong- Maonan/SWL1536/ 2019 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)		6	Turkey (5) Guinea pig (4)	0	6	In-house generated ferret sera
2277	A(H1) A/Guangdong- Maonan/SWL1 536/2019 (H1N1)-like	A(H3) A/Darwin/9/2021- like	B/Austria/13594 17/2021-like (B/Victoria/2/87 (del162-164B)- lineage)	A(H3) A/Darwin/9/2021 -like		A(H1) A/Victoria/2570/20 19 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	A(H3) A/Hong Kong/2671/2019 (H3N2)-like	7	Human (8)	0	7	WHO CC London ferret sera
2278	A(H1) A/Victoria/257 0/2019 (H1N1)-like	OTHER: low reactor A/Darwin/9/2021 (H3N2)-like	OTHER: low reactor B/Austria/13594 17/2021 - like	A(H3) A/Darwin/9/2021 -like		OTHER: low reactor A/Sydney/5/2021- like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	A(H3) A/Hong Kong/45/2019 (H3N2)-like	7	Human (8)	0	7	WHO CC Atlanta sheep sera
3442							B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)		1	Guinea pig (8)	0	1	WHO CC London ferret sera
10007		A(H3) A/Darwin/9/2021- like					B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)		2	Turkey (4) Guinea pig (4)	0	2	WHO CC London ferret sera
10115	A(H1) A/Sydney/5/2 021 (H1N1)- like	A(H3) A/Darwin/9/2021- like		A(H3) A/Darwin/9/2021 -like		A(H1) A/Guangdong- Maonan/SWL1536/ 2019 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	A(H3) A/Hong Kong/45/2019 (H3N2)-like	6	Turkey (4) Guinea pig (5)	3	3	WHO CC London ferret sera

Individual antigenic characterisation results										Antigenic characterisation method ²			
Specimen EISN_INF23_	01	02	03	04	05	06	07	08	Total number of virus isolates subjected to characteri- sation	RBC used in HA-assay ²	HI-assay		
Expected Result:	A(H1)pdm09 A/Victoria/2 570/2019 (H1N1)-like	A(H3) not attributed to category	B(Vic) lineage not attributed to category	A(H3) A/Darwin/9/2 021 -like	neg	A(H1)pdm09 A/Guangdong- Maonan/SWL153 6/2019 (H1N1)- like	B/Austria/135941 7/2021-like	A(H3) not attributed to category		Species (n viruses)	Oseltamivir used (n viruses)		Sera (source and species)
											Yes	No	
Participant ID ¹													
10464		A(H3) A/Darwin/9/2021- like		OTHER: A/Stockholm/5/2 021-like		A(H1) A/Guangdong- Maonan/SWL1536/ 2019 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	A(H3) not attributed to category	5	Turkey (4) Guinea pig (3)	0	5	WHO CC London ferret sera
10466	A(H1) A/Victoria/257 0/2019 (H1N1)-like	A(H3) A/Darwin/9/2021- like	FAILED	A(H3) A/Darwin/9/2021 -like		A(H1) A/Victoria/2570/20 19 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)		7	Turkey (7)	6	0	In-house generated ferret sera
11111	OTHER: A(H1) A/Denmark/32 80/2019 (H1N1)-like			A(H3) A/Darwin/9/2021 -like		A(H1) A/Guangdong- Maonan/SWL1536/ 2019 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)	OTHER: (H3) A/Singapore/INFIM H-16-0019/2016- like	5	Guinea pig (8) Rooster (8)	0	5	WHO CC London ferret sera, In- house generated rooster sera
11119		A(H3) A/Darwin/9/2021- like		A(H3) A/Darwin/9/2021 -like		A(H1) A/Guangdong- Maonan/SWL1536/ 2019 (H1N1)-like	B/Austria/1359417/20 21-like (B/Victoria/2/87 (del162-164B)- lineage)		6	Turkey (8) Guinea pig (8)	0	4	WHO CC London ferret sera

RBC =red blood cells

Expected result categories are indicated with dark green shading. Results close to expected results are indicated in light green.

¹ Cell with orange shading = laboratory located in an EU/EEA country. Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by WHO Regional Office for Europe.

² Because it was not explicitly asked, we assume that laboratories use the same type of RBC for antigenic characterisation if not otherwise indicated.

Table A14. Summary by panel specimen of reported reference sera and viruses used to antigenically characterise viruses isolated from the panel specimens

Panel specimen	Subtype/lineage	Number of labs that reported information	Number of sera/viruses used to characterise panel viruses			Number of labs that used sera/viruses included in expected results (n; % of total labs)	
			median	min	max		
EISN_INF23-01	A(H1N1)pdm09	8	3	1	6	8	100%
EISN_INF23-02	A(H3N2)	11	4	1	8	11	100%
EISN_INF23-03	B/Vic	5	2	1	8	3	60%
EISN_INF23-04	A(H3N2)	11	4	1	8	11	100%
EISN_INF23-06	A(H1N1)pdm09	10	3	1	6	8	80%
EISN_INF23-07	B/Vic	13	3	1	8	13	100%
EISN_INF23-08	A(H3N2)	8	4	3	6	7	88%

Table A15. Overview of genetic characterisation results using genetic category assignment with performance score and used methodology

Individual genetic category results ¹										Genetic characterisation						
Sample	01	02	03	04	05	06	07	08	Overall score ³	Total number of specimens genetically characterised	On specimen type			Technique		
EISN_INF23_	Expected result:	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	Negative	A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021			A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	Simulated clinical specimen	Virus isolate	Both	Sanger	NGS
Participant ID ²																
95	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	0	7	7	0	0	0	7	
200	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) attributed to recognised group in current guidance but not listed here	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		OTHER: A/Belgium/S1111/2022	OTHER: B(Vic)-lineage B/Bishkek/11/2022	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	1	7	7	0	0	1	6	
1159	A(H1)pdm09 group 6B.1A.5a.2 representative A/Victoria/2570/2019	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	1	7	7	0	0	7	0	
1299	A(H1)pdm09 group 6B.1A.5a.2 representative A/Victoria/2570/2019	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	2	7	1	0	6	0	7	
1643	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	1	7	7	0	0	0	7	
2126	A(H1)pdm09 group 6B.1A.5a.2 representative A/Sydney/5/2021	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	B(Vic)-lineage clade V1A.3 repr, B/Washington/02/2019	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1b representative A/Hong Kong/2671/2019	4	7	7	0	0	0	7	
2271	A(H1)pdm09 group 6B.1A.5a.2 representative A/Victoria/2570/2019	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020		A(H3) attributed to recognised group in current guidance but not listed here		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019		A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	2	5	5	0	0	5	0	

Individual genetic category results ¹										Genetic characterisation					
Sample	01	02	03	04	05	06	07	08	Overall score ³	Total number of specimens genetically characterised	On specimen type			Technique	
EISN_INF23_											Simulated clinical specimen	Virus isolate	Both	Sanger	NGS
Expected result:	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	Negative	A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019							
Participant ID ²															
2278	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	0	7	0	3	3	7	0
3442	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) attributed to recognised group in current guidance but not listed here		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	1	7	7	0	0	0	7
3558	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	FAILED	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	FAILED	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	2	5	7	0	0	7	0
10007	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	1	7	7	0	0	0	7
10023	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	1	7	7	0	0	0	7
10053	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	0	7	7	0	0	0	7
10080	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4005/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/11267/2022	A(H3) attributed to recognised group in current guidance but not listed here		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536/2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417/2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/2019	1	7	7	0	0	0	7

Individual genetic category results ¹										Genetic characterisation					
Sample	01	02	03	04	05	06	07	08	Overall score ³	Total number of specimens genetically characterised	On specimen type			Technique	
EISN_INF23_											Expected result:	Simulated clinical specimen	Virus isolate	Both	Sanger
Participant ID ²															
10104	A(H1)pdm09 group 6B.1A.5a.2 representative A/Sydney/5/2021	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417 /2021	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019	B(Vic)-lineage clade V1A.3 repr, B/Washington/02/ 2019	A(H3) group 3C.2a1b.1b representative A/Hong Kong/2671/2019							
10115	A(H1)pdm09 group 6B.1A.5a.2 representative A/Sydney/5/2021	A(H3) attributed to recognised group in current guidance but not listed here	B(Vic)-lineage clade V1A.3 representative B/Netherlands/112 67/2022	A(H3) attributed to recognised group in current guidance but not listed here		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417 /2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/ 2019	3	7	7	0	0	1	7
10144	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/ 2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/400 5/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/112 67/2022	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417 /2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/ 2019	0	7	7	0	0	0	7
10461	FAILED	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	B(Vic)-lineage clade V1A.3 repr, B/Washington/02/ 2019	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	FAILED	FAILED	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417 /2021	A(H3) group 3C.2a1b.1b representative A/Hong Kong/2671/2019	6	5	8	0	0	0	8
10462	A(H1)pdm09 not attributed to clade	A(H3) not attributed to clade	B(Vic) lineage not attributed to clade	A(H3) not attributed to clade		A(H1)pdm09 not attributed to clade	B(Vic) lineage not attributed to clade	A(H3) not attributed to clade	7	7	7	0	0	0	7
10464	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/ 2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/400 5/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/112 67/2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/4 005/2020		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417 /2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/ 2019	1	7	3	4	0	7	0
10466	A(H1)pdm09 group 6B.1A.5a.2 representative A/Norway/25089/ 2022	A(H3) group 3C.2a1b.2a.2 repr, A/Bangladesh/400 5/2020	B(Vic)-lineage clade V1A.3 representative B/Netherlands/112 67/2022	A(H3) attributed to recognised group in current guidance but not listed here		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417 /2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/ 2019	1	7	0	7	0	0	7

Individual genetic category results ¹										Genetic characterisation					
Sample	01	02	03	04	05	06	07	08	Overall score ³	Total number of specimens genetically characterised	On specimen type			Technique	
EISN_INF23_											Simulated clinical specimen	Virus isolate	Both	Sanger	NGS
Expected result:															
Participant ID ²															
10498	A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019		A(H3) group 3C.2a1b.1a representative A/Denmark/3264/ 2019	2	5	0	5	0	0	5
11119	A(H1)pdm09 group 6B.1A.5a.2 representative A/Sydney/5/2021	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021	B(Vic)-lineage clade V1A.3 repr, B/Washington/02/ 2019	A(H3) group 3C.2a1b.2a.2 representative A/Darwin/9/2021		A(H1)pdm09 group 6B.1A.5a.1 representative A/Guangdong-Maonan/SWL1536 /2019	B(Vic)-lineage clade V1A.3a.2 repr, B/Austria/1359417 /2021	A(H3) group 3C.2a1b.1a representative A/Denmark/3264/ 2019	3	7	7	0	0	0	7

Expected result categories are indicated with dark green shading. Results close to expected results are indicated in light orange. Results that are considered as wrong or not precise enough are indicated in dark orange.

¹ All strain indications refer to representative strain as indicated in the guidance for TESSy genetic categories.

² Cell with orange shading = laboratory located in an EU/EEA country. Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by World Health Organization Regional Office for Europe.

³ Scoring: category as expected or close 0; category not as expected 1.

Table A16. Overview of genetic characterisation results using WHO clade assignment with performance score

Sample EISN_INF23_	01	02	03	04	05	06	07	08	Overall score ²
Expected results	A(H1)pdm09 - 6B.1A.5a.2a.1	A(H3) - 3C.2a1b.2a.2b	B/Vic - V1A.3	A(H3) - 3C.2a1b.2a.2a.3	Negative	A(H1)pdm09 - 6B.1A.5a.1	B/Vic - V1A.3a.2	A(H3) - 3C.2a1b.1a	
Participant ID ¹									
95	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
200	6B.1A.5a.2	3C.2a1b.2a.2	V1A.3	3C.2a1b.2a.2		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	3
1159	6B.1A.5a.2	3C.2a1b.2a.2	V1A.3	3C.2a1b.2a.2		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	3
1299	6B.1A.5a.2	3C.2a1b.2a.2	V1A.3	3C.2a1b.2a.2		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	3
1643	6B.1A.5a.2a.1	3C.2a1b.2a.2	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	1
2126	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
2271	6B.1A.5a.2a.1	3C.2a1b.2a.2b		3C.2a1b.2a.2a.3		6B.1A.5a.1		3C.2a1b.1a	0
2278	6B.1A.5a.2	3C.2a1b.2a.2	V1A.3	3C.2a1b.2a.2		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	3
3442	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
3558	6B.1A.5a.2a.1	3C.2a1b.2a.2b	FAILED	3C.2a1b.2a.2a.3		6B.1A.5a.1	FAILED	3C.2a1b.1a	2
10007	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
10023	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
10053	6B.1A.5a.2	3C.2a1b.2a.2	V1A.3	3C.2a1b.2a.2		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	3
10080	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
10104	6B.1A.5a.2a	3C.2a1b.2a.2b	V1A.3a.2	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3	3C.2a1b.1a	3
10115	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
10144	6B.1A.5a.2a.1	3C.2a1b.2a.2	V1A.3	3C.2a1b.2a.2		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	2
10461	FAILED	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3	FAILED	FAILED	V1A.3a.2	3C.2a1b.1a	3
10462	FAILED	FAILED	FAILED	FAILED		FAILED	FAILED	FAILED	7
10464	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2b		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	1
10466	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0
10498	6B.1A.5a.1	3C.2a1b.2a.2b		3C.2a1b.2a.2a.3		6B.1A.5a.1		3C.2a1b.1a	1
11119	6B.1A.5a.2a.1	3C.2a1b.2a.2b	V1A.3	3C.2a1b.2a.2a.3		6B.1A.5a.1	V1A.3a.2	3C.2a1b.1a	0

Expected results are indicated with dark green shading. Results that are considered as wrong or not precise enough are indicated in dark orange.

¹ Cell with orange shading = laboratory located in an EU/EEA country. Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by World Health Organization Regional Office for Europe.

² Scoring: WHO clade as expected 0; WHO clade not as expected 1.

Table A17. Overview of neuraminidase inhibitors and baloxavir genetic antiviral susceptibility testing results with performance score and used methodology

Specimen:	EISN_AV23-01					EISN_AV23-02					EISN_AV23-03				
Subtype	A(H1N1)pdm09					A(H3N2)					A(H3N2)				
Result type: Participant ID ¹	Genotype		Interpretation ²			Genotype		Interpretation ²			Genotype		Interpretation ²		
	NA	PA	Oseltamivir	Zanamivir	Baloxavir	NA	PA	Oseltamivir	Zanamivir	Baloxavir	NA	PA	Oseltamivir	Zanamivir	Baloxavir
Expected Result	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	Del245-248	None	AAHRI	AARI	AANS
112	H275Y	L28P	AARI	AANI	AARS	None	E23G	AANI	AANI	AARS	del245-248; R292K; N294S	None	AAHRI	AAHRI	AANS
200	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	del245-248	None	AAHRI	AARI	AANS
1159	H275Y		AAHRI	AANI		None		AANI	AANI		G244del, N245del, A246del, T247del		NO	NO	
1299	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	Del 245-248	none	AARI	AANI	AANS
1643	H275Y	None	AAHRI	AANI	AANS	N329S	E23G	AANI	AANI	AANS	None	None	AANI	AANI	AANS
2126	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	del244-247	None	AAHRI	AARI	AANS
2816	H275Y	None	AAHRI	AANI	AANS	None	None	AANI	AANI	AANS	N2:del245-248	None	AAHRI	AARI	AANS
3442	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	aa245-248deletion	None	AAHRI	AARI	AANS
3558	H275Y	None	AAHRI	AANI	NO	none	E23G	AANI	AANI	NO	245del;246del;247del;248del	None	AAHRI	AARI	NO
10007	H275Y	None	AAHRI	AANI	AANS	N329S	E23G	AANI	AANI	AARS	None	None	AANI	AANI	AANS
10023	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	Del245-248	None	AAHRI	AARI	AANS
10053	H275Y		AAHRI	AANI		N329S		NO	NO		Del 245-248		AAHRI	NO	
10080	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	del245-248	None	AAHRI	AARI	AANS
10115	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	deletion amino-acid 244-248	None	AAHRI	AARI	AANS
10142	H275Y for A(H1N1)pdm09	None	AAHRI	AANI	AANS	None	E23G for A(H3N2)	AANI	AANI	AARS	None	None	AANI	AANI	AANS
10144	H275Y		AAHRI	AANI							del245-248	None	AAHRI	AARI	AANS
10205	H275Y		AAHRI	AANI		None		AANI	AANI		Del245-248		AAHRI	AARI	
10461	None		NO	NO		None		AANI	AANI		A246del for A(H3N2)		AARI	AARI	
10462															
10464	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	Del 245-248	None	AAHRI	AARI	AANS
10498	H275Y for A(H1N1)pdm09		AAHRI	AANI		R292K for A(H3N2)	E23G for A(H3N2)	AAHRI	NO	AARS		None			AANS
11111						None		AANI	AANI		Del 245-248		AARI	AANI	
11119	H275Y	None	AAHRI	AANI	AANS	None	E23G	AANI	AANI	AARS	G244del; N245del; A246del; T247del	None	AAHRI	NO	AANS

Specimen:	EISN_INF23-01					EISN_INF23-02					EISN_INF23-03				
Subtype	A(H1N1)pdm09					A(H3N2)					B/Victoria				
Result type:	Genotype		Interpretation ²			Genotype		Interpretation ²			Genotype		Interpretation ²		
Participant ID ¹	NA	PA	Oseltamivir	Zanamivir	Baloxavir	NA	PA	Oseltamivir	Zanamivir	Baloxavir	NA	PA	Oseltamivir	Zanamivir	Baloxavir
Expected result	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
112															
200	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None		AANI	AANI	
1159	None		AANI	AANI		None		AANI	AANI		None		AANI	AANI	
1299	none	none	AANI	AANI	AANS	none	none	AANI	AANI	AANS	none	none	AANI	AANI	AANS
1643	None	None	AANI	AANI	AANS	N329S	None	AANI	AANI	AANS	A395T	None	AANI	AANI	AANS
2126	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None		AANI	AANI	
2816	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
3442	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None		AANI	AANI	
3558	None	none	AANI	AANI	NO	None	none	AANI	AANI	NO	None		AANI	AANI	
10007	None	None	AANI	AANI	AANS	N329S	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10023	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10053	None		AANI	AANI		N329S		NO	NO		None		AANI	AANI	
10080	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10115	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10142															
10144	None		AANI	AANI		None		AANI	AANI		None		AANI	AANI	
10205															
10461						None		AANI	AANI		None		AANI	AANI	
10462	A/California/7/2009		AANI	AANI		A/Kansas/14/2017		AANI	AANI		B/Brisbane/60/2008		AANI	AANI	
10464	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10498	None		AANI	AANI		None	None	AANI	AANI	AANS					
11111						None		AANI	AANI						
11119	None	None	AANI	AANI	AANS	H347Q	None	AANI	AANI	AANS	None	E194X	AANI	AANI	AARS

Specimen:	EISN_INF23-04					EISN_INF23-06					EISN_INF23-07				
Subtype	A(H3N2)					A(H1N1)pdm09					B/Victoria				
Result type:	Genotype		Interpretation ²			Genotype		Interpretation ²			Genotype		Interpretation ²		
Participant ID ¹	NA	PA	Oseltamivir	Zanamivir	Baloxavir	NA	PA	Oseltamivir	Zanamivir	Baloxavir	NA	PA	Oseltamivir	Zanamivir	Baloxavir
Expected Result	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
112															
200	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None		AANI	AANI	
1159	None		AANI	AANI		None		AANI	AANI		None		AANI	AANI	
1299	none	none	AANI	AANI	AANS	none	none	AANI	AANI	AANS	none	none	AANI	AANI	AANS
1643	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	A395V	None	AANI	AANI	AANS
2126	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
2816	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
3442	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
3558	None	none	AANI	AANI	NO	None	none	AANI	AANI	NO	None		AANI	AANI	
10007	None	None	AANI	AANI	AANS	None		AANI	AANI		None	None	AANI	AANI	AANS
10023	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10053	None		AANI	AANI		None		AANI	AANI		None		AANI	AANI	
10080	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10115	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10142															
10144	None	None	AANI	AANI	AANS	None		AANI	AANI		None		AANI	AANI	
10205															
10461	S150R for A(H3N2)		AAHRI	AANI							None		AANI	AANI	
10462	A/Kansas/14/2017		AANI	AANI		A/California/7/2009		AANI	AANI		B/Brisbane/60/2008		AANI	AANI	
10464	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS
10498	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS					
11111	None		AANI	AANI		None		AANI	AANI						
11119	S150R	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS	None	None	AANI	AANI	AANS

Specimen:	EISN_INF23-08					Overall score	Methodology				Sequences uploaded	
Subtype	A(H3N2)						SNP RT-PCR	Pyrosequencing	Partial sequencing	Full length sequencing		
Result type:	Genotype		Interpretation ²									
Participant ID ¹	NA	PA	Oseltamivir	Zanamivir	Baloxavir						NA	PA
Expected Result	None	None	AANI	AANI	AANS							
112						8	0	0	0	3	Yes	Yes
200	None	None	AANI	AANI	AANS	0	0	0	0	10	Yes	Yes
1159	None		AANI	AANI		5	0	0	10	0	Yes	No
1299	none	none	AANI	AANI	AANS	3	0	0	0	10	Yes	Yes
1643	None	None	AANI	AANI	AANS	16	0	0	0	10	Yes	Yes
2126	None	None	AANI	AANI	AANS	1	0	0	0	10	Yes	Yes
2816	None	None	AANI	AANI	AANS	4	0	0	0	10	Yes	Yes
3442	None	None	AANI	AANI	AANS	0	0	0	0	10	Yes	Yes
3558	None	None	AANI	AANI	NO	16	0	0	0	10	Yes	Yes
10007	N329S	None	AANI	AANI	AANS	12	0	0	0	10	Yes	Yes
10023	None	None	AANI	AANI	AANS	0	0	0	0	10	Yes	Yes
10053	N329S		NO	NO		20	0	0	0	10	No	No
10080	None	None	AANI	AANI	AANS	0	8	0	0	10	Yes	Yes
10115	None	None	AANI	AANI	AANS	1	0	0	0	10	Yes	Yes
10142						6	0	0	0	3	Yes	Yes
10144	None		AANI	AANI		0	0	3	0	9	Yes	Yes
10205						0	0	0	3	0	Yes	No
10461	R150S		AAHRI	AANI		16	0	0	0	8	Yes	No
10462	A/Kansas/14/2017		AANI	AANI		14	0	0	0	7	No	No
10464	None	None	AANI	AANI	AANS	0	0	0	0	10	Yes	Yes
10498	None	None	AANI	AANI	AANS	6	0	0	0	7	Yes	Yes
11111	None		AANI	AANI		3	0	0	6	0	Yes	No
11119	R150S	None	AANI	AANI	AANS	13	5	0	0	10	Yes	Yes

Expected results are indicated with dark green shading. Results close to expected results are indicated in light orange and light green. Results that are considered as wrong are indicated in dark orange.

¹ Cell with orange shading = laboratory located in EU/EEA country. Cell with white shading = laboratory located in non-EU/EEA country with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in non-EU/EEA country in WHO European Region with EEIQAP participation supported by World Health Organization Regional Office for Europe.

² None = no amino acid substitution previously associated with (highly) reduced inhibition identified. 'Sequence' column based on the assessment at National Institute for Public Health and the Environment, the Netherlands (RIVM) using the reported sequence and 'Report' column contains the reported result by the laboratory based on their own assessment using the obtained sequence from the panel specimen. NA = Neuraminidase

PA = Polymerase acidic

Neuraminidase inhibitors interpretation:

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;

HRI = highly reduced inhibited (fold-change IC_{50} ; A >100; B >50);

NI = normal inhibited (fold-change IC_{50} ; A <10; B <5);

NO = no interpretation possible due to partial NA segment information (SNP PCR, partial- or pyrosequencing);

RI = reduced inhibited (fold-change IC_{50} ; A ≥ 10 & ≤ 100 ; B A ≥ 5 & ≤ 50).

Baloxavir marboxil interpretation:

AANS = has no amino acid substitutions previously associated with reduced susceptibility (RS) (highest fold-change in range < 3 in WHO Table)

AARS = has amino acid substitutions previously associated with reduced susceptibility (RS) (fold-change ≥ 3 in WHO Table)

Scoring system used:

EISN_AV23-01 substitutions – NA-H275Y found (green shading), 0; any other (orange shading), 1. Not tested, not scored

EISN_AV23-01 interpretation oseltamivir: AAHRI (green shading), 0; AARI (light orange shading), 1; rest (orange shading), 2. Interpretation zanamivir: AANI (green shading), 0; rest (orange shading), 2.

Interpretation baloxavir:

AANS, 0; rest, 2. Not tested, not scored.

EISN_AV23-02 substitutions: PA-E23G found (green shading), 0; rest (orange shading), 2. Not tested, not scored.

EISN_AV23-02 interpretation oseltamivir: AANI (green shading), 0; rest (orange shading), 2. Interpretation zanamivir: AANI (green shading), 0; rest (orange shading), 2. Interpretation baloxavir: AARS (green shading), 0; rest (orange shading), 2. Not tested, not scored.

EISN_AV23-03 substitutions: NA-Del245-248 found (green shading), 0; partial deletions (light orange shading), 1; rest (orange shading), 2. Not tested, not scored.

EISN_AV23-03 interpretation oseltamivir: AAHRI (green shading), 0; AARI (light orange shading), 1; rest (orange shading), 2. Interpretation zanamivir: AARI (green shading), 0; AAHRI (light orange shading), 1; rest (orange shading), 2. Interpretation baloxavir: AANS (green shading), 0; rest (orange shading), 2. Not tested, not scored.

EISN_INF23-01 – 08 (except 05) substitution: none found, 0; any other (orange shading), 2. Not tested, not scored.

EISN_INF23-01 – 08 (except 05) interpretation oseltamivir and zanamivir: AANI (green shading), 0; any other (orange shading), 2. Not tested, not scored.

Table A18. Overview of neuraminidase inhibitor phenotypic antiviral susceptibility testing results with performance score and used methodology (assay type only)

Specimen	EISN_AV23-01				EISN_AV23-02				EISN_AV23-03			
Subtype	A(H1N1)pdm09 ¹				A(H3N2) ⁵				A(H3N2) ⁶			
Antiviral	Oseltamivir		Zanamivir		Oseltamivir		Zanamivir		Oseltamivir		Zanamivir	
Expected result ²	HRI		NI		NI		NI		HRI		RI	
Participant ID ³ /result	IC ₅₀ (nM) ³		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)	
200		RESISTANT	0.38	NORMAL_INHIBITION	7.8	NORMAL_INHIBITION	0.4	NORMAL_INHIBITION	1226.39	HIGHLY_REDUCED_INHIBITION	6.76	REDUCED_INHIBITION
1159	97.1	HIGHLY_REDUCED_INHIBITION	3.1	NORMAL_INHIBITION	0.1	NORMAL_INHIBITION	5.1	NORMAL_INHIBITION	10.5	REDUCED_INHIBITION	25	REDUCED_INHIBITION
1299	214	HIGHLY_REDUCED_INHIBITION	0.4	NORMAL_INHIBITION	0.68	NORMAL_INHIBITION	0.79	NORMAL_INHIBITION	100.88	REDUCED_INHIBITION	11.81	NORMAL_INHIBITION
1643	291.7	HIGHLY_REDUCED_INHIBITION	0.7	NORMAL_INHIBITION	0.15	NORMAL_INHIBITION	0.4	NORMAL_INHIBITION	21.1	NORMAL_INHIBITION	6.9	NORMAL_INHIBITION
2271	342.9	HIGHLY_REDUCED_INHIBITION	0.6	NORMAL_INHIBITION	0.2	NORMAL_INHIBITION	0.3	NORMAL_INHIBITION	29.3	REDUCED_INHIBITION	5.7	REDUCED_INHIBITION
2275	233	HIGHLY_REDUCED_INHIBITION	0.13	NORMAL_INHIBITION	0.11	NORMAL_INHIBITION	0.12	NORMAL_INHIBITION	15.82	REDUCED_INHIBITION	3.1	NORMAL_INHIBITION
2816	1000	HIGHLY_REDUCED_INHIBITION	0.4	NORMAL_INHIBITION	0.6	NORMAL_INHIBITION	0.6	NORMAL_INHIBITION	1000	HIGHLY_REDUCED_INHIBITION	5.3	NORMAL_INHIBITION
3442	235.92	HIGHLY_REDUCED_INHIBITION	0.65	NORMAL_INHIBITION	0.3	NORMAL_INHIBITION	1.32	NORMAL_INHIBITION	68.69	HIGHLY_REDUCED_INHIBITION	8.83	REDUCED_INHIBITION
3558	258.6	HIGHLY_REDUCED_INHIBITION	0.3	NORMAL_INHIBITION	0.14	NORMAL_INHIBITION	0.33	NORMAL_INHIBITION	16.06	HIGHLY_REDUCED_INHIBITION	3.06	NORMAL_INHIBITION
10023	226.97	HIGHLY_REDUCED_INHIBITION	0.28	NORMAL_INHIBITION	0.1	NORMAL_INHIBITION	0.22	NORMAL_INHIBITION	17.27	HIGHLY_REDUCED_INHIBITION	3.8	REDUCED_INHIBITION
10080	370	HIGHLY_REDUCED_INHIBITION	1	NORMAL_INHIBITION	0.18	NORMAL_INHIBITION	0.96	NORMAL_INHIBITION	24	HIGHLY_REDUCED_INHIBITION	17	REDUCED_INHIBITION
10115	226.64	HIGHLY_REDUCED_INHIBITION	0.59	NORMAL_INHIBITION	0.25	NORMAL_INHIBITION	0.68	NORMAL_INHIBITION	19.68	REDUCED_INHIBITION	2.36	NORMAL_INHIBITION
10261	401.1	HIGHLY_REDUCED_INHIBITION	0.7	NORMAL_INHIBITION	0.5	NORMAL_INHIBITION	1.2	NORMAL_INHIBITION	43.2	HIGHLY_REDUCED_INHIBITION	10.5	REDUCED_INHIBITION
10464	408.8	HIGHLY_REDUCED_INHIBITION	0.5	NORMAL_INHIBITION	0.5	NORMAL_INHIBITION	0.5	NORMAL_INHIBITION	44	REDUCED_INHIBITION	7.6	NORMAL_INHIBITION
10466	399.3	HIGHLY_REDUCED_INHIBITION	0.3	NORMAL_INHIBITION	0.5	NORMAL_INHIBITION	0.4	NORMAL_INHIBITION	25.1	REDUCED_INHIBITION	2.7	NORMAL_INHIBITION
11111	247.54	HIGHLY_REDUCED_INHIBITION	0.15	NORMAL_INHIBITION	0.74	NORMAL_INHIBITION	0.41	NORMAL_INHIBITION	43.4	REDUCED_INHIBITION	5.13	NORMAL_INHIBITION
11119	357.83	HIGHLY_REDUCED_INHIBITION	0.34	NORMAL_INHIBITION	0.18	NORMAL_INHIBITION	0.31	NORMAL_INHIBITION	27.82	HIGHLY_REDUCED_INHIBITION	5.24	NORMAL_INHIBITION

Specimen	EISN_INF23-01				EISN_INF23-02				EISN_INF23-03				EISN_INF23-04			
Subtype	A(H1N1)pdm09				A(H3N2)				B/Victoria				A(H3N2)			
Antiviral	Oseltamivir		Zanamivir		Oseltamivir		Zanamivir		Oseltamivir		Zanamivir		Oseltamivir		Zanamivir	
Expected Result ²	NI		NI		NI		NI		NI		NI		NI		NI	
Participant ID ¹ / result	IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)	
200					8.99	NORMAL_INHIBITION	0.37	NORMAL_INHIBITION					8.53	NORMAL_INHIBITION	0.33	NORMAL_INHIBITION
1159		SENSITIVE_NO_IC50		SENSITIVE_NO_IC50		SENSITIVE_NO_IC50		SENSITIVE_NO_IC50	1.2	NORMAL_INHIBITION	21.7	NORMAL_INHIBITION		SENSITIVE_NO_IC50		SENSITIVE_NO_IC50
1299	0.56	NORMAL_INHIBITION	0.48	NORMAL_INHIBITION	1.34	NORMAL_INHIBITION	1.06	NORMAL_INHIBITION					0.74	NORMAL_INHIBITION	0.5	NORMAL_INHIBITION
1643																
2271																
2275	0.35	NORMAL_INHIBITION											0.19	NORMAL_INHIBITION		
2816																
3442																
3558																
10023	0.2	NORMAL_INHIBITION	0.17	NORMAL_INHIBITION	0.18	NORMAL_INHIBITION	0.31	NORMAL_INHIBITION	15.19	NORMAL_INHIBITION	1.76	NORMAL_INHIBITION	0.1	NORMAL_INHIBITION	0.17	NORMAL_INHIBITION
10080	0.78	NORMAL_INHIBITION	0.69	NORMAL_INHIBITION	0.45	NORMAL_INHIBITION	1.4	NORMAL_INHIBITION	40	NORMAL_INHIBITION	13	NORMAL_INHIBITION	0.28	NORMAL_INHIBITION	1.2	NORMAL_INHIBITION
10115	0.3	NORMAL_INHIBITION	0.41	NORMAL_INHIBITION	0.44	NORMAL_INHIBITION	0.9	NORMAL_INHIBITION					0.31	NORMAL_INHIBITION	0.86	NORMAL_INHIBITION
10261																
10464					0.3	NORMAL_INHIBITION	0.2	NORMAL_INHIBITION					0.3	NORMAL_INHIBITION	0.2	NORMAL_INHIBITION
10466	1.3	NORMAL_INHIBITION	0.1	NORMAL_INHIBITION	1.5	NORMAL_INHIBITION	1	NORMAL_INHIBITION	26.2	NORMAL_INHIBITION	8.2	NORMAL_INHIBITION	0.4	NORMAL_INHIBITION	0.4	NORMAL_INHIBITION
11111	0.67	NORMAL_INHIBITION	0.09	NORMAL_INHIBITION									0.34	NORMAL_INHIBITION	0.1	NORMAL_INHIBITION
11119					0.37	NORMAL_INHIBITION	0.71	NORMAL_INHIBITION	25.84	NORMAL_INHIBITION	0.92	NORMAL_INHIBITION	0.14	NORMAL_INHIBITION	0.38	NORMAL_INHIBITION

Specimen	EISN_INF23-06				EISN_INF23-07				EISN_INF23-08				Overall score ⁷	Method used
Subtype	A(H1N1)pdm09				B/Victoria				A(H3N2)					
Antiviral	Oseltamivir		Zanamivir		Oseltamivir		Zanamivir		Oseltamivir		Zanamivir			
Expected result ²		NI		NI		NI		NI		NI		NI		
Participant ID ¹ / result	IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)		IC ₅₀ (nM)			
200					1681.98	REDUCED_INHIBITION	1.56	NORMAL_INHIBITION	7.89	NORMAL_INHIBITION	0.34	NORMAL_INHIBITION	3	NA Fluor
1159	0.6	NORMAL_INHIBITION	5.5	NORMAL_INHIBITION	2.1	NORMAL_INHIBITION	9.3	NORMAL_INHIBITION		SENSITIVE_NO_IC50		SENSITIVE_NO_IC50	9	NA XTD
1299					57.82	HIGHLY_REDUCED_INHIBITION	3.15	NORMAL_INHIBITION	0.82	NORMAL_INHIBITION	0.83	NORMAL_INHIBITION	5	NA-STAR
1643													4	MUNANA In-house
2271													1	MUNANA In-house
2275					25.24	REDUCED_INHIBITION	7.98	REDUCED_INHIBITION					7	MUNANA In-house
2816													2	MUNANA In-house
3442					32.99	NORMAL_INHIBITION	3.73	NORMAL_INHIBITION					0	MUNANA In-house
3558													2	NA Fluor
10023	0.27	NORMAL_INHIBITION	0.17	NORMAL_INHIBITION	15.24	NORMAL_INHIBITION	1.88	NORMAL_INHIBITION	0.12	NORMAL_INHIBITION	0.32	NORMAL_INHIBITION	0	MUNANA In-house
10080	0.82	NORMAL_INHIBITION	0.82	NORMAL_INHIBITION	51	NORMAL_INHIBITION	14	NORMAL_INHIBITION	0.28	NORMAL_INHIBITION	1.2	NORMAL_INHIBITION	0	MUNANA In-house
10115	0.59	NORMAL_INHIBITION	0.69	NORMAL_INHIBITION	14.54	NORMAL_INHIBITION	1.48	NORMAL_INHIBITION	0.43	NORMAL_INHIBITION	1.25	NORMAL_INHIBITION	3	MUNANA In-house
10261													0	MUNANA In-house
10464	0.6	NORMAL_INHIBITION	0.2	NORMAL_INHIBITION	29.1	NORMAL_INHIBITION	2	NORMAL_INHIBITION	0.2	NORMAL_INHIBITION	0.2	NORMAL_INHIBITION	3	MUNANA In-house
10466	1.3	NORMAL_INHIBITION	0.2	NORMAL_INHIBITION	54.9	NORMAL_INHIBITION	5.4	NORMAL_INHIBITION	0.4	NORMAL_INHIBITION	0.4	NORMAL_INHIBITION	3	MUNANA In-house
11111	1.17	NORMAL_INHIBITION	0.19	NORMAL_INHIBITION	86.11	HIGHLY_REDUCED_INHIBITION	3.6	NORMAL_INHIBITION	0.39	NORMAL_INHIBITION	0.16	NORMAL_INHIBITION	5	MUNANA In-house
11119	0.57	NORMAL_INHIBITION	0.34	NORMAL_INHIBITION	3.17	NORMAL_INHIBITION	3.93	NORMAL_INHIBITION	0.32	NORMAL_INHIBITION	0.54	NORMAL_INHIBITION	2	NA Fluor

Expected results are indicated with dark green shading. Results close to expected results are indicated in light orange. Results that are considered as wrong are indicated in dark orange.

¹ Cell with orange shading = laboratory located in EU/EEA country. Cell with white shading = laboratory located in non-EU/EEA country with EEIQAP participation supported by ECDC. Cell with blue shading = laboratory located in non-EU/EEA country in WHO European Region with EEIQAP participation supported by World Health Organization Regional Office for Europe.

² HRI = highly reduced inhibited (fold-change IC₅₀; A >100; B >50)

NI = normal inhibited (fold-change IC₅₀; A <10; B <5)

RI = reduced inhibited (fold-change IC₅₀; A ≥10 & ≤100; B A ≥5 & ≤50)

³ Reported IC_{50} levels. If an IC_{50} value for a particular participant ID is not shown, this is either because the laboratory did not isolate the virus or did not report the IC_{50} .

⁴ Fold-change compared to median IC_{50} of recent Dutch A(H1N1)pdm09 viruses for oseltamivir determined at 561 (HRI) and for zanamivir at 1.6 (NI) at the Dutch NIC location - National Institute for Public Health and the Environment.

⁵ Fold-change compared to median IC_{50} of recent Dutch A(H3N2) viruses for oseltamivir determined at 0.8 (NI) and for zanamivir at 1.1 (NI) at the Dutch NIC location - National Institute for Public Health and the Environment.

⁶ Fold-change compared to median IC_{50} of recent Dutch A(H3N2) viruses for oseltamivir determined at 109 (HRI) and for zanamivir at 19 (RI) at the Dutch NIC location - National Institute for Public Health and the Environment.

⁷ Scoring system used:

- EISN_AV23-01: oseltamivir: HRI (green shading), 0; Resistant (light orange shading), 1; other (orange shading), 2; zanamivir: NI (green shading), 0; other (orange shading), 2.
 - EISN_AV23-02: oseltamivir: NI (green shading), 0; other (orange shading), 2; zanamivir: NI (green shading), 0; other (orange shading), 2.
 - EISN_AV23-03: oseltamivir: HRI (green shading), 0; other (orange shading), 2; zanamivir: NI (green shading), 0; other (orange shading), 2.
 - EISN_INF23-01 - 08 (except 05): oseltamivir and zanamivir: NI (green shading), 0; Sensitive No IC_{50} (light orange shading), 1; other (red shading), 1.
- Score only for the specimens tested.

Table A19. Heatmap of reported HA sequences with rating of sequence quality

	EISN_INF23-01	EISN_INF23-02	EISN_INF23-03	EISN_INF23-04	EISN_INF23-06	EISN_INF23-07	EISN_INF23-08
Virus	A(H1N1)pdm09	A(H3N2)	B/Victoria	A(H3N2)	A(H1N1)pdm09	B/Victoria	A(H3N2)
Participant ID							
95							
200							
1159							
1299							
1643							
2126							
2271				small part			
2278							
3442							
3558							
10007							
10023							
10053			frame shift				
10080							
10104							
10115							
10144							
10461							
10462			codon extra				
10464				wrong clade			
10466							
10498	wrong clade						
11119							

Colour coding

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

Complete = from Adenine-Thymine Guanine (ATG) through stop codon

Nearly complete = small stretches missing at 3' and or 5' end

Incomplete = large part of the sequence missing

Identical = less than six mismatches with original throughout the sequence

Poor = many stretches of Ns and/or many mismatches with original, including insertions and deletions

Participant ID with orange shading = laboratory located in an EU/EEA country.

Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC.

Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by the WHO Regional Office for Europe.

Table A20. Heatmap of reported NA sequences with rating of sequence quality

	EISN_AV23-01	EISN_AV23-02	EISN_AV23-03	EISN_INF23-01	EISN_INF23-02	EISN_INF23-03	EISN_INF23-04	EISN_INF23-06	EISN_INF23-07	EISN_INF23-08
Virus	A(H1N1)pdm09	A(H3N2)	A(H3N2)	A(H1N1)pdm09	A(H3N2)	B/Victoria	A(H3N2)	A(H1N1)pdm09	B/Victoria	A(H3N2)
Antiviral status	NA-H275Y	PA-E23G	NA-245-8del	WT	WT	WT	WT	WT	WT	WT
Participant ID										
112										
200										
1159										
1299										
1643										
2126										
2271										
2816										
3442										
3558										
10007										
10023			NNN at del							
10080										
10115										
10142			KAXG at del							
10144										
10205										
10461										
10464										
10498										
11111										
11119										

Colour coding

	No sequence reported
	Complete; identical to original
	Nearly complete; identical to original
	Incomplete; identical to original
	(Nearly) complete; poor
	Incomplete; poor
	More incomplete; poorer

Complete = from Adenine-Thymine Guanine (ATG) through stop codon

Nearly complete = small stretches missing at 3' and/or 5' end

Incomplete = large part of the sequence missing

Identical = less than six mismatches with original throughout the sequence

Poor = many stretches of Ns and/or many mismatches with original, including insertions and deletions

WT = antiviral susceptibility wildtype

Participant ID with orange shading = laboratory located in an EU/EEA country.

Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC.

Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by the WHO Regional Office for Europe.

Table A21. Heatmap of reported polymerase acidic (PA) sequences with rating of sequence quality

	EISN_AV23-01	EISN_AV23-02	EISN_AV23-03	EISN_INF23-01	EISN_INF23-02	EISN_INF23-03	EISN_INF23-04	EISN_INF23-06	EISN_INF23-07	EISN_INF23-08
Virus	A(H1N1)pdm09	A(H3N2)	A(H3N2)	A(H1N1)pdm09	A(H3N2)	B/Victoria	A(H3N2)	A(H1N1)pdm09	B/Victoria	A(H3N2)
Antiviral status	NA-H275Y	PA-E23G	NA-245-8 del	WT	WT	WT	WT	WT	WT	WT
Participant ID										
112										
200										
1299										
1615										
1643										
2126										
2271										
2313										
2816										
3442										
3558										
10007										
10023										
10080										
10115										
10142										
10144										
10464										
10498										
11119										

Colour coding

	No sequence reported
	Complete; identical to original
	Nearly complete; identical to original
	Incomplete; identical to original
	(Nearly) complete; poor
	Incomplete; poor
	More incomplete; poorer

Complete = from Adenine-Thymine Guanine (ATG) through stop codon

Nearly complete = small stretches missing at 3' and/or 5' end

Incomplete = large part of the sequence missing

Identical = less than six mismatches with original throughout the sequence

Poor = many stretches of Ns and/or many mismatches with original, including insertions and deletions

WT = antiviral susceptibility wildtype

Participant ID with orange shading = laboratory located in an EU/EEA country.

Cell with white shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by ECDC.

Cell with blue shading = laboratory located in a non-EU/EEA country in WHO European Region with EEIQAP participation supported by the WHO Regional Office for Europe.