Schweizerische Eidgenossenschaft
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Hôpitaux Universitaires Genève

Influenza virus surveillance in Switzerland
Season 2018–2019

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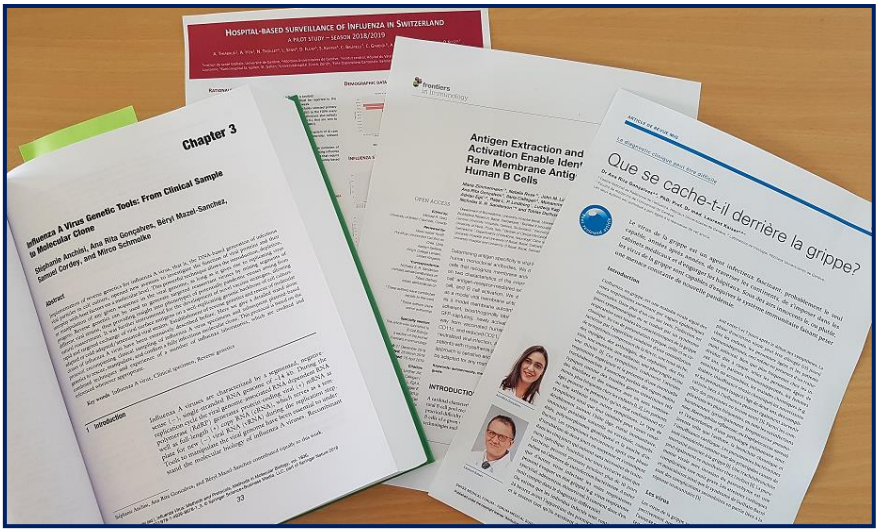

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Cover: Contribution of the National Reference Centre of Influenza to the influenza field.

Contents

Abbreviations and Acronyms

CDC:	Centers for Disease Control and Prevention
CPE:	cytopathic effect
Ct:	cycle threshold
ECDC:	European Centre for Disease Prevention and Control
EEA:	European Economic Area
EEIQAP:	European External Influenza Virus Quality Assessment Programme
EQAP:	(WHO) External Quality Assessment Programme (for the Detection of Influenza Viruses)
EU:	European Union
FOPH:	Federal Office of Public Health
HA:	hemagglutinin
HEF:	hemagglutinin-esterase-fusion
HI:	hemagglutination inhibition
H/LPAI:	high/low pathogenic avian influenza
ILI:	influenza-like illness
M:	matrix
MDCK:	Madin-Darby canine kidney cells
MDCK-SIAT1:	sialic acid-enriched MDCK cells
MN:	microneutralization
MUNANA:	2'-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid
NA:	neuraminidase
NAI:	neuraminidase inhibitor
NEP:	nuclear export protein
NRCI:	National Reference Centre of Influenza
NS:	non-structural
PA:	acidic protein
PB:	basic protein
RBC:	red blood cells
RFU:	relative fluorescent units
RNA:	ribonucleic acids
RNP:	ribonucleoprotein
rRT-PCR:	real-time reverse-transcription polymerase chain reaction
USA:	United States of America
Vic, Yam:	Victoria, Yamagata
WHO:	World Health Organization
WIC:	Worldwide Influenza Centre

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Résumé – Zusammenfassung – Summary

Résumé

En Suisse, l'épidémie de grippe de 2018/19 a duré onze semaines, de la semaine 2/2019 à la semaine 12/2019, avec un pic pendant la semaine 6/2019. Mille et un prélèvements nasopharyngés ont été dépistés pour la grippe, parmi ces derniers 40,1% étaient positifs. Les virus de l'influenza A étaient largement dominants cette saison, seuls deux virus de l'influenza B ont été détectés. Les virus de influenza A(H1N1)pdm09 étaient prévalents de la semaine 45/2018 à la semaine 7/2019. La souche A(H3N2) devenant dominante dès la semaine 8/2019. La plupart des virus de l'influenza A(H1N1)pdm09 étaient antigéniquement proches de la souche vaccinale A/Michigan/45/2015 (clade 6B.1); avec une majorité des isolats (64,7%) se regroupant dans le sous-groupe génétique 6B.1A5. Les données antigéniques obtenues avec des panels de sera humains indiquent que les virus portant la substitution S183P HA1 sont moins bien inhibés par ces derniers. De ce fait, la souche d'influenza A(H1N1)pdm09 contenue dans les vaccins antigrippaux a été mise à jour pour la saison 2019/20. Les souches vaccinales recommandées étant des virus antigéniquement similaires à la souche A/Brisbane/02/2018.

Tous les virus A(H3N2) isolés en Suisse étaient antigéniquement proches de la souche A/Singapore/INFIMH-16-0019/2016 (3C.2a1). Cependant, la reconnaissance était réduite pour certains isolats. Au niveau génétique, la plupart des isolats de A (H3N2) appartenaient au sous-groupe 3C.2a1b du clade 3C.2a1. Quelques virus 3C.3a « récents » et 3C.2a2 ont également été observés. Comme les virus 3C.3a «récents» se sont révélés antigéniquement distincts des virus 3C.2a1 et 3C.3a « antérieurs », le composant A(H3N2) du vaccin antigrippal pour 2019/20 a également été mis à jour pour. La souche A/Singapore/INFIMH-16-0019/2016 sera remplacée par un virus antigéniquement similaire à A/Kansas/14/2017. Le seul virus de l'influenza B appartenant à la lignée B/Yamagata/16/1988 isolé au cours de cette saison était antigéniquement similaire à la souche vaccinale 2018/19, B/Phuket/3073/2013 (clade 3).

Aucun des isolats testés au Centre National de Référence de l'influenza dans le cadre de la surveillance saisonnière de l'influenza n'a présenté d'inhibition réduite par l'oseltamivir et le zanamivir. Cependant, un isolat provenant d'un patient hospitalisé

sous traitement à l'oseltamivir portait la substitution H275Y associée à une inhibition fortement réduite par ce médicament antiviral.

Peu de cas humains d'infection par des virus de l'influenza aviaire hautement/faiblement pathogène ont été identifiés depuis 2017/18.

Zusammenfassung

Dieses Jahr hat die Grippeepidemie in der Schweiz 11 Wochen gedauert. Das heisst von Woche 2/2019 bis Woche 12/2019, mit einem Maximum im Verlauf der Woche 6/2019. Insgesamt wurden 1001 Proben auf Influenza-Viren untersucht, 40,1% davon waren positiv. Influenza-A-Viren waren weitgehend dominant, nur zwei Influenza-B-Viren wurden nachgewiesen. Influenza A (H1N1)pdm09 Viren waren von Woche 45/2018 bis Woche 7/2019 verbreitet, dann wurden A(H3N2) der dominierende Stämme. Die meisten A(H1N1)pdm09-Viren waren dem Impfstoffstamm A/Michigan/45/2015 (Klade 6B.1) antigenisch ähnlich; mit einer Mehrheit von Isolaten (64,7%), die sich in der genetischen Untergruppe 6B.1A5 gruppieren. Antigendaten, die mit Humanseren erhalten wurden, zeigten, dass Viren mit der S183P HA1-Substitution, weniger gut gehemmt wurden. Die A(H1N1)pdm09 Impfstoffkomponente für 2019/20 wurde daher auf A/ Brisbane/02/2018 aktualisiert.

Alle A(H3N2)-Viren, die in der Schweiz isoliert wurden, waren antigenisch verwandt mit dem Stamm A/Singapore/INFIMH-16-0019/2016 (3C.2a1). Die Erkennung war jedoch für einige Isolate vermindert. Auf genetischer Ebene gehörten die meisten A(H3N2)-Isolate zur Subklasse 3C.2a1b der Klasse 3C.2a1. "Aktuelle" 3C.3a- und 3C.2a2-Viren wurden auch bei niedrigeren Fallzahlen beobachtet. Da gezeigt wurde, dass sich die „aktuellen“ 3C.3a-Viren antigenisch von 3C.2a1- und „früheren“ 3C.3a-Viren unterscheiden, wurde auch die Impfstoffkomponente A(H3N2) für 2019/20 auf A/Kansas/14/2017 aktualisiert. Das einzige Influenza-B/Yamagata/16/1988-Abstammungsvirus, das während dieser Saison isoliert wurde, war dem 2018/19 Impfstoffstamm, B/Phuket/3073/2013 (Klade 3) antigenisch ähnlich.

Keines der am National Referenz Zentrum von Influenza im Rahmen der Influenza-Saisonüberwachung getesteten Isolate zeigte eine verminderte Hemmung durch Oseltamivir und Zanamivir. Ein Isolat, das von einem Krankenhauspatienten unter Oseltamivir-Behandlung stammte, trug jedoch die H275Y-Substitution, welche mit einer stark reduzierten Empfindlichkeit gegenüber Oseltamivir verbunden ist.

Summary

The Swiss 2018/19 influenza epidemic lasted 11 weeks, from weeks 2/2019 to 12/2019, with a peak at week 6/2019. Of 1001 samples screened for influenza, 40.1% were positive. Influenza A viruses were largely dominant and only two influenza B viruses were detected. The influenza A(H1N1)pdm09 virus was prevalent from weeks 45/2018 to 7/2019, and then A(H3N2) became the dominant strain. Most A(H1N1)pdm09 viruses were antigenically similar to the A/Michigan/45/2015 vaccine strain (clade 6B.1), with a majority of isolates (64.7%) clustering in the 6B.1A5 genetic subgroup. Antigenic data obtained with human sera indicated that viruses carrying the S183P HA1 substitution were less well inhibited. The A(H1N1)pdm09 strain for 2019/20 vaccine was therefore updated to A/Brisbane/02/2018.

All of the A(H3N2) viruses isolated in Switzerland were antigenically related to the A/Singapore/INFIMH-16-0019/2016 strain (3C.2a1). However, recognition was reduced for some isolates. At the genetic level, most A(H3N2) isolates belonged to the subclade 3C.2a1b of clade 3C.2a1. “Recent” 3C.3a and 3C.2a2 viruses were also observed at lower numbers. As “recent” 3C.3a viruses were shown to be antigenically distinct from 3C.2a1 and former 3C.3a viruses, the A(H3N2) strain for 2019/20 vaccine was also updated to A/Kansas/14/2017. The only influenza B/Yamagata/16/1988-lineage virus isolated during this season was antigenically similar to the 2018/19 vaccine strain, B/Phuket/3073/2013 (clade 3).

None of the isolates tested at the National Reference Centre of Influenza in the context of influenza seasonal surveillance exhibited a reduced inhibition by oseltamivir and zanamivir. However, one isolate originating from a hospitalized patient under oseltamivir treatment carried the H275Y substitution associated with highly-reduced inhibition by oseltamivir. Only a few human cases of low and highly pathogenic avian influenza infections have been identified since 2017/18.

1 Introduction

Influenza virus infections are a major clinical and economic burden worldwide.[1] In Switzerland, the Sentinel surveillance system is a community-based network of primary care medical practitioners who report suspected cases of influenza or influenza-like illness (ILI) to the Federal Office of Public Health (FOPH). A subgroup of Sentinel practitioners collects respiratory samples from patients diagnosed with ILI that are sent to the National Reference Centre of Influenza (NRCI) in Geneva for further characterization. This report summarizes the demographic, epidemiologic and virus characterization data gathered from samples processed and analyzed by the NRCI during the 2018/19 influenza season.

2 The influenza virus

Influenza viruses are orthomyxoviruses, a family of enveloped, negative, single-stranded ribonucleic acid (RNA) viruses (Figure 1), known to be causative agents of respiratory tract infections and referred to as influenza disease or “flu”. Influenza viruses are divided into four genera, A, B, C and D.[1]

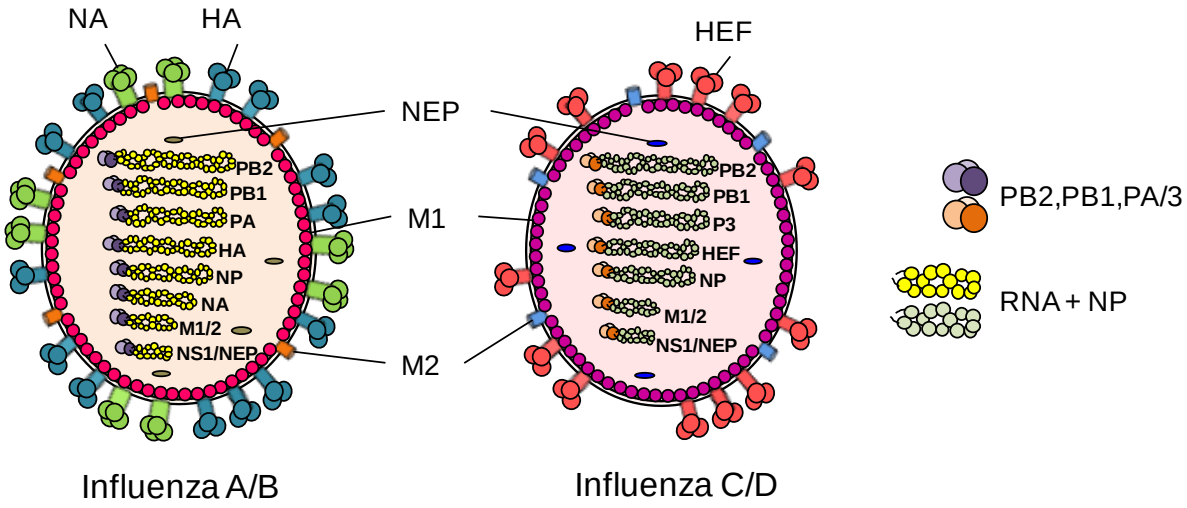

Figure 1. The structure of influenza viral particles. Basic protein 2 (PB2), 1 (PB1) and acidic protein or 3 (PA or P3) form a complex that corresponds to the RNA-dependent polymerase. The hemagglutinin (HA) and the hemagglutinin-esterase-fusion (HEF) play a role in virus attachment to sialic acids present at the surface of host cells and in fusion. The neuraminidase (NA) is crucial for virion detachment from the cellular surface by cleaving the HA on the virus surface. In influenza B, the NA gene also encodes the NB ion channel (not shown). The matrix protein 1 (M1) protein forms the viral capsid. The ion channel M2 allows virion acidification required for fusion. The nuclear export protein (NEP), also named “non-structural protein 2”, is implicated in the export of the virus polymerase – RNA + nucleoprotein (NP) complex – to the cell nucleus. The RNA + NP is also called ribonucleoprotein RNP. The RNA segments PB1, PB2, PA/3, HA or HEF, NP, NA (not present in influenza C and D), M and NS are present inside the viral capsid, protected by NPs. Only non-structural protein 1 is not present in the viral particle, but it is expressed upon infection of the host cell. Influenza D is structurally closer to influenza C than to A and B.

Influenza A viruses have a wide host tropism, while influenza B viruses are mainly found in humans[2] and in harbour seals (human origin).[3] These two influenza types are responsible for the annual influenza epidemics. Influenza C viruses can be isolated from swine and humans, in whom they can cause mostly limited symptoms, and the epidemiological pattern is not well studied. Influenza D viruses are mainly found in swine and cattle.[4] Even if the pathogenic potential of influenza D virus in humans remains unknown, a recent study estimated that specific influenza D antibodies could be found in approximately 1.3% of the general human population.[5]

3 Methodology

3.1 Clinical identification of influenza cases

During the Swiss influenza surveillance period, starting at week 40 and lasting until week 16 of the following year, 150 to 200 primary care practitioners participate in the epidemiological national influenza surveillance network. They are requested to notify ILI cases on a weekly basis. Within the Swiss sentinel system, ILI cases are defined as sudden fever (>38°C) onset and cough or sore throat. The presence of other symptoms such as malaise, myalgia, joint pain and headache, as well as gastrointestinal symptoms is optional. Patients presenting with a secondary disease (pneumonia, bronchitis, otitis, etc.) consecutive to an influenza not yet notified are also expected to be reported.

A subgroup of Sentinel practitioners collects nasopharyngeal swabs from patients with ILI for subsequent viral detection and characterization. The sampling procedure of specimens is performed according to the following protocol:

1) during the pre- and post-epidemic phases: when the number of ILI reported by Sentinel practitioners remains below the annual pre-defined epidemic threshold, screening for influenza viruses is performed in all cases that fulfill the case definition;

2) during the epidemic phase, defined as when the number of ILI is above the epidemic threshold: screening is only performed in a subgroup of cases; in general, every fifth ILI case per practitioner is sent to the NRCI and screened for the presence of influenza.

The threshold value is defined by the FOPH based on data collected over the past 10 years (excluding the pandemic season 2009/10). For the 2018/19 influenza season, it corresponded to 68 suspected influenza cases per 100,000 inhabitants.

3.2 Sampled population data

The Sentinel practitioners who send samples to the NRCI are asked to complete a case report form to collect the following data: sample type; age; gender; time of symptoms onset; pneumonia; hospitalization; travel within the previous 14 days; and influenza vaccination status.

3.3 Virological detection of influenza viruses[1]

Nasopharyngeal swabs received at the NRCI are submitted to virus screening and subtyping tests. For screening, a one-step real-time reverse transcription polymerase chain reaction (rRT-PCR) adapted from the 2009 USA Centers for Disease Prevention and Control (CDC) protocol is used to detect the presence of influenza A and B viral genomes in the clinical samples. The duplex rRT-PCR targets are the M protein and the non-structural (NS) protein genes for influenza A and B viruses, respectively.

Influenza A positive samples are subtyped since the 2017/18 season using an in-house-developed quadruplex rRT-PCR targeting the HA (H1 and H3) and the NA (N1 and N2) genes in order to discriminate between influenza A(H1N1)pdm09 and A(H3N2) strains. This new assay is a mix of already validated (in-house H1 and H3 CDC) and newly-designed ($N2_2$) rRT-PCR combinations, adapted from the one used in the study by Henritzi et *al*[6] (N1). The quadruplex detection limit is similar to that of the diagnostic rRT-PCR. The N1 combination was able to detect the $H1N1v_3$, $swH1N1_4$ and $H5N1_5$ isolates tested during the assay validation process. The H3 and N2 rRT-PCR combinations are also able to detect the A/Wisconsin/12/2010 H3N2

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[1] The evaluation of the proficiency of the Laboratory of Virology at Geneva University Hospitals in performing molecular detection of influenza viruses is accessed through the World Health Organization (WHO) External Quality Assessment Programme for the Detection of Influenza Viruses by RT-PCR initiated in 2007 by the WHO. https://www.who.int/influenza/gisrs_laboratory/external_quality_assessment_project/en/

[2] Human N2 sequences from 2009-2017 were used for the N2 rRT-PCR design.

[3] H1N1v: A/Switzerland/***2244/2011 and A/Berne/****6552/2017, variants isolated from Swiss pig breeders.

[4] swH1N1 35 (2008): virus isolated from a Swiss pig.

[5] H5N1: A/Hong Kong/6841/2010 (EQAP panel 16) and A/goose/Qinghai/1A/05*A/PR8/34(INT).

triple reassortant (H3N2tr),[7] although the latter virus is not known to circulate in Switzerland. Nevertheless, if needed, additional tests are available at the NRCI to discriminate seasonal H3N2 from H3N2tr viruses. Influenza B Yamagata (Yam) and B Victoria (Vic) lineages are determined using a duplex rRT-PCR.

The quality of the NRCI influenza A/B detection and subtyping was successfully evaluated during 2018 by the European External Influenza Virus Quality Assessment Programme 2018 (EEIQAP 2018) and the WHO External Quality Assessment Programme for the Detection of Influenza Viruses by RT-PCR (EQAP panel 17).

During the pre- and post-epidemic phases, the majority of rRT-PCR-negative specimens are inoculated on cells for viral culture. This strategy allows to detect potential influenza strains that may have “escaped” rRT-PCR detection. For example, this could be the case in the presence of drifted viruses carrying mutations in the genomic regions targeted by rRT-PCR screening.

3.4 Antigenic and genetic characterization of influenza viruses

During the season, selection of influenza viruses are submitted to phenotypic and genotypic characterization (Figure 2). In brief, during the pre- and post-epidemic phases, all positive samples with sufficient HA titers are phenotypically characterized using the HA inhibition (HI) assay, which evaluates the antigenic similarity between the reference and circulating influenza strains. HIs are performed with glutaraldehyde-fixed guinea pig (Charles River, Lyon, France) red blood cells (RBC). Of note, a microneutralization (MN) test can be used for samples that do not (or only poorly) hemagglutinate RBC. Reference antisera (Annex 8) and corresponding viral reference strains used for the HI and MN are kindly provided by the WHO Collaborating Centre Reference Laboratory at the Francis Crick Worldwide Influenza Centre (WIC), London, United Kingdom. Reference virus stocks for the current influenza season are produced on cells (Madin-Darby canine kidney [MDCK] and MDCK-sialic acid-enriched [MDCK-SIAT]). During the epidemic phase, up to 5 positive samples per week with a cycle threshold (Ct) value ≤30 and sufficient HA titers are analyzed. When judged relevant, samples with Ct values ≥30 can also be selected for characterization.

To assess the phylogeny of the circulating strains and to determine how genetically close they are to vaccine strains, the HA1 part of the HA gene is sequenced.

Samples previously chosen for phenotypic characterization or judged to be of interest are submitted to Sanger sequencing (up to five per week, generally with a Ct ≤30). The corresponding NA genes are also sequenced. Whenever needed, additional genes as influenza A M and influenza B NS genes can also sequenced. The NA gene sequence allows to detect key mutations previously described as conferring resistance to NA inhibitors (NAI). M and NS gene sequencing allow to control the adequacy of rRT-PCR influenza A and B screening, respectively. In addition, we are currently working on a sequencing protocol for the PA gene, in order to identify amino acids substitutions associated with a reduced susceptibility to PA inhibitors as baloxavir marboxyl.

3.4.1 Cell culture

As mentioned in chapter 3.3, most of the received samples, both positive and negative, are cultured on MDCK and MDCK-SIAT1 cells in parallel during the pre- and post-epidemic phases. This allows, to some extent, to control that a low positivity rate observed outside the epidemic phase is not due to a rRT-PCR detection default.

As HI analysis requires a sufficient concentration of influenza virus, a viral amplification step is performed by inoculating the clinical samples on MDCK and MDCK-SIAT1 cells in parallel. A sampling of five rRT-PCR positive specimens per week with Ct values <30 are inoculated on cells. When judged pertinent, positive samples with Ct values ≥30 can also be selected for culture.

In brief, 0.4 ml of transport medium containing nasopharyngeal swab are incubated for seven days under 5% CO_2 at 33°C on MDCK cells and 37°C on MDCK-SIAT1. The presence of virus is confirmed by the presence of a cytopathic effect (CPE) under visible light (Nikon®, Tokyo, Japan) and/or by an immunofluorescence test using monoclonal influenza A and B antibodies combined with mouse fluorescein isothiocyanate-conjugate (Merck-Millipore, Chemicon®, Schaffhausen, Switzerland). Positive samples are submitted to a hemagglutination test in order to determine the virus titer. The hemagglutination and HI assays are dependent on the ability of the viral HA to bind to sialic acids present on the surface of RBC.

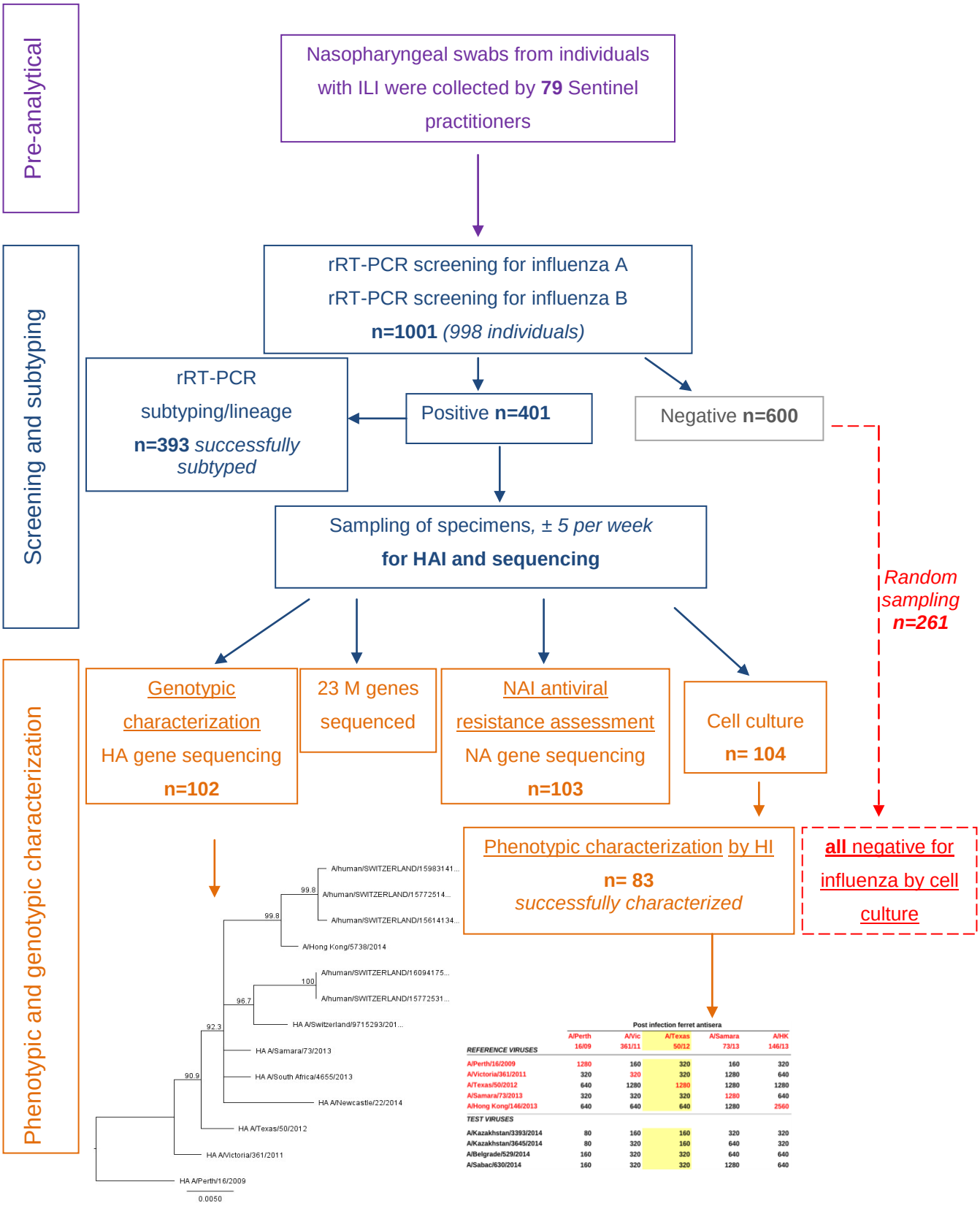


Figure 2. Flow chart of Sentinel sample collection and processing. Numbers (n) represent the number of samples submitted to the described step during the 2018/19 season.

3.4.2 Hemagglutination inhibition assay

A two-fold serial dilution is performed using 50 µl of viral suspension in SALK buffer and 25 µl of glutaraldehyde-fixed guinea pig RBC (1.5%) are added for 1 h incubation at 4°C. HA titer is defined as the last dilution in which the complete HA is still observed. After titer determination, HI is performed according to the following procedure: 25 µl of reference antisera are added in the first two wells of a 96-well plate. Two-fold dilutions are prepared by adding 25 µl of SALK buffer in the second well. Twenty-five µl are then collected from the same well and the procedure is repeated to the end of each line. Twenty-five µl of viral suspension containing four HA units are added to the ferret antisera dilution and incubated for 1 h at room temperature. Then, 25 µl of guinea pig RBC are added to each well and the plates are incubated for 1 h at 4°C. The HI titer corresponds to the last antiserum dilution for which HA is still inhibited. This titer is compared to the homologous titer obtained with reference strains submitted to their corresponding ferret antisera (antigenic table). The antigenic tables are influenza strain-specific (Figure 3) and are therefore adjusted each year. As the ferret serum is initially diluted 1/8, the titers provided in Figure 3 and Annexes 2 to 4 should be multiplied by eight to obtain the final titers.

Since we started using fixed RBCs (more than 10 years' expertise) instead of fresh ones, the requirement for the addition of 20 nM oseltamivir during the HI test in order to prevent NA-mediated hemagglutination of A(H3N2) viruses, is evaluated at the beginning of each season. It is also checked regularly during the season or in the case of unexpected test results.

3.4.3 Antiviral resistance

The evolution of influenza viruses is known to be very rapid, thus allowing them to escape from immune responses and/or infection inhibition by therapeutic molecules. Known mutations conferring antiviral resistance to a given influenza type/subtype/lineage can be monitored by sequencing the NA genes for NAI resistance and M genes for the M2 inhibitors. Viral sequences are manually and semi-automatically (FluSurver: http://flusurver.bii.a-star.edu.sg/) screened for the presence of mutations known to be associated with antiviral resistance as reported in the WHO "Summary table of neuraminidase amino acid substitutions associated with reduced inhibition by neuraminidase inhibitors" (https://www.who.int/influenza

/gisrs_laboratory/antiviral_susceptibility/NAI_Reduced_Susceptibility_Marker_Table_WHO.pdf?ua=1).

New antiviral resistance to NAIs can be identified by combining NA genotyping/sequencing and phenotypic NA enzyme-inhibitor (NAI) assays. At the NRCI, phenotypic antiviral resistance of influenza stains are performed if needed and/or upon request using the NA-Fluor™ Influenza Neuraminidase Assay Kit (Thermo Fisher Scientific, Ecublens, Switzerland). In brief, a titration of the viral NA activity is performed for each test by serial two-fold dilutions. The optimum virus dilution to be used in subsequent inhibition assays is determined by plotting the virus dilutions against the relative fluorescent units (RFU) minus background values. In black 96-well plates, 25 µl of each NAI dilution to be tested are mixed with 25 µl of diluted virus; the plates are then covered and incubated for 30 min at 37°C. After incubation, 50 µl of 200 µM NA-Fluor™ substrate working solution are added to each well and the plates incubated again for 1 h at 37°C. The substrate-enzyme reaction is terminated by adding 100 µl of NA-Fluor™ Stop Solution to each well. The plates are read using a Fluoroskan Ascent™ FL Microplate Fluorometer (Thermo Fisher Scientific, Ecublens, Switzerland). The excitation/emission wavelengths were 355 nm and 460 nm, respectively. Data are plotted as the log inhibitor concentration against fluorescence inhibition and the IC50s are read from the graph.

The quality of the NRCI sequencing and antiviral resistance (also phenotypic) assessment was successfully evaluated during 2018 by the European External Influenza Virus Quality Assessment Programme 2018 (EEIQAP 2018) and the WHO External Quality Assessment Programme for the Detection of influenza Viruses by RT-PCR (EQAP panel 17).

a. H1N1pdm09 / antisera	A/California/7/09	A/Michigan/45/15	A/St Petersburg/27/11
A/California/7/09	**128**	128	128
A/Michigan/45/15	64	**64**	64
A/St Petersburg/27/11	128	128	**128**

b. H3N2 / antisera	A/Switzerland/9715293/13	A/Hong Kong/4801/14	A/Singapore/INFIM-16-0019/16
A/Switzerland/ 9715293/13	**128**	128	128
A/Hong-Kong/4801/14	32	**64**	64
A/Singapore/INFIM-16-0019/16	128	128	**128**

c. B / antisera	B/Wisconsin /1/10	B/Novosibirsk/ 1/12	B/Phuket/307 3/13	B/Brisbane /60/08	B/Hong Kong/ 514/11 (1B)	B/Johannesburg/ 3964/12	B/Colorado/0 6/2017 (1AΔ2)
B/Wisconsin/1/10	**128**	64	64	<16			
B/Novosibirsk/1/12	128	**256**	128				
B/Phuket/3073/13	128	64	**64**				
B/Brisbane/60/08	<16			**512**	64	128	64
B/Hong Kong/514/11 (1B)				64	**64**	64	64
B/Johannesburg/3964/12				128	128	**128**	128
B/Colorado/06/2017 (1AΔ2)				128	32	64	**128**

Figure 3. Antigenic tables for the 2018/19 influenza season. These tables correspond to the HI titers of reference influenza strains incubated with ferret reference antisera. The HI reaction is performed as described in the methodology section. HI titers correspond to the highest dilution where an inhibition is still observed. The titer obtained after incubation of a given strain with the corresponding ferret antiserum is known as the homologous titer (in bold). In red: 2018/19 influenza vaccine strains. a, b and c correspond to A(H1N1pdm09), A(H3N2) and B influenza virus antigenic tables, respectively. The first line and column of each influenza type/subtype corresponds to the ferret antiserum and virus strain tested, respectively.

Antigenic similarity

A strain is considered as being antigenically related to a reference strain when the ratio “titer of the tested isolate/homologous titer” is ≤ four-fold. If the ratio is > four-fold, the tested strain is considered as antigenically different from the reference strain (also referred to as “low reactors”).

4 2018/19 influenza season

The 2018/19 influenza surveillance started on 30 September 2018 (week 40/2018) and ended on 20 April 2019 (week 16/2019). The first influenza positive case of the season was detected during week 44 (Annex 1). The epidemic threshold of 68 ILI cases reported per 100,000 inhabitants was exceeded from weeks 2/2019 to 12/2019. The epidemics peak was reached during week 6/2019. The epidemic phase of the 2018/19 influenza season lasted 11 weeks and was four weeks shorter than in 2017/18.

4.1 Sentinel population demographics

A total of 998 individuals presenting with ILI in the community were sampled during the 2018/19 influenza season. Among them, 505 (50.6%) were female and 493 were male. Four hundred and one participants (40.2%) were positive for influenza A or B (Table1). Three individuals were sampled twice during the surveillance period.

Data on age were available for 994 of 998 individuals (median, 37 years [range, 5 months to 92 years]; 95% CI, 35-39). Median age of females was 39 years (range, 8 months to 90 years; 95% CI, 36-42) and 35 years (range, 5 months to 92 years; 95% CI, 32-39) for males. Individuals were stratified into different age groups defined by the FOPH, i.e. 0-4 years (50; 5%), 5-14 years (127;12.8%), 15-29 years (203; 20.4%), 30-64 years (514; 51.7%), and ≥65 years (100; 10.1%) (Table 1).

Most individuals were sampled three days (median) after first symptoms onset (Table 1). Twenty-four (14 influenza positive and 10 negative) were reported as having a swab collection time of more than 10 days after symptoms onset. Nineteen individuals were reported as having pneumonia, among those seven were influenza positive (four A(H1N1)pdm09 and three A(H3N2)).

Twenty-nine of 994 subjects experienced an ILI episode within 14 days of their return from a stay outside Switzerland. Twenty were returning from European countries, three from the Caribbean, three from Thailand, two from Africa, and one from Brazil. Among these individuals, seven were A(H1N1)pdm09 (clade 6B.1) positive and three A(H3N2) clade (3C.2a1b) positive.

One hundred and twenty-six (12.6%) subjects were vaccinated against influenza during the 2018/19 season. Fifty-four (42.9%) out of 126 revealed to be influenza-positive (Table 1), and 22 (40.7%) out of 54 individuals were ≥65 years old (Figure 9).

During the 2018/19 season, only 41.8% (n=98) of individuals ≥65 years old in the Sentinel population were vaccinated (Figure 9). No information on vaccination status was provided for 39 participants.

Table 1. Description of the subgroup of the Sentinel population whose samples were submitted to laboratory confirmation for influenza

	Influenza A-positive	Influenza B-positive	Negative for influenza	Total
Gender				
Female	209		296	505
Male	190	2	301	493
Total	**399**	**2**	**597**	**998**
Age group distribution (y), n=994				
0-4	18		32	50
5-14	58	1	68	127
15-29	72		131	203
30-64	205	1	308	514
≥65	44		56	100
Age was missing for 4 individuals.				
Time to disease onset median, (range, days)	3 (1-30) *n=385*	5 (3 and 7) *n=2*	3 (1-30) *n=580*	3 (1-30) *n=967*
Information was lacking for 31 individuals.				
Vaccination status, n=959				
Vaccinated	54		72	126
Non-vaccinated	332	2	499	833
No information on the vaccination status was provided for 39 individuals.				

4.2 Detection of influenza in nasopharyngeal samples

A total of 1001 samples were screened for influenza at the NRCI during the 2018/19 season. Overall, 401 (40.1%) swabs were positive for influenza by rRT-PCR (Figure 4a; Annex 1). Almost all samples (99.5%) were influenza A-positive; 217 (54.4%) were A(H3N2) and 175 (43.9%) were A(H1N1)pdm09 strains (Figure 4b). Seven (1.8%) influenza A positive samples could not be subtyped due to a low viral load (Figure 4b). Only two (0.2% of the total and 0.5% of positive samples) swabs were influenza B-positive, one B/Yamagata/16/88 and the other remained unsubtyped due to low viral load.

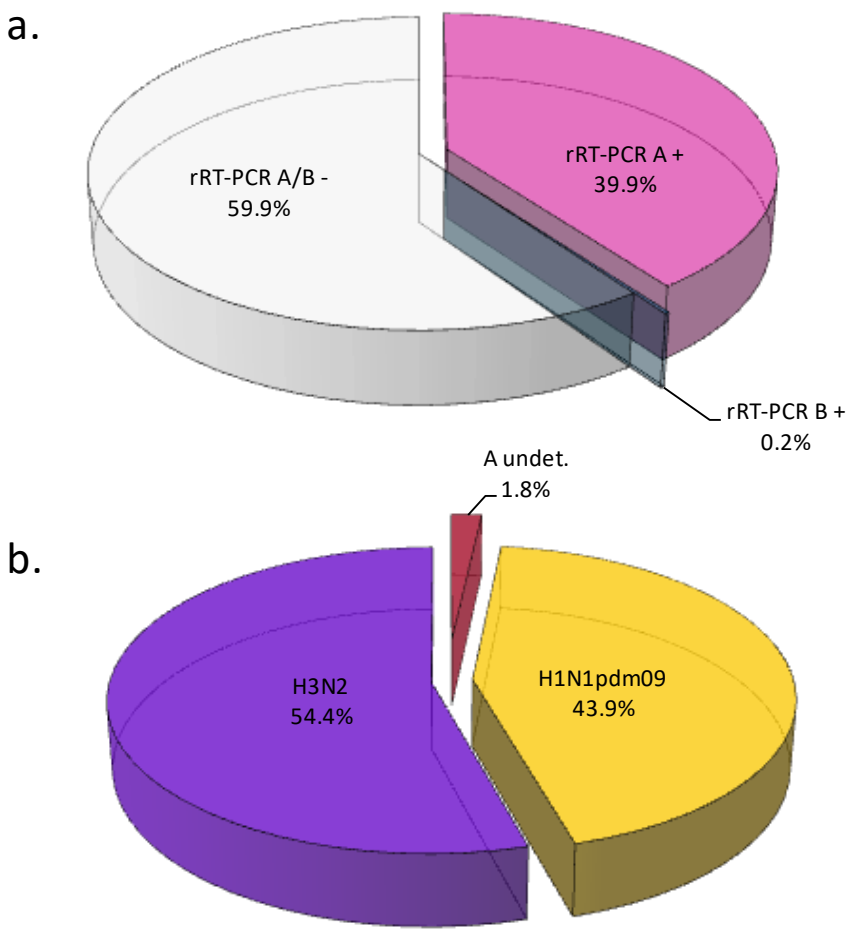


Figure 4. Distribution of influenza viruses detected in nasopharyngeal specimens collected during the 2018/19 season. a) Percentage of rRT-PCR A and B-positive (+) versus rRT-PCR-negative (-) specimens (n=1001). b) Distribution (%) of the different influenza A subtypes (n=399). All positive samples were submitted to subtyping. A undet.: subtype not determined (negative subtyping).

Frequent detection of positive samples (positivity rate >10%) started at week 48/2018 and lasted until week 15/2019. A maximum number of 93 (positivity rate, 64.5%) samples were received during week 6/2019, which also corresponded to the peak of ILI reported per 100,000 inhabitants. A positivity rate ≥50% was observed from weeks 4/2019 to 11/2019, with a peak at 66.7% during weeks 7 and 8/2019. From weeks 45/2018 to 8/2019, the number of influenza A(H1N1)pdm09-positive viruses outnumbered the A(H3N2). The latter then became the dominant strain. Two influenza B viruses were detected during weeks 50/2018 and 5/2019 respectively. (Figure 5).

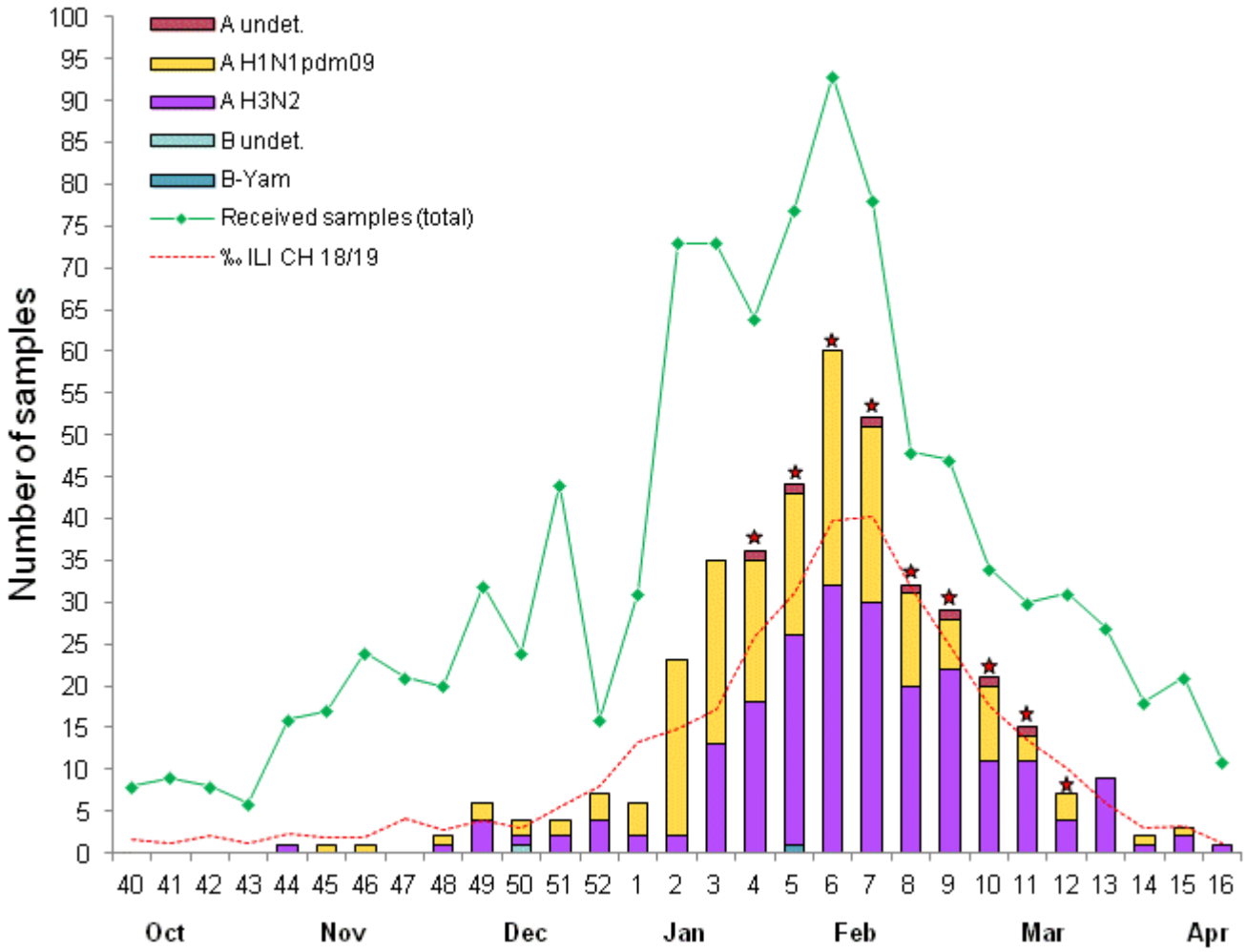


Figure 5. Schematic illustration of the 2018/19 influenza season. A undet.: influenza A, but the type could not be determined. B undet.: influenza B, but the type could not be determined; B-Yam: influenza B of Yamagata lineage; ILI 18/19: ILI cases reported during the 2018/19 season (‰); red stars (sampling period): indicate the weeks when Sentinel practitioners were requested to send only 1 out of 5 ILI case samples for influenza screening (weeks 4 to 12/2019).

4.3 Epidemiology of influenza viruses detected by the Sentinel network

4.3.1 Stratification by gender and age

The samples received were analyzed by gender and age. No significant differences in the numbers of positive or negative samples were observed among males and females (data not shown). The positivity rate, as well as the distribution of negative and positive samples, was similar among age groups (Figures 6 & 7). Information about age was lacking for four swabs (two positive and two negative).

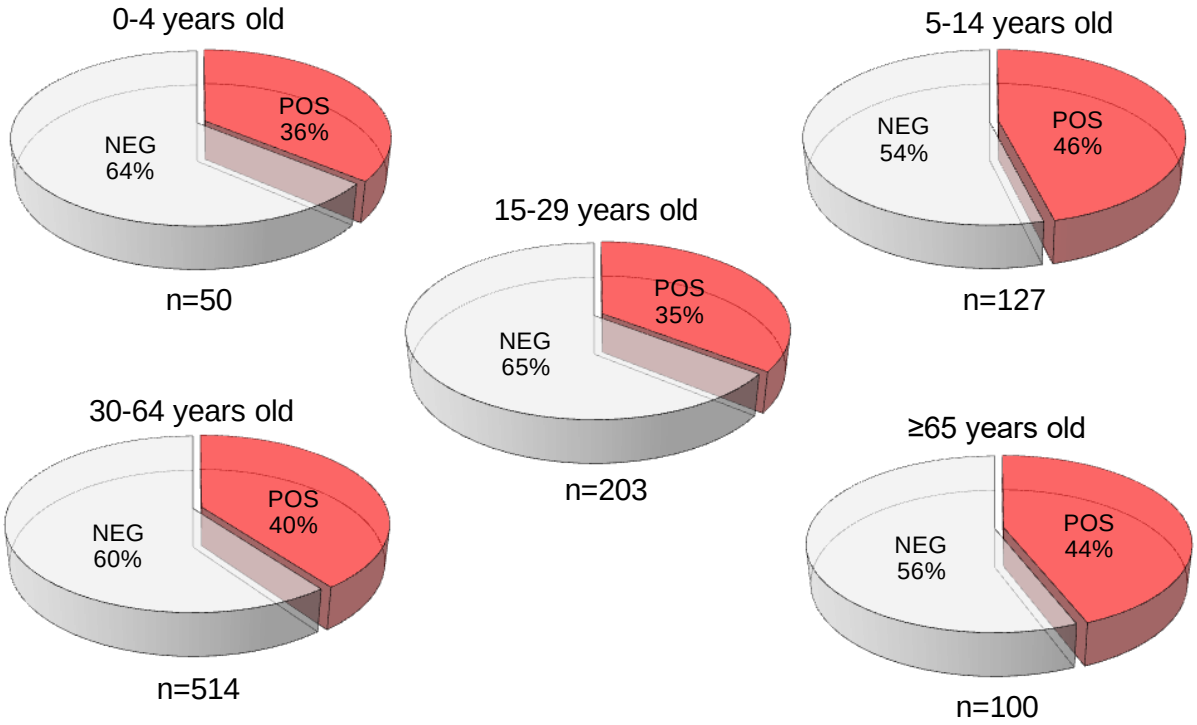


Figure 6. Influenza prevalence per age group. POS: positive; NEG: negative

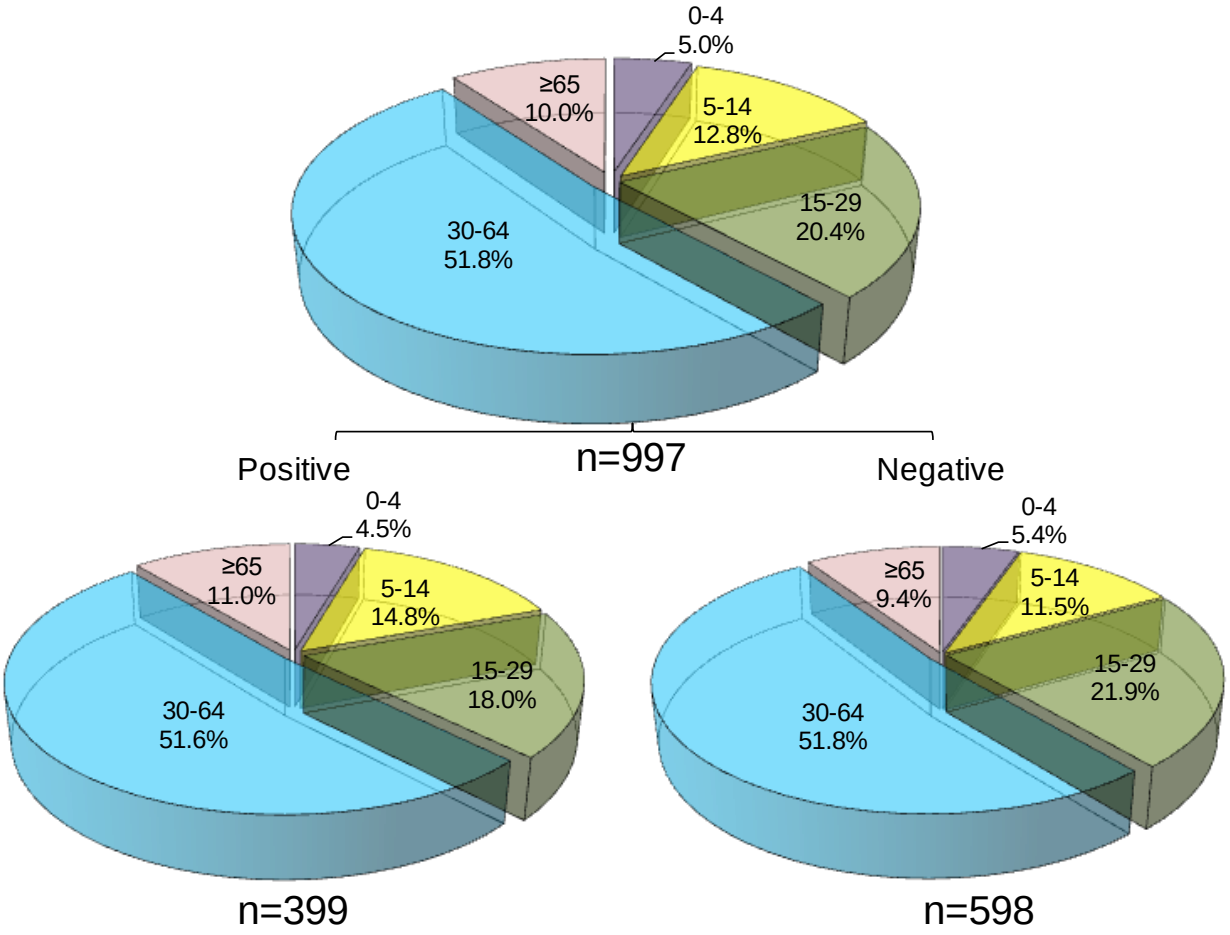


Figure 7. Distribution of the total influenza-positive and -negative samples in the different age groups.

Influenza A(H3N2) was dominant in the age groups ≥65 years (79.5%; n=44), 15-29 years (59.7%; n=72) and 5-14 years (57.6%; n=58). Equivalent proportions of A(H1N1)pdm09 and A(H3N2) were observed for the 30-64 years' group (49.3% and 47.8%, respectively; n=205). Influenza A(H1N1)pdm09 was the most prevalent strain in the 0-4 years' (72.2%; n= 18) group (Figure 8). Influenza B viruses were observed in the groups 5-14 years (n=1) and 30-64 years (n=1) (Figure 8).

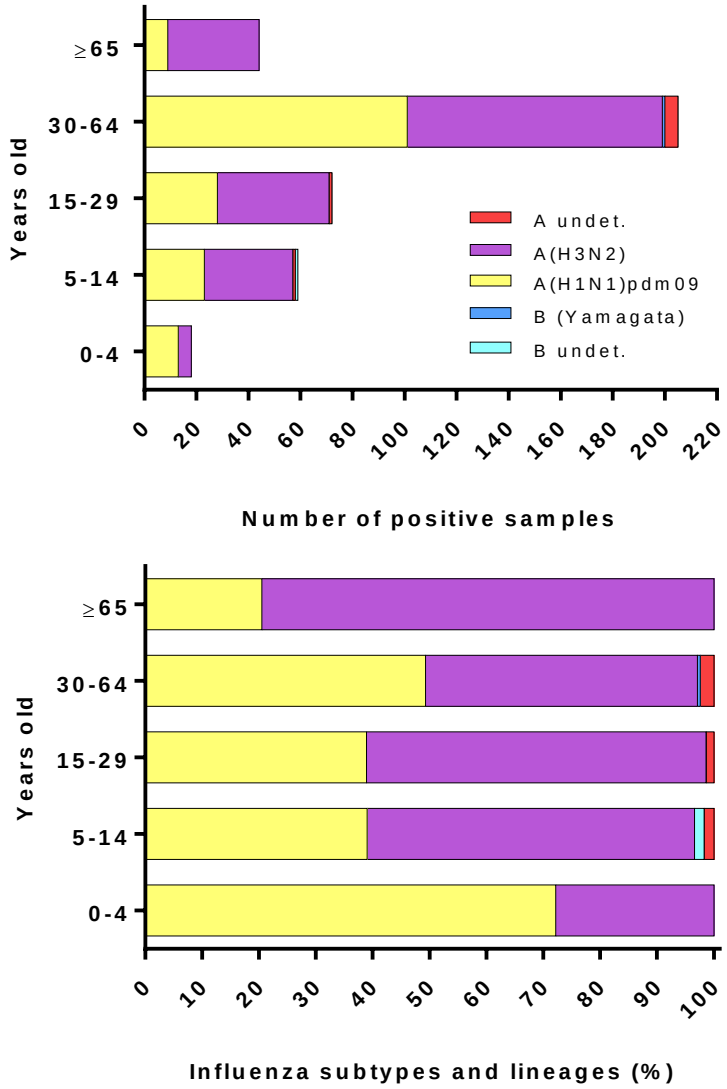


Figure 8. Distribution of influenza virus subtypes/lineages per age group. Upper panel: number of positive samples per subtype per age group. Lower panel: subtypes/lineages proportions per age group (%); A/B undet.: not able to be subtyped.

4.3.2 Stratification by influenza vaccination status

Vaccination status was available for 959 individuals, among whom 126 were reported as being vaccinated (Table 1). As mentioned previously, 54 (42.9%) of 126 were influenza-positive; 38 (70.4%) were A(H3N2) and 16 (29.6%) A(H1N1)pdm09 (data not shown). Of the 22 positive swabs originating from vaccinated participants of ≥65 years old (Figure 9), 90.9% (n=20) were A(H3N2) and 9.1% (n=2) A(H1N1)pdm09). Among the 24 positive samples isolated from vaccinated individuals of the age group 30-64 years (Figure 9), eleven were identified as A(H1N1)pdm09 and thirteen as A(H3N2) viruses.

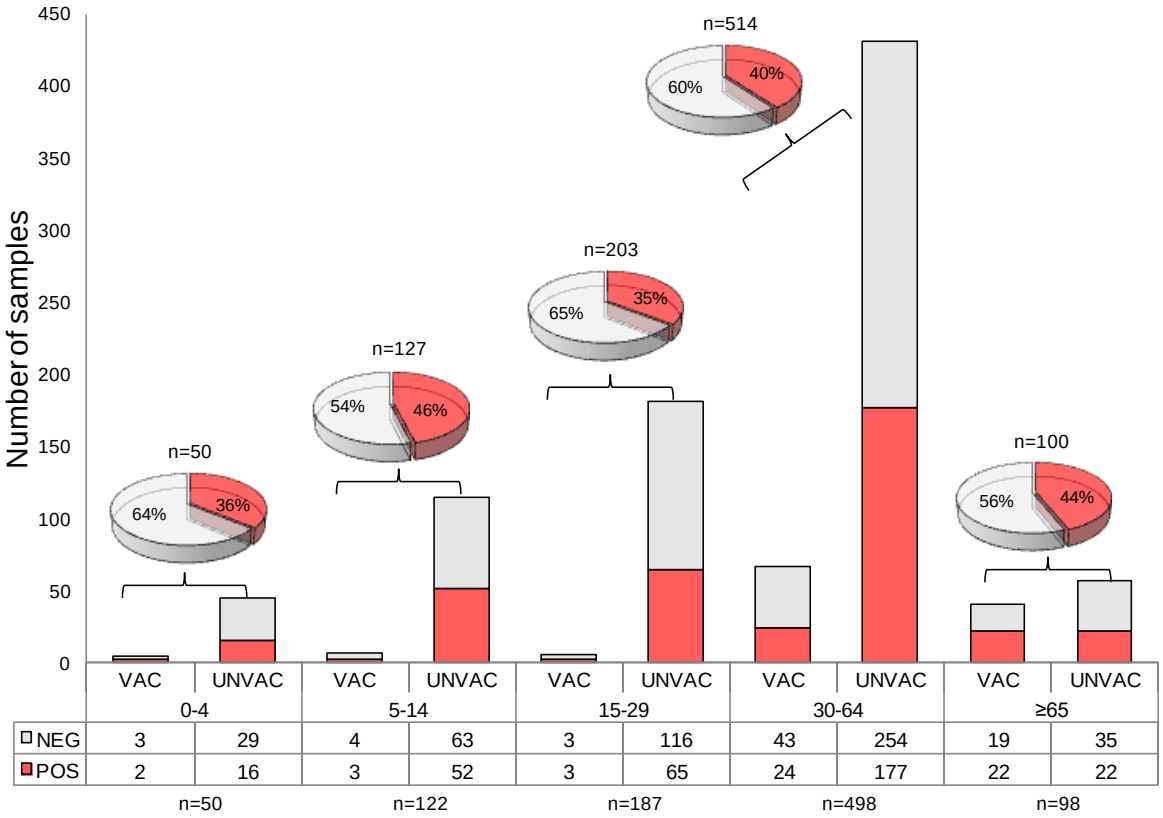


Figure 9. Number of positive and negative samples, per age group and per individuals' vaccination status. The pie charts correspond to Figure 6. VAC: vaccinated, UNVAC: unvaccinated. POS: positive. NEG: negative. 0-4, 5-14, 15-29, 30-64 and ≥65 years old groups. n=x below the table corresponds to the total number of samples, per age group, from individuals for whom a vaccination status was reported. The vaccination status was not available for 39 participants.

Only few individuals from age groups 0-4, 5-14 and 15-29 years were reported as being vaccinated (Figure 9).

4.4 Antigenic and genetic characterization of influenza viruses

One hundred and four influenza-positive samples were cultured on MDCK and MDCK-SIAT cells. Among these, 83 (43 A(H1N1)pdma09, 39 A(H3N2) and 1 B/Yamagata/16/1988) grew on MDCK and/or MDCK-SIAT cells and were submitted to antigenic characterization by HI. All isolates were successfully subtyped (Figure 10; Annexes 2 to 4).

One hundred and four samples were chosen for sequencing of the HA and NA genes (52 A(H1N1)pdm09 and 52 A(H3N2)). One hundred and two HA (51 A(H1N1)pdm09 and 51 A(H3N2)) and 103 NA (51 A(H1N1)pdm09 and 52 A(H3N2)) sequences were successfully recovered. One sample could not be sequenced at all due to a low viral load and sample degradation.

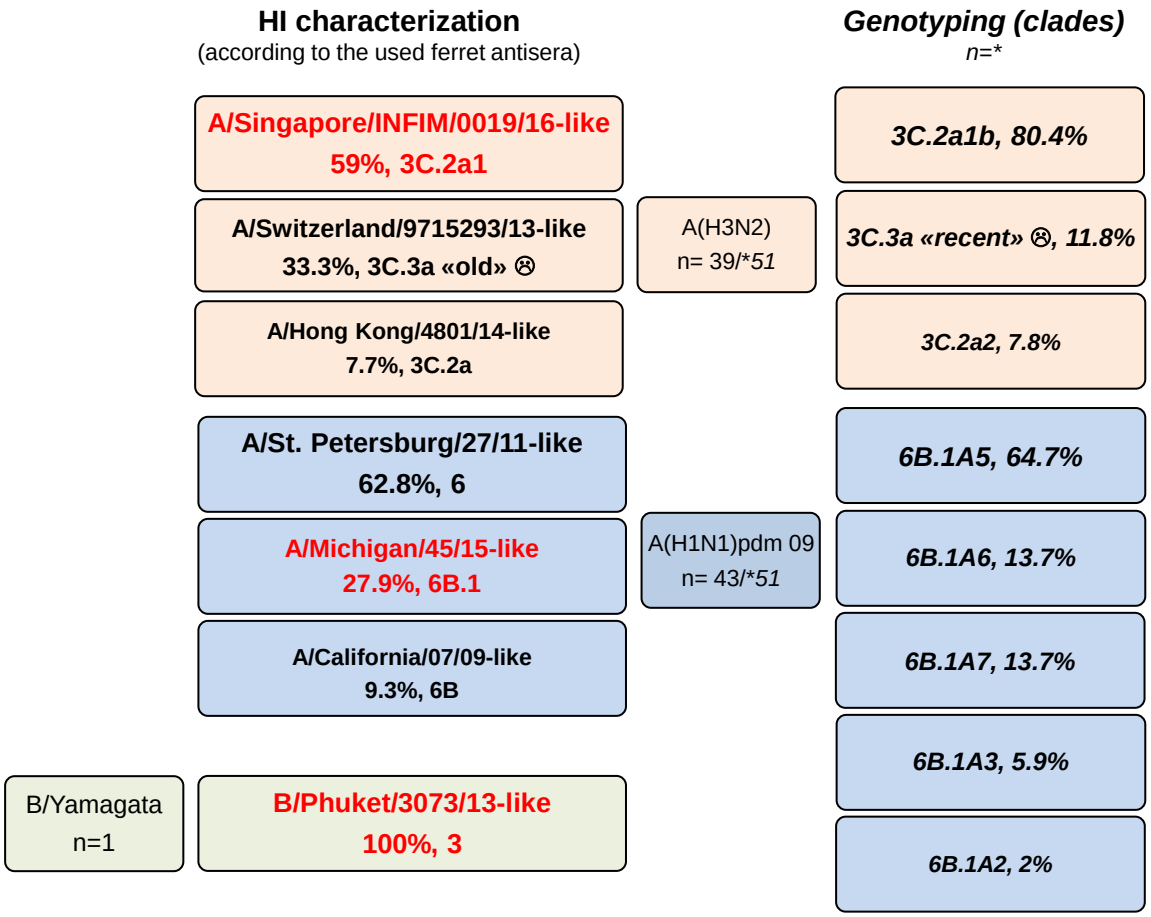


Figure 10. Antigenic and genetic characterization of selected influenza viruses isolated during the 2018/19 season. a) Antigenic characterization by HI (n=83 culture positive samples). The antigenic characterization was assessed on the basis of the antigenic tables for 2018/19 season (Figure 3). Red : 2018/19 vaccine strains. b) Genetic characterization by HA1 sequence analysis (n=102 positive influenza samples). Reference viruses for the genetic subgroups are found in the HA1 phylogenic trees (Figures 11 & 13). A(H3N2) 3C.2a1 (subclade of the 2018/19 northern hemisphere vaccine strain), 3C.2a1b and 3C.2a2 (subclade of the 2019 southern hemisphere vaccine strain) are genetic subgroups of subclade 3C.2a corresponding to the 2016/17 northern hemisphere vaccine strain. ☹ : recent and old 3C.3a viruses are antigenically distinct. A(H1N1)pdm09 6B, 6B.1, 6B.1A1-7 are genetic subgroups of clade 6. B/Yamagata clade 3 corresponds to the 2018/19 vaccine strain genetic group.

Twenty-three M genes (11 A(H1N1)pdm09 and 12 A(H3N2)) were also partially sequenced. As observed during the 2017/18 season, no significant changes were observed in the sequenced M region. Of note, one mismatch (C/T) in the fifth nucleotide from the 3' end of the forward primer (InfA-CDC) of the diagnostic rRT-PCR (A/B-CDC) has been observed with increasing frequency since 2015 in A(H3N2) strains only. This substitution does not have a visible impact on our diagnostic capacity. However, this event will be closely monitored during the forthcoming seasons.

A total of 40 Sentinel samples (20 in January and 20 in April 2019) were sent to the Francis Crick Worldwide Influenza Centre (WIC) in London, UK, which is also a WHO

Collaborating Centre for Reference and Research on Influenza. One A(H1N1)pdm09 positive sample (A/Switzerland/1860/2019) originating from a pig breeder swabbed in April 2019 was also sent. Preliminary results for the first shipment are available in the document: “Report prepared for the WHO annual consultation on the composition of influenza vaccine for Northern hemisphere 2019-2020”.[8] However, the results for the second dispatch are still pending as of July 2019.

4.4.1 Characterization of influenza A(H1N1)pdm09

Forty-three (H1N1)pdm09 strains were successfully characterized by HI (Figure 10; Annexes 2a-c). On the basis of our antigenic tables, 27 isolates were defined as A/Saint-Petersburg/27/2011-like, 12 as A/Michigan/45/2015-like, and four as A/California/07/2009-like. Thirty-three (76.8%) isolates were recognized by the antiserum (F32/16) directed against the currently-used vaccine strain A/Michigan/45/2015 as equal, two-fold or four-fold higher the homologous titer. Interestingly, five (11.6%) isolates were recognized as eight-fold higher than the homologous titer by the antiserum raised against A/Michigan/45/2015 and equal to two-fold the homologous titer of the antisera (F07/16 and F23/11, respectively) targeting A/California/07/2009 and A/Saint-Petersburg/27/2011 strains. Finally, five (11.6%) isolates had titers two- to four-fold lower than the homologous titer of A/Michigan/45/2015 antiserum. These five isolates were also poorly recognized by antisera against A/California/07/2009 and A/Saint-Petersburg/27/2011 strains.

Fifty-one HA1 genes were successfully sequenced (Figures 10 & 11). All isolates belonged to subclades (6B.1A2 [2%], 6B.1A3 [5.9%], 6B.1A5 [64.7%], 6B.1A6 [13.7%] or 6B.1A7 [13.7%]) of clade 6B.1, the current A/Michigan/45/2015 vaccine strain genetic group, defined by the presence of S84N, S162N and I216T HA1 amino acid substitutions (Figures 10 & 11). Clade 6B.1A isolates bear S74R, S164T (glycosylation is altered from positions 162 to 164) and I295V additional mutations compared to A/Michigan/45/2015. All, except the isolate A/Switzerland/5541/19, carried the substitution S183P. Isolates of the 6B.1A5 subgroup carried substitutions N260D in combination with N129D plus (all but one) T185I, or N260D plus E235D as the reference strain A/Switzerland/3330/17. Viruses from 6B.1A3 and 6B.1A6 subgroups carried the T120A substitution, and those in the subclade 6B.1A7, the mutation K302T.

Fifty-one NA genes were successfully sequenced (Figure 12). All isolates clustered similarly to the respective HA1 genes. As for last year, when compared to A/Michigan/45/2015, all isolates carried the additional substitutions G77R, V81A and N449D; mutations I188T, D416N and I389K were also observed among some of the samples analyzed (Figure 12).

Data from WIC: A(H1N1)pdm09 viruses[8]

Of 13 A(H1N1)pdm09 viruses from the first NRCI dispatch (January 2019), 12 were recovered and tested by HI assay. All viruses were recognized well by A/Michigan/45/15 Egg (6B.1) and A/Switzerland/3330/17 Egg (6B.1A5) antisera. At the genetic level, NRCI isolates sent to the WIC clustered in the subclades 6B.1A5 and 6B.1A6.

All of the A(H1N1)pdm09 isolates (worldwide origin) tested by the WIC were antigenically similar to the egg-propagated A/Michigan/45/15 (clade 6B.1) (Annex 5) and all were divided into genetic subgroups of clade 6B.1A (data not shown).

A(H1N1)pdm09 viruses

All A(H1N1)pdm09 viruses isolated during the 2018/19 influenza season were antigenically and genetically similar to the vaccine strain A/Michigan/45/2015 (clade 6B.1); 64.7% of the Swiss isolates clustered in the 6B.1A5 genetic subgroup.

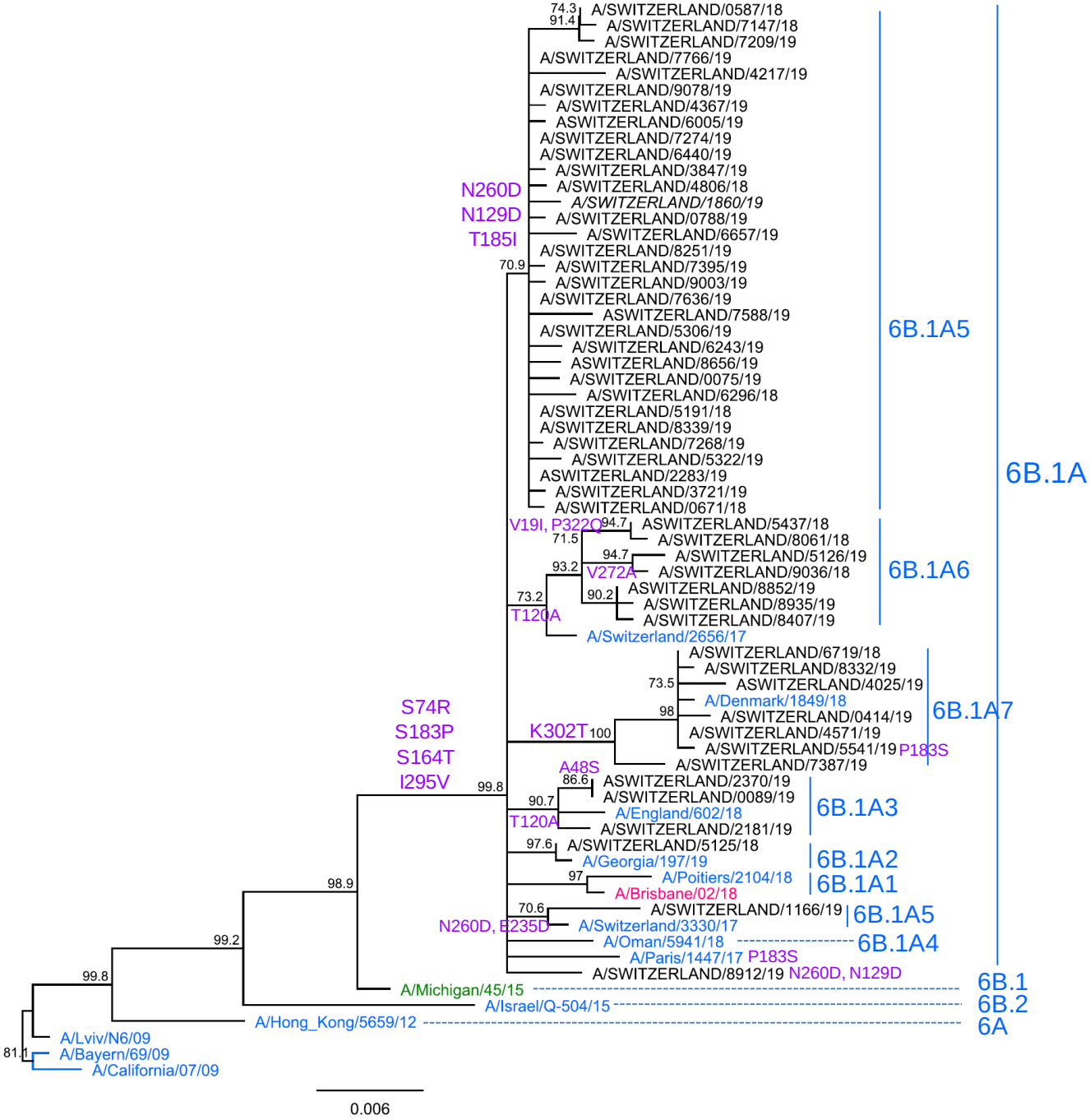


Figure 11. Phylogenetic analysis of the HA1 genes of A(H1N1)pdm09 viruses. Black: influenza virus detected in the Sentinel network during the 2018/19 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/19). Green: 2018/19 vaccine strain. Pink: 2019/20 vaccine strain. Blue: reference strains. 6A, 6B, 6B.1 (A1-7) and 6B.2: A(H1N1)pdm09 genetic clades and subclades. Purple: typical mutations described by the WIC and/or observed at the NRCI. *Italic*: non-Sentinel sample isolated from a pig breeder. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

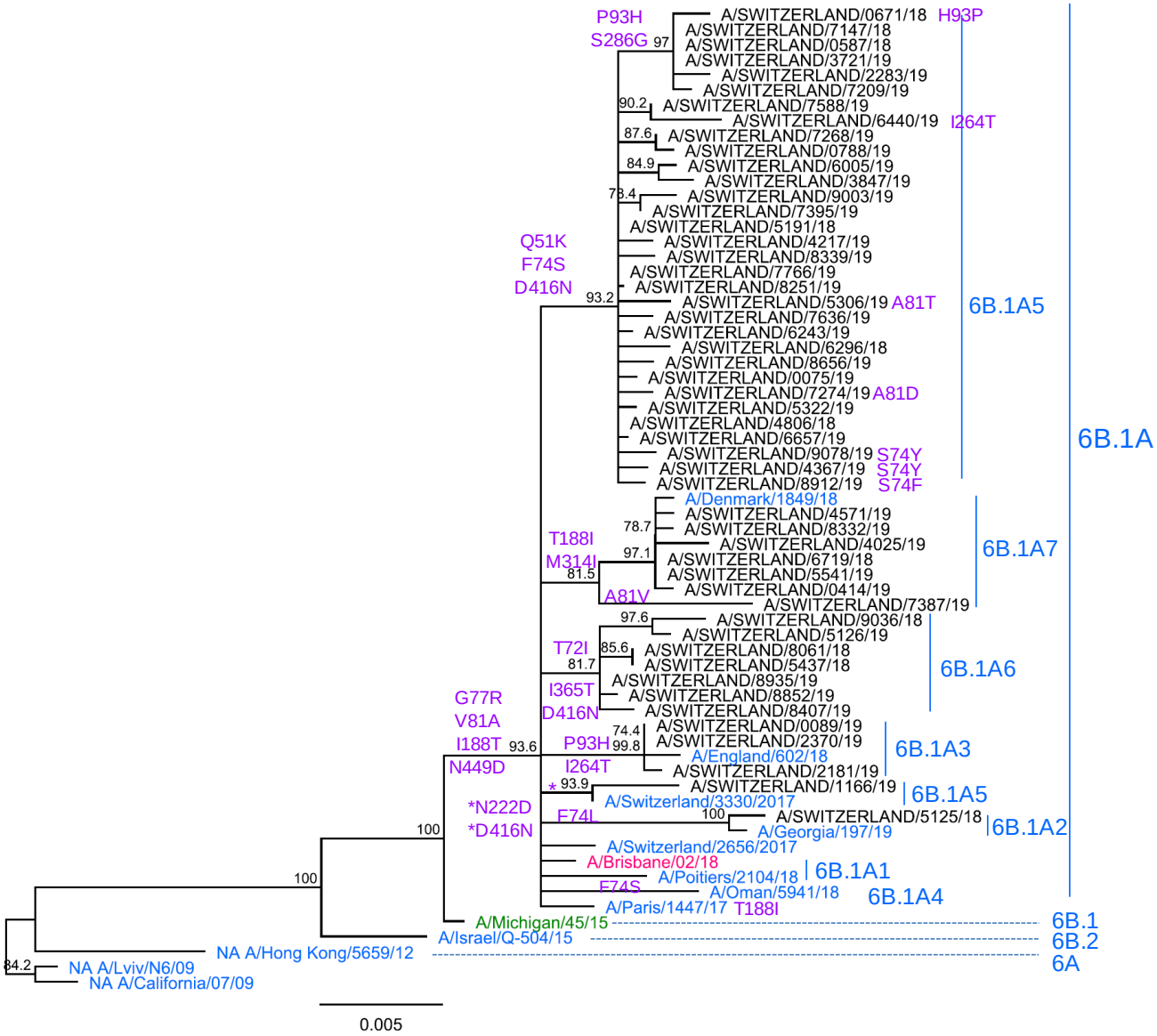


Figure 12. Phylogenetic analysis of the NA genes of A(H1N1)pdm09 viruses. Black: influenza virus detected in the Sentinel network during the 2018/19 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/19). Green: 2018/19 vaccine strain. Pink: 2019/20 vaccine strain. Blue: reference strains. 6A, 6B, 6B.1 (A1-7) and 6B.2: A(H1N1)pdm09 genetic clades and subclades. Purple: typical mutations described by the WIC and/or observed at the NRCI. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

4.4.2 Characterization of influenza A(H3N2)

Similar to the previous influenza seasons, WIC reported that antigenic characterization of A(H3N2) viruses by HI was difficult due to a variable agglutination of RBC from guinea pig, turkey and humans, and the NA-mediated agglutination of RBCs. As for the last season, all A(H3N2) viruses could successfully be tested by HI using our glutaraldehyde-fixed RBCs.

Thirty-nine A(H3N2) viruses were analyzed using the HI assay. All were successfully characterized (Figure 10). Twenty-three (59%) of 39 were identified as A/Singapore/INFIM-16-0019/16-like viruses, the current vaccine strain. Thirteen were (33.3%) classified as A/Switzerland/9715293/13-like, the 2015/16 vaccine component. The three (7.7%) remaining isolates were A/Hong Kong/4801/14-like viruses, the 2016/18 vaccine strain. All viruses were recognized by the egg-propagated A/Singapore/INFIM-16-0019/16 antiserum (F46/17); 15 with equivalent, 19 within two-fold (15 reduced two-fold) and five within four-fold (four reduced four-fold) of the titer obtained with the A/Singapore/INFIM-16-0019/16 (E5/E2 10^{-4}/MDCK-SIAT1) virus. Of note, test viruses classified as A/Switzerland/9715293/13-like were recognized by antiserum (F46/17) at titers systematically two- to four-fold lower than the homologous virus.

Fifty-one HA1 genes were successfully sequenced (Figures 10 & 13). Most (88.2%) isolates belonged to different subclades of the 3C.2a genetic group, with 80.4% of 3C.2a1b a 3C.2a1 subclade (reference: A/Singapore/INFIM-16-0019/16) and 7.8% of 3C.2.a2 viruses (reference: A/Switzerland/8060/17, the 2019 Southern Hemisphere vaccine strain). Finally, 11.8% of the test viruses belonged to the “recent” 3C.3a clade (references: A/Switzerland/9715293/13 for the “old” 3C.3a, A/England/538/18 and A/Kansas/14/17 for the “recent 3C.3a strains). A HA1-based phylogenic tree, with representative mutations (in violet) of A(H3N2) clades and subclades is shown as Figure 13. Viruses of the genetic group 3C.2a usually carry substitutions L3I, N128T, N144S, N145S, F159Y, K160T, P198S, F219S, N225D and Q311H in their HA1; those of subclade 3C.2a1b have the additional K62R, R142G and H311Q substitutions in HA1. T128A and T135K mutations were also present in a subgroup of the 3C.2a1b genetic subgroup. Isolates from the “recent 3C.3a clade are mainly characterized by mutations L3I, S91N, N144K, F193S and K326R, in addition to the “old” 3C.3a HA1 substitutions T128A, A138S and R142G (Figure 13).

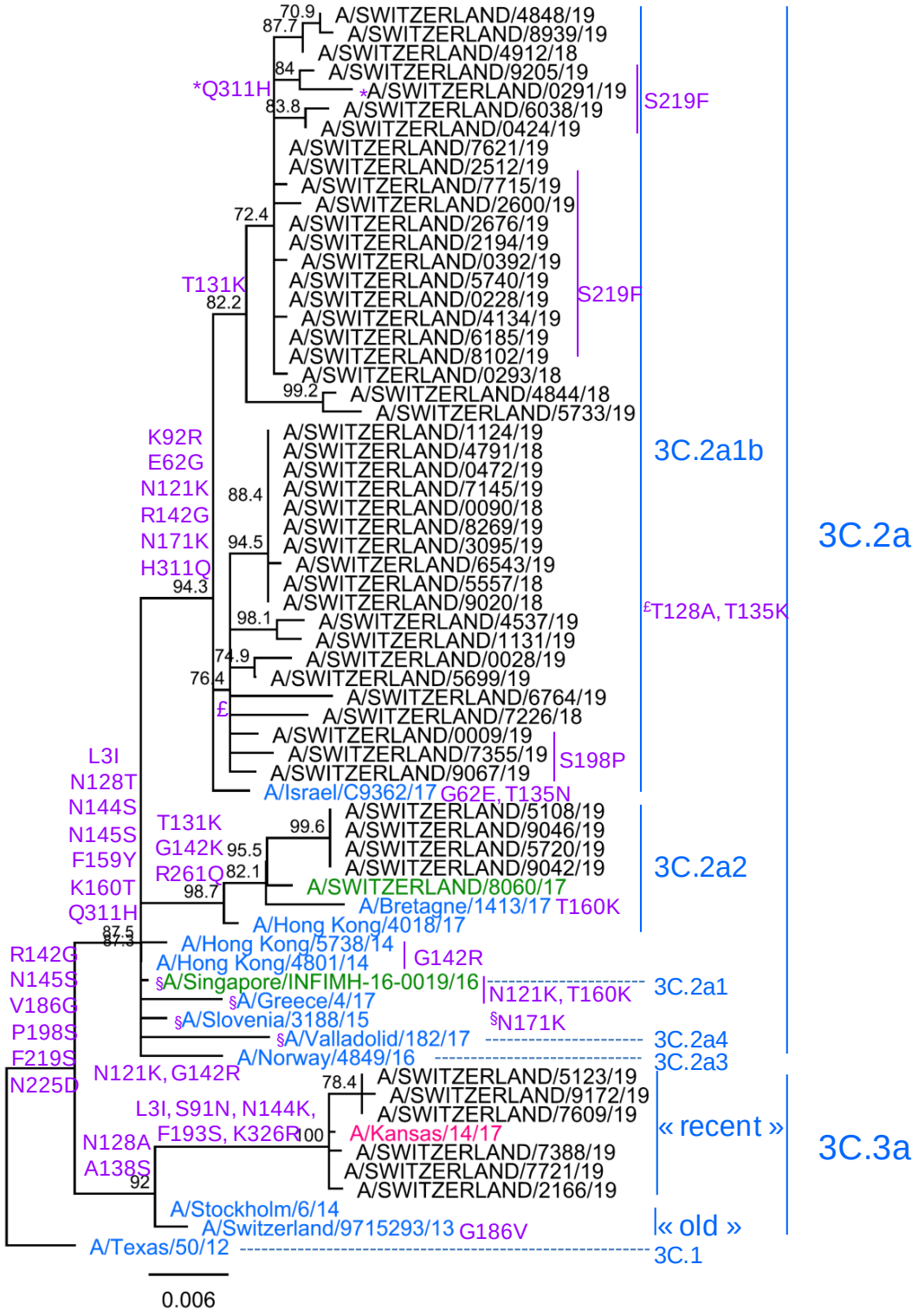


Figure 13. Phylogenetic analysis of the HA1 gene of A(H3N2) viruses. Black: influenza virus detected in the Sentinel network during the 2018/19 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/19). Green: 2018/19 Northern hemisphere and 2019 Southern Hemisphere vaccine strains. Pink: 2019/20 vaccine strain. Blue: reference strains. Purple: typical mutations described by the WIC and/or observed at the NRCI. 3C.2a, 3C2a1 to 4 and 3C3a are A(H3N2) genetic clades and subclades. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

Fifty-two NA genes were successfully sequenced (Figure 14). Most of the NA and HA1 genes clustered similarly. NA substitutions K220N, V303I and N329S were observed in subclade 3C.2a1b viruses, while NA mutation P386S was present in some 3C.2a1b viruses and all 3C.2a2 isolates. K75R, Y155H, L141I, N329T and E344K mutations were carried by 3C.3a isolates. All Swiss 3C.3a isolates also carried N161S and P386S substitutions. (Figure 14)

Data from the WIC: A(H3N2) viruses[8]

All seven A(H3N2) viruses sent to the WIC during the first dispatch (January 2019) were recovered and analyzed (Annexes 6a-c). Only isolate A/Switzerland/293/2018 was submitted to HI assay. The six remaining isolates, as well as isolate A/Switzerland/293/2018, were tested by plaque reduction neutralization. A/Switzerland/293/2018 virus was recognized at a titer four-fold lower than the homologous titre by the antiserum raised against the egg-propagated A/Singapore/16-0019/2016. Antisera raised against the cell culture-propagated 3C.2a1b viruses, A/Rioja/23202/2018 and A/Norway/3275/2018, recognized the test virus at titers of 160 and 360, respectively. There were no homologous titers for the two latter antisera since the reference viruses were unable to agglutinate RBCs. When tested by plaque reduction neutralization, all NRCI isolates, except the A/Switzerland/293/2018 virus, were recognized by the antiserum A/Singapore/INFIMH-16-0019/2016 SIAT (F45/17) at titers equivalent or two- to four-fold reduced compared to the homologous virus. NRCI isolates were poorly recognized by antiserum A/Singapore/INFIMH-16-0019/2016 egg-propagated (F46/17) and none was recognized by antiserum (F29/15) raised against the egg-propagated A/Switzerland/9715293/2013 clone 123. The seven NRCI isolates were attributed to the 3C.2a1b genetic subgroup.

The majority of viruses of worldwide origin tested by HI at the WIC were recognized by the antiserum (F30/14) raised against the cell-propagated A/Hong Kong/5738/2014 virus (clade 3C.2a); and 50% by the egg-propagated A/Singapore/INFIMH-16-0019/2016 (2018/19 vaccine strain, clade 3C.2a1) at titers ≤ four-fold reduced compared to the homologous titer. In plaque reduction neutralization assays, antiserum (F29/15) recognized only 21% of the tested isolates within four-fold of the homologous titer. Genetic group 3C.2a1b viruses were predominant.

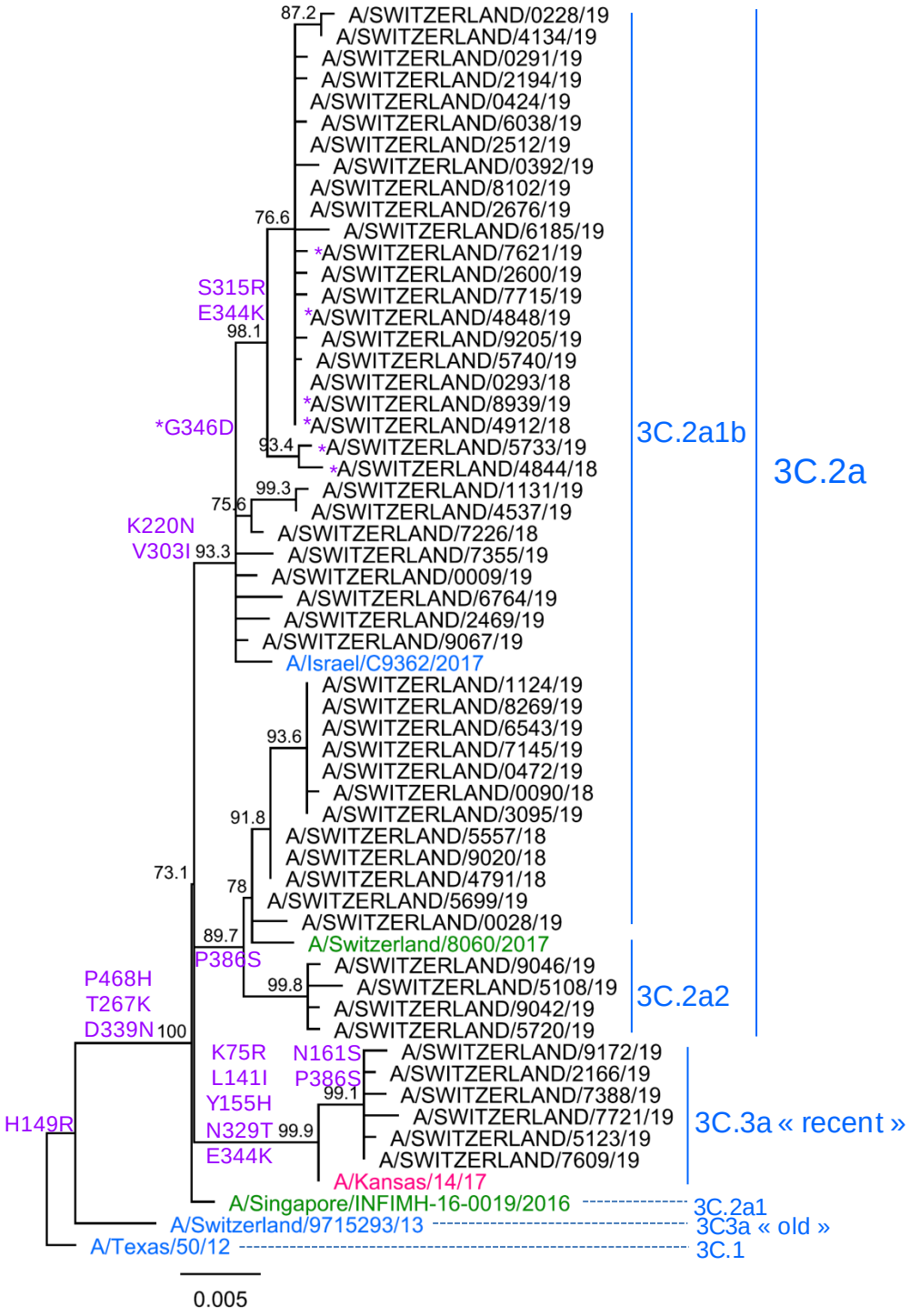


Figure 14. Phylogenetic analysis of the NA gene of A(H3N2) viruses. Black: influenza virus detected in the Sentinel network during the 2018/19 season; strain names are A/Switzerland/isolate number/year (e.g. A/Switzerland/5214/19). Green: 2018/19 Northern hemisphere and 2019 Southern Hemisphere vaccine strains. Pink: 2019/20 vaccine strain. Blue: reference strains. Purple: typical mutations described by the WIC and/or observed at the NRCI. 3C.2a, 3C2a1 to 4 and 3C3a are A(H3N2) genetic clades and subclades. Sequences were aligned using Geneious 6.1.8 MAFFT alignment (v7.017) with default settings. A consensus tree was built from 1000 original trees in ML (70% support threshold) constructed using Geneious 6.1.8 PHYML default settings.

However, an increasing number of viruses belonging to a 3C.3a subgroup, antigenically drifted from “old” 3C.3a viruses (A/Switzerland/9715293/2013, 2015/16 vaccine component) are being observed.

A(H3N2) viruses

All of the A(H3N2) viruses isolated in Switzerland were antigenically related to the 2018/19 vaccine strain A/Singapore/INFIMH-16-0019/2016 (genetic clade 3C.2a1). However, recognition by ferret antisera was reduced for some isolates.

At the genetic level, most Sentinel A(H3N2) isolates belonged to the subclade 3C.2a1b (reference strain A/Norway/2620/18) of clade 3C.2a1. “Recent” 3C.3a (reference strain A/England/538/18) and 3C.2a2 (reference strain A/Switzerland/8060/17) viruses were also observed in lower numbers.

4.4.3 Characterization of influenza B viruses

Only two influenza viruses were detected during the 2018/19 season. Both had low viral titers and only one, B/Switzerland/7847/19, could be further attributed to the B/Yamagata/16/1988 lineage.

B/Switzerland/7847/19 was recognized as two- to four-fold lower than the homologous titer of the three antisera tested, i.e. B/Wisconsin/1/10, B/Novosibirsk/1/12 and B/Phuket/3073/13, the latter being the current and next season vaccine strain (Annex 4).

Data from the WIC: B viruses[8]

Due to the low viral load of the only two viruses isolated at the NRCI, no influenza B isolates were shared with the WIC.

Only a few B/Yamagata/16/1988 and B/Victoria/2/1987 lineage viruses of worldwide origin were received at the WIC. Most of the B/Yamagata/16/1988 lineage isolates fell within genetic clade 3 (reference: B/Phuket/3073/2013, 2018/19 vaccine strain) and were well recognized by antisera raised against egg-propagated clade 3 viruses.

B/Victoria/2/1987 lineage viruses were divided into four genetic groups of the clade 1A. None, double and triple deleted viruses were co-circulating. All subgroups were shown to be distinct antigenically.

Influenza B viruses

The only Influenza B/Yamagata/16/1988-lineage virus isolated during this season was antigenically similar to the 2018/19 vaccine strain, B/Phuket/3073/13.

4.5 Antiviral resistance

4.5.1 Sentinel isolates

None of the fifty-two NA analyzed carried substitutions known to be associated with reduced susceptibility to oseltamivir and zanamivir. These results were concordant with the antiviral resistance data available from the WIC.

One A(H1N1)pdm09 isolate of the 218 tested was identified with the H275Y mutation at the WIC. None of the A(H3N2) and influenza B viruses from both lineages analysed at the WIC showed reduced susceptibility to oselatmivir and zanamivir.

4.5.2 Non-Sentinel isolates

During the 2018/19 influenza season, the NRCI was asked to test two isolates from two hospitalized patients for NAI susceptibility. One sample came from the “Hôpital du Valais - Institut Central” and the other from the “Centre hospitalier universitaire vaudois” (Lausanne). Only a post-treatment sample was available for each patient and no information was provided on the patients’ immune status. Both samples were A(H1N1)pdm09 positive. Both patients were under oseltamivir treatment, but did not show clinical improvement. The substitution H275Y in the NA gene was present in one isolate. Both isolates shown normal inhibition by zanamivir.

NAIs sensitivity

None of the isolates tested at the NRCI in the context of influenza seasonal surveillance exhibited a reduced inhibition by oseltamivir and zanamivir.

One isolate, originating from a hospitalized patient under oseltamivir treatment, carried the H275Y substitution associated with highly-reduced inhibition by oseltamivir.

5 WHO recommendation for the composition of influenza virus vaccines for the 2019/20 influenza season

Influenza vaccine recommendations are made on the basis of the Global Influenza Surveillance Response System network data, virus antigenic and genetic characterization data, human serology data, virus fitness forecasting data, antiviral resistance data, vaccine effectiveness, and the availability of candidate vaccine viruses.

The A(H1N1)pdm09 and A(H3N2) vaccine components will be updated for the next influenza season. The vaccine strains recommended for the 2019/20 Northern hemisphere influenza vaccine by the WHO experts are[9]:

Table 2. Recommended influenza vaccine composition for the 2019/20 influenza season.

	Virus strains
A(H1N1)pdm09	A/Brisbane/02/2018 (H1N1)pdm09-like
A(H3N2)	A/Kansas/14/2017 (H3N2)-like
B/Yamagata/16/1988 lineage	B/Colorado/06/2017-like
B/Victoria/2/1987 lineage	B/Phuket/3073/2013-like

**Only B strain included in the trivalent vaccine*

6 A(H1N2) viruses

As during the 2017/18 influenza epidemic in the Netherlands,[10] two A(H1N2) reassortment events between A(H1N1)pdm09 and A(H3N2) viruses occurred in Sweden and Denmark. The Swedish virus had an A(H3N2) NA segment with all the other segments derived from A(H1N1)pdm09.[11] The isolate from Denmark harboured a similar segment reassortment as the Swedish virus.[12] This subtype is not considered as being a major threat, or at least no more than the other human subtypes, as it results from the reassortment of circulating human viruses. Similar to the one observed during the 2017/18 season, these viruses were genetically distinct from the A(H1N2) viruses observed in Switzerland during the 2002/03 influenza season.

7 Human infection with animal influenza viruses

A(H1N1) and A(H3N2) influenza strains are responsible for the seasonal human influenza outbreaks observed worldwide. However, transmission of influenza viruses of animal origin to humans, notably avian and porcine, can potentially lead to severe epidemics and, in a worst-case scenario, to pandemics. Even if non-human influenza

strains appear to require a close contact with infected animals for spread and do not (or at least not efficiently) sustain human-to-human transmission as yet, they can be responsible for confined outbreaks. In addition, recombination events between porcine/avian and human viruses due to concomitant circulation with seasonal influenza A strains could lead to the human adaptation of avian strains. To allow the early identification and rapid containment of new potential animal-to-human transmission events, several countries, including Switzerland, have introduced the regular screening of animals (mainly poultry/wild birds and farm pigs) for the presence of the respective influenza strains.

7.1 Swine-to-human influenza virus transmission

In Switzerland, veterinarians contribute to swine influenza surveillance by collecting specimens from farm pigs with respiratory symptoms. These samples are then analyzed at the National Veterinarian Institute (Vetvir, Zurich). In parallel, they send samples to the NRCI from consenting pig breeders (or their employees) who have been in contact with influenza-infected animals and present ILI symptoms. The presence of porcine influenza A viruses in human samples is then assessed using a rRT-PCR with the capacity to distinguish influenza A viruses of human and animal origin, both avian and porcine, and confirmed by sequencing. During the 2018/19 influenza season, four samples from two different farms were sent to the NRCI for swine influenza testing. One swab was positive for human A(H1N1)pdm09 (A/Switzerland/1860/19; Figure 11) and three (from the same farm) were negative (Table 3). One pig, sampled in the farm belonging to the human influenza positive pig breeder, was reported as positive (N1 gene) for the typical swine influenza circulating in Swiss pigs.

Table 3. Pig breeders influenza rRT-PCR results.

Sample ID	Age	Sex	Result	Origin	Sender	Sample date
1860	59	M	A(H1N1)pdm09	Zürich	SUISAG-Büro Zürich	12.04.19
5219	22	M	IA/IB NEG	Ruswil	Tierartzpraxis Bühlmann	18.04.19
5256	23	M	IA/IB NEG	Ruswil	Tierartzpraxis Bühlmann	18.04.19
5306	42	F	IA/IB NEG	Ruswil	Tierartzpraxis Bühlmann	18.04.19

IA/IB:influenza A/B, NEG: negative.

Influenza A viruses of porcine origin, but isolated from human cases, are identified as “variant” viruses and denoted with a letter “v”, such as A(H3N2)v, A(H1N1)v and A(H1N2)v. Since 2005, the systematic reporting of all human infections with variant viruses is mandatory in the USA. Since 2010, 465 (430 A(H3N2)v, 10 A(H1N1)v and 25 A(H1N2)v) human cases of variant influenza have been reported within several States.[13]

7.2 Avian influenza A subtypes in humans

At the NRCI, we received a request for an A(H5N1) analysis from a private laboratory located in Eastern Switzerland (patient sampled on the 04.03.2019; female, 62 years old). After contacting the laboratory for more information, it turned out that the exact reason for this analysis request was unknown and is most probably due to a lack of knowledge about the circulating influenza subtypes. The sample was nevertheless tested and was positive for seasonal A(H3N2). As expected the A(H5N1) rRT-PCR was negative.

As of June 2019, a total of 860 laboratory-confirmed human cases of A(H5N1), including 454 deaths, have been reported to WHO.[14] No cases were reported in 2018 and only one case, unfortunately fatal, was identified in Nepal since the start of 2019.

Since February 2014, 24 cases of highly pathogenic avian influenza (HPAI) A(H5N6), including 15 deaths, have been identified in Mainland China. All cases belonged to the genetic group 2.3.4.4 of Asian origin. No human cases have been reported so far in 2019.[14]

Since February 2013, 1568 human cases of A(H7N9) infection have been reported; among those, 616 had a fatal outcome. All cases were of Chinese origin and most were isolated in China (Figure 15). On January 2017, HPAI A(H7N9) viruses started to be detected in poultry and environmental samples and in human isolates. Human infection with low (LPAI) or HPAI viruses has no impact on disease severity, but mortality and NAI resistance seems to be more elevated in HPAI-infected individuals.[15] Of note, after a peak in the number of human cases reported during 2016/17 (fifth wave), only few cases were identified during 2017/18 (sixth wave) and 2018/19 (seventh wave).

Figure 15. A(H7N9) reported cases. Human cases are depicted in the geographic location where they were reported. (http://www.fao.org/ag/againfo/programmes/en/empres/H7N9/img/wave_7/map_2019_06_05.jpg).

Fifty-four confirmed human cases of A(H9N2) infections have been reported since 1998, mainly in Mainland China. The most recent case of human A(H9N2) infection was reported from China in February 2019. Since October 2018, five new cases have been described.[14]

8 Avian influenza A in animals[14]

The reservoir for L/HPAI influenza A viruses are wild birds. Both virus types can cause moderate to large outbreaks in poultry worldwide. While it could be expected to find virtually all existing influenza A subtypes within the bird population, most of the detected outbreaks are due to viruses of the H5, H7 and H9 subtypes.

Since 2017, 54% and 46% of the avian influenza outbreaks identified in Europe have been due to A(H5N6) and A(H5N8) viruses, respectively. Interestingly, the vast majority of A(H5N6) outbreaks concerned wild birds, while A(H5N8) outbreaks mostly occurred in poultry. From November 2018 to February 2019, several European countries reported outbreaks of avian influenza. Bulgaria experienced two outbreaks of HPAI A(H5N8) in poultry; Denmark had to deal with two outbreaks of HPAI A(H5N6) in wild birds; and the Netherlands reported one outbreak of LPAI A(H5N3) in domestic birds. A(H5N6) viruses detected in European countries clustered (2.3.4.4b) with A(H5N6) viruses that have been circulating in Europe since 2017 and are distinct from those present in Asia (2.3.4.4c).

From November 2018 to February 2019, Russia, Asia and Africa also experienced outbreaks of HPAI A(H5N1), A(H5N2), A(H5N6) and A(H5N8). India and Taiwan reported the largest numbers with 19 HPAI A(H5N1) and 14 HPAI A(H5N2), respectively. Of note, no A(H7N9) virus outbreaks have been reported since June 2018 worldwide. LPAI A(H9N2) viruses were detected in both domestic and wild birds in Asia, the Middle East and North Africa.

2018/19 season overview worldwide

Influenza A viruses were dominant during the 2018/19 influenza season worldwide. Both A(H1N1)pdma09 and A(H3N2) viruses co-circulated. Few influenza B viruses of both lineages were detected, with the majority in the USA.

A(H1N1)pdm09 viruses with the S183P HA substitution, present in most NRCI isolates, showed reduced inhibition by human post-vaccination antisera. Therefore, the 2019/20 vaccine component was updated to A/Brisbane/02/2018 (H1N1)pdm09-like.

An increasing number of recent 3C.3a A(H3N2) viruses are being detected. As those were shown to be antigenically drifted from 3C.2 isolates and reference viruses, the 2019/20 vaccine component was updated to A/Kansas/14/2017 (H3N2)-like.

Few viruses with substitutions associated with antiviral resistance were identified during the 2018/19 epidemic, mostly in treated patients.

Despite the fact that influenza outbreaks are being regularly reported in wild birds and poultry, only a few human cases of LPAI and HPAI infections have been identified, mostly in Mainland China.

9 Discussion

The Swiss 2018/19 influenza epidemic lasted 11 weeks. It was 4 weeks shorter than in 2017/18, but equivalent to the 2016/17 and 2015/16 epidemics. An influenza activity peak was observed during week 6/2019 in Switzerland and during week 05/2019 in Europe in general. In the USA, the 2018/19 influenza season started earlier and was unusually long compared to previous epidemics. It lasted for 21 weeks, but peaked in mid-February, similar to Europe.[16] However, in contrast to Europe, the epidemic peak in USA lasted for approximately 6 weeks (weeks 6 to 11/19). Interestingly, despite this extended epidemic, there were no reports of higher disease severity and hospitalization rates were even lower for adults than during 2017/18. Of note, hospitalization rates for children remained similar to those observed during 2017/18.[16]

Influenza A viruses were clearly dominant during the 2018/19 Northern hemisphere season. Several countries, including Switzerland, reported a majority of A(H1N1)pdm09 viruses from October to mid-January or –February, with the end of the epidemic being dominated by A(H3N2) strains. During the extended USA influenza epidemic, the influenza activity peak was characterized by a first spike of A(H1N1)pdm09 cases closely followed by a second of A(H3N2).[16] Only a few influenza B isolates were detected during this season in most reporting countries.

The number of individuals and samples tested at the NRCI during the 2018/19 epidemics was slightly lower than last year, but comparable to previous seasons. The female/male ratio was close to one (1.02), and there was no significant difference in positivity rates between males and females.

Seasonal distribution of the tested individuals among the different age groups was comparable to 2017/18, with 51.7%, 30-64 years, 20.4%, 15-29 years, 12.8%, 5-14 years, 10.1%, ≥65 years, and 5%, 0-4 years. The overall influenza positivity rate was lower (40.1%) during the 2018/19 season than 2017/18 (57.6%). It was below 50% across all groups (46% for 5-14 years old, 44% for ≥65 years old, 40% for 30-64 years old 36% 0-4 years old and 35% for 15-29 years old group). In contrast to the previous season, there was only a slight difference in positivity rates between age groups. The influenza positivity rate tends to fluctuate from one epidemic to another and it can be postulated that this variation may depend on the clinical features of the circulating strains, previous exposures to different influenza strains depending on the

participants' age, the prevalence of other respiratory viruses matching the ILI-case definition, the sensitivity of the chosen ILI-case definition,[17,18] and the medical practitioners' compliance with the case definition when addressing patients for laboratory confirmation. Of note, a low positivity rate can also result from a detection failure, emphasizing the requirement of performing regular quality assessments. Only marginal differences were observed in the distribution of positive versus negative samples among age groups. Not surprisingly, influenza A(H3N2) viruses were more prevalent in individuals ≥65 years (79.5%) and A(H1N1)pdm09 was the most frequent strain detected in the 0-4 years (72.2%) group. A(H1N1)pdm09 and A(H3N2) viruses were co-circulating in the three other age groups with A(H3N2) strains in 59.7% and 57.6% of those 15-29 years and 5-14 years respectively, and 49.3% of A(H1N1)pdm09 in the 30-64 years' group. To some extent, a similar age group distribution of influenza A subtypes was observed in Europe,[19] as well as in the USA,[16] particularly the dominance of A(H3N2) viruses in elderly individuals.

A similarly low number of individuals tested in the context of the Sentinel surveillance network were vaccinated during the 2018/19 (126) and 2017/18 (133) seasons. The percentage of vaccinated subjects was slightly higher in 2018/19 (12.6%) than in 2017/18 (10%); 31.7% were aged ≥65 years. This season, 41.8% of the tested elderly, with a known vaccination status, were vaccinated; among whom 53.7% were positive for influenza. The vast majority of the vaccinated elderly, who were also positive for influenza, carried an A(H3N2) strain. The vaccine effectiveness for the latter virus subtype has regularly been shown to be low[20,21] despite the HI results. Of note, as shown in the recent European Centre for Disease Prevention and Control (ECDC) report based on a survey conducted by the Vaccine European New Integrated Collaboration Effort III in collaboration with the ECDC, vaccination rates remain low in most European countries,[22] including in Switzerland.

Influenza A viruses were dominant worldwide during the 2018/19 influenza epidemic,[16,23] while influenza B viruses were only sporadically detected. Both influenza A subtypes co-circulated in many countries, including Switzerland.

The vast majority of the characterized A(H1N1)pdm09 viruses were antigenically close to the egg-propagated A/Michigan/45/2015 virus, the vaccine strain for the Northern hemisphere 2018/19 influenza season. Similar results were also observed with the antiserum raised against the cell-propagated counterpart. Concordantly, all tested viruses belonged to subclades (6B.1A, A1-A5) of the A/Michigan/45/2015

genetic group 6B.1. Of note, in studies performed with panels of post-vaccination human (children, adults and the elderly) antisera, A(H1N1)pdm09 viruses carrying the S183P substitution in HA1 were less well recognized. For this reason, the A(H1N1)pdm09 vaccine component for the Northern hemisphere 2019/20 influenza season was substituted by an A/Brisbane/02/2018-like strain (containing the HA1 S183P mutation). As mentioned, all Swiss isolates, except the isolate A/Switzerland/5541/19, carried the substitution S183P.

Less than 1% of the A(H1N1)pdm09 viruses tested worldwide for NAI and endonuclease inhibitor resistance exhibited a (highly) reduced sensitivity to the tested drug.[8,9,16] The selection of resistance mutations, particularly in immunosuppressed patients treated with antiviral drugs, is well documented.[24]

In general, most of the A(H3N2) viruses belonging to subclades of the 3C.2a genetic group were recognized within four-fold of the homologous titer by antisera raised against cell culture-propagated A/Singapore/INFIMH-16-0019/2016 (3C.2a1). A(H3N2) virus recognition was weaker by the antisera raised against egg-propagated A/Singapore/INFIMH-16-0019/2016 and A/Switzerland/8060/2017 (3C.2a2) viruses. Even if some cross-recognition could be observed in both HI and plaque reduction assays among 3C.2a1b, 3C.2a2, and recent and old 3C.3a viruses by the respective antisera, the overall data showed that these viruses are antigenically drifted. Similar to results with ferret antisera, 3C.2a1b viruses were recognized well by panels of post-vaccination human sera. However, recent 3C.3a viruses reacted poorly with post-vaccination human antibodies.[8] The latter observation, in addition to the increasing detection of recent 3C.3a viruses, motivated the update of the 2019/20 A(H3N2) vaccine component to a A/Kansas/14/2017-like strain.[8]

Among the A(H3N2) viruses tested for NAI and endonuclease inhibitor resistance worldwide, less than 0.1% exhibited a reduced susceptibility to oseltamivir but more than 4%, all originating from baloxavir marboxyl-treated Japanese children, carried the PA I38T or I38T/M substitution known to confer resistance to the drug.[9,16]

As the only influenza B/Yamagata/16/1988 lineage virus identified in Switzerland, isolates characterized worldwide were recognized well by antisera raised against the B/Phuket/3073/2013 vaccine component. B/Yamagata/16/1988 lineage viruses belonged to the genetic clade 3.[8,9,16] No B/Victoria/2/1987-lineage viruses were isolated at the NRCI, but data from the WIC showed that most viruses circulating worldwide belonged to genetic clade 1A. Genetic and antigenic diversity is increasing

significantly among B/Victoria/2/1987-lineage viruses. While the great majority of viruses with the 162-163 amino acids HA1 deletion reacted well with post-infection ferret antisera raised against B/Colorado/06/2017-like viruses, the 2018/19 vaccine component, “wild-type” viruses, as well as isolates with the 162-164 amino acids HA deletion, reacted poorly with these antisera. Titers observed for both influenza B lineages with post-vaccination human serum panels were systematically, even if not significantly, reduced compared to those obtained with post-infection ferret antisera.[8] Less than 0.5% of the influenza B viruses tested for antiviral resistance worldwide exhibited reduced susceptibility to oseltamivir, zanamivir or peramivir.[8,16]

Almost 99% of all influenza A viruses, A(H1N1)pdm09 and A(H3N2), are suspected of carrying substitutions in the M gene associated with resistance to M2 inhibitors. Not surprisingly, this was the case of all NRCI isolates for which the M gene was sequenced.

Despite the fact that avian influenza subtypes (H5 and H7) continue to circulate in wild birds and poultry, only a few human cases have been reported since 2017. All were of Asian origin and exhibited a genetic composition distinct from the corresponding subtypes currently circulating in birds in Europe. However, a tight surveillance of influenza viruses circulating in animals remains necessary in order to identify early animal-to-human transmissions of pandemic potential.

As of July 2019, influenza activity started to increase earlier this season in Australia, Chile, South Africa and New Zealand, with A(H3N2) viruses being predominant in Oceania and South Africa and influenza A(H1N1)pdm09 viruses in temperate South America.

10 Other activities of the NRCI

10.1 Validation and/or evaluation of assays

During the 2018/19 season, we performed a “post-validation” evaluation of the quadruplex rRT-PCR that we are using as a routine test for the subtypization of influenza A viruses since 2017/18. Of note, the 2017/18 influenza epidemic was dominated by influenza B and it was therefore difficult to assess the quadruplex performance. We know from the validation process that the N1 and H3 targets of the rRT-PCR can be less sensitive than the N2 and H1 targets. Indeed, we lost H3 and N1 detection in 5% and 8% of samples, respectively.

Fifty-six isolates (14% of the positive subtyped samples) representing 28 A(H1N1)pdm09 and 28 A(H3N2), were negative for N1 or H3 targets, respectively. Apart from eight samples (five A(H1N1)pdm09 and three A(H3N2), a decreased sensitivity was observed in samples with screening rRT-PCR Ct values > 30 (median of 33 for both targets). Fifteen and 13 of the 28 A(H1N1)pdm09 viruses and A(H3N2) (Ct values <35) with a negative N1 or H3, respectively, were submitted to sequencing. All were recovered successfully with sequences similar to “fully” rRT-PCR subtyped isolates. No significant differences could be found at the sequence level that could explain the loss of sensitivity. N1 or H3 negative samples did not cluster together and were distributed along N1 or H3 positive ones. The initial sample quality, coupled with the lower PCR efficiency of the N1 and H3 targets, may certainly account for the loss in sensitivity.

Seven influenza A remained unsubtyped this season; all had screening rRT-PCR Ct values >34.5. All were tested with the former H1 and H3 rRT-PCRs and only three would have been further subtyped.

Overall, the new quadruplex rRT-PCR allowed to “fully” subtype 84.2% of influenza A positive samples. On the basis of these results, the new quadruplex will be retained as the main rRT-PCR subtyping tool for influenza A positive samples. Of note, the H1 and H3 rRT-PCRs remain available if needed.

10.2 Sharing of influenza cell-cultured isolates and/or reference strains

1. Shared material: *Reference strain A/California/07/09 and MDCK-SIAT cells.*

   With whom: *Professor Caroline Tapparel, Department of Medicine and Molecular Microbiology, University of Geneva Faculty of Medicine.*

   Project: *"Nanomaterials coated with a2.6 linked sialic acid trisaccharide were previously tested against different influenza virus strains including H1N1, H3N2 and influenza B with good inhibitory activities. We evidenced also a switch in receptor dependence from a2.6 to a2.3 if the virus was previously serially passaged in eggs. The aim of testing recently isolated clinical strains is to verify if the protection shown against the laboratory strain is maintained against circulating strains with minor adaptation to cell lines."*

2. Shared material: *Reference strains A/Switzerland/9715293/2013, B/Phuket/ 3073/2013, B/Brisbane/60/2008 and A/Hong Kong/4801/2014*

   With whom*: Dr. Stefan Kuster (PI), Department of Infectious Diseases and Hospital Epidemiology, University Hospital of Zürich; and Professor Alexandra Trkola (Co-PI), Institut für Medizinische Virologie, University of Zürich.*

   Project: *"Influenza neutralization assays covering the strains circulating during 2015/16 and 2016/17 seasons in the context of an SNF project on flu transmission in the hospital setting."*

3. Shared material: *Isolates A/Switzerland/7197/12 and A/Switzerland/446/12; reference strains A/Switzerland/9715293/2013, A/Victoria/361/11 and A/Texas/50/12; MDCK and MDCK-SIAT cells.*

   With whom*: Dr Christoph Berger, Translational Immunology, Department of Biomedicine, University Hospital Basel.*

   Project: *"Examination of influenza immune escape at hemagglutinin position 128: The goal of our research is to study how viral mutations in the influenza hemagglutinin affect immune recognition by the vaccine-induced antibody response in humans. We address this in clinical cohorts of healthy volunteers vaccinated with the seasonal influenza vaccine at the University Hospital Basel. The study is approved by the ethics committee of Northwestern Switzerland (EKNZ)."*

4. Shared material: *Reference strains A/New Caledonia/20/99, A/California/07/09, A/Texas/50/12 and A/Singapore/INFIM-0016-19/16; MDCK and MDCK-SIAT cells.*

   With whom*: Professor Bruno Correia, Laboratory of Protein Design & Immunoengineering, Ecole polytechnique fédérale de Lausanne*

   Project: *"Epitope-focused immunogen design toward the development of an 'universal' influenza vaccine: using computational design and experimental improvement, we are generating small protein scaffolds, called epitope-focused immunogens, presenting isolated broadly neutralizing epitopes of influenza HA. The selected epitopes are highly conserved and targeted in varying ways by a variety of antibodies originating from different germ lines and effective against various strains. By combining the different epitope-focused immunogens and multimerizing them on nanoparticles, we aim to induce and boost the production*

of broadly neutralizing antibodies in a scenario of pre-existing immunity. This subunit vaccine represents a promising approach for the development of a secure and highly specific way to induce a broad and long-lasting protection."

Onsite training (2.5 days at the NRCI): Sarah Wehrle, Doctoral Assistant, Laboratory of Protein Design & Immunoengineering, in the context of this project (March 2019).

10.3 Collaborative projects/publications

1. Poster submitted for the WHO conference: "Moving influenza burden estimate to policy decisions and estimating the whole influenza burden pyramid", 25-27 June 2019; and for "OPTIONS X for the control of influenza", 28 August - 1 September 2019 (accepted for both conferences).

   "Hospital-based surveillance of influenza in Switzerland – a pilot study"
   Amaury Thiabaud, Anne Iten, Nicolas Troillet, Laurence Senn, Domenica Flury, Stefan Kuster, Carlo Balmelli, Céline Gardiol, Ana Rita Gonçalves Cabecinhas, Laurent Kaiser, Olivia Keiser.

2. Poster submitted for the "Réunion annuelle commune 2019 SSMI | SSinf | SSHH | SSMTP | SSMTV" (accepted).

   "Managing influenza in a nursing home: insights from the 2019 Delémont outbreak"
   S Meylan; M Burr; AR Gonçalves; C Nusbaumer; L Brockhaus.

3. Influenza A virus genetic tools: from clinical sample to molecular clone

   Stéphanie Anchisi, Ana Rita Gonçalves, Béryl Mazel-Sanchez, Samuel Cordey, and Mirco Schmolke
   https://link.springer.com/protocol/10.1007%2F978-1-4939-8678-1_3

4. "Que se cache-t-il derrière la grippe ?"

   Ana Rita Gonçalves and Laurent Kaiser
   https://medicalforum.ch/fr/article/doi/smf.2019.08066/

5. Antigen extraction and B cell activation enable identification of rare membrane antigen specific human B cells

   Maria Zimmermann, Natalie Rose, John M. Lindner, Hyein Kim, Ana Rita Gonçalves, Ilaria Callegari, Mohammedyaseen Syedbasha, Lukas Kaufmann, Adrian Egli, Raija L. P. Lindberg, Ludwig Kappos, Elisabetta Traggiai, Nicholas S. R. Sanderson and Tobias Derfuss
   https://www.frontiersin.org/articles/10.3389/fimmu.2019.00829/full

6. Submitted SNF grant (2019), by Professor Olivia Keiser: "Online analytical platform for influenza surveillance"

   International collaborative project (Switzerland-Brazil).

10.4 Work in progress

1. The validation of a new rRT-PCR H5 combination (duplex) is being currently finalized. It will be added to the list of "available tests" for the 2019/20 influenza season and a validation description will be provided in the 2019/20 annual report. The rationale behind this development came from the observation that our current H5 rRT-PCR sensitivity was decreasing, particularly for the A(H5N6) strains (WHO EQAP panels).

   As A(H5N8) viruses are circulating in European birds, including Switzerland, and A(H5N6) strains are found in humans, rRT-PCRs specific for N6 and N8 genes are already available and functional at the NRCI and these will be submitted to our institutional validation process in the near future. We expect these rRT-PCRs to be part of "routine" methods for 2020/21 at the latest.

   Of note, if not initiated in response to the emergence of a new pandemic virus, institutional validation of new rRT-PCRs for viruses of animal origin (H5, H7, H9…) is only performed when no human influenza viruses are being processed in the laboratory (June-August).

2. We are currently finalizing the sanger sequencing procedure for the assessment of the presence of amino acid substitutions associated with PA inhibitors resistance. The test will be validated/used during 2019/20 influenza season. We are also planning to develop a complementary phenotypic, test normally during June-August 2020.

3. Specific whole genome sequencing implementation process at the NRCI is currently being finalized and the institutional validation process will take place during the 2019/20 season. It is foreseen to be coupled with a newly-updated Sanger sequencing strategy, which is currently under investigation. Sanger sequencing will remain the main sequencing method for the 2019/20 influenza season. However the use of whole genome sequencing for surveillance purposes may be addressed in the future.

4. The Institute of Medical Virology in Zürich has been identified as the "partner laboratory" for the NRCI in the case of a new influenza pandemic. The final version of the document describing each partner's tasks and duties ("diagnostic concept in the case of an influenza pandemic") is expected to be finalized at the

end of 2019-beginning 2020. A working group meeting of NRCI, IMV and FOPH partners will be held on 08.08.2019 at the Liebefeld FOPH Campus.

5. We have and will continue to participate to the new project initiated by the FOPH : “Hospital-based surveillance of influenza in Switzerland – a pilot study”. The goal of the project will be to establish a sustainable hospital-based sentinel surveillance for severe influenza in Switzerland. This will complement the follow up of hospitalized influenza cases, we have been performing, for many years, at the Geneva University Hospitals in collaboration with the Hospital Infection Prevention and Control Unit.

6. A new influenza type, influenza D, has been shown to infect cattle. Switzerland has an important cattle population and there is serologic evidence, particularly in the USA, of transmission of Influenza D virus to humans, even if the infective potential of this virus remains unknown. We aim to validate a rRT-PCR targeting influenza D in order to screen human nasopharyngeal samples for the presence of this virus. This could be a collaborative project with Professor Ronald Dijkman, University of Bern.

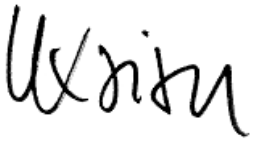
7. We are currently working with the Geneva Centre for Emerging Viral Diseases in order to establish a shared serobank of characterized human sera that will be especially useful in order to update current serology tests for influenza in particular, but not only (deadline to be discussed).

Geneva, 31 July 2019

Ana Rita Gonçalves Cabecinhas, PhD

M[rs] Patricia Suter-Boquete

Professor Laurent Kaiser, MD



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Annex 1: Weekly report of influenza virus detection and virus characteristics

Sentinel Surveillance, Season 2018-19														
Weeks	Dates		‰ ILI	Samples received	Influenza A				Influenza B				Total virus (n)	% pos
					Undet.	A (H1N1) pdm09	A (H3N2) seasonal	Total	Undet.	Bvic	Byam	Total		
40	30-Sep-18	6-Oct-18	1.7	8	0	0	0	0	0	0	0	0	0	0.00
41	7-Oct-18	13-Oct-18	1.2	9	0	0	0	0	0	0	0	0	0	0.00
42	14-Oct-18	20-Oct-18	2	8	0	0	0	0	0	0	0	0	0	0.00
43	21-Oct-18	27-Oct-18	1.1	6	0	0	0	0	0	0	0	0	0	0.00
44	28-Oct-18	3-Nov-18	2.4	16	0	0	1	1	0	0	0	0	1	6.25
45	4-Nov-18	10-Nov-18	1.9	17	0	1	0	1	0	0	0	0	1	5.88
46	11-Nov-18	17-Nov-18	1.9	24	0	1	0	1	0	0	0	0	1	4.17
47	18-Nov-18	24-Nov-18	4.1	21	0	0	0	0	0	0	0	0	0	0.00
48	25-Nov-18	1-Dec-18	2.7	20	0	1	1	2	0	0	0	0	2	10.00
49	2-Dec-18	8-Dec-18	4	32	0	2	4	6	0	0	0	0	6	18.75
50	9-Dec-18	15-Dec-18	2.9	24	0	2	1	3	1	0	0	1	4	16.67
51	16-Dec-18	22-Dec-18	5.5	44	0	2	2	4	0	0	0	0	4	9.09
52	23-Dec-18	29-Dec-18	8.1	16	0	3	4	7	0	0	0	0	7	43.75
1	30-Dec-18	5-Jan-19	13.3	31	0	4	2	6	0	0	0	0	6	19.35
2	6-Jan-19	12-Jan-19	14.8	73	0	21	2	23	0	0	0	0	23	31.51
3	13-Jan-19	19-Jan-19	17.2	73	0	22	13	35	0	0	0	0	35	47.95
4	20-Jan-19	26-Jan-19	25.9	64	1	17	18	36	0	0	0	0	36	56.25
5	27-Jan-19	2-Feb-19	31.2	77	1	17	25	43	0	0	1	1	44	57.14
6	3-Feb-19	9-Feb-19	39.8	93	0	27	33	60	0	0	0	0	60	64.52
7	10-Feb-19	16-Feb-19	40.2	78	1	21	30	52	0	0	0	0	52	66.67
8	17-Feb-19	23-Feb-19	31.7	48	1	11	20	32	0	0	0	0	32	66.67
9	24-Feb-19	2-Mar-19	25	47	1	6	22	29	0	0	0	0	29	61.70
10	3-Mar-19	9-Mar-19	17.7	34	1	9	11	21	0	0	0	0	21	61.76
11	10-Mar-19	16-Mar-19	13.6	30	1	3	11	15	0	0	0	0	15	50.00
12	17-Mar-19	23-Mar-19	10.1	31	0	3	4	7	0	0	0	0	7	22.58
13	24-Mar-19	30-Mar-19	5.9	27	0	0	9	9	0	0	0	0	9	33.33
14	31-Mar-19	6-Apr-19	3.1	18	0	1	1	2	0	0	0	0	2	11.11
15	7-Apr-19	13-Apr-19	3.3	21	0	1	2	3	0	0	0	0	3	14.29
16	14-Apr-19	20-Apr-19	1.2	11	0	0	1	1	0	0	0	0	1	9.09
					7	**175**	**217**		**1**	**0**	**1**			
				1001	**399**				**2**				**401**	

‰ ILI: Medical consultations for influenza-like illness (‰)

Undet.: Not determined or insufficient viral load

A(H1N1)pdm09 : Influenza A (H1N1) pandemic 2009

BVic: Influenza B Victoria lineage

BYam: Influenza B Yamagata lineage

Annex 2a: Hemagglutination inhibition data of influenza A(H1N1)pdm09 viruses

			Antisera		
		Reference viral isolates	A/California/07/09	A/Michigan/45/15	A/St. Petersburg/27/11
		A/California/07/09	**128**	128	128
		A/Michigan/45/15*	64	**64**	64
		A/St Petersburg/27/11	128	128	**128**
Isolates	**HA titre**	**Typisation**			
6719	**16**	**A/California/7/09-like**	128	512	128
5437	**64[§]**	**A/Michigan/45/15-like**	64	128	64
6296[@]	**64[§]**	**A/St Petersburg/27/11-like**	64	128	128
5125	**16**	**A/St Petersburg/27/11-like**	64	256	128
4806[@]	**16[§]**	**A/Michigan/45/15-like**	128	64	128
7147[@]	**32[§]**	**A/St Petersburg/27/11-like**	256	256	128
8061[@]	**64[§]**	**A/St Petersburg/27/11-like**	128	256	128
9036[@]	**128**	**A/St Petersburg/27/11-like**	128	128	128
0671[@]	**32[§]**	**A/Michigan/45/15-like**	64	128	64
5541	**64**	**A/Michigan/45/15-like**	32	32	32
5126	**32**	**A/California/7/09-like**	128	512	256
7209	**64**	**A/Michigan/45/15-like**	16	16	16
4217	**32**	**A/St Petersburg/27/11-like**	128	256	128
4367	**32**	**A/St Petersburg/27/11-like**	128	256	128
4553	**32**	**A/St Petersburg/27/11-like**	128	256	128
4571	**32**	**A/St Petersburg/27/11-like**	256	512	256
4642	**64**	**A/St Petersburg/27/11-like**	128	128	128
7105	**64**	**A/Michigan/45/15-like**	32	64	32

HA titers were established in MDCK-SIAT cells (S/1) or [§]MDCK cells (MD/1). **HI titers should be multiplied by 8.** [@]also sent to the WIC. Vaccine strain.

Annex 2b: Hemagglutination inhibition data of influenza A(H1N1)pdm09 viruses

			Antisera		
		Reference viral isolates	A/California/07/09	A/Michigan/45/15	A/St. Petersburg/27/11
		A/California/07/09	**128**	128	128
		A/Michigan/45/15*	64	**64**	64
		A/St Petersburg/27/11	128	128	**128**
Isolates	HA titre	Typisation			
7274	32	A/St Petersburg/27/11-like	128	512	128
7414	64	A/St Petersburg/27/11-like	256	256	128
8639	64	A/St Petersburg/27/11-like	256	512	256
8775	32	A/California/7/09-like	128	256	256
8840	32	A/St Petersburg/27/11-like	256	256	128
7438	128	A/St Petersburg/27/11-like	512	256	128
1166	128	A/St Petersburg/27/11-like	256	128	128
5133	64	A/Michigan/45/15-like	64	128	64
7081	32	A/St Petersburg/27/11-like	128	128	128
8677	128	A/Michigan/45/15-like	32	32	32
0714	64	A/St Petersburg/27/11-like	256	256	256
0964	64	A/California/7/09-like	128	256	256
2283	128	A/Michigan/45/15-like	16	32	32
0788	128	A/Michigan/45/15-like	64	64	64
9718	64	A/Michigan/45/15-like	64	64	64
9902	64	A/St Petersburg/27/11-like	128	128	128
0099	128	A/St Petersburg/27/11-like	128	128	128
9972	64	A/St Petersburg/27/11-like	128	128	128

HA titers were established in MDCK-SIAT (S/1) or [§]MDCK cells (MD/1). **HI titers should be multiplied by 8.** Vaccine strain.

Annex 2c: Hemagglutination inhibition data of influenza A(H1N1)pdm09 viruses

			Antisera		
		Reference viral isolates	A/California/07/09	A/Michigan/45/15	A/St. Petersburg/27/11
		A/California/07/09	**128**	128	128
		A/Michigan/45/15*	64	**64**	64
		A/St Petersburg/27/11	128	128	**128**
Isolates	**HA titre**	**Typisation**			
8251	**64**	**A/St Petersburg/27/11-like**	128	128	128
4025	**64**	**A/St Petersburg/27/11-like**	128	128	128
6005	**128**	**A/St Petersburg/27/11-like**	128	128	128
5306	**32**	**A/St Petersburg/27/11-like**	512	256	128
7268	**32**	**A/Michigan/45/15-like**	16	32	32
7387	**32**	**A/St Petersburg/27/11-like**	256	256	256
8332	**64**	**A/St Petersburg/27/11-like**	256	128	256

HA titers were established in MDCK-SIAT (S/1) or [§]MDCK cells (MD/1). **HI titers should be multiplied by 8.** Vaccine strain.

Annex 3a: Hemagglutination inhibition data of influenza A(H3N2) viruses

			Antisera		
		Reference viral isolates	*A/Hong Kong/4801/14*	*A/Switzerland/9715293/13*	*A/Singapore/INFIM-16-0019/16**
		A/Hong Kong/4801/14	**128**	128	128
		A/Switzerland/9715293/13	32	**64**	64
		A/Singapore/INFIM-16-0019/16	128	128	**128**
Isolates	**HA titre**	**Typisation**			
0293	**64[§]**	**A/Singapore/INFIM-16-0019/16-like**	64	<16	256
4912	**64**	**A/Switzerland/9715293/13-like**	64	64	64
4791	**64[§]**	**A/Singapore/INFIM-16-0019/16-like**	128	128	128
0090	**64**	**A/Singapore/INFIM-16-0019/16-like**	256	256	256
9020	**64**	**A/Switzerland/9715293/13-like**	32	32	32
5699	**64**	**A/Singapore/INFIM-16-0019/16-like**	256	512	128
5733	**32**	**A/Singapore/INFIM-16-0019/16-like**	256	256	256
7145	**32**	**A/Hong Kong/4801/14-like**	256	512	512
1150	**128**	**A/Singapore/INFIM-16-0019/16-like**	256	256	256
1184	**128**	**A/Switzerland/9715293/13-like**	64	32	32
5172	**64**	**A/Singapore/INFIM-16-0019/16-like**	256	256	256
7609	**128**	**A/Singapore/INFIM-16-0019/16-like**	128	128	128
8647	**128**	**A/Switzerland/9715293/13-like**	64	64	64
8745	**128**	**A/Singapore/INFIM-16-0019/16-like**	64	128	128
2166	**128[§]**	**A/Switzerland/9715293/13-like**	64	64	64
5204	**128**	**A/Switzerland/9715293/13-like**	64	64	128
5108	**128**	**A/Singapore/INFIM-16-0019/16-like**	32	32	32
5720	**32**	**A/Hong Kong/4801/14-like**	128	256	256

HA titers were established in MDCK-SIAT (S/1) cells or [§]MDCK (MD/1). **HI titers should be multiplied by 8**. Vaccine strain.

Annex 3b: Hemagglutination inhibition data of influenza A(H3N2) viruses

			Antisera		
		Reference viral isolates	*A/Hong Kong/4801/14*	*A/Switzerland/9715293/13*	*A/Singapore/INFIM-16-0019/16**
		A/Hong Kong/4801/14	**128**	128	128
		A/Switzerland/9715293/13	32	**64**	64
		A/Singapore/INFIM-16-0019/16	128	128	**128**
Isolates	**HA titre**	**Typisation**			
7821	**128**	**A/Switzerland/9715293/13-like**	64	64	64
9707	**64**	**A/Switzerland/9715293/13-like**	32	32	32
0009	**64**	**A/Switzerland/9715293/13-like**	64	64	64
0472	**64**	**A/Singapore/INFIM-16-0019/16-like**	256	64	64
7945	**128**	**A/Singapore/INFIM-16-0019/16-like**	128	128	128
0538	**64**	**A/Switzerland/9715293/13-like**	64	64	64
5196	**64[§]**	**A/Singapore/INFIM-16-0019/16-like**	256	128	128
5555	**64[§]**	**A/Switzerland/9715293/13-like**	32	32	64
4995	**64[§]**	**A/Switzerland/9715293/13-like**	128	64	64
5032	**64[§]**	**A/Switzerland/9715293/13-like**	64	32	64
5254	**64**	**A/Hong Kong/4801/14-like**	128	128	64
5580	**64**	**A/Singapore/INFIM-16-0019/16-like**	64	128	128
8269	**32**	**A/Singapore/INFIM-16-0019/16-like**	128	256	128
0029	**64**	**A/Singapore/INFIM-16-0019/16-like**	128	256	128
0424	**16**	**A/Singapore/INFIM-16-0019/16-like**	128	256	128
6543	**64**	**A/Singapore/INFIM-16-0019/16-like**	256	256	256
5740	**64**	**A/Singapore/INFIM-16-0019/16-like**	256	256	256
6968	**64**	**A/Singapore/INFIM-16-0019/16-like**	128	128	128

HA titers were established in MDCK-SIAT (S/1) cells or [§]MDCK (MD/1). **HI titers should be multiplied by 8**. Vaccine strain.

Annex 3c: Hemagglutination inhibition data of influenza A(H3N2) viruses

			Antisera		
		Reference viral isolates	*A/Hong Kong/4801/14*	*A/Switzerland/9715293/13*	*A/Singapore/INFIM-16-0019/16**
		A/Hong Kong/4801/14	**128**	128	128
		A/Switzerland/9715293/13	32	**64**	64
		A/Singapore/INFIM-16-0019/16	128	128	**128**
Isolates	**HA titre**	**Typisation**			
6997	**64**	**A/Singapore/INFIM-16-0019/16-like**	128	128	128
6764	**64**	**A/Singapore/INFIM-16-0019/16-like**	64	128	128
5424	**64**	**A/Singapore/INFIM-16-0019/16-like**	128	128	128

HA titers were established in MDCK-SIAT (S/1) cells or [§]MDCK (MD/1). **HI titers should be multiplied by 8**. Vaccine strain.

Annex 4: Hemagglutination inhibition data of influenza B Yamagata lineage viruses

			Antisera		
		Reference viral isolates	B/Wisconsin/1/10	B/Novosibirsk/1/12	B/Phuket/3073/13
		B/Wisconsin/1/10	128	64	64
		B/Novosibirsk/1/12	128	256	128
		B/Phuket/3073/13	128	64	64
Isolates	HA titer	Typisation			
7847	128	B/Phuket/3073/13-like	32	128	32

HA titers were established MDCK cells. **HI titers should be multiplied by 8**. Vaccine strain.

Annex 5: Hemagglutination inhibition data of influenza A(H1N1)pdm09 viruses, WIC 22.01.2019

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre – Post-infection ferret antisera									
					A/Mich 45/15 Egg NIB F42/16*1	A/Cal 7/09 Egg F07/16*1	A/Bayern 69/09 MDCK F09/15*1	A/Lviv N6/09 MDCK F14/13*1	A/Astrak 1/11 MDCK F22/13*1	A/HK 5659/12 MDCK F17/15*1	A/Slov 2903/2015 Egg F02/16*1	A/Paris 1447/17 MDCK F03/18*2	A/Swit 2656/17 Egg F20/18*1	A/Swit 3330/17 Egg F23/18*1
		Genetic group			6B.1				5	6A	6B.1	6B.1A	6B.1A	6B.1A5
REFERENCE VIRUSES														
A/Michigan/45/2015		6B.1	2015-09-07	E3/E3	320	320	320	320	320	320	640	1280	320	320
A/California/7/2009	clone 38-32		2009-04-09	E3/E3	320	320	320	640	320	640	640	1280	640	320
A/Bayern/69/2009	G155E		2009-07-01	MDCK5/MDCK1	<	<	320	160	<	<	40	160	40	<
A/Lviv/N6/2009	G155E, D222G		2009-10-27	MDCK4/SIAT1/MDCK3	40	40	640	320	<	40	80	320	160	80
A/Astrakhan/1/2011		5	2011-02-28	MDCK1/MDCK7	320	320	640	320	160	320	640	1280	320	320
A/Hong Kong/5659/2012		6A	2012-05-21	MDCK4/MDCK2	320	160	160	160	160	320	640	1280	160	160
A/Slovenia/2903/2015	clone 37	6B.1	2015-10-26	E4/E2	320	320	160	160	320	320	640	1280	320	320
A/Paris/1447/2017		6B.1A	2017-10-20	MDCK1/MDCK3	160	160	160	80	160	160	640	1280	160	160
A/Switzerland/2656/2017		6B.1A	2017-12-21	E5/E2	640	640	640	320	640	640	1280	2560	1280	640
A/Switzerland/3330/2017	clone 35	6B.1A5	2017-12-20	E6/E2	320	160	160	160	160	160	640	1280	320	640
TEST VIRUSES														
A/Ghana/3763/2018		6B.1A	2018-11-07	C2/MDCK1	1280	640	320	160	320	640	1280	2560	640	640
A/Oman/6052/2018		6B.1A	2018-11-14	MDCK2	320	80	160	80	80	80	320	640	160	160
A/Estonia/118321/2018		6B.1A	2018-12-20	MDCK1	640	320	320	160	160	320	1280	1280	640	320
A/Estonia/118316/2018		6B.1A	2018-12-20	MDCK1	160	80	80	40	80	80	320	640	160	80
A/Estonia/118371/2018		6B.1A	2018-12-27	MDCK2	320	<	160	80	80	80	320	640	160	160
A/Bulgaria/028/2019		6B.1A	2019-01-07	MDCK1	320	320	160	80	160	320	640	1280	320	320
A/Bulgaria/041/2019		6B.1A	2019-01-08	MDCK1	640	320	320	160	160	320	1280	2560	640	320
A/Ghana/3865/2018		6B.1A5	2018-10-02	MDCK1	320	160	160	80	80	160	640	1280	320	160
A/Oman/5965/2018		6B.1A5	2018-11-11	MDCK1	640	80	160	160	160	160	640	1280	320	160
A/Lyon/CHU-R18.112.40/2018		6B.1A5	2018-11-15	MDCK2/MDCK1	320	160	160	80	80	160	640	1280	160	160
A/Lyon/2119/2018		6B.1A5	2018-11-22	MDCK2/MDCK1	640	160	320	160	160	320	640	1280	320	320
A/Oman/6319/2018		6B.1A5	2018-11-23	MDCK1	320	1280	160	160	160	320	640	1280	320	320
A/Oman/6338/2018		6B.1A5	2018-11-24	MDCK1	320	160	160	80	160	320	1280	1280	320	320
A/Ireland/84720/2018	D187X(D/N), S190X(S/R)	6B.1A5	2018-11-29	MDCK2/MDCK1	160	40	80	40	40	160	320	640	80	80
A/Clermont-Ferrand/2180/2018		6B.1A5	2018-12-03	MDCK2/MDCK1	160	80	80	80	80	160	320	640	80	80
A/Ireland/85958/2018		6B.1A5	2018-12-04	MDCK2/MDCK1	640	160	320	320	320	320	1280	2560	640	320
A/Ireland/85950/2018		6B.1A5	2018-12-04	MDCK2/MDCK1	640	160	320	160	320	320	1280	1280	320	640
A/Switzerland/4806/2018		6B.1A5	2018-12-10	MDCK2/MDCK1	640	320	320	320	320	320	1280	2560	640	640
A/Switzerland/6296/2018		6B.1A5	2018-12-12	MDCK1/MDCK1	320	40	160	160	80	160	640	1280	160	160
A/Lithuania/MB36587/2018		6B.1A5	2018-12-17	MDCK1/MDCK1	640	320	320	320	320	640	1280	2560	640	640
A/Switzerland/7147/2018		6B.1A5	2018-12-18	MDCK1/MDCK1	640	320	320	160	320	320	1280	1280	640	320
A/Estonia/118306/2018		6B.1A5	2018-12-19	MDCK1	640	320	320	160	160	320	640	1280	320	320
A/Estonia/118323/2018		6B.1A5	2018-12-20	MDCK1	640	160	160	160	160	160	640	1280	160	160
A/Estonia/118355/2018		6B.1A5	2018-12-21	MDCK1	1280	320	640	320	320	640	2560	2560	640	640
A/Switzerland/671/2018		6B.1A5	2018-12-27	MDCK1/MDCK1	320	160	160	160	160	320	640	1280	320	320
A/Bulgaria/043/2019		6B.1A5	2019-01-08	MDCK1	320	160	160	160	80	160	640	640	320	160
A/Oman/6133/2018		6B.1A7	2018-11-18	MDCK1	640	160	160	80	160	320	640	1280	320	320
A/Ireland/84816/2018		6B.1A7	2018-11-29	MDCK1/MDCK1	640	160	320	160	160	320	640	1280	320	160
A/Denmark/1848/2018		6B.1A7	2018-12-03	MDCK3/MDCK1	320	160	160	80	80	160	640	1280	320	320
A/Denmark/1839/2018		6B.1A7	2018-12-03	MDCK3/MDCK1	320	80	160	160	80	160	640	1280	320	320
A/Denmark/1849/2018		6B.1A7	2018-12-05	MDCK3/MDCK1	320	80	80	40	80	160	320	640	160	160
A/Denmark/1850/2018		6B.1A7	2018-12-06	MDCK3/MDCK1	320	40	160	80	80	160	640	640	320	160
A/Poitiers/2104/2018		6B.1A1	2018-11-13	MDCK3/MDCK1	320	80	160	40	80	160	320	1280	160	80
A/Oman/5755/2018		6B.1A2	2018-11-05	MDCK1	320	160	160	80	160	160	640	1280	320	160
A/Bulgaria/023/2019		6B.1A2	2019-01-07	MDCK1	320	160	160	80	160	160	640	1280	320	160
A/Oman/5940/2018		6B.1A6	2018-11-11	MDCK1	160	80	80	80	80	160	320	640	160	160
A/Macon/2132/2018		6B.1A6	2018-11-27	MDCK2/MDCK1	320	160	160	80	160	160	640	1280	320	160
A/Ireland/84630/2018		6B.1A6	2018-11-28	MDCK2/MDCK1	640	320	320	160	320	320	1280	2560	320	320
A/Saint-Etienne/2167/2018		6B.1A6	2018-11-28	MDCK2/MDCK1	320	160	160	80	160	160	640	1280	320	320
A/Denmark/1832/2018		6B.1A6	2018-11-29	MDCK3/MDCK1	320	<	160	160	80	160	640	1280	320	160
A/Denmark/1841/2018	S106X(S/N), F111X(F/I)	6B.1A6	2018-12-03	MDCK3/MDCK1	160	<	80	80	80	80	320	640	160	80
A/Switzerland/8061/2018		6B.1A6	2018-12-19	MDCK1/MDCK1	320	160	160	80	160	160	640	1280	320	320
A/Switzerland/9036/2018		6B.1A6	2018-12-21	SIAT1/MDCK1	160	80	80	80	80	160	320	640	160	160
				Vaccine										

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40; 2 < = <80

Annex 6a: Hemagglutination inhibition data of influenza A(H3N2) viruses, WIC 08.02.2019

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre – Post-infection ferret antisera: A/Stock 6/14 SIAT F14/14*1 3C.3a	A/HK 5738/14 MDCK F30/14*1 3C.2a	A/Bretagne 1413/17 SIAT F01/18*1 3C.2a2	A/Singapore 0019/16 Egg 10-4 F46/17*1 3C.2a1	A/La Rioja 2202/18 SIAT F26/18*1 3C.2a1b	A/Switz 8060/17 Egg F27/18*1 3C.2a2	A/Eng 538/18 SIAT F31/18*1 3C.3a	A/Neth 10260/18 Egg F02/19*1 3C.2a1b	A/Nor 3275/18 SIAT F03/19*1 3C.2a1b
REFERENCE VIRUSES													
A/Stockholm/6/2014		3C.3a	2014-02-06	SIAT1/SIAT3	320	40	80	320	80	160	320	<	80
A/Hong Kong/5738/2014		3C.2a	2014-04-30	MDCK1/MDCK2/SIAT1	160	80	160	320	160	160	160	40	160
A/Bretagne/1413/2017		3C.2a2	2017-10-09	MDCK1/SIAT4	160	40	640	320	80	640	160	40	160
A/Singapore/INFIMH-16-0019/2016		3C.2a1	2016-04-14	E5/E3	40	<	40	640	160	80	80	<	40
A/Switzerland/8060/2017	clone 57	3C.2a2	2017-12-12	E7/E1	80	80	2560	1280	160	1280	160	40	160
A/England/538/2018		3C.3a	2018-02-26	MDCK1/SIAT3	160	640	80	320	40	80	1280	<	40
A/Netherlands/10260/2018		3C.2a1b	2018-02-15	E5	40	<	80	160	320	160	80	1280	160
TEST VIRUSES													
A/Switzerland/293/2018			2018-11-01	MDCK1/SIAT3	160	40	80	160	160	160	160	<	320
A/Nizhny Novgorod/14645V/2018			2018-11-20	MDCK1/SIAT2	<	<	<	40	80	<	40	<	40
A/Hungary/5/2019			2018-12-19	Cx/SIAT1	160	<	<	80	160	<	40	<	160
A/Hungary/28/2019			2019-01-03	Cx/SIAT1	80	<	<	80	80	<	40	<	80
A/Hungary/26/2019			2019-01-07	Cx/SIAT1	160	40	40	80	320	80	80	40	80
A/Netherlands/10002/2019			2019-01-07	MDCK-MIX2/SIAT1	80	<	40	40	<	<	640	<	<
A/Armenia/91/2019			2019-01-14	SIAT1	160	<	40	160	160	40	80	<	80
A/Neth/10006/2019			2019-01-14	MDCK-MIX2/SIAT1	40	<	40	40	<	<	640	<	<
A/Albania/9142/2019			2019-01-19	SIAT1	80	<	<	80	80	40	40	<	40
								Vaccine SH 2018 NH 2018-19		Vaccine SH 2019			

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used) [1] < = <40

(Guinea pig RBCs with 20nM oseltamivir)

Annex 6b: Plaque reduction neutralization data of influenza A(H3N2) viruses, (MDCK-SIAT), WIC 24.01.2019

Viruses	Passage history / Ferret number / Genetic group	Collection Date	Passage History	Neutralisation Titre[1] Post-infection ferret antisera: A/Singapore INFIMH-16-0019/16 EGG 10^{-4} F46/17 3C.2a1		A/Singapore INFIMH-16-0019/16 SIAT F45/17 3C.2a1		A/Switzerland 8060/17 Egg F27/18 3C.2a2		A/HK 656/18 SIAT F25/18 3C.2a2		A/La Rioja 2202/18 SIAT F26/18 3C.2a1b		HA1 substitutions for 3C.2a(1) viruses compared to A/Hong Kong/4801/2014: Egg adaptation HA substitutions compared to the corresponding cell isolate	NA Polymorphism
				2-fold	Read	2-fold	Read	2-fold	Read	2-fold	Read	2-fold	Read		
REFERENCE VIRUSES															
A/Singapore/INFIMH-16-0019/2016	3C.2a1		E5/E2 10^{-4}	1280	1186	320	262	160	222	160	182	640	590	N121K, R142G, T160K (-CHO), N171K, L194P, D225G	
A/Singapore/INFIMH-16-0019/2016	3C.2a1		MDCK1/SIAT3/SIAT3	80	66	1280	1236	80	92	160	156	160	120	N121K, R142G, N171K	
A/Switzerland/8060/2017 DEF1	3C.2a2		E6(Am2Al4)c57 10^{-5}	1280	1180	320	244	2560	3812	5120	3907	160	124	N96S, T131K, R142K, R261Q, T160K (-CHO), L194P	
A/Hong Kong/656/2018	3C.2a2		MDCK1/SIAT1 10^{-1}	80	70	640	576	1280	1807	2560	2255	40	46	T131K, R142K, N158X (-CHO), A212T, R261Q, I300V	
A/La Rioja/2202/2018	3C.2a1b		SIAT1/SIAT1 10^{-1}	80	74	640	567	<	3	80	63	320	291	E62G, K92R, N121K, T128A (-CHO), T135K (-CHO), R142G, N171K, H311Q	
TEST VIRUSES															
A/Switzerland/293/2018	3C.2a1b	2018-11-01	MDCK1/SIAT1	<	10	160	206	40	49	80	67	40	46	E62G, K92R, N121K, T131K, R142G, N171K, H311Q	D151X
A/Switzerland/4912/2018	3C.2a1b	2018-11-28	MDCK1/SIAT1	40	20	640	495	80	99	160	135	80	68	E62G, K92R, N121K, T131K, R142G, N171K, H311Q	
A/Hong Kong/3165/2018	3C.2a1b	2018-12-06	MDCK1/SIAT1	40	40	320	313	<	0	<	3	40	20	E62G, K92R, N121K, T128A (-CHO), T135K (-CHO), S137F, A138S, R142G, N171K, F193S, H311Q	
A/Switzerland/4791/2018	3C.2a1b	2018-12-10	MDCK1/SIAT1	40	47	640	498	<	3	40	46	320	254	E62G, K92R, N121K, T128A (-CHO), T135K (-CHO), R142G, N171K, H311Q	
A/Bulgaria/1493/2018	3C.2a1b	2018-12-14	SIAT1	40	43	320	471	160	125	160	137	80	103	I34T, E62G, K82E, K92R, N121K, T131K, R142G, N171K, H311Q	
A/Switzerland/90/2018	3C.2a1b	2018-12-20	MDCK1/SIAT1	40	57	640	744	160	131	80	63	640	501	E62G, K92R, N121K, T128A (-CHO), T135K (-CHO), R142G, N171K, H311Q	
A/Hong Kong/46/2019	3C.2a1b	2018-12-24	MDCK1/SIAT1	<	10	160	122	640	712	<	10	80	103	D53N, E62G, K92R, N121K, T128A (-CHO), T135K (-CHO), I140L, R142G, N171K, P221L, H311Q	
A/Hong Kong/126/2019	3C.2a1b	2018-12-28	MDCK1/SIAT1	40	48	320	280	80	77	80	99	160	113	E62G, N81D, K92R, N121K, T131K, R142G, N171K, S219F, H311Q	
A/Hong Kong/118/2019	3C.2a1b	2018-12-28	MDCK1/SIAT1	80	91	320	458	80	107	160	140	160	147	E62G, K92R, N121K, T131K, R142G, N171K, Q197R, S219F, H311Q	
A/Hong Kong/123/2019	3C.2a1b	2019-01-02	MDCK1/SIAT1	40	56	320	348	<	1	<	<	160	237	D53N, E62G, K92R, N121K, T135K (-CHO), R142G, N171K, S209N, H311Q	
A/Hong Kong/122/2019	3C.2a1b	2019-01-02	MDCK1/SIAT1	40	44	320	340	<	0	40	40	320	244	D53N, E62G, K92R, N121K, T135K (-CHO), R142G, N171K, S209N, H311Q	
A/Bulgaria/018/2019	3C.2a1b	2019-01-04	SIAT1	40	40	320	403	160	117	160	125	80	87	E62G, K83E, K92R, N121K, T131K, R142G, N171K, H311Q	
A/Bulgaria/1344/2018	3C.2a2	2018-12-01	SIAT1	80	94	1280	960	5120	6400	5120	5954	80	76	R33Q, T131K, R142K, A212T, R261Q, D291E	
A/Bulgaria/1409/2018	3C.2a2	2018-12-04	SIAT1	160	123	1280	1167	5120	5565	5120	6244	160	150	R33Q, N126D (-CHO), T131K, R142K, A212T, R261Q, D291E	
A/Lithuania/MB35528/2018	3C.2a4	2018-12-07	SIAT2/SIAT1	80	61	640	498	80	62	80	71	320	437	N31S, I34V, D53N, R142G, S144R, Q173H, I192T, Q197H, I242L	
A/Ghana/792/2018	Pending	2018-12-04	SIAT1	80	70	320	351	160	196	40	43	320	262	HA incomplete	
A/Ghana/4101/2018	Pending	2018-12-08	SIAT1	80	79	320	356	40	40	40	58	320	251	HA incomplete	
				Vaccine SH 2018 NH 2018-19				Vaccine SH 2019							

[1] Antiserum dilution value (2-fold), equivalent to HI reading, closest to the actual computer read value from a digitized image (Read) causing 50% reduction in plaque formation

Annex 6c: Plaque reduction neutralization data of influenza A(H3N2) viruses, (MDCK-SIAT), WIC 28.01.2019

Viruses	Passage history Ferret number Genetic group	Collection Date	Passage History	Neutralisation Titre[1] Post-infection ferret antisera A/Singapore INFIMH-16-0019/16 EGG 10^{-4} F46/17 3C.2a1		A/Singapore INFIMH-16-0019/16 SIAT F45/17 3C.2a1		A/Switzerland 8060/17 Egg F27/18 3C.2a2		A/La Rioja 2202/18 SIAT F26/18 3C.2a1b		A/Norway 3275/18 SIAT F03/19 3C.2a1b		A/Netherlands 10260/18 Egg F02/19 3C.2a1b		HA1 substitutions for 3C.2a(1) viruses compared to A/Hong Kong/4801/2014: Egg adaptation HA substitutions compared to the corresponding cell isolate
				2-fold	Read	2-fold	Read	2-fold	Read	2-fold	Read	2-fold	Read	2-fold	Read	
REFERENCE VIRUSES																
A/Singapore/INFIMH-16-0019/2016	3C.2a1		E5/E2 10^{-4}	1280	1186	320	262	160	222	640	590	40	40	<	10	N121K,R142G,T160K (-CHO), N171K,L194P,D225G
A/Singapore/INFIMH-16-0019/2016	3C.2a1		MDCK1/SIAT3/SIAT3	80	66	1280	1236	80	92	160	120	1280	1222	40	20	N121K,R142G,N171K
A/Switzerland/8060/2017 DEF1	3C.2a2		E6(Am2Al4)c57 10^{-5}	1280	1180	320	244	2560	3812	160	124	160	151	40	40	N96S, T131K, R142K, T160K (-CHO), L194P, R261Q
A/La Rioja/2202/2018	3C.2a1b		SIAT1/SIAT1 10^{-1}	80	74	640	567	<	3	320	291	80	72	40	40	E62G, K92R, N121K, T128A (-CHO), T135K (-CHO), R142G, N171K, H311Q
A/Norway/3275/2018	3C.2a1b		SIAT1	<	10	320	271	80	90	40	54	640	524	<	6	E62G, K92R, A106V, N121K, T131K, R142G, N171K, H311Q
A/Netherlands/10260/2018	3C.2a1b		E5 10^{-5}	80	78	320	313	80	82	320	359	160	160	5120	4998	E62G, K92R, N121K, T128A (-CHO), T135K (-CHO), R142G, T160K (-CHO), N171K, G186V, R261L, S219F, D225G, H311Q
TEST VIRUSES																
A/Brasov/239416/2018	3C.2a1b	2018-12-18	SIAT1/SIAT1	80	112	1280	997	80	95	320	303	1280	1564	80	116	K2E, E62G, K92R, N121K, T131K, R142G, N171K, P227S, H311Q
A/Brasov/239414/2018	3C.2a1b	2018-12-18	SIAT1/SIAT1	40	59	640	498	160	172	320	281	320	254	<	5	Q57R, E62G, K92R, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, H311Q
A/Bucuresti/239376/2018	3C.2a1b	2018-12-18	SIAT1/SIAT1	40	56	320	433	<	0	320	395	320	351	<	10	E62G, K92R, A106V, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, S198P, H311Q
A/Linkoping/72/2018	3C.2a1b	2018-12-21	MDCK0/SIAT1	40	20	320	309	80	65	40	54	640	556	<	0	K2E, E62G, K92R, N121K, T131K, R142G, N171K, H311Q
A/Bucuresti/239696/2018	3C.2a1b	2018-12-23	SIAT1/SIAT1	80	61	320	451	<	10	160	169	160	217	<	0	E62G, K92R, A106V, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, S198P, H311Q
A/Switzerland/5733/2018	3C.2a1b	2018-12-27	SIAT1	80	70	1280	1157	320	411	320	267	2560	3151	80	70	K2E, E62G, K92R, N121K, T131K, R142G, N171K, D188N, H311Q
A/Switzerland/5699/2018	3C.2a1b	2018-12-27	SIAT1	40	54	640	558	<	10	320	384	160	204	40	20	E62G, K92R, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, H311Q
A/Bucuresti/3586-4138/2018	3C.2a1b	2018-12-27	SIAT1/SIAT1	<	10	320	253	40	58	40	50	640	667	<	10	K2E, E62G, K92R, N121K, T131K, R142G, N171K, H311Q
A/Switzerland/7145/2019	3C.2a1b	2019-01-03	SIAT1	40	40	320	384	<	5	320	292	160	158	<	10	E62G, K92R, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, H311Q
A/Slovenia/11/2019	3C.2a1b	2019-01-03	SIATx/SIAT1	40	56	640	486	<	<	320	256	160	227	<	10	E62G, K92R, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, H311Q
A/Iasi/239544/2019	3C.2a1b	2019-01-04	SIAT1/SIAT1	80	63	640	512	<	5	320	473	160	237	40	20	E62G, K92R, A106V, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, S198P, H311Q
A/Slovenia/71/2019	3C.2a1b	2019-01-07	SIATx/SIAT1	80	77	640	563	<	1	640	524	160	219	40	20	E62G, K92R, N121K, T128A (-CHO), T135K(-CHO), R142G, N171K, I192V, A304T, H311Q
A/Slovenia/99/2019	3C.2a1b	2019-01-08	SIATx/SIAT1	40	20	320	290	40	45	40	40	640	786	40	20	D53N, E62G, K83E, K92R, N121K, T131K, R142G, N171K, K276N, H311Q
A/Bosnia and Herzegovina/69/2018	3C.2a2	2018-12-27	SIAT1	40	51	640	587	1280	1690	80	80	1280	1171	40	40	N122T, T131K, R142K, R261Q
				Vaccine SH 2018 NH 2018-19				Vaccine SH 2019								

[1] Antiserum dilution value (2-fold), equivalent to HI reading, closest to the actual computer read value from a digitized image (Read) causing 50% reduction in plaque formation

Annex 7: List of reference antisera provided by the WIC for the 2018/19 season

Reference antisera	Source
A/California/7/2009	F07/16
A/Michigan/45/2015	F32/16
A/St Petersburg/27/2011	F23/11
A Hong Kong/4801/2014 egg	F41/15
A/Switzerland/9715293/2013 clone123 egg	NIB F43/16
A/Singapore/INFIMH-16-0019/2016 egg	F46/17
B/Wisconsin/1/2010	F10/13
B/Novosibirsk/1/2012	F17/14
B/Phuket/3073/2013	NIB F51/16
B/Brisbane/60/2008	F52/16
B/Hong Kong/514/2009	F9/13
B/Johannesburg/3964/2012	F04/16
B/Hong Kong/269/17 egg	F50/17
B/Norway/2409/2017	F20/17
B/Colorado/6/17 egg	F10/18

Annex 8: Sequencing primers used during the 2018/19 season

Primers used for classical RT-PCR detection of influenza viruses				
Influenza virus	**Target gene**	**Primer or probe**		**Origin and reference**
A(H1N1)pdm09	Hemagglutinin (H1)	Forward	cswHAF31	R.Daniel, MRC-NIMR Feb 2011
		Reverse	AH1p873	
		Reverse	cswHAR1263	
		Reverse	cswAH1p1313R	
	Neuraminidase (N1)	Forward	cswN1F1	R.Daniel, MRC-NIMR
		Forward	cswN1F401	
		Reverse	cswN1R424	
		Forward	cswN1F1076	
		Reverse	cswN1R1099	
		Reverse	cswN1R1424	
		Reverse	cswN1R1440	
	Matrix	Forward	M93c	Y. Thomas, CNRI, Aug 2009
		Reverse	MF821Y	
A/H3N2 seasonal	Hemagglutinin (H3)	Forward	AH3G	J. Ellis London Jan 2006
		Reverse	AH3H	
		Forward	AH3B	
		Reverse	AH3CII	
		Reverse	AH3I	
		Forward	H3HAF567	
		Reverse	H3HAR650	
	Neuraminidase (N2)	Forward	H3N2F1	V. Gregory , MRC-NIH Modified by Y. Thomas, Mar 2011
		Reverse	N2R410	
		Forward	N2F387	
		Reverse	N2R1104	
		Forward	N2F1083	
		Reverse	N2R1447	
	Matrix	Forward	M93c	Y.Thomas, CNRI, Feb 2007
		Reverse	MF820R	
*B seasonal	Hemagglutinin	Forward	BHA1F1	V.Gregory, MRC-NIMR Jan 2006
		Reverse	BHA1R1	
		Forward	BHAF	
		Forward	BHA25	
		Forward	BHAF458	
		Reverse	BHAR652	
	Neuraminidase	Forward	BNAF5	V. Gregory , MRC-NIMR Modified by Y.Thomas, 2011
		Forward	BNAF310	
		Forward	BNAF725	
		Forward	BNAF1496	
		Reverse	BNAR1487	
		Reverse	BNAR1119	
		Reverse	BNAR748	

*Except for the WHO EQAP panel 18, no influenza B viruses were sequenced during the 2018/19 influenza season.